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Direct and indirect effects of elevated CO_2 on leaf respiration in a forest ecosystem

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ABSTRACT

We measured the short-term direct and long-term indirect effects of elevated CO_2 on leaf dark respiration of loblolly pine (*Pinus taeda*) and sweetgum (*Liquidambar styraciflua*) in an intact forest ecosystem. Trees were exposed to ambient or ambient + 200 μmol mol^{-1} atmospheric CO_2 using free-air carbon dioxide enrichment (FACE) technology. After correcting for measurement artefacts, a short-term 200 μmol mol^{-1} increase in CO_2 reduced leaf respiration by 7–14% for sweetgum and had essentially no effect on loblolly pine. This direct suppression of respiration was independent of the CO_2 concentration under which the trees were grown. Growth under elevated CO_2 did not appear to have any long-term indirect effects on leaf maintenance respiration rates or the response of respiration to changes in temperature (Q_{10}, R_0). Also, we found no relationship between mass-based respiration rates and leaf total nitrogen concentrations. Leaf construction costs were unaffected by growth CO_2 concentration, although leaf construction respiration decreased at elevated CO_2 in both species for leaves at the top of the canopy. We conclude that elevated CO_2 has little effect on leaf tissue respiration, and that the influence of elevated CO_2 on plant respiratory carbon flux is primarily through increased biomass.

Key-words: *Liquidambar styraciflua*; *Pinus taeda*; free-air carbon dioxide enrichment (FACE); loblolly pine; sweetgum.

INTRODUCTION

To predict the influence of elevated CO_2 on plant metabolism and carbon budgets, one must understand the response of both photosynthesis and leaf respiration. Experiments using growth chambers, open-top chambers and free-air carbon dioxide enrichment (FACE) rings have shown that photosynthesis increases in response to elevated CO_2 (Gunderson & Wullschleger 1994; Curtis & Wang 1998; Saxe, Ellsworth & Heath 1998; Norby *et al*. 1999); however, there is no consensus on the effects on leaf respiration (Norby *et al*. 1999). Complicating any interpretation of the response of leaf respiration is the possibility of both short-term direct and long-term indirect effects (acclimation) (Amthor 1991; Amthor 1997).

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Several reports have suggested that elevated CO_2 may suppress leaf respiration rate immediately through direct interaction with respiratory enzymes (Thomas & Griffin 1994; Amthor 1997; Drake *et al*. 1999; McDowell *et al*. 1999; Baker *et al*. 2000). However, a number of researchers have failed to find a suppression (Amthor 1997; Tjoelker, Oleksyn & Reich 1999a; Amthor 2000a). The existence of a direct effect continues to be controversial because a number of measurement artefacts – such as the diffusion of CO_2 into or out of the measurement cuvette – can produce such observations (Amthor 1997; McDermitt *et al*. 2001). Further proposed mechanisms, such as carbamylation of proteins or direct inhibition of respiratory enzymes, cannot adequately explain the amount of suppression observed (Gonzàlez-Meler, Drake & Azcón-Bieto 1996; Drake *et al*. 1999; Gonzàlez-Meler & Siedow 1999).

Elevated CO_2 may also influence leaf respiration indirectly by altering growth rate, non-structural carbohydrate concentration and other changes in tissue composition. Recent meta-analyses have found that elevated CO_2 reduces leaf respiration rates by an overall 18% when expressed on a mass basis (μmol CO_2 g leaf tissue^{-1} s^{-1}) (Curtis & Wang 1998; Wang & Curtis 2000). Nevertheless, in individual studies, leaf respiration is often unresponsive or even increases with elevated CO_2 (Amthor 1997; Norby *et al*. 1999; Tjoelker *et al*. 1999a). Thus, it is impossible to predict either direct or indirect effects of elevated CO_2 on leaf respiration at the individual or the ecosystem level at present.

One approach to scaling leaf respiration to the ecosystem level is developing predictive relationships between respiration and tissue nitrogen concentration similar to those for photosynthesis (Peterson *et al*. 1999). In this analysis, respiration is conceptually partitioned into separate components for growth and maintenance (McCree 1970; Thornley 1970; Amthor 2000b). Growth respiration is the amount of CO_2 respired to produce new tissue, and maintenance respiration is the amount of CO_2 respired to maintain extant tissue. A strong relationship between maintenance respiration and tissue nitrogen content has been found when several species are considered together (Ryan 1995; Reich *et al*. 1998; Tjoelker, Reich & Oleksyn 1999b), leading to use of such relationships in scaling respiration to the ecosystem level (Ryan 1991a; Ryan 1991b). Nevertheless, many studies have found either a very weak relationship or no relationship at all between maintenance respiration and leaf nitrogen for individual species (Roberntz & Stockfors 1998; Mitchell, Bolstad & Vose 1999).

The growth component of respiration (construction respiration) can be estimated using calorimetric techniques (Williams *et al*. 1987; Griffin 1994; Carey, DeLucia & Ball 1996). Experiments with seedlings in growth chambers suggest that elevated CO_2 may reduce construction costs of pine foliage (Griffin, Thomas & Strain 1993; Griffin, Winner & Strain 1996b), although there have been no studies on mature trees.

The purpose of this study was to quantify the short- and long-term effects of elevated atmospheric CO_2 on leaf dark respiration of trees growing in an intact forest ecosystem. For long-term effects, we partitioned leaf respiration into growth and maintenance components to see whether or not elevated CO_2 affected these components differently. We compared one evergreen (*Pinus taeda*) and one deciduous (*Liquidambar styraciflua*) species native to the south-eastern United States growing under ambient and elevated atmospheric CO_2 in a FACE experiment.

MATERIALS AND METHODS

Site and species

The study site was the forest atmosphere carbon dioxide transfer and storage (FACTS-1) research facility, a 17-year-old loblolly pine (*Pinus taeda* L.) plantation in the Piedmont region of North Carolina (35°58′N, 79°05′W). In this plantation, an understorey of deciduous trees has established naturally, with sweetgum (*Liquidambar styraciflua* L.) occasionally reaching the canopy. Three 30-m-diameter FACE rings are fumigated continuously with CO_2 to raise the atmospheric concentration 200 μmol mol^{-1} above the current ambient concentrations; three fully instrumented rings serve as controls (Hendrey *et al*. 1999). For our measurements, we selected three individuals each of codominant loblolly pine and canopy-emergent sweetgum from each ring. Fumigation with elevated CO_2 started in August 1996, and as loblolly pine needles live an average of 18 months, needles from treatment plots measured in this study developed under elevated CO_2 concentrations.

Gas exchange

To access the long-term indirect effect of exposure to elevated CO_2 on leaf dark respiration, we measured respiration rates of leaves at average night-time CO_2 concentrations in the ambient (400 μmol mol^{-1}) and elevated (600 μmol mol^{-1}) plots. Average night-time CO_2 concentrations in the rings between 1 June 1999 and 30 September 1999 were 404 μmol mol^{-1} [standard deviation (SD) = 41·2] in the ambient plots and 595 μmol mol^{-1} (SD = 60·7) in the elevated plots. We selected one fully expanded leaf (sweetgum) or 8–12 fascicles (loblolly pine) from the top and bottom of the canopy for each individual. Respiration measured on these fully expanded leaves represents maintenance respiration. Dark respiration was measured at 5 week intervals (mid-June, average night-time temperature 19·7 °C; late July, average night-time temperature 23·5 °C; early September, average night-time temperature 20·8 °C) on detached leaves using an open gas-exchange system with a conifer cuvette (LI 6400; LiCor Inc., Lincoln, NE, USA) and flow rates of 100 μmol sec^{-1}. Actual leaf area in the cuvette was 17–40 cm^2 for sweetgum and 22–47 cm^2 for pine. Leaves were stored in humidified plastic bags and all measurements were made within 20 min of detaching. Prior to this experiment, we measured respiration rates of leaves in growth chambers and in the field before and after detaching and found that respiration rates were unaffected for several hours. Night-time measurements of respiration were not comparable to measurements made during the daytime on darkened leaves, even when corrected for temperature differences. Therefore, all measurements were made between 2100 and 0400 h (EST). We checked for possible changes in respiration rate at a given temperature over the course of a night and found none.

To eliminate leaks and minimize diffusion of CO_2 through the gas exchange cuvette, all gaskets in the cuvette were covered with a thick layer of silicone putty (Permagum; Virginia KMP Corp., Dallas, TX, USA) and the entire cuvette covered with plastic film (Saran wrap; S.C. Johnson, Racine, WI, USA) (McDermitt *et al*. 2001). To account for any residual diffusion, the respiration rate of each leaf or group of needles was recalculated using correction factors, measured by sealing an empty cuvette and measuring the difference between CO_2 in the sample and reference chambers for the reference CO_2 concentration used (400 or 600 μmol mol^{-1}). Correction factors used were an average of three independent runs of an empty sealed cuvette. After respiration measurement, leaf tissue was sampled from the sweetgum for determination of specific leaf area (SLA); leaf tissue was dried at 70 °C and weighed. Pine needles were trimmed to the actual amount of tissue in the cuvette, dried and weighed. Projected leaf area of pine needles was calculated according to Naidu *et al*. (1998). Dried leaf tissue was analysed for carbon and nitrogen using a micro-Dumas CHN analyser (model NA1500; Carlo Erba Strumentazione, Milan, Italy).

To measure the effect of short-term exposure to elevated CO_2 on leaf respiration, we measured respiration rates at four different CO_2 concentrations (200, 400, 600, 800 μmol mol^{-1}) in early September on leaves from the top and the bottom of the canopy. After changing the measurement CO_2 concentration, respiration stabilized within 5–10 min. All measurements were corrected for residual diffusion as above.

The temperature response of leaf respiration (Q_{10}) in both species was measured in mid-June for leaves from the top of the canopy. Respiration was measured at four temperatures, from about 22 °C to 32 °C, and was described as $R_t = R_0[\exp(t \times \ln Q_{10})/10)]$, where R_t is the respiration rate at temperature t and R_0 is the respiration rate at 0 °C (Johnson & Thornley 1985; Ryan 1991a). We started the measurements at ambient temperature (~ 26 °C), then at approximately 4 °C below and two temperatures above ambient. There was no evidence of hysteresis in the measurements.

Construction respiration

Leaf tissue for measurements of construction cost and construction respiration was collected in late July. Samples were dried and ground to a fine powder and ash-free heat of combustion was determined using a microbomb calorimeter (Gentry Instruments, Aiken, NC, USA). Ash fraction was obtained by combusting samples in a muffle furnace at 500 °C. Construction cost (in g glucose g tissue^{-1}) was calculated according to Williams *et al*. (1987) and converted to CO_2 units. Construction respiration (an estimate of g carbon respired g tissue^{-1}) was calculated from construction cost by subtracting the carbon content of the tissue (Nobel, Alm & Cavelier 1992; Carey *et al*. 1996; Carey, Callaway & DeLucia 1997).

Statistical analysis

Statistical comparisons of the indirect effects of CO_2 were performed with analysis of variance (ANOVA) (Proc Mixed version 6·12; SAS Institute, Cary, NC, USA) using a repeated measures mixed model design with treatment, species, canopy position and day as fixed factors, block as a random factor and leaf nitrogen as a covariate. Respiration rates calculated on a leaf area and leaf mass basis were analysed separately. For the analysis of direct effects, we performed a regression of respiration versus CO_2 for each leaf (Proc Reg version 6·12, SAS Institute), and then analysed the regression slopes using ANOVA. The model for construction respiration and construction cost used treatment, species and canopy position as fixed factors, with block as a random factor. The Q_{10} model used treatment and species as fixed factors and block as a random factor. Using block as a random factor is more conservative than a fixed-factor analysis, but it allows for generalization to the forest as a whole. Respiration rates and leaf nitrogen were transformed to meet the assumptions of the tests. In all analyses, we started with a model including all main effects and two-way interactions. The final model was determined by sequentially removing terms from the starting model (starting with the largest *P*-values first) until only significant factors remained.

RESULTS

Direct effects of CO_2 on leaf respiration

For loblolly pine, there was essentially no direct effect of CO_2 on leaf respiration rate (Fig. 1c). After correcting the raw rates of CO_2 production (Fig. 1a) for chamber diffusion (Fig. 1b), the slope of respiration versus reference CO_2 concentration was $6{\cdot}224 \times 10^{-5}$ μmol CO_2 m^{-2} s^{-1}(μmol mol^{-1} CO_2)$^{-1}$, producing a 1·5–2·5% stimulation in respiration rate for a 200 μmol mol^{-1} increase in CO_2. This slope was statistically significant (d.f. = 5; t = –4·70; P = 0·0053), but should be regarded with caution because an effect of this magnitude is beyond the accuracy of the instrument.

For sweetgum, there was a small residual suppression of leaf respiration rate by CO_2 (Fig. 1f). The corrected slope of respiration versus reference CO_2 concentration was $-1{\cdot}328 \times 10^{-4}$ μmol CO_2 m^{-2} s^{-1}(μmol mol^{-1} CO_2)$^{-1}$ (d.f. = 5; t = 10·02; P = 0·0002); a 200 μmol mol^{-1} increase in CO_2 decreased respiration rates by 7–14% depending on measurement date and canopy position. For both loblolly pine and sweetgum, the effect of CO_2 on respiration was independent of the CO_2 concentrations under which trees were grown (F = 0·72, P = 0·48).

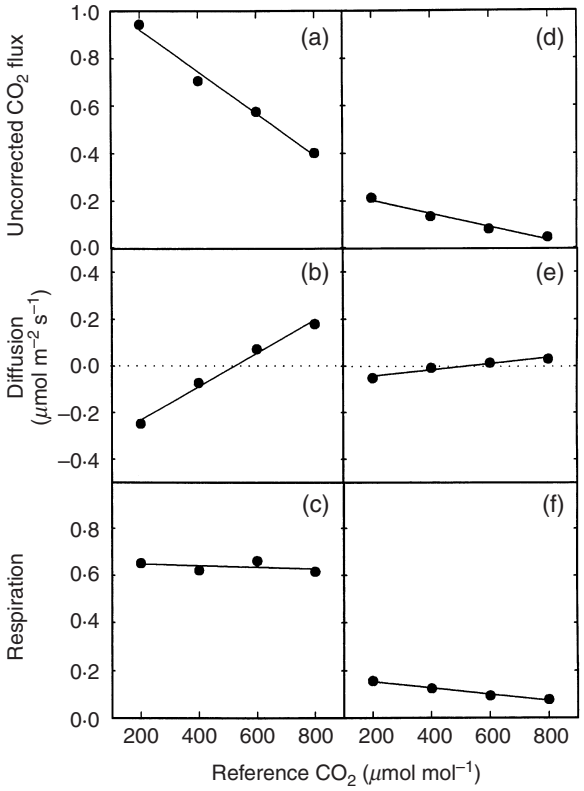

Figure 1. Example of correcting the direct effect of CO_2 on leaf respiration for one loblolly pine and one sweetgum tree: apparent response of leaf respiration to increasing CO_2 without correcting for diffusion between cuvette and the surrounding air (a, d); diffusion of CO_2 into and out of sealed, empty cuvette (b, e); corrected response of respiration to ambient CO_2 (c, f). Concentration of CO_2 in the room was approximately 560 μmol mol^{-1}. All respiration measurements were adjusted to 28 °C using our measured Q_{10} values.

Indirect effects of CO_2 on leaf respiration

There was no evidence that trees grown in elevated CO_2 had different rates of mass-based or area-based leaf respiration than those grown in ambient CO_2 (Table 1, Fig. 2). For both species, respiration was significantly higher in leaves from the top of the canopy than in those from the bottom (Table 1, Fig. 2).

Both leaf nitrogen concentration (g N g leaf^{-1}) and content (g N m^2 leaf^{-1}) changed through time, peaking in July (Table 2). Leaf nitrogen content was higher for leaves at the top of the canopy for both species (sweetgum P = 0·0001;

Table 1. Final statistical model for the indirect effect of elevated atmospheric CO_2 on leaf respiration measured on an area or mass basis. Three trees of each species were sampled in each ring

	NDF	DDF	*F*	*P*
Area-based respiration				
Sweetgum				
Treatment	1	17·2	2·38	0·14
Time	2	63·3	20·84	0·0001
Canopy position	1	57·1	38·14	0·0001
Square root nitrogen	1	87	25·22	0·0001
Loblolly pine				
Treatment	1	4	0·00	0·9733
Time	2	99	20·76	0·0001
Canopy position	1	99	69·09	0·0001
Mass-based respiration				
Sweetgum				
Treatment	1	1·99	0·00	0·9802
Time	2	86·5	46·24	0·0001
Canopy position	1	86·5	51·91	0·0001
Loblolly pine				
Treatment	1	4·01	0·15	0·7169
Time	2	97	13·02	0·0001
Canopy position	1	97	36·88	0·0001
Canopy position ×day	2	97	5·23	0·0070

NDF, numerator degrees of freedom; DDF, denominator degrees of freedom.

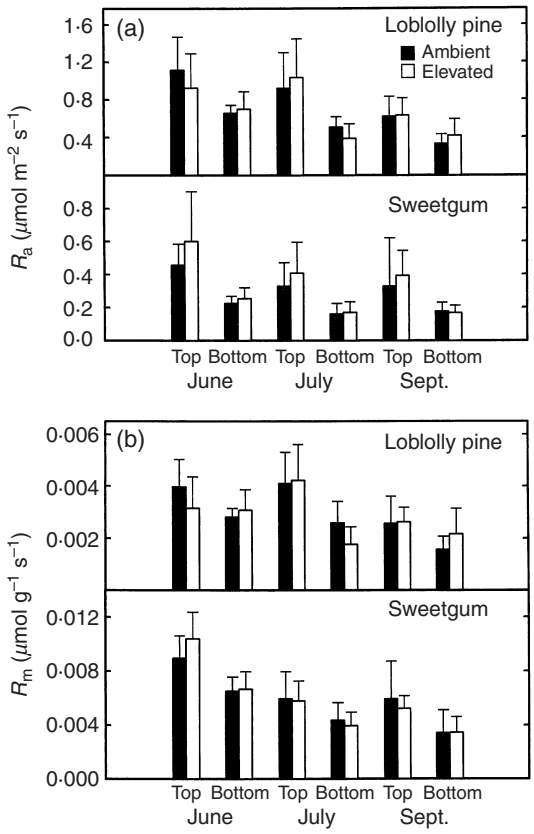

Figure 2. Indirect effect of CO_2 on leaf respiration adjusted to 28 °C using our measured Q_{10} values: area-based respiration rates (R_a) (a) and mass-based respiration rates (R_m) (b). Each leaf was measured at its respective night-time growth CO_2 concentration (solid bar, 400 μmol mol^{-1}; open bar, 600 μmol mol^{-1}). Each bar represents an average of nine measurements. Error bars: 1 SD.

pine P = 0·0001), but nitrogen concentration did not vary with canopy position. Averaged across the three measurement dates, there was a small reduction in total leaf nitrogen concentration under elevated CO_2 for sweetgum (0·0175 versus 0·0159 g N g leaf^{-1}; P = 0·001) but not for loblolly pine (0·0124 versus 0·0113 g N g leaf^{-1}; P = 0·29). Leaf nitrogen content increased under elevated CO_2 for sweetgum (0·788 versus 0·989 g N m^2 leaf^{-1}; P = 0·0001) but was unchanged in pine (2·97 versus 2·79 g N g leaf^{-1}; P = 0·6017). For sweetgum, leaf nitrogen content correlated positively with area-based respiration rates (Table 1, Fig. 3), with nearly identical relationships between ambient and elevated treatments. There were no significant relationships between leaf nitrogen concentration and mass-based respiration rates.

Table 2. Leaf characteristics of loblolly pine and sweetgum growing in the FACTS-1 research facility

		Projected SLA (cm^2 g^{-1})		% C		Projected g N m^2 leaf^{-1}		g N g leaf^{-1}	
		Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated
Loblolly pine									
June	Top	34·75	32·88	49·14	48·21	3·32	3·48	0·011	0·011
	Bottom	42·85	43·42	53·08	48·60	2·82	2·42	0·012	0·009
July	Top	44·00	39·61	45·87	49·04	3·55	3·14	0·015	0·012
	Bottom	50·38	43·97	47·46	50·07	3·33	2·84	0·016	0·013
September	Top	40·44	41·43	48·73	48·41	2·79	2·80	0·011	0·011
	Bottom	46·35	48·84	48·56	48·85	2·37	2·31	0·011	0·011
Sweetgum									
June	Top	182·81	114·60	48·52	54·23	1·03	1·64	0·018	0·018
	Bottom	352·56	332·19	45·97	46·46	0·54	0·55	0·017	0·018
July	Top	160·23	97·88	43·90	48·72	1·10	1·62	0·017	0·015
	Bottom	320·36	276·37	47·15	44·93	0·63	0·62	0·019	0·017
September	Top	142·64	108·95	46·81	47·91	1·77	1·33	0·018	0·014
	Bottom	293·12	246·28	44·14	43·29	0·52	0·59	0·015	0·014

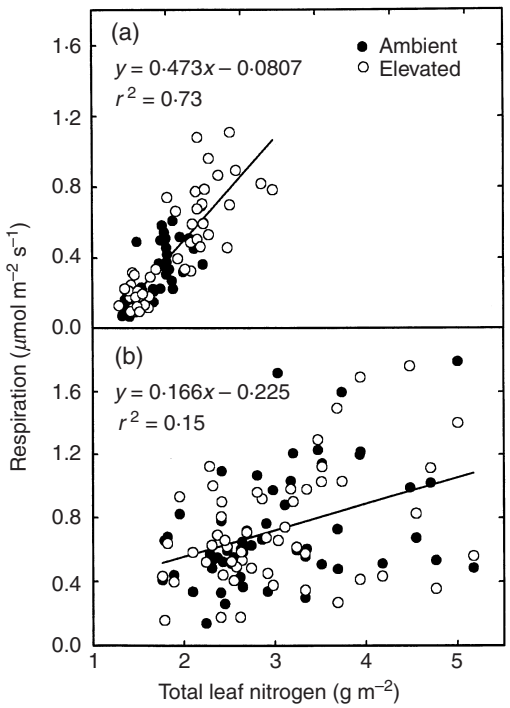

Figure 3. Relationship between leaf nitrogen content and area-based respiration rate (R_a) for sweetgum (a) and loblolly pine (b) growing in ambient and elevated CO_2. Respiration rates were adjusted to 28 °C using our measured Q_{10} values.

Temperature response of leaf respiration

The Q_{10} of loblolly pine and sweetgum leaves averaged 2·71 and 2·05, respectively, with no differences between CO_2 treatments (Table 3). Basal respiration rates (R_o) were higher for sweetgum than for loblolly pine when expressed on either a mass or area basis. For sweetgum, area-based R_o was significantly higher for trees grown in ambient CO_2.

Leaf construction costs

There were no differences in leaf construction costs between trees in ambient or elevated CO_2 for either loblolly pine or sweetgum (Table 4). Also, there were no differences between the top and bottom of the canopy for loblolly pine, although there was an effect of canopy height on construction cost for sweetgum. Sweetgum leaves at the top of the canopy showed a trend for higher fraction of total carbon in elevated compared with ambient plots (elevated 0·49 ± 0·01 versus ambient 0·44 ± 0·02 g C g leaf^{-1}), but leaves at the bottom of the canopy were similar (elevated 0·45 ± 0·02 versus ambient 0·47 ± 0·01 g C g leaf^{-1}). For loblolly pine, carbon concentrations of leaves at the top (elevated 0·49 ± 0·01 g C g leaf^{-1}; ambient 0·46 ± 0·03 g C g leaf^{-1}) and bottom of the canopy (elevated 0·50 ± 0·02 g C g leaf^{-1}; ambient 0·47 ± 0·03 g C g leaf^{-1}) had similar differences between ambient and elevated plots. Differences in carbon fraction between treatments were not due to differences in ash content because there was no effect of elevated CO_2 on ash content (data not shown). Our estimates of leaf construction respiration were significantly lower in elevated compared with ambient plots for both species at the top of the canopy but were not different between treatments at the bottom of the canopy (Table 4).

DISCUSSION

Direct effects of CO_2 on leaf dark respiration

Short-term elevation of CO_2 suppressed leaf dark respiration by about 10% for a 200 μmol mol^{-1} increase in CO_2 in sweetgum and had essentially no effect on loblolly pine. If we had not accounted for the diffusion of CO_2, we would have concluded erroneously that this effect was much larger. In an extensive study, Amthor (2000a) concluded that direct effects of CO_2 on leaf respiration are small (averaging 1·5% decrease for a 400 μmol mol^{-1} increase in CO_2). Our study supports this conclusion for loblolly pine, but sweetgum showed a larger suppression. The existence of a direct effect of CO_2 on leaf respiration may have implications on daily plant carbon budgets, as ambient CO_2 concentrations change between day and night. For example, as CO_2 concentrations increase at night, total night-time respiratory losses of sweetgum could be reduced as leaf respiration is suppressed. However, a direct effect of CO_2 on leaf respiration will probably not alter plant carbon budgets as global atmospheric CO_2 increases because there appears to be no long-term effect of CO_2 on leaf respiration (Fig. 2).

Indirect effects of CO_2 on leaf dark respiration

Maintenance respiration

Leaf maintenance respiration did not appear to be altered by long-term exposure to elevated CO_2 (Table 1, Fig. 2).

Table 3. Temperature response of leaf maintenance respiration (Q_{10}) and basal leaf respiration rate (R_o). Each value is an average of nine independent measurements

	Q_{10}		R_o (μmol m^{-2} s^{-1})		R_o (μmol g^{-1} s^{-1})	
	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated
Pine	2·79	2·62^{ns}	0·0754	0·0855^{ns}	0·000261	0·000282^{ns}
Sweetgum	2·15	1·96^{ns}	0·0669	0·145*	0·00118	0·00159^{ns}

^{ns} Mean values between treatments that were not statistically different ($P > 0·05$).
* $P = 0·012$.

Table 4. Growth component of respiration in late July measured by calorimetry. Construction cost includes the carbon atoms incorporated into the tissue; construction respiration includes only carbon respired to construct tissue

	Heat of combustion (kJ g^{-1})		Construction cost (mol CO_2 kg $tissue^{-1}$)		Construction respiration (mol CO_2 kg $tissue^{-1}$)	
	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated
pine top	$20{\cdot}03^{aA}$	$20{\cdot}21^{aA}$	$50{\cdot}20^{aA}$	$50{\cdot}35^{aA}$	$11{\cdot}97^{aA}$	$9{\cdot}48^{aB}$
pine bottom	$19{\cdot}90^{aA}$	$20{\cdot}51^{aB}$	$49{\cdot}54^{aA}$	$50{\cdot}63^{aA}$	$9{\cdot}99^{bA}$	$8{\cdot}90^{aA}$
sweetgum top	$19{\cdot}55^{aA}$	$19{\cdot}40^{aA}$	$48{\cdot}15^{aA}$	$47{\cdot}81^{aA}$	$11{\cdot}74^{aA}$	$7{\cdot}19^{aB}$
sweetgum bottom	$18{\cdot}48^{bA}$	$18{\cdot}04^{bA}$	$45{\cdot}18^{bA}$	$43{\cdot}92^{bA}$	$5{\cdot}74^{bA}$	$6{\cdot}48^{aA}$

Letters indicate significant differences ($P < 0{\cdot}05$) between columns (capital letters) or rows (lower-case letters) within a species.

Inconsistency in the literature does not allow confidence in predictions of long-term responses of leaf respiration to elevated CO_2, but the generalization is that indirect effects are related to changes in leaf tissue composition (Amthor 1997; Saxe *et al*. 1998; Norby *et al*. 1999). That is, lower respiration rates are often linked to reduced nitrogen concentrations resulting from accumulation of carbohydrates. In this study, sweetgum showed reduced concentrations of total leaf nitrogen under elevated CO_2, but this did not translate into a difference in leaf maintenance respiration rate. Also, there did not appear to be a relationship between leaf respiration rate and total leaf nitrogen concentration.

A positive correlation between respiration and leaf nitrogen concentration has been found for tree seedlings (Griffin, Ball & Strain 1996a; Tjoelker *et al*. 1999b) and when several species are used in the same analysis to obtain wide variation in nitrogen (Ryan 1995; Reich *et al*. 1998); but for mature trees within a species, there is often no relationship (Ryan 1995; Roberntz & Stockfors 1998; Mitchell *et al*. 1999). The absence of a correlation between maintenance respiration and leaf nitrogen concentration may be explained, in part, by amounts of respiratory enzymes generally being in excess of those required for observed rates of respiration (Amthor 1991). In addition, leaf maintenance respiration may be related directly to protein turnover instead of just the total amount of protein (Amthor 2000b). Thus, leaf nitrogen concentration may not predict leaf maintenance respiration in forest stands, and should be used only if it is first shown to be predictive.

When we expressed leaf nitrogen on a leaf area basis (g N cm^2 $leaf^{-1}$) instead of a concentration basis (g N g $leaf^{-1}$), we found a significant relationship with respiration for sweetgum (Fig. 3). This reflected a relationship with SLA and area-based respiration rates instead of an underlying physiological relationship between respiration and leaf nitrogen.

The Q_{10} measured in mid-June was 30% higher in loblolly pine compared with sweetgum (Table 3). This indicates the importance of species-specific measurements for stand scaling of leaf respiration rates. It has been shown that Q_{10} varies seasonally, peaking in cold months and reaching a minimum during the summer (Paembonan, Hagihara & Hozumi 1991; Criddle *et al*. 1994; Stockfors & Linder 1998; Atkin, Holly & Ball 2000). However, several other studies have not found a seasonal affect on Q_{10} (Benecke 1985; Cropper & Gholz 1991; Tjoelker *et al*. 1999b; Gunderson, Norby & Wullschleger 2000). Growth at elevated CO_2 appeared to have no effect on this temperature response and did not affect mass-based R_0. The higher area-based basal respiration rate of leaves grown in elevated CO_2 is probably a result of lower specific leaf area (Table 2) in the elevated CO_2 treatments. Consequently, there was more respiring mass per leaf area for high-CO_2-grown leaves, producing higher basal respiration rates.

Indirect effects of CO_2 on leaf dark respiration

Growth respiration

We found no evidence that elevated CO_2 altered leaf construction costs for loblolly pine or sweetgum (Table 4). At higher concentrations of elevated CO_2 than we used in this experiment (300–350 μmol mol^{-1} increase), changes in non-structural carbohydrates sometimes reduce construction costs of leaves (Griffin *et al*. 1993; Griffin *et al*. 1996b; Wullschleger *et al*. 1997). Even in these cases, however, the reduction is only about 3%.

We found that growth under elevated CO_2 reduced construction respiration by 21% for loblolly pine and by 39% for sweetgum for leaves at the top of the canopy. We know of no other estimates of the effects of elevated CO_2 on leaf construction respiration using calorimetric methods. However, estimates of the construction component of respiration (called growth respiration) from chemical composition (Poorter *et al*. 1997) or from regression techniques (Wullschleger & Norby 1992; Wullschleger, Norby & Gunderson 1992) have found reductions in leaf construction respiration under elevated CO_2 of 10–20%. This reduction was found to be primarily a result of reduced protein concentration, although accumulation of total non-structural carbohydrates (TNC) also played a role. Reduced protein concentrations can lower growth respiration because synthesis of proteins are accompanied by large CO_2 production, whereas increased concentrations of TNC can reduce growth respiration because compounds such as starch can be formed with little CO_2 production (Poorter *et al*. 1997).

In our case, lower construction respiration for sweetgum leaves at the top of the canopy under elevated CO_2 can be explained partly by lower nitrogen concentrations in elevated compared with ambient plots, and partly by higher fractions of total carbon in elevated compared with ambient plots for leaves at the top of the canopy but not for those at the bottom. For loblolly pine, we found a trend for reduced nitrogen and higher-fraction total carbon for leaves at both the top and bottom of the canopy under elevated CO_2. These differences were enough to allow for statistically significant reduced construction respiration at the top of the canopy but not at the bottom.

CONCLUSIONS

Increasing atmospheric CO_2 concentration by 200 μmol mol^{-1} did not appear to have a large impact on leaf dark respiration of mature loblolly pine or sweetgum trees growing in an intact forest ecosystem. There appeared to be a short-term direct suppression of respiration by elevated CO_2 in sweetgum, but not in loblolly pine. We found no evidence for an indirect long-term effect on leaf maintenance respiration. Although elevated CO_2 reduced leaf construction respiration for leaves at the top of the canopy, construction respiration is a small fraction of maintenance respiration on a yearly basis (Ryan *et al*. 1996). Leaf nitrogen concentration did not predict leaf respiration and should be used with caution for scaling to the stand level. Because elevated atmospheric CO_2 did not appear to influence leaf-tissue-specific respiration rates, the effects of elevated CO_2 on plant respiratory carbon flux are primarily at the whole-plant level through increased biomass.

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