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Acclimation of photosynthesis and respiration to elevated atmospheric CO_2 in two Scrub Oaks

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Abstract

For two species of oak, we determined whether increasing atmospheric CO_2 concentration (C_a) would decrease leaf mitochondrial respiration (R) directly, or indirectly owing to their growth in elevated C_a, or both. In particular, we tested whether acclimatory decreases in leaf-Rubisco content in elevated C_a would decrease R associated with its maintenance. This hypothesis was tested in summer 2000 on sun and shade leaves of *Quercus myrtifolia* Willd. and *Quercus geminata* Small. We also measured R on five occasions between summer 1999 and 2000 on leaves of *Q. myrtifolia*. The oaks were grown in the field for 4 years, in either current ambient or elevated (current ambient + 350 μmol mol^{-1}) C_a, in open-top chambers (OTCs). For *Q. myrtifolia*, an increase in C_a from 360 to 710 μmol mol^{-1} had no direct effect on R at any time during the year. In April 1999, R in young *Q. myrtifolia* leaves was significantly higher in elevated C_a—the only evidence for an indirect effect of growth in elevated C_a. Leaf R was significantly correlated with leaf nitrogen (N) concentration for the sun and shade leaves of both the species of oak. Acclimation of photosynthesis in elevated C_a significantly reduced maximum RuBP-saturated carboxylation capacity ($V_{c\,max}$) for both the sun and shade leaves of only *Q. geminata*. However, we estimated that only 11–12% of total leaf N was invested in Rubisco; consequently, acclimation in this plant resulted in a small effect on N and an insignificant effect on R. In this study measurements of respiration and photosynthesis were made on material removed from the field; this procedure had no effect on gas exchange properties. The findings of this study were applicable to R expressed either per unit leaf area or unit dry weight, and did not support the hypothesis that elevated C_a decreases R directly, or indirectly owing to acclimatory decreases in Rubisco content.

Keywords: elevated CO_2, leaf nitrogen, leaf respiration, open-top chambers, photosynthetic acclimation, scrub oaks

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Introduction

It is predicted that atmospheric CO_2 concentration (C_a) is to double within the next 100 years (Schimel *et al*. 1996). Under this scenario a substantial stimulation of C_3 photosynthesis could increase ecosystem carbon uptake (Drake *et al*. 1997; Norby *et al*. 1999). However, long-term effects on ecosystem carbon storage may, in part, depend on whether rates of mitochondrial respiration (R) are affected by increased atmospheric C_a and associated climate changes (Ryan 1991; Amthor 1995; Drake *et al*. 1999).

A direct, reversible inhibition of foliar dark respiration has often been observed when C_a is increased (Amthor *et al*. 1992; Mousseau 1993; Teskey 1995; Gonzalez-Meler *et al*. 1996; Ryan *et al*. 1996). However, many studies also report no direct effect of increasing C_a on R (Ryle *et al*. 1992; Ziska & Bunce 1994; Mitchell *et al*. 1995; Tjoelker *et al*. 1999; Amthor 2000). Generalization about a direct effect of C_a on respiration is further complicated by the

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absence of a mechanism capable of accounting for the magnitude of the direct effects that have been observed (Gonzalez-Meler & Siedow 1999).

In addition to having a direct effect on R, growth in elevated C_a may change leaf chemical composition, particularly concentrations of leaf nitrogen (N) and total nonstructural carbohydrates (TNC) which may indirectly affect R. Tissue N concentration is highly correlated to R (Wullschleger *et al.* 1992; Ryan 1995; Reich *et al*. 1998) and commonly decreases in plants grown in elevated C_a. This decrease is often consistent with an acclimatory decrease in N in photosynthetic enzymes—particularly Rubisco (Nie *et al*. 1995; Rogers *et al*. 1998; Li *et al*. 1999; Griffin *et al*. 2000). Given that up to 25% of leaf N can be invested in Rubisco (Drake *et al*. 1997), substantial reductions in Rubisco could reduce maintenance respiration associated with its synthesis and turnover. Conversely, concentrations of nonstructural carbohydrates generally increase in elevated C_a, increasing R (Hrubec *et al*. 1985; Thomas *et al*. 1993; Thomas & Griffin 1994; Mitchell *et al*. 1995; Tjoelker *et al*. 1999). Clearly, the indirect effects of decreasing N and increasing TNC in leaves growing in elevated C_a could have opposite effects on R (Tjoelker *et al*. 1999).

Recent literature reviews have concluded that a reduction in R is often the consequence of exposure to, or growth in, elevated C_a. A meta-analysis by Curtis & Wang (1998) concluded that R per unit mass was reduced by 18% in woody plants grown at elevated C_a. Drake *et al*. (1999) concluded that doubling of present C_a would directly reduce R per unit mass by 15–18%. While foliar respiration is but one component of ecosystem respiration, and is commonly a much smaller component than root and microbial soil respiration (Valentini *et al*. 2000), the effects of elevated C_a on foliar respiration could be significant at the regional or global scale. Drake *et al*. (1999) showed with a model of global carbon balance that a 15% decrease in foliar respiration would significantly reduce global annual respiratory CO_2 flux by 3 Gt.

In this study we tested the hypothesis that growth in elevated C_a will reduce R, both directly, and indirectly owing to acclimatory decreases in leaf-Rubisco content. The study site was a Florida scrub-oak ecosystem, within which open-top chambers (OTCs) were used to create the test atmosphere of either current ambient or elevated C_a for vegetation that had regenerated after fire for 4 years. On five occasions between summer 1999 and 2000, we measured R in detached leaves of *Quercus myrtifolia* Willd. exposed to a step change in C_a to determine the direct effect on R. By comparing R of leaves measured at a common C_a, we tested for the indirect effect of C_a on R. The maximum RuBP-saturated carboxylation capacity ($V_{c\,max}$) of *Quercus geminata* Small consistently decreases in elevated C_a. For *Q. myrtifolia*, $V_{c\,max}$ typically does not decrease in elevated C_a. Given that $V_{c\,max}$ can provide a surrogate measure of leaf Rubisco content (Long & Drake 1992), this situation presented the possibility of testing whether acclimatory decreases in leaf Rubisco content will decrease mitochondrial R of leaves growing in elevated C_a.

Materials and methods

The site

The Smithsonian Environmental Research Center OTC project was sited in the scrub-oak palmetto ecosystem of coastal central Florida. The project was on Merritt Island (28°38′N, 80°42′W), within NASA's Kennedy Space Center. Two burns, one in August 1995 and the other in January 1996 cleared the site prior to the installation of the OTCs. In May 1996, growth inside the OTCs was cut back to ground level and fumigation began. Since then the ecosystem has regenerated in 16 large OTCs (9.42 m^2 ground area and 1.76 m high) (Li *et al*. 1999; Hungate *et al*. 2000). Eight of the OTCs were maintained at current ambient C_a and eight at elevated C_a. Carbon dioxide concentration was measured in each of the 16 OTCs every 11 min. For 1999, average CO_2 concentrations were $380 \pm 1\,\mu mol\ mol^{-1}$ and $690 \pm 4\,\mu mol\ mol^{-1}$ during the photoperiod, and $441 \pm 38\,\mu mol\ mol^{-1}$ and $800 \pm 10\,\mu mol\ mol^{-1}$ during the night, for the ambient and elevated OTCs, respectively.

Leaf mitochondrial dark respiration

A custom-designed respiration circuit was used to measure R. The circuit passed air through eight stainless steel cylindrical chambers, each of which contained up to 100 cm^2 of leaf material, and one identical empty sealed reference chamber. All the chambers were immersed in a water bath during measurements. This design had two important benefits:

1. The large leaf area increased measurement sensitivity and enabled high flow rates through the system, thereby maintaining positive pressure and reducing the possibility of air leaking into the circuit.
2. By placing the chambers into a water bath, any leaks out of, or into, the chambers could be easily observed.

Dry ambient air from a pressurized cylinder entered the respiration circuit at a flow rate of 12 L min^{-1} through 4 mm diameter tubing (Impolene, Imperial Eastman, USA). The airflow was then conditioned to a set C_a in two stages. First it was passed through a soda-lime column and scrubbed of CO_2, then CO_2 was added from a pressurized cylinder containing 10 000 μmol mol^{-1} CO_2, balance 21 kPa O_2 in N_2 (Boggs Gases, Titusville, FL, USA). The air flowed sequentially through four 1 L mixing volumes to ensure mixing and constant C_a. Once conditioned, the airflow was divided between the eight sample, and one reference, chambers. Each of the eight sample chambers and the reference chamber received a constant flow rate of 1 L min^{-1}, measured using separate flow meters (MMA 0.5–5, Dwyer, Marietta, GA, USA). The leaf material was placed on a wire-mesh support in the middle of each chamber. This enabled air flowing into the chambers to circulate under and around the respiring leaves before exiting the chamber. Airflow left the reference and one sample chamber in separate lengths of tubing and passed through a second flow meter, then into separate cells of an infra-red gas analyzer (IRGA; LI 6262, LICOR, Lincoln, NB, USA) set in differential mode that had been calibrated against a water vapour generator (LI 610, LICOR, Lincoln, NB, USA) and standard CO_2 concentration of 700 μmol mol^{-1} (Boggs Gases, Titusville, FL, USA). Airflow from the reference cell passed through a second IRGA, which continuously measured the absolute reference CO_2 and H_2O mol fraction and fed this value into the differential analyzer, which could then correct the measured CO_2 (ΔCO_2) and H_2O (ΔH_2O) differentials for changes in background C_a. For each measurement a solenoid valve switched the airflow between reference and sample cells of the differential IRGA. In each configuration ΔCO_2 was measured. Using the mean of these two ΔCO_2 accounted for any physical differences between the IRGA cells, or differences resulting from their ageing. When not being sampled air continued to flow through the sample chambers at the rate of 1 L min^{-1}.

From a known ΔCO_2 (μmol mol^{-1}) and flow rate (μmol s^{-1}), R was calculated and expressed either per unit leaf area (R_{LA}) or per unit dry mass (R_{DW}).

$$R_{LA}(\mu mol\ m^{-2} s^{-1}) = (\Delta CO_2 \times u)/s$$

where s is leaf surface area (m^{-2}).

$$R_{DW}(\eta mol\ g\,s^{-1}) = (\Delta CO_2 \times u)/DW$$

where DW is the leaf dry weight (g).

For the sun leaves of *Q. myrtifolia*, R was measured in September and November 1999, March, April, and July 2000. For the sun and shade leaves of *Q. myrtifolia* and *Q. geminata*, R was measured in July and August 2000, respectively. Each sample chamber contained 20 leaves, four from each of five different plants, from one OTC. Data sets were collected over two consecutive days. Shade leaves were removed from the lowest branch of the shoot. All the leaves were harvested within an hour after sunrise when leaf carbohydrate levels were at a minimum. After being placed in the sample chambers, the leaves were maintained at a set C_a for at least 1 h before measurements were made, and C_a changed. All measurements were made at an air temperature (T_{air}) of 25 °C measured using copper constantan thermocouples (Omega Engineering, Stanford, CT, USA) located where air left the leaf chambers. The temperature of the air flowing through the system tracked T_{air} within the lab, which was controlled at 25 °C and buffered against changes by circulating it through a water bath also set at 25 °C.

Testing IRGA accuracy

The calculation of ΔCO_2 by the IRGA relies on a series of calibration constants unique to each analyzer and determined on initial factory calibration. As the components of the IRGA age, it is possible that these calibration constants may become inappropriate leading to erroneous measurements of ΔCO_2. We tested this possibility in April 2000, midway through our experiments. A known, stable ΔCO_2 was generated and measured at a series of background C_a of 376, 696 and 1000 μmol mol^{-1} on the IRGA used in these experiments. Each of these three measurements were made on the IRGA after the span calibration had been performed at a C_a of 376, 696 and 1000 μmol mol^{-1}.

Light-saturated photosynthetic capacity

In July 2000, for *Q. myrtifolia*, and August 2000, for *Q. geminata*, entire branches were harvested at the same time as the leaves for the measurements of respiration. These branches were cut under water, transferred to a controlled environment, and maintained in low light intensity until analyzed. Analyses were usually made within 2–3 h after harvesting. The response of light-saturated photosynthesis (A_{sat}) to substomatal C_a (C_i) was made using a portable gas exchange system (LI-6400, LICOR, Lincoln, NB, USA). Measurements were made at a leaf temperature (T_{leaf}) of 25 °C and vapour pressure deficit (VPD) of 1.4 kPa, in 21 kPa O_2 and at a photosynthetically-active photon flux density (PPFD) of 1200 μmol m^{-2} s^{-1}, found to be saturating for photosynthesis. Photosynthetic induction was at the growth C_a, thereafter steady-state photosynthesis was measured with stepwise decreases in C_a. A second measurement at the growth C_a was made after a measurement at 5 Pa, followed by measurements with stepwise increases in C_a up to 150 Pa. The $V_{c\,max}$ and maximum capacity for electron transport contributing to RubP regeneration (J_{max}) were determined from the A_{sat} vs. C_i response curve using the equations and constants of McMurtrie & Wang (1993).

Leaf N analysis

To determine leaf N concentrations, leaves (collected as described above) were dried at 60 °C to constant weight, ground to 40 mesh, and subsamples of the ground tissue were analyzed for C and N concentrations using an elemental analyzer (CE 2100, Elantech, Lukewood, NJ, USA). Leaf N content was expressed both per unit dry weight (N_{DW} g g^{-1}) and per unit leaf area (N_{LA} g m^{-2}). Analyses were conducted at the Colorado Plateau Stable Isotope Laboratory at Northern Arizona University. External analytical precisions (st dev, $n = 10$ duplicate samples), were < 0.10% N and < 0.40% C for these analyses.

Estimating the fraction of leaf N invested in Rubisco

An estimation of leaf Rubisco content was calculated from measured $V_{c\,max}$ as shown below:

$$\text{Rubisco (mol m}^{-2}) = (V_{c\,max}/1\,000\,000)/(8 \times K_{cat})$$

where eight was the number of Rubisco active sites (Raines *et al*. 1991). The carboxylation capacity of Rubisco active sites (K_{cat}) was 3.3 (mol CO_2 s^{-1} site^{-1}) (Woodrow & Berry 1988). Leaf Rubisco content (mol m^{-2}) was multiplied by the molecular mass of Rubisco (Raines *et al*. 1991) and Avogadros number, to convert it to g m^{-2}. Finally, by knowing the proportion of Rubisco that was N (Steer *et al*. 1968), we estimated the amount of leaf N in Rubisco and ultimately the fraction of leaf N invested in Rubisco (*f Rubisco*).

Comparing R, $V_{c\,max}$ and J_{max} measured in the lab and in situ

On three occasions, once in each of June, July and August 2000, we compared R measured on detached leaves, using the procedure described above, to R measured in the field on attached leaves. These measurements were made at predawn in June and July 2000 and after sunset in August 2000. Measurements in the field were made using a portable gas exchange system (LI-6400, LICOR, Lincoln, NB, USA) with a large leaf chamber (LI-6400–05, LICOR, Lincoln, NB, USA). The leaf chamber was secured around a shoot and typically accommodated up to 60 cm leaf area, plus stem. For the purpose of comparing R, measurements made in the field were corrected for the proportion of total biomass; that was stem. This was typically 10%, and that stem respiration per unit dry weight was 30% of leaf respiration per unit dry weight (data not shown). In July 1997 we compared $V_{c\,max}$ and J_{max} of five leaves on excised stems determined from A/C_i curves measured as described above, with $V_{c\,max}$ and J_{max} determined from A/C_i curves measured *in situ*. Measurements were made predawn over 3 days. For all gas exchange comparisons the environmental conditions within the lab were maintained as similar as possible to those in the field.

Statistical analysis

Two-factor analysis of variance (ANOVA) was used to test for an effect of C_a exposure (direct effect) and C_a growth (indirect effect) on R of *Q. myrtifolia* sun leaves for the individual sampling dates between summer 1999 and 2000. Two-factor ANOVA was also used to test for the effect of growth C_a and leaf position on measurements of A_{sat}, $V_{c\,max}$, J_{max}, N and *f Rubisco* made on leaves of *Q. myrtifolia* and *Q. geminata* in summer 2000. Three-factor ANOVA tested for the effect of species, leaf position, and growth C_a on R of the leaves of *Q. myrtifolia* and *Q. geminata* in summer 2000. For leaves of both the species, a *t*-test was used to examine the straight-line dependence of R on N. An effect has been described as statistically significant when $P < 0.05$. All statistical analyses were performed using a software package (SYSTAT 9.0, Systat inc, Evanstone, IL, USA).

Results

Throughout this study the effect of elevated C_a on R was the same whether the rate was expressed as R_{LA} or R_{DW} (Figs 1 and 3; Tables 1 and 3). Consequently, the symbol R is used throughout except when we wish to distinguish between the two different methods for normalizing the results.

Seasonal measurements on Q. myrtifolia sun leaves

For measurements made in September and November 1999, March, April, and July 2000 on the sun leaves of *Q. myrtifolia* grown in both current ambient and elevated C_a, R was unaffected by instantaneous increases in C_a from 360 to 710 μmol mol^{-1} (Fig. 1; Table 1). In April 2000, R was significantly higher for leaves grown in elevated C_a when exposed to both 360 and 710 μmol mol^{-1} C_a—indicating an indirect effect of growth in elevated C_a (Fig. 1; Table 1). There were no clear effects of leaf age on R. For the leaves that flushed in spring 1999, and were measured in September 1999 when they were 5-month old, November 1999 at 7-month old and March 2000 at 11-month old, R was similar during each measurement period. For the new flush of leaves measured in April 2000, R was again similar for leaves grown at ambient C_a, however, R was higher for the leaves grown in elevated C_a. The lowest rates of R occurred in July 2000 when leaves were 3-month old.

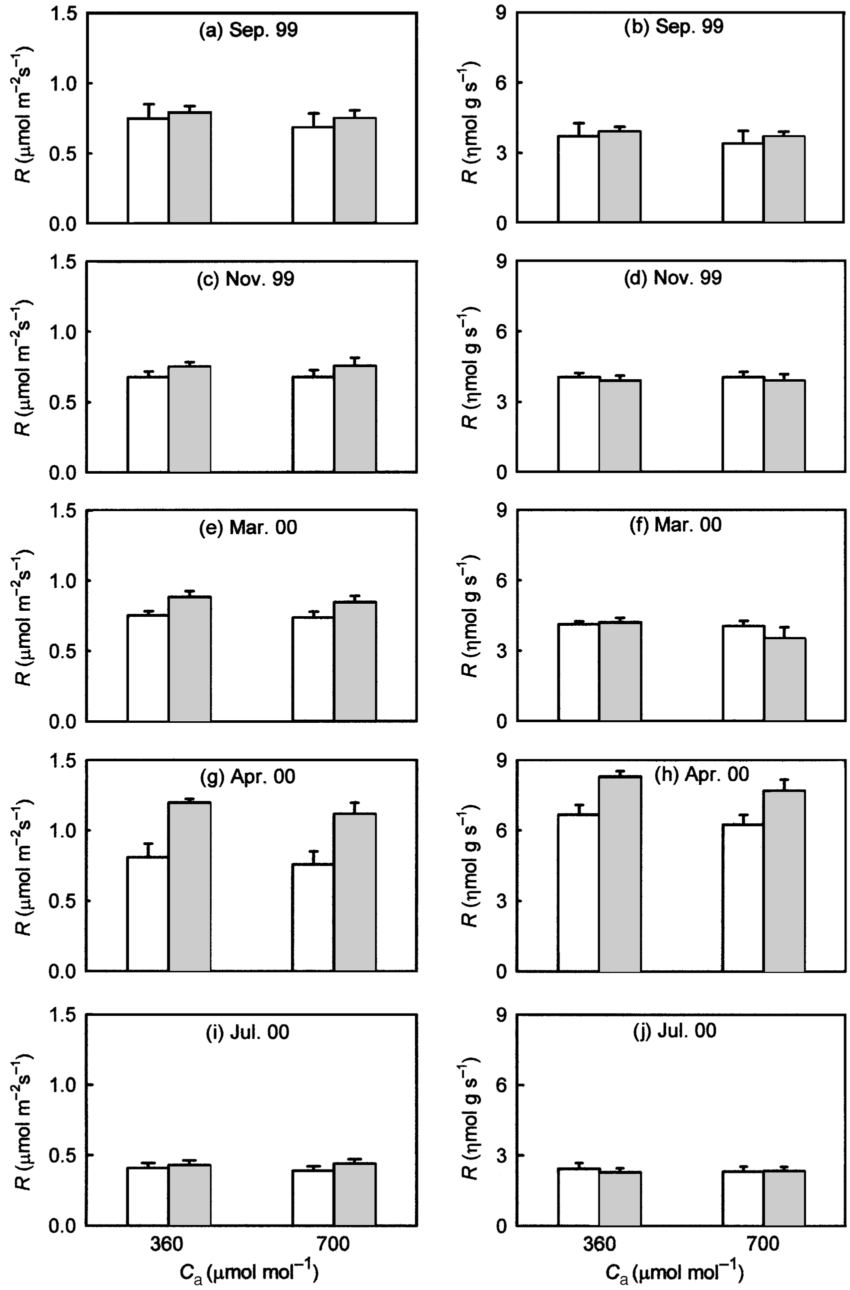

Fig. 1 Seasonal leaf dark respiration. Leaf respiration (R) of the sun leaves of *Q. myrtifolia* measured in September 1999 (a, b), November 99, (c, d), March 2000 (e, f), April 2000 (g, h), and July 2000 (i, j). Leaves were grown in either ambient (white bars) or elevated (grey bars) C_a and exposed to both 360 and 700 μmol mol^{-1} CO_2. Leaf respiration is expressed per unit leaf area (a, c, e, g & i) and per g dry weight (b, d, f, h & j). Each bar is the mean of $n \geq 6$, $\pm$ 1 SE.

Indirect effects of growth in elevated C_a and light environment on photosynthesis and R

Growth in elevated C_a and leaf light environment caused changes in light-saturated photosynthetic capacity. For *Q. myrtifolia* and *Q. geminata*, both $V_{c\,max}$ and J_{max} were significantly lower in the shade leaves than in the sun leaves (Fig. 2; Table 2). *Quercus myrtifolia* and *Q. geminata* displayed contrasting responses of $V_{c\,max}$ and J_{max} to growth in elevated C_a. For *Q. myrtifolia*, $V_{c\,max}$ and J_{max} of both the sun and shade leaves were unaffected by growth in elevated C_a. For *Q. geminata* grown in elevated C_a, $V_{c\,max}$ was significantly reduced by 33 and 45% in both the sun and shade leaves, respectively, and J_{max} was significantly reduced by 20 and 17% in the sun and shade leaves, respectively (Fig. 2; Table 2). For *Q. myrtifolia*, A_{sat} measured at the respective growth C_a was significantly stimulated by 78% in both the sun and shade leaves growing in elevated C_a. For both the sun and shade leaves of *Q. geminata*, acclimatory reductions in both $V_{c\,max}$ and J_{max} removed any stimulation of A_{sat} in elevated C_a (Table 2).

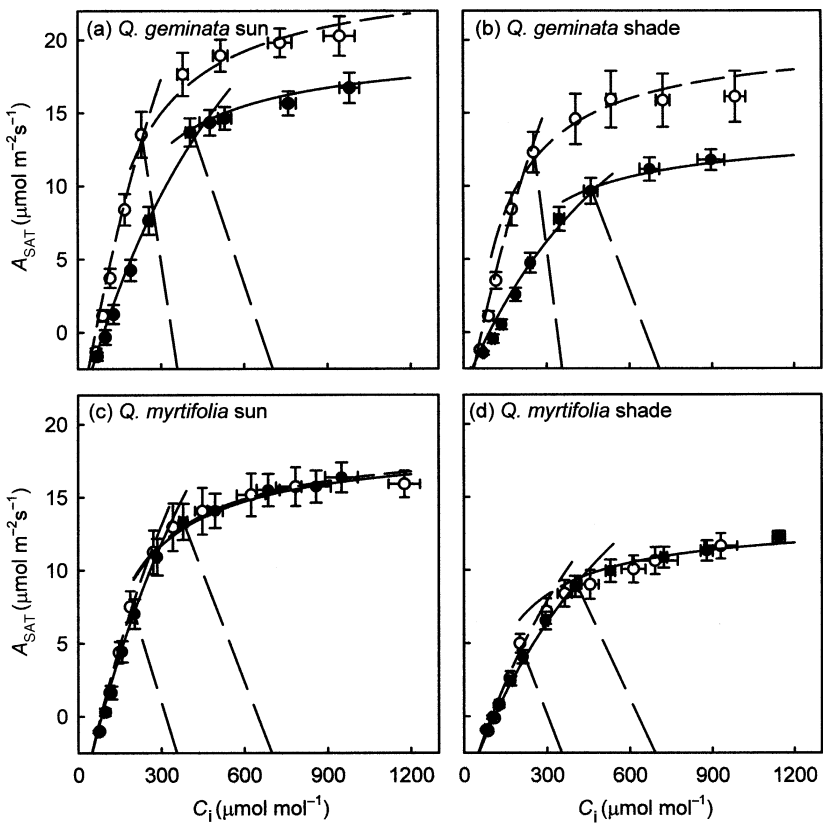

Fig. 2 Light-saturated photosynthesis. Plot of A_{sat} against C_i for *Q. geminata* sun (a) and shade (b) leaves, and *Q. myrtifolia* sun (c) and shade (d) leaves. Measurements were made in July (*Q. myrtifolia*) and August (*Q. geminata*) 2000, on leaves of excised branches grown in elevated (solid symbols, solid lines) or ambient (open symbols, dashed lines) C_a treatments. Also shown are the supply functions for each curve, the C_i obtained for a given g_s with varying A_{sat} (dashed line). The operating point is at the intersection of the supply function and the A/C_i curve for each treatment. The A/C_i curves for $V_{c\ max}$ and J_{max} are fitted to the data points illustrated by maximum-likelihood regression following the functions of McMurtrie & Wang (1993). Data points shown are the means ($\pm$ 1 SE) for measurements made in at least seven replicate OTCs.

Table 1 Seasonal rates of R. Per unit leaf area (R_{LA}) and per unit dry weight (R_{DW}) are shown for the sun leaves of *Q. myrtifolia* grown in either ambient or elevated C_a, and exposed to both 360 and 710 μmol mol^{-1} CO_2. Data shown are means ($\pm$ 1 SE) for measurements made in ≥six replicated OTCs on five occasions between summer 1999 and 2000. Two-factor ANOVA was used to test the effect of exposure C_a, and growth C_a on R_{LA} and R_{DW}. Bold figures indicate a statistically significant effect at $P < 0.05$

		Ambient		Elevated		ANOVA		
		360	710	360	710	C_a expose	C_a grow	Int
Sep. 99	R_{LA}	0.62 ± 0.05	0.57 ± 0.04	0.79 ± 0.04	0.75 ± 0.05	$F_{1,26} = 0.5$	$F_{1,26} = 0.5$	$F_{1,26} = 0.1$
	R_{DW}	3.66 ± 0.55	3.30 ± 0.33	3.90 ± 0.21	3.68 ± 0.21	$F_{1,26} = 0.4$	$F_{1,26} = 0.5$	$F_{1,26} = 0.2$
Nov. 99	R_{LA}	0.68 ± 0.04	0.68 ± 0.04	0.75 ± 0.03	0.76 ± 0.06	$F_{1,28} = 0.01$	$F_{1,28} = 2.8$	$F_{1,28} = 0.01$
	R_{DW}	4.1 ± 0.17	4.05 ± 0.23	3.89 ± 0.23	3.89 ± 0.28	$F_{1,28} = 0.01$	$F_{1,28} = 0.5$	$F_{1,28} = 0.01$
Mar. 2000	R_{LA}	0.75 ± 0.03	0.74 ± 0.04	0.88 ± 0.04	0.85 ± 0.04	$F_{1,28} = 0.4$	$F_{1,28} = 2.5$	$F_{1,28} = 0.1$
	R_{DW}	4.10 ± 0.13	4.07 ± 0.23	4.05 ± 0.23	3.52 ± 0.48	$F_{1,28} = 1.8$	$F_{1,28} = 0.6$	$F_{1,28} = 1.1$
Apr. 2000	R_{LA}	0.81 ± 0.10	0.76 ± 0.10	1.20 ± 0.03	1.12 ± 0.08	$F_{1,20} = 0.7$	$\mathbf{F_{1,20} = 22.3}$	$F_{1,20} = 0.1$
	R_{DW}	6.66 ± 0.42	6.25 ± 0.41	8.29 ± 0.23	7.69 ± 0.48	$F_{1,20} = 1.6$	$\mathbf{F_{1,20} = 15.0}$	$F_{1,20} = 0.1$
Jul. 2000	R_{LA}	0.41 ± 0.04	0.39 ± 0.03	0.43 ± 0.03	0.44 ± 0.03	$F_{1,28} = 0.9$	$F_{1,28} = 0.3$	$F_{1,28} = 0.7$
	R_{DW}	2.43 ± 0.25	2.32 ± 0.21	2.29 ± 0.17	2.35 ± 0.17	$F_{1,28} = 0.01$	$F_{1,28} = 0.1$	$F_{1,28} = 0.2$

R_{LA} is expressed in μmol m^{-2}s^{-1}, R_{DW} is expressed in ηmol g s^{-1}.

For the sun and shade leaves of *Q. myrtifolia*, R was unaffected by growth in elevated C_a. For *Q. geminata*, growth in elevated C_a decreased R by 7 and 9% in the sun and shade leaves, respectively—neither decrease was statistically significant (Fig. 3; Table 3). Leaf position in the canopy had statistically significant effects on R. For *Q. geminata*, R_{LA} and R_{DW} were lower in the shade leaves than in the sun leaves by 40 and 22%, respectively, in leaves grown in ambient C_a, and for leaves grown in elevated C_a by 40 and 22%, respectively. Similarly, for

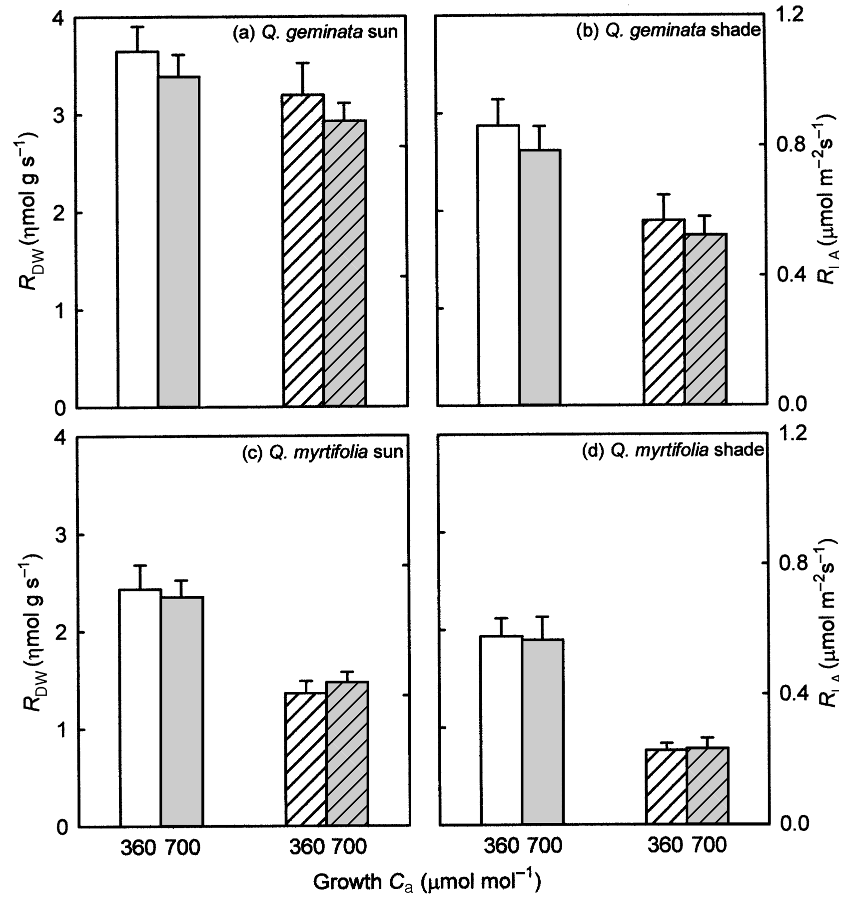

Fig. 3 Indirect effects on leaf respiration. Leaf respiration (R) of *Q. geminata* sun (a) and shade (b) leaves and *Q. myrtifolia* sun (c) and shade (d) leaves. Each plot shows both R_{DW} (unhatched bars) and R_{LA} (hatched bars) for the leaves grown at either 360 μmol mol^{-1} CO_2 (white bars) or 700 μmol mol^{-1} CO_2 (grey bars) and exposed to a common C_a of 360 μmol mol^{-1}. Each bar is the mean of eight replicated OTCs (± 1 SE).

Table 2 Light-saturated photosynthesis and leaf properties. A_{sat} was measured at the growth C_a for ambient and elevated C_a treatments. ($V_{c\,max}$) and J_{max} were derived from the equations and constants of McMurtrie & Wang (1993). All values are the means (± 1 SE) for plants from at least seven open top chambers. Two-factor ANOVA was used to test the effect of growth C_a and leaf position on the parameters measured. Bold figures indicate a statistically significant effect at $P < 0.05$

		Sun Leaves		Shade Leaves		ANOVA		
		Ambient	Elevated	Ambient	Elevated	C_a	Position	Int
Q. myrtifolia	A_{sat}	7.5 ± 1.1	13.3 ± 1.2	5.0 ± 0.6	8.9 ± 0.7	$\mathbf{F_{1,24} = 13.5}$	$\mathbf{F_{1,24} = 25.9}$	$F_{1,24} = 1.0$
	$V_{c\,max}$	66.1 ± 7.1	61.1 ± 4.2	39.3 ± 3.3	31.42 ± 3.2	$F_{1,24} = 1.9$	$\mathbf{F_{1,24} = 35.5}$	$F_{1,24} = 0.1$
	J_{max}	113.9 ± 7.5	113.5 ± 5.7	82.9 ± 6.1	76.6 ± 3.9	$F_{1,24} = 0.3$	$\mathbf{F_{1,24} = 32.5}$	$F_{1,24} = 0.2$
	N_{DW}	11.9 ± 0.3	10.6 ± 0.5	12.1 ± 0.3	10.7 ± 0.4	$\mathbf{F_{1,28} = 12.3}$	$F_{1,28} = 0.2$	$F_{1,28} = 0.1$
	f Rubisco	11.0 ± 1.0	10.44 ± 0.9	9.29 ± 0.8	8.23 ± 0.8	$F_{1,24} = 0.8$	$\mathbf{F_{1,24} = 4.9}$	$F_{1,24} = 0.1$
	SLA	5.97 ± 0.21	5.33 ± 0.15	8.46 ± 0.20	8.17 ± 0.13	$\mathbf{F_{1,28} = 7.0}$	$\mathbf{F_{1,28} = 233}$	$F_{1,28} = 0.3$
Q. geminata	A_{sat}	14.1 ± 1.2	14.8 ± 0.6	12.3 ± 1.4	9.65 ± 0.9	$F_{1,26} = 0.9$	$\mathbf{F_{1,26} = 11.1}$	$F_{1,26} = 2.6$
	$V_{c\,max}$	93.5 ± 4.7	62.4 ± 2.9	72.9 ± 7.7	40.1 ± 3.1	$\mathbf{F_{1,26} = 19.4}$	$\mathbf{F_{1,26} = 43.0}$	$F_{1,26} = 0.03$
	J_{max}	150.2 ± 7.0	120.0 ± 5.3	105.3 ± 13.2	88.0 ± 4.8	$\mathbf{F_{1,26} = 8.7}$	$\mathbf{F_{1,26} = 22.7}$	$F_{1,26} = 0.6$
	N_{DW}	11.5 ± 0.2	10.8 ± 0.3	10.5 ± 0.3	9.6 ± 0.2	$\mathbf{F_{1,28} = 7.8}$	$\mathbf{F_{1,28} = 5.8}$	$F_{1,28} = 4.2$
	f Rubisco	11.0 ± 0.6	8.0 ± 0.5	12.2 ± 1.1	7.1 ± 0.6	$\mathbf{F_{1,26} = 31.0}$	$F_{1,26} = 0.2$	$F_{1,26} = 3.5$
	SLA	5.29 ± 0.21	5.04 ± 0.15	3.90 ± 0.17	3.87 ± 0.10	$\mathbf{F_{1,28} = 46.0}$	$F_{1,28} = 0.52$	$F_{1,28} = 0.31$

A_{sat}, $V_{c\,max}$ and J_{max} are expressed in μmol m^{-2}s^{-1}, N_{DW} in mg g^{-1}. *f Rubisco* is the percentage of leaf N invested in Rubisco. SLA is expressed in m^2 kg^{-1}.

Table 3 Effect of species, leaf position and growth in elevated C_a on R. Per unit leaf area (R_{LA}) and per unit dry weight (R_{DW}) are shown for the sun and shade leaves of *Q. myrtifolia* and *Q. geminata* grown at either ambient or elevated C_a and exposed to a common Ca of 360 μmol mol^{-1}. Data shown are means ± 1SE for eight replicated OTCs. Three-way ANOVA was used to test for the effect of species, leaf position, and growth C_a on R_{LA} and R_{DW}. None of the interactions between the three factors was statistically significant, therefore *F*-values for the three factors only are shown. Bold figures indicate a statistically significant effect at $P < 0.05$

	Q. myrtifolia				*Q. geminata*				ANOVA		
	Sun		Shade		Sun		Shade				
	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Species	Position	C_a
R_{LA}	0.41 ± 0.04	0.44 ± 0.03	0.23 ± 0.02	0.23 ± 0.03	0.95 ± 0.1	0.88 ± 0.06	0.57 ± 0.08	0.52 ± 0.06	$\mathbf{F_{1,56} = 100}$	$\mathbf{F_{1,56} = 49.4}$	$F_{1,56} = 0.3$
R_{DW}	2.35 ± 0.17	2.43 ± 0.25	1.93 ± 0.18	1.89 ± 0.24	3.64 ± 0.26	3.39 ± 0.22	2.87 ± 0.27	2.62 ± 0.25	$\mathbf{F_{1,56} = 35.9}$	$\mathbf{F_{1,56} = 14.7}$	$F_{1,56} = 0.9$

R_{LA} is expressed in μmol $m^{-2}s^{-1}$, R_{DW} is expressed in ηmol g s^{-1}.

Q. myrtifolia R_{LA} and R_{DW} were also lower in the shade leaves than in the sun leaves by 44 and 21%, respectively in leaves grown in ambient C_a, and in leaves grown in elevated C_a by 48 and 20%, respectively. For both the species, grown in both ambient and elevated C_a, growth in the shade decreased the leaf density by between 30 and 53% (Table 2). Decreased leaf density in the shade leaves accounted for the fact that the decrease in R_{LA} in the shade leaves was greater than the decrease in R_{DW}. For both the sun and shade leaves, R was significantly lower in *Q. myrtifolia* than *Q. geminata* (Fig. 3; Table 3).

Comparing N, R, $V_{c\,max}$ and f Rubisco

For the sun and shade leaves of both the species, growth in elevated C_a resulted in small but statistically significant decreases in N of *c*a. 10% when it was expressed per unit dry weight. There was no effect of elevated C_a on N_{LA} for the leaves of either species (Table 2). For all the leaves the difference in the decrease in N_{DW} and N_{LA} was consistent with increases in leaf density in elevated C_a. The similar decreases in N for both the species were irrespective of the fact that $V_{c\,max}$ of *Q. myrtifolia* was unaffected by elevated C_a, whereas $V_{c\,max}$ was significantly decreased in *Q. geminata* (Table 2). The significant relationship between R_{LA} and N_{LA}, was unaffected by C_a for *Q. myrtifolia* ($t_{38} = 1.03$; $P = 0.31$) and *Q. geminata* ($t_{38} = 0.42$; $P = 0.69$) (Fig. 4). For *Q. myrtifolia*, *f Rubisco* was unaffected by elevated C_a in both the sun and shade leaves (Table 2). However, acclimation in *Q. geminata* significantly decreased *f Rubisco* from 11 to 8% in the sun leaves, and from 12.2 to 7.1% in the shade leaves (Table 2).

Comparing R, $V_{c\,max}$ and J_{max} measured in the lab and in situ

Leaf respiration measured in the lab on detached leaves was not different from R measured *in situ* in June ($t_6 = 0.25$; $p = 0.81$), July ($t_6 = 2.4$; $p = 0.06$) and August 2000 ($t_8 = 0.54$; $p = 0.61$) (Fig. 5a). For the measurements made in July 1997 both $V_{c\,max}$ ($t_8 = 0.3$; $p = 0.77$) and J_{max} ($t_8 = 0.4$; $p = 0.70$) measured on leaves attached to stems, but that had been removed from trees, were not different from $V_{c\,max}$ and J_{max} measured *in situ* (Fig. 5b).

Testing IRGA accuracy

The IRGA used in these experiments proved to be able to measure a known ΔCO_2 of 20 μmol mol^{-1} to within ± 1% accuracy, over a range of background C_a from 376 to 1000 μmol mol^{-1}, as long as the background C_a was known (Fig. 6). If the background C_a was not known, the IRGA could only measure ΔCO_2 at the background C_a at which it had been calibrated. For an IRGA calibrated at a C_a of 1000 μmol mol^{-1} measuring a ΔCO_2 at a background C_a of 376 μmol mol^{-1} the ΔCO_2 measured was 30% too high.

Discussion

This study tested two hypotheses: (1) Elevated C_a directly inhibits leaf mitochondrial R; and (2) Growth in elevated C_a will indirectly decrease leaf mitochondrial R, specifically because of the acclimatory decreases in leaf Rubisco content. For *Q. myrtifolia* and *Q. geminata*, the results of this 1-year study do not support either of the hypotheses (Figs 1, 2 and 3). Increased C_a had no direct effect on R for leaves grown in either ambient or elevated C_a. An indirect effect was observed only in young leaves just after the first-leaf flush of the year in which R was stimulated but not inhibited. In this study, R was measured on detached leaves and photosynthesis on leaves of detached stems. Neither of these procedures affected the gas exchange properties of the leaves (Fig. 5).

Recent reviews conclude that growth in elevated C_a will decrease R_{DW} by between 15 and 18% (Amthor

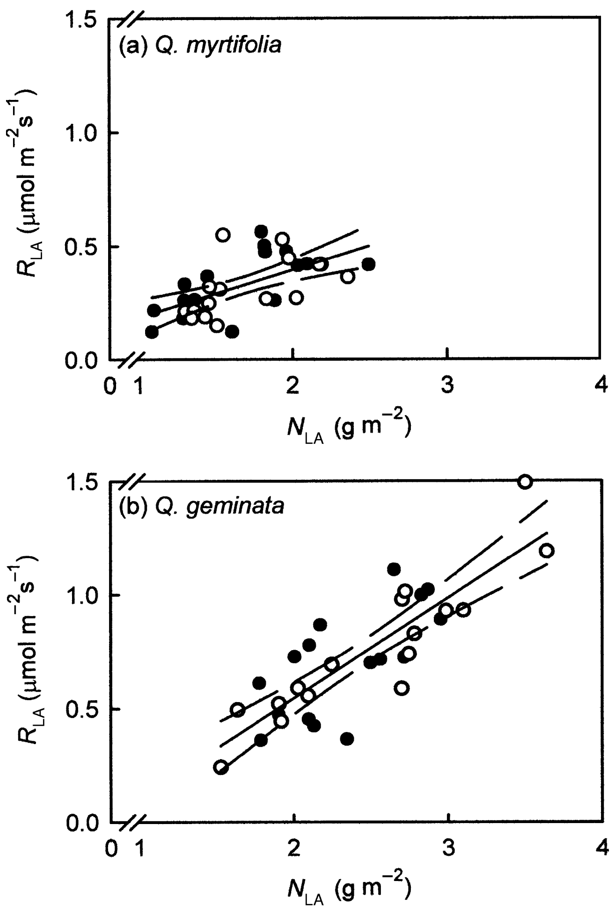

Fig. 4 Relationships between R_{LA} and N_{LA}. Plots of R_{LA} against N_{LA} for *Q. myrtifolia* (a) and *Q. geminata* (b). Each plot shows data from both the sun and shade leaves combined for both the leaves grown at elevated (solid symbols) and ambient C_a (open symbols). Least squares, linear regressions and 95% confidence limits are shown.

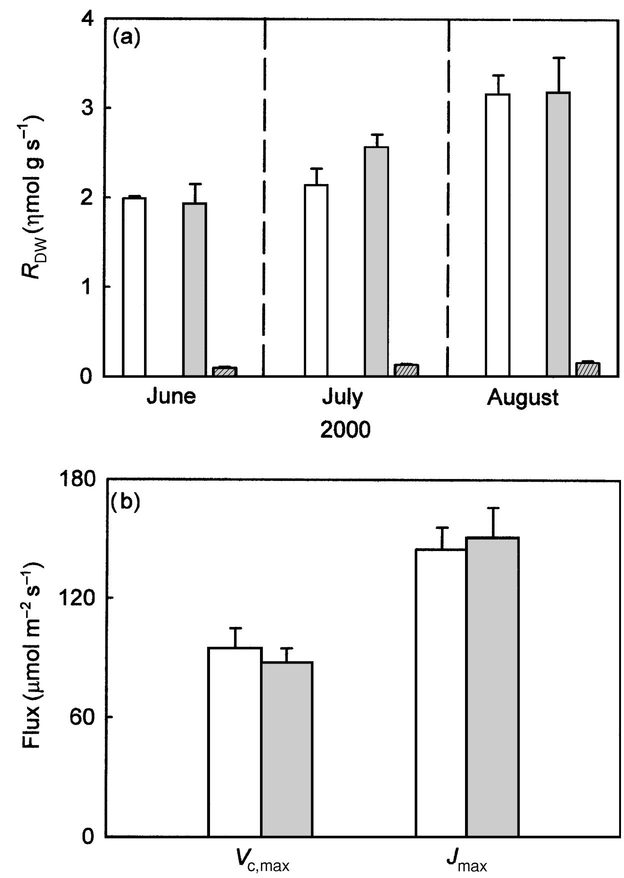

Fig. 5 Comparing R, $V_{c\,max}$ and J_{max} measured in the lab and *in situ*. (a) Plots of R_{DW} measured in June, July and August 2000 on detatched leaves (white bars) and *in situ* (grey bars). *In situ* measurements of R were partitioned between leaf R (solid grey bars) and stem R (hatched grey bars). Bars shown represent the mean ($\pm$ 1 SE) for four replicate measurements in June and July, and five replicate measurements in August 2000. (b) Plots of $V_{c\,max}$ and J_{max} measured on the leaves on excised stems (white bars) and *in situ* (grey bars). Plots shown are the mean ($\pm$ 1 SE) for five replicate measurements conducted in July 1997.

1997; Drake *et al*. 1997; Curtis & Wang 1998; Drake *et al*. 1999). However, these figures mask large variability. Gonzalez-Meler & Siedow (1999) reviewed literature reporting a direct effect of elevated C_a on R, and found that R ranged from an inhibition of up to 60% in shoots of *Castanea sativa* (El Kohen *et al*. 1991); no effect on *Pinus ponderosa* seedlings (Griffin *et al*. 1996); to a 20% stimulation in some crop species (Ziska & Bunce 1994). Both the magnitude and direction of the direct effect may depend on many interacting factors. Tissue type, species, growth temperature, C_a during growth, N supply, and activity of the alternative pathway which is not inhibited by elevated C_a, may all potentially influence the direct effect of elevated C_a on R (Gonzàlez-Meler & Siedow 1999). When elevated C_a directly inhibits R it may be owing to the inhibition of the activity of specific mitochondrial enzymes (Gonzàlez-Meler *et al*. 1996). However, this mechanism is not thought to be capable of accounting for the magnitude of the inhibition often observed, because the control of the overall rate of respiration exerted by these enzymes is too small (Gonzalez-Meler & Siedow 1999). For the leaves of *Q. myrtifolia* at 1, 4, 6, 8 and 11 month of age, and for leaves from plants that had grown in the field for up to 4 years in either current ambient or elevated C_a, we found no evidence that increasing C_a directly decreases R (Fig. 1).

This study also focused on the possibility that photosynthetic acclimation, which decreases leaf-Rubisco content, may decrease N and indirectly decrease R (Drake *et al*. 1999). As often observed, even R was strongly correlated to N (Fig. 4) (Wullschleger *et al*. 1992; Ryan 1995; Reich *et al*. 1998). However, for the sun and shade leaves

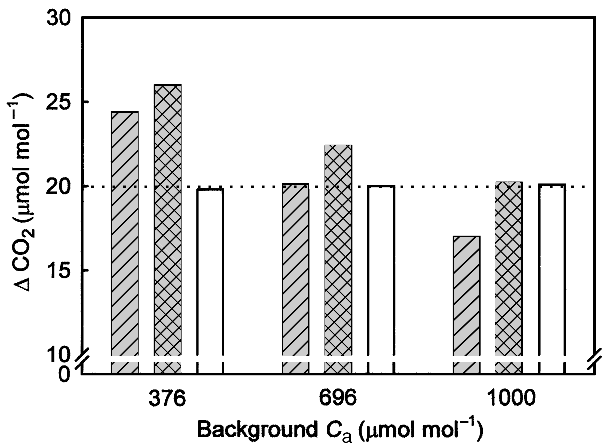

Fig. 6 Testing IRGA accuracy. Differential CO_2 concentrations (ΔCO_2) measured at three different background C_a of 376, 696 and 1000 μmol mol^{-1}. The IRGA was calibrated at a C_a of either 696 (grey, hatched bars) or 1000 (grey, cross-hatched bars) μmol mol^{-1} and uncorrected for changes in background C_a, or wired into an absolute analyzer to correct for changes in background C_a and calibrated at 696 μmol mol^{-1} (white bars). The dashed line represents the true ΔCO_2.

of *Q. geminata*, large acclimatory decreases in $V_{c\,max}$ in elevated C_a resulted in small (< 10%) decreases in N and R—only the decrease in N was statistically significant (Fig. 3; Tables 2, 3). We can conceive of four reasons why large decreases in $V_{c\,max}$ were not paralleled by large decreases in N and R:

1. Photosynthesis at low C_i and light saturation was not limited by Rubisco. This would be unusual, however, for the shade leaves it has been shown to be possible (Sage *et al*. 1990).
2. Decreases in $V_{c\,max}$ were not reflective of decreases in leaf Rubisco content. Elevated C_a often decreases leaf-Rubisco content (Drake *et al*. 1997), however, Rubisco activity can also be decreased by decreases in Rubisco activation state in elevated C_a (Drake *et al*. 1997).
3. Decreases in $V_{c\,max}$ were reflective of decreases in leaf Rubisco content, however, N released from Rubisco remained in the leaf. Nitrogen released from Rubisco in elevated C_a may remain in the leaf and be made available to N-limited sinks (Sage 1994; Stitt 1991; Woodrow 1994; Medlyn 1996). There is little consensus as to whether such a process occurs in the leaves growing in elevated C_a and experimental evidence to suggest that it does not (Medlyn 1996).
4. Decreases in $V_{c\,max}$ were reflective of decreases in leaf-Rubisco content and N released from Rubisco was translocated out of the leaf, however, *fRubisco* was such a small proportion of total N that decreases in leaf Rubisco content had small effects on N and R.

For *Q. geminata* we estimated *fRubisco* to be 11 and 12% in the sun and shade leaves, respectively (Table 2). Estimating *fRubisco* is not new (Tissue *et al*. 1996) however, the estimate requires inputs for both K_{cat} and the number of Rubisco active sites. We used 3.3 for K_{cat}—a figure obtained from Spinach (Woodrow & Berry 1988). This value may be high for the subtropical trees in this study. In addition, we assumed that Rubisco has eight active sites (Raines *et al*. 1991). It has also been shown that Rubisco has 6.5 active sites (Sage *et al*. 1993). Decreases in both K_{cat} and the number of Rubisco active sites will increase *fRubisco*. If Rubisco has 6.5 active sites and K_{cat} was 2.5 then our estimate of *fRubisco* increases to 17 and 20% in the sun and shade leaves of *Q. geminata*, respectively. If we assume that the decreases in $V_{c\,max}$ of 33 and 45% in the sun and shade leaves in elevated C_a, respectively, were reflective of decreases in leaf Rubisco content, and that N released was translocated out of the leaf, then from our lower estimates of *fRubisco* we would expect N to be decreased by 4 and 6% in the sun and shade leaves in elevated C_a, respectively, These estimates are less than the observed decreases in N of 6 and 9% in these leaves (Table 2). Only if we use the higher estimates of *fRubisco*, we would predict the same decreases in N that we observed. These findings provide good evidence that the reason that acclimatory decreases in leaf-Rubisco content did not significantly decrease N or R in this study was because *fRubisco* was low.

When measuring CO_2-fluxes into or out of leaves; leaks, diffusion of air through porous tubing, adsorption or absorption of CO_2 by tubing, may cause experimental artifacts (Long & Hällgren 1993; Amthor 2000). These errors may be compounded when small fluxes are being measured, as may be the case with respiratory fluxes from leaves. In this study we measured respiration using a system that had been designed to protect against many of these potential artifacts. Most importantly, measurements were made in cuvettes that could accommodate a large leaf area, typically 100 cm^2. This enabled the generation of a large quantity of ΔCO_2 whilst maintaining high flow rates through the system, which in turn protected against leaks of outside air into the system without compromising measurement sensitivity. Instrument ageing may change the differential sensitivity of the IRGA and render factory calibration constants inappropriate—again leading to measurement artifacts. We found that ageing of our IRGA and its components had no effect on its ability to accurately measure ΔCO_2 over a range of background C_a (Fig. 6).

In conclusion, respiratory carbon losses can be important for determining whether natural ecosystems are a net source or sink for CO_2 (Valentini *et al*. 2000). In an elevated C_a world, effects on specific rates of leaf respiration are predicted to have important consequences for ecosystem-carbon sequestration (Drake *et al*. 1999). This study was performed on plants growing in a natural ecosystem that had been exposed *in situ* to elevated C_a for between 3 and 4 years. The year of data presented in this study provided no evidence to support the hypothesis that elevated C_a decreases leaf R either directly, or indirectly owing to the acclimatory decreases in Rubisco content.

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