Impacts of drought on leaf respiration in darkness and light in *Eucalyptus saligna* exposed to industrial-age atmospheric CO_2 and growth temperature

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Summary

- Our study assessed the impact of a wide range of industrial-age climate scenarios on leaf respiration (*R*) in *Eucalyptus saligna*.
- Well-watered or sustained drought-treated plants were grown in glasshouses differing in atmospheric CO_2 concentration ($[CO_2]$) (280, 400 and 640 μl l^{-1}) and temperature (26 and 30°C). Rates of *R* in darkness (R_{dark}) and light (R_{light}), photosynthesis (*A*) and related leaf traits (mass : area relationships, and nitrogen, phosphorus, starch and sugar concentrations) were measured.
- Light inhibited *R* in all cases ($R_{light} < R_{dark}$) (well-watered: 40%; drought-treated: 73%). Growth $[CO_2]$ and temperature had little impact on area-based rates of R_{dark} or R_{light}, with R_{light} exhibiting minimal thermal acclimation. By contrast, sustained drought resulted in reduced R_{dark}, R_{light} and *A*, with the inhibitory effect of drought on *A* and R_{light} (*c.* 50–70%) greater than that on R_{dark} (*c.* 15%). Drought effects were fully reversible after watering. Variability in R_{light} appeared to be dependent on the underlying rate of R_{dark} and associated Rubisco activity.
- Collectively, our data suggest that there is an asynchronous response of leaf carbon metabolism to drought, and a tighter coupling between R_{light} and *A* than between R_{dark} and *A*, under both past and future climate scenarios. These findings have important implications for ecosystem/global models seeking to predict carbon cycling.

Introduction

Climate-dependent changes in leaf respiration (*R*) are now accepted as being important for the functioning of individual plants (Amthor, 1989) and whole ecosystems (Reichstein *et al.*, 2007), and for the extent to which atmospheric CO_2 will be sequestered by the terrestrial biosphere (King *et al.*, 2006). Although we know that rates of leaf *R* may vary in response to sustained future increases in atmospheric CO_2 concentration ($[CO_2]$) (Poorter *et al.*, 1992; Gonzàlez-Meler *et al.*, 2004), there is uncertainty about whether rates of leaf *R* have changed in response to increases in atmospheric $[CO_2]$ from subambient, pre-industrial levels (*c.* 280 μl l^{-1}) to the current *c.* 390 μl l^{-1}. Moreover, although we know that leaf *R* is dependent on water availability (Flexas *et al.*, 2005; Ribas-Carbó *et al.*, 2005; Galmés *et al.*, 2007) and growth temperature (Atkin & Tjoelker, 2003; Tjoelker *et al.*, 2009; Ow *et al.*, 2010), little is known about the extent to which rates of leaf *R* are dependent on the interactive effects of atmospheric $[CO_2]$ (past and future), growth temperature and water availability.

There is growing evidence that photosynthesis would have been CO_2-limited during the pre-industrial Holocene epoch (Sage & Reid, 1992; Tissue *et al.*, 1995; Sage & Coleman, 2001; Campbell & Sage, 2006; Ghannoum *et al.*, 2010b). Given that rates of leaf *R* and photosynthesis are often coupled (Reich *et al.*, 1998; Gifford, 2003; Loveys *et al.*, 2003; Whitehead *et al.*, 2004; Atkin *et al.*, 2006; Wertin & Teskey, 2008), one possibility is that leaf *R* might be lower in plants grown under subambient atmospheric $[CO_2]$. Alternatively, because plants grown under low $[CO_2]$ often exhibit higher concentrations of leaf nitrogen (N) (Körner & Diemer, 1994; Tissue *et al.*, 1995;

Campbell & Sage, 2006), and because leaf R is often positively correlated with leaf N (Ryan *et al.*, 1996; Tissue *et al.*, 2002; Atkin *et al.*, 2008; Reich *et al.*, 2008), growth under subambient atmospheric [CO_2] might be associated with higher leaf R.

In assessing the impact of climate-dependent changes in leaf R on plant productivity and ecosystem CO_2 exchange, we need to better understand the extent to which leaf R continues in the light (Wohlfahrt *et al.*, 2005; Mercado *et al.*, 2007). In most studies, rates of nonphotorespiratory mitochondrial CO_2 release in the light (R_{light}) are usually lower than the rate of leaf R in the dark (R_{dark}) (Hurry *et al.*, 2005), with the degree of inhibition ranging from 16 to 77%. Light inhibition is reported to be greater in leaves exposed to 365 μl l^{-1} atmospheric [CO_2] compared with plants grown under elevated [CO_2] (730 μl l^{-1}) (Wang *et al.*, 2001; Shapiro *et al.*, 2004). However, no study has yet investigated the impact of low, pre-industrial atmospheric [CO_2] on rates of R_{light}. Moreover, it is uncertain whether the impacts of pre-industrial/subambient and future/elevated atmospheric [CO_2] on light inhibition are affected by growth temperature and/or water availability. Failure to account for variations in light inhibition of leaf R can lead to large overestimates of daily respiration in individual leaves, branches and whole ecosystems, and hence gross primary productivity (Wohlfahrt *et al.*, 2005; Wingate *et al.*, 2007). It can also have important implications for our understanding of the processes controlling the rate of net CO_2 assimilation in the light (A_{net}).

One factor that might contribute to light inhibition of leaf R is photorespiration (i.e. O_2 uptake by Rubisco (v_o) and subsequent reactions in peroxisomes and mitochondria). The degree of light inhibition often *increases* under conditions in which rates of photorespiration are also high (Atkin *et al.*, 1998a,b, 2000; Hurry *et al.*, 2005; Zaragoza-Castells *et al.*, 2007), reflecting photorespiration-dependent reductions in mitochondrial pyruvate decarboxylase (PDC) activity (Randall *et al.*, 1990) and transition to a partial tricarboxylic acid (TCA) cycle in the light (Igamberdiev *et al.*, 2001; Tcherkez *et al.*, 2005). Changes in cellular energy status associated with photorespiration may be responsible for both factors (Hurry *et al.*, 2005). Substantial uncertainty remains, however, about the linkage between light inhibition and photorespiration, as Tcherkez *et al.* (2008) found that the degree of light inhibition of R_{light} *decreases* when *Xanthium strumarium* leaves are exposed to low atmospheric [CO_2] for short periods (i.e. which increase demand for TCA cycle intermediates associated with the recovery of photorespiratory cycle intermediates in the peroxisome). Thus, while the available literature strongly suggests a functional link between the degree of light inhibition of leaf R and photorespiration, the directionality of that link remains unclear.

The objective of our study was to investigate the interactive effects of atmospheric [CO_2], drought and growth temperature on leaf respiratory metabolism (R_{dark} and R_{light}) and associated leaf traits of a fast-growing evergreen tree (*Eucalyptus saligna*). Comparisons were made using plants grown under three atmospheric CO_2 concentrations (pre-industrial/subambient, ambient and future/elevated [CO_2]), two watering regimes (well watered and sustained drought) and two growth temperatures (ambient and ambient plus 4°C). Our study is the first to investigate both the main and interactive effects of atmospheric [CO_2], water availability and growth temperature on leaf R_{dark} and R_{light}. The specific aims of the study were as follows. First, we aimed to establish the response of leaf R_{dark} and R_{light} and associated leaf traits to a wide environmental envelope that encompasses past and future climate scenarios. Secondly, we aimed to determine the extent to which leaf R and photosynthesis remained coupled in leaves grown in these climate scenarios. Past studies have reported strong coupling between leaf R and photosynthesis over a limited range of environmental conditions but have not established how robust this coupling is when plants are challenged with a wide range of abiotic treatments, or the extent to which leaf R_{dark} and R_{light} differ in their degree of coupling to photosynthesis. Our third aim was to quantify the impact of environment-dependent variations in photorespiration (resulting from differences in atmospheric [CO_2], water availability and/or growth temperature) on the degree of light inhibition of leaf R.

Materials and Methods

Plant material and growing conditions

Seeds of Sydney blue gum (*Eucalyptus saligna* Sm.) were obtained from Ensis (Australian Tree Centre, Canberra, ACT, Australia). Seeds were sown on trays containing seed raising mix (Plugger Custom; Debco Pty Ltd, Berkshire Park, NSW, Australia) in late September 2008, and then placed in a temperature-controlled cabinet provided with ambient [CO_2]. Seedlings were then transplanted after 1 month into pots (15 cm diameter × 40 cm length PVC pipes) that contained 9 kg of dry loamy-sand with low organic matter and water holding capacity. Further details on soil properties are provided in Ghannoum *et al.* (2010a). Pots were then randomly placed in six adjacent glasshouse compartments (each 3.0 m (width) × 5.0 m (length) × 3.5 m (height)) located in the grounds of the University of Western Sydney, Richmond, NSW, Australia. The glasshouses were CO_2 and temperature controlled. Initially, two to six seedlings were transplanted into each pot – these were later thinned to one per pot. Seedlings were irrigated daily and periodically supplied with a nutrient solution of a commercial fertilizer (General Purpose, Thrive Professional, Yates Australia, Padstow, NSW, Australia) at a concentration of 0.2 g N l^{-1}).

Of the six glasshouse compartments, three were set to simulate the 30-yr average growing season (November–May) night : day temperature regime experienced in Richmond, NSW (18 : 26°C night : day; hereafter termed the 'ambient temperature' treatment). The other three glasshouse compartments experienced a daily temperature cycle that was 4°C higher than that of the ambient temperature regime (i.e. 22°C : 30°C night : day; hereafter termed the 'high temperature' treatment). Air temperature was thoroughly monitored using thermocouples and was adjusted continuously as described in Ghannoum *et al.* (2010a). For the three glasshouses in each temperature treatment, [CO_2] treatments were applied as subambient [CO_2] (target 280 μl l^{-1}), ambient [CO_2] (target 400 μl l^{-1}), and elevated [CO_2] (target 640 μl l^{-1}); details are provided in Ghannoum *et al.* (2010a). The average relative humidity was 70% during the experimental period. Peak midday photosynthetically active radiation (PAR) was measured by a nearby weather station and averaged 1200–1300 μmol photons m^{-2} s^{-1} over the growing period; maximum photosynthetically active radiation (PAR) values exceeded 2300 μmol photons m^{-2} s^{-1}. Within the glasshouse compartments, direct sunlight was attenuated by 10–15% compared with that outside (Ghannoum *et al.*, 2010a).

Drought treatment

In February 2009 (5 months after seed sowing), eight plants were randomly selected in each of the three [CO_2] and two temperature treatments and divided into two groups of four replicate plants. Four plants were watered daily to field capacity ('well-watered controls'), while the other set of four plants were subjected to a sustained period (1 month, starting on 9 February 2009) of water stress ('drought-treated' plants). Drought was achieved via initial cessation of daily watering followed by controlled addition of small amounts of water to maintain low stomatal conductance (g_s), which was measured each morning under saturating irradiance (1800 μmol photons m^{-1} s^{-1}) using a Li-Cor 6400 portable photosynthesis system (Li-Cor Inc., Lincoln, NE, USA). Leaf g_s provided an integrative indication of the degree of water stress in leaves (Gulias *et al.*, 2002; Medrano *et al.*, 2002). When g_s was < 0.05 mol H_2O m^{-2} s^{-1}, water was added to each pot in the morning such that morning-measured g_s values remained in the 0.05–0.10 mol H_2O m^{-2} s^{-1} range.

For each [CO_2] and temperature treatment combination, measurements of leaf gas exchange were conducted a few days after initiation of the drought treatment (the 'initial drought phase') and 3–4 wk after cessation of watering (the 'sustained drought phase'). Leaf gas exchange was also measured several days after re-watering of the drought-treatment plants to field capacity (the 'recovery phase').

Soil water potential was determined based upon weighing pots each morning, determining the soil water content of the soil, and converting soil water content to soil water potential using an empirically determined soil moisture release curve. Soil water potentials in drought-treatment plants during the initial drought phase (1.21 ± 0.40 kPa) and the sustained drought phase (0.97 ± 0.17 kPa) were similar ($P > 0.05$), but significantly lower than in well-watered plants (0.07 ± 0.05 kPa) during those periods. The soil water potential in drought-treatment plants in the recovery phase (0.07 ± 0.02 kPa) was similar to that in well-watered plants (0.07 ± 0.05 kPa) during that period. There were no [CO_2] or temperature treatment effects on soil water potential during the experimental period.

Leaf gas exchange measurements

Light response curves of net CO_2 exchange (A_{net}) were measured on the most recently fully expanded leaves using a Li-Cor 6400 portable photosynthesis system (Li-Cor Inc.) equipped with a CO_2 controller and a 6-cm^2 chamber with a red-blue light source (6400-02B). Light response curve measurements were conducted at 26°C, irrespective of the growth temperature. In all cases, light-response measurements were conducted at the respective growth [CO_2]. Light-saturated photosynthesis (A_{sat}) was measured at 1800 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) and a relative humidity of 60–70% *c.* 3 h after sunrise, and after leaves had been exposed to saturating irradiance in the cuvette for 10 min. After measurement of A_{sat}, the irradiance response of net CO_2 exchange was measured, beginning at 100 μmol m^{-2} s^{-1}, followed by 90, 80, 70, 60, 55, 50, 45,40, 35, 30, 25 and 20 μmol m^{-2} s^{-1}, and ending at 0 μmol m^{-2} s^{-1} (i.e. darkness) (Supporting Information Fig. S1). An equilibration period of 5 min was allowed at each irradiance level before gas exchange was recorded. Additional measurements of net CO_2 exchange in darkness were conducted after a further 10 min of darkness. The flow rate was kept at 500 μmol s^{-1} for measurements made at saturating irradiance and 300 μmol s^{-1} for the low irradiance (100–0 μmol m^{-2} s^{-1}). Typically, light-response curve measurements were completed by mid-afternoon.

We used the Kok (1948) method to estimate R_{light} because measurements could be conducted at the prevailing growth atmospheric [CO_2], which was not possible with the Laisk (1977) method. First order regressions were fitted to the linear region between 20 and 60 μmol m^{-2} s^{-1} (data were often curvilinear at irradiances above 70 μmol m^{-2} s^{-1} PPFD; Fig. S1). Averaged across growth [CO_2] and temperature treatments, the r^2 values of well-watered and drought-treated plants were 0.97 ± 0.01 and 0.95 ± 0.01, respectively.

When using the Kok method, consideration needs to be given to the fact that internal CO_2 concentrations (c_i) increase as measuring irradiance decreases (Fig. S2). The rise in c_i suppresses photorespiration and increases carboxylation, resulting in a concomitant relative increase in the rate of net assimilation in the linear region (Villar *et al.*, 1994). As a result, the slope of the linear region plotted through observed data is less than it would be if c_i remained constant (Kirschbaum & Farquhar, 1987; Villar *et al.*, 1994), with the result that R_{light} may be underestimated. Accordingly, R_{light} was estimated after correcting for changes in internal CO_2 concentration (c_i) (Kirschbaum & Farquhar, 1987), whereby rates of R_{light} are adjusted (by iteration) to ensure that the intercept of plots of photosynthetic electron transport (J) against irradiance are minimized. J was calculated according to Farquhar & von Caemmerer (1982):

$$J = \frac{\left[\left(4 \times \left(A_{net} + R_{light}\right)\right) \times \left(c_i + 2\Gamma_*\right)\right]}{\left(c_i - \Gamma_*\right)}, \quad \text{Eqn 1}$$

(c_i, the intercellular CO_2 concentration; Γ_*, the CO_2 compensation point in the absence of R_{light} (von Caemmerer & Farquhar, 1981).) Rates of oxygenation and carboxylation by Rubisco (v_o and v_c, respectively) at any given irradiance were calculated according to Farquhar & von Caemmerer (1982):

$$v_c = \frac{1}{3} \times \left[(J/4) + 2(A_{net} + (R_{light}))\right] \quad \text{Eqn 2}$$

and:

$$v_o = \frac{2}{3} \times \left[(J/4) - (A_{net} + (R_{light}))\right] \quad \text{Eqn 3}$$

An underlying assumption in our approach to calculating R_{light} using the Kok method is that the concentration of CO_2 at sites of carboxylation (c_c) is the same as that in the internal airspace (c_i). In leaves with moderate to high internal conductance (g_i; mol m^{-2} s^{-1}), this assumption is likely to be valid. However, in illuminated leaves exhibiting low internal conductance, c_c will be lower than c_i, with the relative difference between c_c and c_i declining with decreasing measuring irradiance (Fig. S1). Low g_i thus has implications for the Kirschbaum & Farquhar (1987) correction procedure noted above. To explore how variations in g_i impact on c_c (and thus predicted rates of R_{light}), we estimated g_i based on data presented in Evans & von Caemmerer (1996) comparing rates of A_{sat} with corresponding g_i values:

$$g_i = 0.012A_{sat} \quad \text{Eqn 4}$$

Subsequently, c_c was calculated according to:

$$c_c = c_i - \frac{A_{net}}{g_i} \quad \text{Eqn 5}$$

Finally, R_{light} was estimated via application of the Kirschbaum & Farquhar (1987) correction procedure after replacing c_i with c_c.

Leaf structural traits and chemical composition

In the sustained drought phase, leaves utilized in gas exchange were collected and leaf area was measured using a portable leaf area meter (LI-3100A; Li-Cor). Subsequently, leaf fresh mass was determined, and leaves were frozen in liquid N_2 and stored in a −85°C freezer, freeze-dried for 24 h, and then weighed for dry mass. Leaf samples were ground in a ball mill and analysed for tissue N and phosphorus (P) using a Technicon Auto-analyzer II (Bran + Luebbe Pty. Ltd, Norderstedt, Germany) and Kjeldahl acid digests.

In addition, two leaves adjacent to gas exchange leaves were collected to determine ratios of leaf dry mass to leaf area (LMA), leaf fresh mass to leaf area (FMA) and leaf dry matter content (DMC; the ratio of leaf dry mass to leaf fresh mass).

The additional leaves were also used to analyse sugars, starch and total nonstructural carbohydrates (TNCs) as described previously (Loveys *et al.*, 2003).

Statistical analyses

Statistical analyses were conducted using SPSS (version 16.0; SPSS, Chicago, IL, USA). Data were tested for normality and homogeneity of variance, and log or arcsine square root transformed where necessary. Data were analysed using analysis of variance and linear and stepwise regression. To compare means, LSD post hoc tests were used.

Results

Leaf gas exchange rates were measured twice over the 1-month drought period, and after re-watering. All gas exchange data were used when assessing relationships between environment-dependent variations in photorespiration and the degree of light inhibition of leaf R, with focus being placed on the impacts of the sustained drought phase on leaf gas exchange, and when considering a broad range of leaf structural, chemical and physiological traits.

Leaf structural and chemical traits

Table 1 shows a range of leaf structural and chemical composition data for leaves harvested during the sustained drought phase, with Table 2 showing the results of a three-

Table 1 Effect of growth temperature, atmospheric CO_2 concentration and water availability on structural and chemical traits of leaves of *Eucalyptus saligna* sampled during the sustained drought period

Growth temperature (°C)	Atmospheric CO_2 concentration (μl l^{-1})	Water treatment	LMA (g DM m^{-2})	FMA (g FM m^{-2})	DMC (g DM g^{-1} FM)	[sugars] (mg g^{-1} DM)	[starch] (mg g^{-1} DM)	[TNC] (mg g^{-1} DM)	[N] (mg g^{-1} DM)	[P] (mg g^{-1} DM)	N : P ratio
26	280	Well-watered	58.2 ± 3.3	154.7 ± 6.1	0.38 ± 0.01	67.5 ± 2.8	51.7 ± 25.0	119.2 ± 23.5	18.1 ± 0.9	1.6 ± 0.3	12.5 ± 2.7
		Drought-treated	60.4 ± 4.6	154.6 ± 9.8	0.39 ± 0.01	58.8 ± 1.9	200.6 ± 21.9	259.6 ± 21.5	14.2 ± 0.7	0.9 ± 0.1	16.1 ± 1.4
	400	Well-watered	57.2 ± 1.2	164.4 ± 4.9	0.35 ± 0.01	65.2 ± 3.0	83.3 ± 29.1	148.4 ± 27.1	19.2 ± 2.1	2.9 ± 0.4	6.7 ± 0.5
		Drought-treated	69.8 ± 7.0	175.4 ± 13.7	0.40 ± 0.01	65.7 ± 2.5	233.7 ± 35.7	299.5 ± 34.2	10.0 ± 0.4	1.5 ± 0.3	7.2 ± 1.1
	640	Well-watered	79.9 ± 11.1	202.7 ± 28.2	0.39 ± 0.01	61.6 ± 3.1	226.7 ± 32.3	288.2 ± 29.4	12.6 ± 0.8	2.1 ± 0.1	6.3 ± 0.8
		Drought-treated	88.6 ± 5.3	202.1 ± 12.5	0.44 ± 0.01	61.2 ± 2.2	282.8 ± 12.4	344.8 ± 11.7	7.6 ± 0.3	1.1 ± 0.2	7.0 ± 0.7
30	280	Well-watered	40.3 ± 3.4	135.2 ± 14.3	0.30 ± 0.01	62.1 ± 3.3	44.5 ± 12.7	106.5 ± 12.7	24.2 ± 2.0	2.1 ± 0.1	11.6 ± 0.5
		Drought-treated	47.4 ± 1.5	146.7 ± 4.9	0.32 ± 0.01	57.8 ± 1.0	144.8 ± 23.0	202.6 ± 23.8	15.6 ± 0.8	1.1 ± 0.1	14.0 ± 0.7
	400	Well-watered	60.7 ± 2.7	172.0 ± 7.9	0.35 ± 0.01	63.7 ± 1.2	95.4 ± 19.8	159.2 ± 19.3	16.5 ± 0.8	1.7 ± 0.1	10.1 ± 0.7
		Drought-treated	70.7 ± 2.4	174.2 ± 3.4	0.41 ± 0.02	71.7 ± 2.8	140.7 ± 49.4	212.4 ± 48.2	11.6 ± 1.1	1.0 ± 0.2	11.7 ± 1.5
	640	Well-watered	63.2 ± 3.9	167.9 ± 9.7	0.38 ± 0.02	55.7 ± 3.7	240.4 ± 32.7	296.1 ± 29.8	13.8 ± 1.2	1.5 ± 0.2	9.3 ± 0.8
		Drought-treated	64.9 ± 7.4	164.8 ± 8.1	0.39 ± 0.03	62.1 ± 2.4	203.5 ± 59.5	265.7 ± 57.4	12.1 ± 1.5	1.3 ± 0.5	11.0 ± 2.3

Values are means (n = 4, ± SE). See Table 2 for statistical analysis results. LMA, leaf mass to leaf area; FMA, fresh mass per unit area; DMC, dry matter content; [sugars], concentration of soluble sugars; [starch], starch concentration; [TNC], concentration of total nonstructural carbohydrates; [N], nitrogen concentration; [P], phosphorous concentration.

way ANOVA considering all treatments, also for leaves harvested during the sustained drought phase. In many cases, there was a significant interaction between growth [CO_2] and temperature (Table 2), suggesting that the response to growth [CO_2] needs to be considered separately for ambient and elevated temperature growth plants (using two-way ANOVAs with growth [CO_2] and water supply as factors).

For plants grown at ambient temperature, leaf structural and chemical composition traits were often similar in plants grown under subambient and ambient [CO_2], with only plants grown under elevated [CO_2] exhibiting markedly different trait values (Tables 1, 2). For example, LMAs were higher in elevated [CO_2], underpinned by large differences in FMA (i.e. elevated [CO_2]-grown leaves were thicker than their ambient and subambient [CO_2]-grown counterparts). By contrast, growth under subambient [CO_2] did result in markedly different leaf trait values (relative to their ambient and elevated [CO_2] counterparts) when plants were grown at elevated temperature (Tables 1, 2). Thus, the impact of subambient [CO_2] on leaf structural and chemical traits appears to be growth temperature dependent.

Leaf N and P concentrations were consistently lower in drought-treated leaves irrespective of growth [CO_2] (two-way ANOVA, $P < 0.001$), with overall N concentrations highest in plants grown under subambient [CO_2] (Table 1). N : P ratios were markedly higher under subambient [CO_2] compared with plants grown under ambient and elevated [CO_2], with N : P ratios highest in drought-treated plants (Tables 1, 2).

Irrespective of the growth temperature, significant effects of growth [CO_2] and water supply on TNC were found, with TNC being greatest under elevated [CO_2] and drought conditions (Tables 1, 2). Variations in TNC were mostly attributable to large variations in the concentration of starch (varying near 6-fold, from 52 to 283 mg g^{-1}) because soluble sugar concentrations were relatively homeostatic across the treatments (range: 56 to 72 mg g^{-1}; Table 1).

Environmental response of leaf gas exchange

The impacts of each treatment combination on a range of area-based leaf gas exchange parameters from the initial drought phase measurements are shown in Table S1. Growth [CO_2] and water availability both had significant effects on photosynthetic traits – by contrast, growth [CO_2] did not significantly affect rates of leaf R_{dark} and R_{light}. While drought treatment significantly reduced R_{light}, R_{dark} was not significantly affected by water availability during this early stage of drought.

During the sustained drought phase, there was no significant effect of growth [CO_2] or temperature on area-based A_{sat} (Fig. 1, Table 3). By contrast, drought-mediated reductions in $g_{s\text{-}sat}$ (Fig. 1) resulted in consistent and significantly

Table 2 Main and interactive effects of atmospheric [CO_2], growth temperature and water availability on structural and chemical traits for leaves of *Eucalyptus saligna* sampled during the sustained drought period

		Main effects			Interactive effects			
Parameter	*n*	CO_2	Temp	H_2O	CO_2 × Temp	CO_2 × H_2O	Temp × H_2O	CO_2 × Temp × H_2O
LMA	4	**0.000**	**0.001**	**0.026**	**0.011**	0.618	0.790	0.721
FMA	4	**0.000**	**0.032**	0.621	*0.083*	0.862	0.993	0.830
DMC	4	**0.000**	**0.000**	**0.000**	**0.001**	0.185	0.791	0.445
Soluble sugars	4	**0.004**	0.378	0.822	0.248	**0.010**	*0.050*	0.921
Starch	4	**0.000**	*0.070*	**0.000**	0.978	**0.043**	**0.034**	0.810
TNC	4	**0.000**	**0.050**	**0.000**	0.997	**0.047**	**0.040**	0.813
[N]	4	**0.000**	**0.000**	**0.000**	**0.037**	*0.083*	0.486	**0.020**
[P]	4	**0.100**	0.120	**0.000**	**0.004**	0.423	0.140	0.256
N : P ratio	4	**0.000**	**0.013**	**0.029**	**0.011**	0.515	0.840	0.774

Number of replicates (*n*) and *P*-values from the three-way ANOVA are presented. Significant values ($P < 0.05$) are shown bold, while those values close to significance are shown in italic. See Table 1 for average values of each parameter. LMA, leaf mass to leaf area; FMA, fresh mass per unit area; DMC, dry matter content; [sugars], concentration of soluble sugars; [starch], starch concentration; [TNC], concentration of total nonstructural carbohydrates; [N], nitrogen concentration; [P], phosphorous concentration.

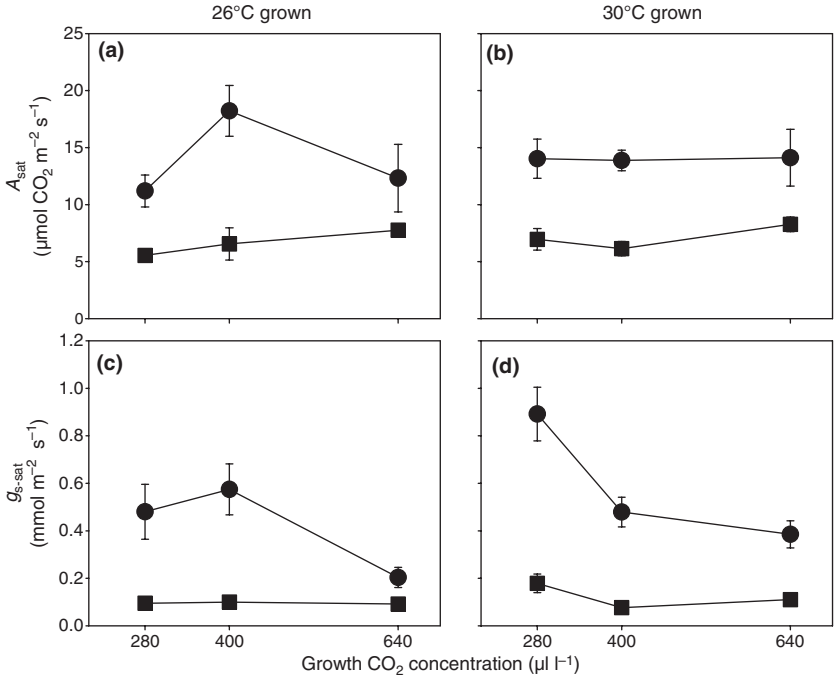

Fig. 1 Effects of growth temperature, atmospheric [CO_2], and water availability on area-based rates of light-saturated photosynthesis (A_{sat}) (a, b) and stomatal conductance ($g_{s\text{-}sat}$) (c, d), using data from the sustained drought phase of the experiment. (a, c) Data for 26°C-grown *Eucalyptus saligna* plants; (b, d) data for 30°C-grown plants. Within each growth temperature: closed circles, well-watered plants; closed squares, drought-treated plants. Values are means ($n = 4$, ± SE).

lower rates of area-based A_{sat} in all growth [CO_2] and growth temperature treatments (Fig. 1, Table 3). Averaged across all [CO_2] treatments, drought reduced mean A_{sat} by 52 and 49% in the ambient and elevated temperature-grown plants, respectively. Associated with the decline in area-based A_{sat} were significant decreases in area-based carboxylation ($v_{c\text{-}sat}$) and increases in oxygenation ($v_{o\text{-}sat}$) (data not shown). Re-watering of drought-treated plants resulted in full recovery of area-based A_{sat} when compared with well-watered controls (Table 3, Fig. S3).

When expressed on a dry mass basis, drought also decreased A_{sat}, irrespective of the growth [CO_2] or temperature (Fig. 2). By contrast, while drought-mediated reductions in N- and P-based rates were evident in most treatment combinations (Table S2), little difference in A_{sat}/[N] and A_{sat}/[P] were found between well-watered and drought-treated plants at elevated [CO_2] in plants grown at 26°C (Fig. 2). The relative difference in A_{sat} between drought-treated and well-watered plants was smaller when considered on a per unit [P] basis than when considered on a per unit dry mass or per unit area basis.

As was the case for A_{sat} during the sustained drought phase, no significant effects of growth [CO_2] or temperature were found on area-based leaf R_{dark} and R_{light} for each

Table 3 Main and interactive effects of atmospheric [CO_2], growth temperature and water availability on leaf gas exchange traits for leaves of *Eucalyptus saligna* sampled during the sustained drought and recovery periods

		Main effects			Interactive effects			
Parameter	*n*	CO_2	Temp	H_2O	CO_2 × Temp	CO_2 × H_2O	Temp × H_2O	CO_2 × Temp × H_2O
(a) Sustained drought phase								
A_{sat}	4	0.285	0.746	**0.000**	0.119	0.126	0.823	0.402
R_{light}	4	0.767	0.363	**0.000**	0.364	0.628	0.907	0.998
R_{dark}	4	0.495	*0.088*	**0.012**	0.233	0.296	0.293	0.870
R_{light} : R_{dark}	4	0.358	0.826	**0.000**	0.421	0.948	0.223	0.919
R_{light} : A_{sat}	4	0.170	*0.092*	0.783	0.403	0.215	0.374	0.515
R_{dark} : A_{sat}	4	0.281	**0.023**	**0.000**	0.214	*0.081*	0.776	0.661
(b) Recovery phase								
A_{sat}	4	**0.017**	0.198	0.339	*0.064*	0.791	0.823	0.474
R_{light}	4	0.102	**0.001**	0.507	0.600	0.898	*0.069*	0.333
R_{dark}	4	**0.035**	**0.004**	0.509	0.201	0.680	**0.015**	0.842
R_{light} : R_{dark}	4	0.140	*0.098*	0.887	0.941	0.293	0.900	0.141
R_{light} : A_{sat}	4	**0.026**	**0.031**	0.124	0.100	0.332	**0.048**	0.166
R_{dark} : A_{sat}	4	**0.029**	*0.097*	0.123	*0.058*	0.231	*0.068*	0.567

Number of replicates (*n*) and *P*-values from the three-way ANOVA are presented. Significant values ($P < 0.05$) are shown bold, while those values close to significance are shown in italic. For average values of each trait during the sustained drought phase, see Fig. 1 (A_{sat}) and Fig. 4 (R_{light}, R_{dark}, R_{light} : R_{dark}, R_{light} : A_{sat} and R_{dark} : A_{sat}), and for data during the recovery phase, see Supporting Information Fig. S1 (A_{sat}) and Fig. 6 (R_{light}, R_{dark}, R_{light} : R_{dark}, R_{light} : A_{sat} and R_{dark} : A_{sat}). A_{sat}, area-based light-saturated photosynthesis; R_{light}, area-based leaf respiration in the light, R_{dark}, area-based respiration in the dark, R_{light} : R_{dark}, ratio of respiration in the light to that in the dark; R_{light} : A_{sat} and R_{dark} : A_{sat}, ratios of respiration in the light and dark to saturated photosynthesis (respectively).

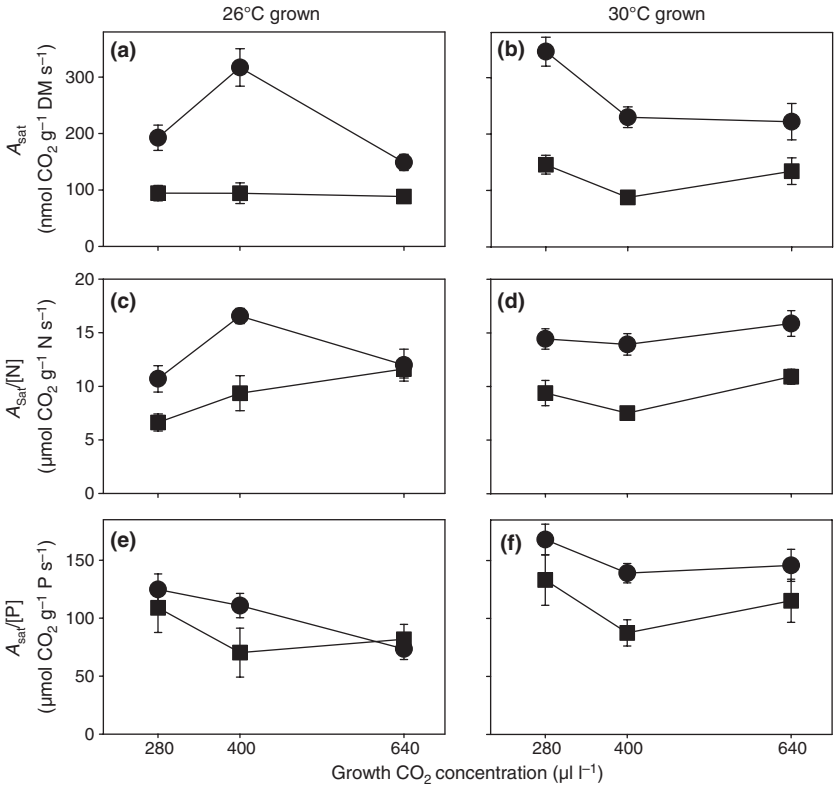

Fig. 2 Effects of growth temperature, atmospheric [CO_2], and water availability on rates of light-saturated photosynthesis (A_{sat}) expressed per unit dry mass, nitrogen and phosphorous, using data from the sustained drought phase of the experiment. (a, c, e) Data for 26°C-grown *Eucalyptus saligna* plants; (b, d, f) data for 30°C-grown plants. Within each growth temperature: closed circles, well-watered plants; closed squares, drought-treated plants. Values are means ($n = 4$, ± SE).

treatment combination (when measured at a common temperature of 26°C) (Fig. 3a,b, Table 3). However, there was a significant inhibitory effect of drought on area-based leaf R_{dark} and R_{light} (Table 3). Averaged across all [CO_2] treatments, drought reduced mean R_{dark} by 17% (ambient temperature) and 16% (elevated temperature), and R_{light}

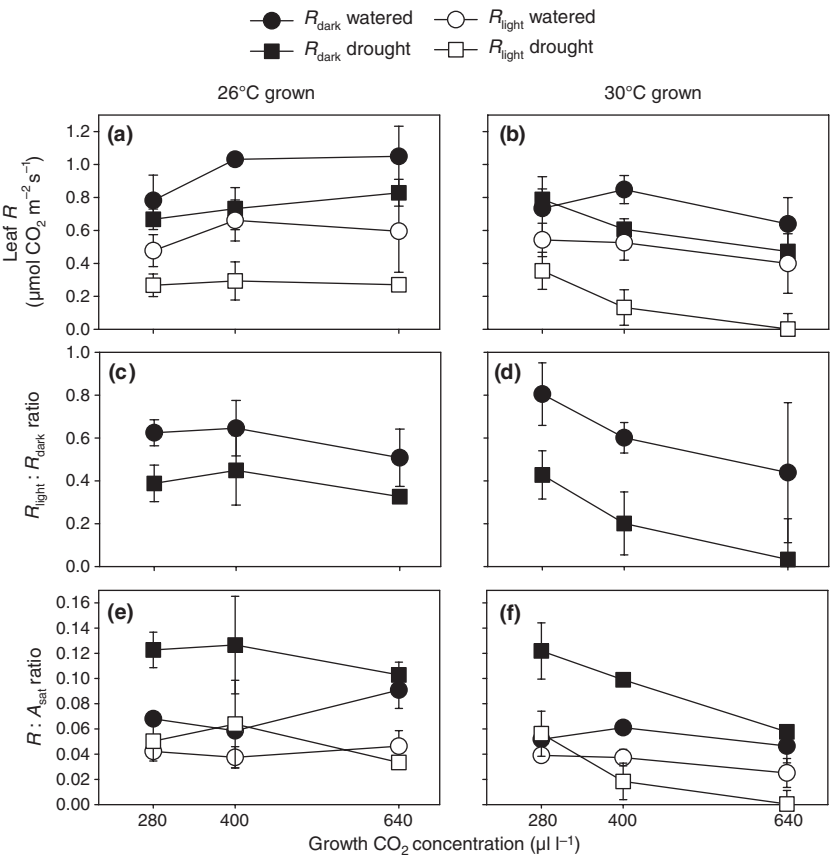

Fig. 3 Effects of growth temperature, atmospheric [CO_2], and water availability on area-based rates of leaf respiration and related traits for gas exchange rates measured during the sustained drought phase of the experiment. Shown are plots of *Eucalyptus saligna* leaf respiration in the light (R_{light}) and in the dark (R_{dark}) (a, b), ratios of respiration in the light to that in darkness (R_{light} : R_{dark}) (c, d), and ratios of respiration (both in the light and in the dark) to saturated photosynthesis (R : A_{sat}) (e, f), each against growth [CO_2]. (a, c, e) Data for 26°C-grown plants; (b, d, f) data for 30°C-grown plants. Dark respiration: closed squares, drought-treated plants; closed circles, well-watered plants. R_{light}: open squares, drought-treated plants; open circles, well-watered plants, within each growth temperature. Values are means (n = 4, ± SE). Note: R_{light} values were calculated assuming infinite internal conductance (i.e. $g_i = \infty$), with rates of R_{light} corrected for irradiance-dependent changes in internal CO_2 concentrations (c_i) (Kirschbaum & Farquhar, 1987). See Fig. 6 and Supporting Information Fig. S5 for comparative values calculated assuming $g_i = 0.012A_{sat}$ (Evans & von Caemmerer, 1996).

by 52% (ambient temperature) and 67% (elevated temperature).

R_{light} was consistently lower than R_{dark} (Figs 3, 4) generating R_{light} : R_{dark} ratios less than unity (Fig. 3c,d). Drought resulted in significantly lower R_{light} : R_{dark} ratios (Table 3), demonstrating a greater drought sensitivity of R_{light} compared with R_{dark}. When averaged across both growth temperatures and [CO_2] treatments, average R_{light} : R_{dark} ratios were 0.60 ± 0.07 (i.e. 40% light inhibition) and 0.27 ± 0.06 (i.e. 73% light inhibition) for well-watered and drought-treated plants, respectively. Moreover, the degree of drought inhibition of R_{light} was similar to that of A_{sat}, as shown by the lack of significant drought effects on R_{light} : A_{sat} ratios (Table 3, Fig. 3e,f). By contrast, drought resulted in significant increases in R_{dark} : A_{sat} because drought had a greater inhibitory effect on A_{sat} than R_{dark}. Rewatering of drought-treated plants resulted in full recovery of leaf R_{dark} and R_{light}, as well as R_{light} : R_{dark}, R_{light} : A_{sat} and R_{dark} : A_{sat} (Table 3, Fig. 5).

Sustained drought significantly decreased leaf R_{light} on a dry mass, N and P basis (Fig. 4, Table S2). Similarly, drought-mediated decreases in leaf R_{dark} were evident on a dry mass and P basis, but not on an N basis (Fig. 4, Table S2). When expressed on a leaf N basis, growth temperature had an overall significant effect on leaf R_{dark}, reflecting higher R_{dark} in 26°C-grown plants (compared with 30°C-grown plants), particularly for plants grown under elevated [CO_2] (Fig. 4).

Accounting for limitations in internal conductance

To explore how variation in g_i might affect predicted rates of R_{light}, we first applied the Kirschbaum & Farquhar (1987) correction after replacing c_i with c_c, using a wide range of *assumed* g_i values to calculate c_c. Fig. S4 shows that predicted R_{light} (using data for subambient [CO_2]/ambient temperature-grown plants from the sustained drought phase) increased markedly at low g_i (particularly below 0.1 mol m^{-2} s^{-1}). Thus, low g_i must be considered when estimating R_{light}.

To obtain estimates of g_i, we calculated g_i using Eqn 4 (Evans & von Caemmerer, 1996) using data from the sustained drought phase (Table 4). We then calculated c_c at each measurement irradiance. As expected, c_c was markedly lower than the corresponding c_i, with the impact of decreasing irradiance being greater on c_c than on c_i (Fig. S2). To assess the consequences for estimated R_{light}, we then applied the Kirschbaum & Farquhar (1987) correction procedure using these c_c values. Fig. 6 shows R_{light} for all plants measured in the sustained drought phase, with R_{light} calculated

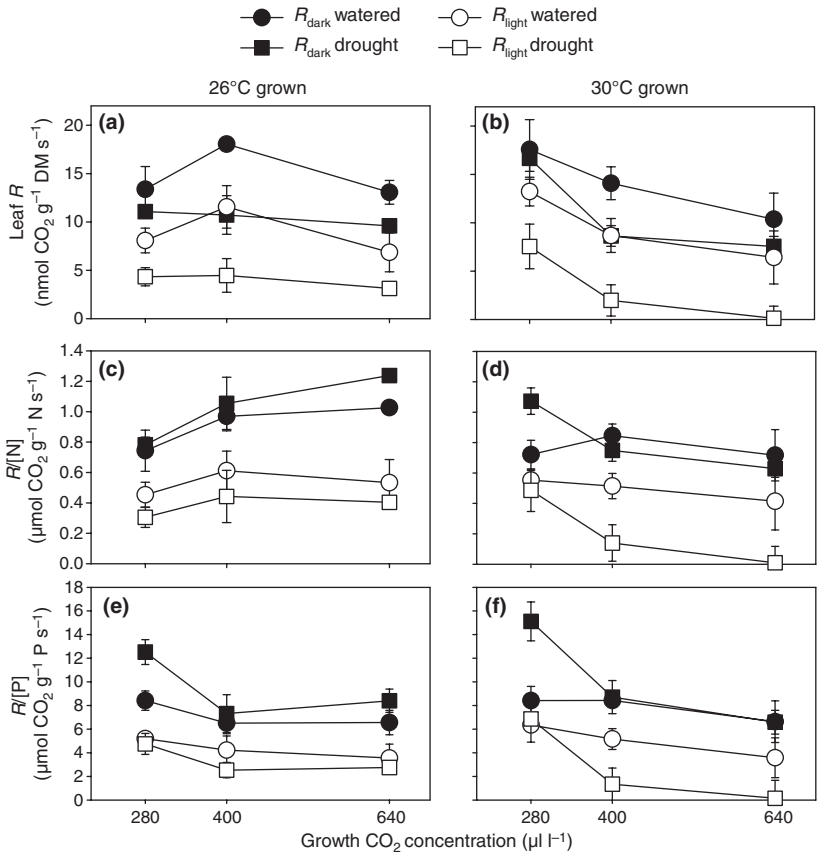

Fig. 4 Effects of growth temperature, atmospheric [CO_2], and water availability on rates of leaf respiration in the light and the dark (R_{light} and R_{dark}, respectively) expressed per unit dry mass, nitrogen and phosphorous, using data from the sustained drought phase of the experiment. (a, c, e) Data for 26°C-grown *Eucalyptus saligna* plants; (b, d, f) data for 30°C-grown plants. R_{dark}: closed squares, drought-treated plants; closed circles, well-watered plants. R_{light}: open squares, drought-treated plants; open circles, well-watered plants, within each growth temperature. Values are means (n = 4, ± SE). See legend of Fig. 3 for further details.

assuming $g_i = \infty$ being plotted against the corresponding R_{light} values calculated assuming $g_i = 0.012A_{sat}$. In some cases R_{light} (and R_{light} : R_{dark}) increased when assuming low g_i. However, overall the difference in predicted R_{light} and corresponding R_{light} : R_{dark} ratios was relatively minor. Moreover, with the exception of drought impacts on subambient [CO_2]/ambient temperature-grown plants, the trends observed in Fig. 3 (i.e. greater proportional reduction in R_{light} than R_{dark}, assuming $g_i = \infty$) were maintained when a similar plot was generated using data calculated assuming $g_i = 0.012A_{sat}$ (Fig. S5). Therefore, conclusions regarding the effect of drought on R_{light} and R_{light} : R_{dark} remain valid when accounting for restricted diffusion of CO_2 within leaves.

Leaf *R* and predictive criteria

One of the aims of our study was to establish whether environment-dependent variation in photorespiration had a predictable impact on leaf R_{light} and the degree of light inhibition of leaf R. To address this aim, we conducted linear and stepwise regressions of all area-based gas exchange data (assuming $g_i = \infty$) from the initial drought phase, the sustained drought phase, and the recovery phase.

Although significant *positive* relationships were found between leaf R_{light} and photorespiration (when v_o was measured at 1800 and 100 μmol m^{-2} s^{-1} PPFD), and between R_{light} : R_{dark} ratios and photorespiration, r^2 values were uniformly low (Table 5). Significant R_{light}–v_c and R_{light} : R_{dark}–v_c relationships were also observed (Table 5). Variations in R_{light} were, however, strongly correlated with variations in R_{dark} (r^2 = 0.522), with slope of the R_{light}–R_{dark} plot being 0.702 (i.e. when considering all data collectively, the average degree of light inhibition of leaf R was 30%). Variations in R_{light} : R_{dark} were more strongly correlated with variations in R_{light} (r^2 = 0.457) than R_{dark} (r^2 = 0.025).

A stepwise regression model including all leaf gas exchange parameters indicated that leaf R_{dark} accounted for 52% of variation in R_{light} (Table 5). Rates of v_c measured at 100 μmol m^{-2} s^{-1} PPFD ($v_{c\text{-}100}$) contributed a further 7% (i.e. a total of 59% was explained in the two-component model, where $R_{light} = -0.452 + 0.654R_{dark} + 0.069\ v_{c\text{-}100}$). Adding other parameters to the model did not significantly increase the explanatory power, suggesting that variations in R_{light} can be largely explained by R_{dark} and the prevailing rate of carboxylation by Rubisco. Importantly, when excluding $v_{c\text{-}100}$ and $v_{c\text{-}1800}$ from the model, 58% of the variation in R_{light} could still be accounted for in a model that included R_{dark} and rates of v_o.

To explore the relationship between R_{light} : R_{dark} and all other leaf functional traits, we conducted a stepwise

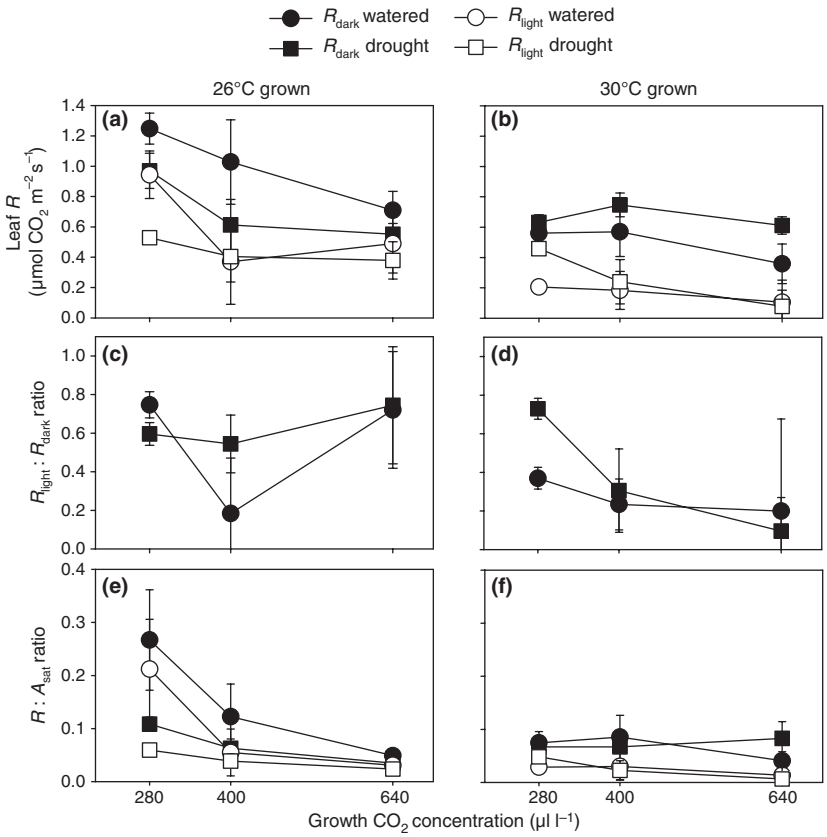

Fig. 5 Effects of growth temperature, atmospheric [CO_2], and water availability on area-based rates of leaf respiration and related traits for gas exchange rates measured during the recovery phase of the experiment on *Eucalyptus saligna* plants. See legend of Fig. 3 for further details.

Table 4 Estimates of internal conductance (g_i) calculated using data from *Eucalyptus saligna* plants measured during the sustained drought phase

Growth temperature (°C)	Atmospheric CO_2 concentration (μl l^{-1})	Water treatment	g_i (mol m^{-2} s^{-1})
26	280	Well-watered	0.134 ± 0.019
		Drought-treated	0.067 ± 0.007
	400	Well-watered	0.219 ± 0.031
		Drought-treated	0.079 ± 0.020
	640	Well-watered	0.148 ± 0.041
		Drought-treated	0.097 ± 0.006
30	280	Well-watered	0.168 ± 0.021
		Drought-treated	0.083 ± 0.011
	400	Well-watered	0.166 ± 0.011
		Drought-treated	0.074 ± 0.008
	640	Well-watered	0.169 ± 0.030
		Drought-treated	0.099 ± 0.008

g_i was calculated assuming $g_i = 0.012A_{sat}$ (Evans & von Caemmerer, 1996) using data shown in Fig. 1.

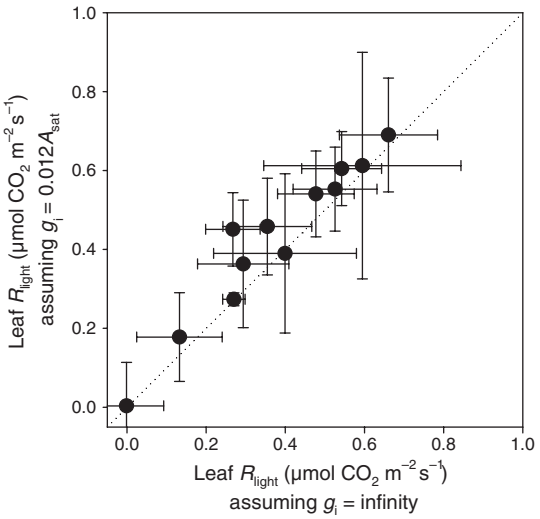

Fig. 6 Rates of respiration in the light (R_{light}) calculated assuming that internal conductance (g_i) is infinite plotted against the corresponding R_{light} values assuming $g_i = 0.012A_{sat}$ (where A_{sat} is net CO_2 assimilation in the light) (Evans & von Caemmerer, 1996). For data on the *y*-axis, we used concentrations of CO_2 at the site of carboxylation (c_c) assuming that $c_c = c_i - A_{net}/g_i$ (where c_i is the CO_2 concentration in the internal airspace). For data on the *x*-axis, R_{light} was estimated using c_i values obtained from the gas exchange measurements (see the Materials and Methods section for details). Data shown are for all growth [CO_2], growth temperature and water availability treatments, using data collected during the sustained drought phase on *Eucalyptus saligna* plants (n = 4; ± SE).

regression analysis which indicated that R_{dark} (when added to a model already incorporating R_{light}) explained a further 12% of the variation in R_{light} : R_{dark} (i.e. a total of 71% was explained in the two-component model). Finally, adding $v_{o\text{-}100}$ to the model explained a significant, but minor (3%) component, with the overall model accounting for 74% of

Table 5 Linear regression of relationships among *Eucalyptus saligna* leaf gas exchange traits using data from the initial drought, sustained drought and recovery phases

y-axis	*x*-axis	r^2	*y*-axis intercept	Slope	*P*
R_{dark}	A_{sat}	0.005	0.725	0.005	0.408
R_{dark}	A_{100}	0.000	0.761	0.001	0.953
R_{dark}	$v_{c\text{-}sat}$	0.011	0.701	0.006	0.218
R_{dark}	$v_{c\text{-}100}$	0.007	0.662	0.022	0.317
R_{dark}	R_{light} : R_{dark}	0.025	0.690	0.161	0.066
R_{light}	A_{sat}	0.032	0.285	0.012	**0.037**
R_{light}	A_{100}	0.011	0.276	0.029	0.235
R_{light}	$v_{c\text{-}sat}$	0.049	0.253	0.012	**0.009**
R_{light}	$v_{c\text{-}100}$	0.110	−0.011	0.082	**0.000**
R_{light}	$v_{o\text{-}sat}$	0.059	0.276	0.033	**0.004**
R_{light}	$v_{o\text{-}100}$	0.065	0.226	0.131	**0.003**
R_{light}	$g_{s\text{-}sat}$	0.025	0.346	0.182	0.066
R_{light}	$g_{s\text{-}100}$	0.074	0.305	0.516	**0.001**
R_{light}	R_{dark}	0.522	−0.146	0.702	**0.000**
R_{light}	R_{light} : R_{dark}	0.457	0.059	0.672	**0.000**
R_{light} : R_{dark}	A_{sat}	0.052	0.360	0.015	**0.007**
R_{light} : R_{dark}	A_{100}	0.012	0.374	0.031	0.199
R_{light} : R_{dark}	$v_{c\text{-}sat}$	0.067	0.333	0.015	**0.002**
R_{light} : R_{dark}	$v_{c\text{-}100}$	0.208	−0.057	0.113	**0.000**
R_{light} : R_{dark}	$v_{o\text{-}sat}$	0.038	0.403	0.026	**0.022**
R_{light} : R_{dark}	$v_{o\text{-}100}$	0.053	0.346	0.119	**0.007**
R_{light} : R_{dark}	R_{light}	0.457	0.231	0.679	**0.000**
R_{light} : R_{dark}	R_{dark}	0.025	0.381	0.154	*0.066*

Data from all atmospheric [CO_2], growth temperature and water availability treatments were used in the analyses. Significant values ($P < 0.05$) are shown in bold, while values close to significance are shown in italic. A_{sat}, area-based light-saturated photosynthesis; A_{100}, area-based photosynthesis at 100 μmol photons $m^{-2}s^{-1}$; R_{light}, area-based leaf respiration in the light; R_{dark}, area-based respiration in the dark; R_{light} : R_{dark}, ratio of respiration in the light to that in the dark; R_{light} : A_{sat} and R_{dark} : A_{sat}, ratios of respiration in the light and the dark to saturated photosynthesis (respectively). $v_{o\text{-}100}$ and $v_{o\text{-}sat}$, area-based rates of photorespiration under low and saturating irradiance, respectively; $v_{c\text{-}100}$ and $v_{c\text{-}sat}$, area-based rates of carboxylation under low and saturating irradiance, respectively.

variation in R_{light} : R_{dark} (with R_{light} : R_{dark} = 0.372 + $1.033R_{light} - 0.515R_{dark} + 0.078v_{o\text{-}100}$); replacing v_c with v_o resulted in little change in the overall model.

Discussion

In this study, we sought to understand how a wide environmental envelope that encompasses past and future climate scenarios affects leaf respiratory metabolism in a fast-growing tree. Therefore, we quantified the effects of subambient to elevated atmospheric [CO_2], water availability and growth temperature on rates of leaf *R* and associated leaf traits of *Eucalyptus saligna*. Unlike most previous studies assessing the impact of multiple environmental gradients on leaf *R* (Ryan, 1991; Amthor, 1994; Bunce, 1994; Tjoelker *et al.*, 1999; Lusk & Reich, 2000; Griffin *et al.*, 2002; Galmés *et al.*, 2007), we measured rates of leaf *R* in both darkness and the light. We found that light inhibited leaf *R* in all cases (i.e. $R_{light} < R_{dark}$), even when accounting for potentially low g_i. Growth [CO_2] had little impact on area-based rates of R_{dark} or R_{light} during the initial and sustained drought periods. Moreover, while there was some evidence that area-based rates of R_{dark} may have thermally acclimated to the 4°C difference in growth temperature (as shown by the lower rates of R_{dark} measured at a common measurement temperature in plants grown at the elevated temperature (Atkin & Tjoelker, 2003)), there was no evidence that R_{light} thermally acclimated. By contrast, sustained drought resulted in reduced R_{dark}, R_{light} and A_{sat}, with the inhibitory effect of drought on A_{sat} and R_{light} being greater than that on R_{dark}. Importantly, the effects of drought on the $R : A_{sat}$ and R_{light} : R_{dark} ratios were fully reversible following re-watering during the recovery phase (Fig. 6, Table 3).

Drought and chemical composition: an explanation for drought inhibition of *R*?

Our finding that leaf *R* (both in the light and in the dark) is reduced under drought is similar to the findings of the majority of studies in which leaf R_{dark} (measured at a common temperature) decreased following imposition of water stress (Flexas *et al.*, 2005, 2006; Atkin & Macherel, 2009). Drought can reduce rates of leaf *R* in three ways: by decreasing the substrate supply to mitochondria as a result of reduced photosynthesis; by decreasing the demand for respiratory energy to support cellular metabolism; and by decreasing the abundance, structure and composition of individual mitochondria (Lawlor & Fock, 1977; Flexas *et al.*, 2005; Atkin & Macherel, 2009). The inhibitory effect of drought on leaf *R* was unlikely to have been caused by limitations in substrate supply, because sugar concentrations were largely homeostatic across all treatments, as has been previously observed (Ghashghaie *et al.*, 2001). Given that *in vitro* activity of several key respiratory enzymes is generally unaffected by mild to moderate water stress (Herppich & Peckmann, 2000), and that respiratory capacity rarely limits respiratory flux when measured at moderate temperatures (Atkin *et al.*, 2005), it seems unlikely that reduced respiratory capacity was responsible for the reduced rates of leaf *R* under drought. Rather, the decrease in leaf *R* under drought was more likely to have been a reflection of reduced demand for respiratory products.

In fully expanded, mature leaves that are no longer growing (as were used in our study), mitochondrial ATP is required for sucrose synthesis, phloem loading and maintenance processes (Lambers, 1985; Bouma *et al.*, 1994, 1995). Our finding that starch concentrations increased under drought (indicating reduced rates of carbohydrate export) suggests that decreases in ATP demand associated

with phloem loading of sugars (which account for *c.* 30% of ATP produced by respiration (Bouma *et al.*, 1995)) could have contributed to the lower rates of R_{dark}. A further factor that may have contributed to a reduced demand for ATP was that, with the exception of plants grown under elevated [CO_2] at 30°C, drought resulted in a marked decrease in [N] in all treatment combinations; lower [N] results in reduced demand for respiratory ATP associated with protein turnover. Similarly, depending on the extent to which the reduced concentrations of total [P] in drought-treated leaves were matched by decreases in cytosolic [P] (depending on the degree to which P was stored in the vacuole), the decrease in [P] may have accentuated the degree of adenylate restriction of leaf *R* (Plaxton & Podesta, 2006).

Although we did not quantify rates of N transport/assimilation in our study, rates of NO_3^- transport from roots to leaves would probably have been lower in drought-treated plants, reflecting a decrease in water transport. In turn, this would have resulted in reduced demand for respiratory products associated with NO_3^- reduction/assimilation, particularly the demand for respiratory NADH (Miflin & Lea, 1976; Beevers & Hageman, 1980; Bloom *et al.*, 2010). Moreover, given that leaf N reduction/assimilation is a light-dependent process, we suggest that the impact of reduced demand for respiratory products would have been most evident in the light; subsequently, the inhibitory effect of drought on leaf R_{light} was greater than that of R_{dark}.

Balance between respiration and photosynthesis

The balance between leaf respiration and photosynthesis is crucial for the carbon budget of individual plants, ecosystem net CO_2 exchange, and the global carbon balance (Gifford, 2003). In many predictive Earth system models, such as those used by the Hadley Centre in the UK, changes in climate (including drought) are assumed to have similar proportional impacts on leaf *R* and A_{sat} (Cox, 2001) (i.e. the leaf $R : A_{sat}$ ratio is invariant). However, both our study and previous work (Flexas *et al.*, 2005, 2006; Atkin & Macherel, 2009) have shown that leaf $R_{dark} : A_{sat}$ ratios increase markedly under drought, with important consequences for rates of predicted net ecosystem CO_2 exchange (Flexas *et al.*, 2006; Reichstein *et al.*, 2007). Importantly, however, our study has shown that drought does not have equal effects on R_{dark} and R_{light} – rather, drought impacts on R_{light} (even when accounting for low g_i values) were markedly greater than those on R_{dark}. Consequently, $R_{light} : A_{sat}$ ratios were similar in well-watered and drought-treated plants. Irrespective of the underlying mechanisms responsible for this homeostasis of $R_{light} : A_{sat}$, this finding has important implications for ecosystem gas exchange models seeking to account for drought-mediated changes in *R* and A_{sat} when simulating ecosystem carbon fluxes and when interfaced with global circulation models (GCMs) to predict the impacts of global climate change on carbon exchange in terrestrial ecosystems (Ryan, 2002).

Light inhibition of leaf R and photorespiration

One of the objectives of our study was to quantify the impact of environment-dependent variations in photorespiration on the degree of light inhibition of leaf *R*. Irrespective of the growth conditions, light often inhibited leaf *R* (i.e. $R_{light} : R_{dark}$ ratios were less than unity, even after taking into account possible effects of low g_i). While $R_{light} : R_{dark}$ ratios were lower in drought-treated plants (where rates of v_o were enhanced as a result of reduced g_s, thus supporting the suggestion that light inhibition increases under conditions of high v_o; Hurry *et al.* (2005)), $R_{light} : R_{dark}$ ratios were not lower in plants grown under subambient atmospheric [CO_2], another environmental condition known to increase rates of v_o. In addition, we did not observe negative slopes in the linear regressions of R_{light} and $R_{light} : R_{dark}$ against v_o. Thus, while photorespiration-dependent reductions in PDC activity may well underpin light inhibition of leaf *R*, it seems unlikely that the degree of light inhibition is increased under conditions of higher photorespiratory flux.

Our stepwise analysis demonstrated that variations in R_{light} can be largely explained by R_{dark} and the prevailing rate of carboxylation (and/or oxygenation) by Rubisco. One explantation is that rates of R_{dark} reflect the underlying capacity of leaf mitochondria to release CO_2 via the TCA cycle. The extent to which R_{light} is lower than R_{dark} is dependent on (1) the degree to which there is a reduction in PDC activity (with the impact of PDC inactivation on TCA activity partially dependent on the control coefficient; Fell, 1997) and (2) the energy status and/or demand for C-skeletons associated with the wider cellular metabolism taking place in the light (Hurry *et al.*, 2005), including the demand for carbon skeletons associated with photorespiratory NH_2 transfer (Tcherkez *et al.*, 2008) and/or demand for respiratory products created by increased rates of CO_2 fixation by Rubisco (e.g. increased demand for mitochondrial ATP to support sucrose synthesis; Krömer, 1995). This may explain why the prevailing rate of Rubisco activity (either carboxylation or oxygenation) could account for a substantive amount of the variation in the rate of R_{light} and the $R_{light} : R_{dark}$ ratio.

Starch concentrations: an indicator of carbon supply and demand

Under well-watered conditions, we found that leaf starch concentrations increased 5-fold over the 280–640 μl l^{-1} CO_2 range. By contrast, growth [CO_2] had little effect on starch concentrations in drought-treated leaves, with starch concentrations remaining relatively high across all [CO_2]

treatments. Consequently, the stimulatory effect of drought on starch concentrations was greater under subambient [CO_2] than under ambient or elevated [CO_2]. Starch typically accumulates under conditions where CO_2 fixation rates exceed rates of carbon export from the chloroplast (Taiz & Zeiger, 2002). Therefore, we expected starch concentrations to be lowest under conditions that reduce photosynthetic CO_2 uptake (i.e. drought conditions and/or under subambient atmospheric [CO_2]). Indeed, starch concentrations are often lower under drought (Chaves & Oliveira, 2004; Slot *et al.*, 2008), but starch may accumulate under extended drought conditions (Tissue & Wright, 1995; Schurr *et al.*, 2000). In *E. saligna*, increased leaf starch during sustained drought probably reflects greater inhibitory effects on carbon export and use (e.g. in response to reduced cell expansion at the growing tips resulting from lower soil moisture availability) than on CO_2 uptake by photosynthesis during drought.

Concluding statements

Estimates of leaf *R* profoundly influence our understanding of ecosystem and Earth system functioning (Valentini *et al.*, 2000; King *et al.*, 2006; Atkin *et al.*, 2008), yet we lack basic information on key determinants of this process. For example, although previous studies have shown that light inhibits leaf *R* by up to 80% (e.g. Atkin *et al.*, 2000) and that failure to account for light inhibition of leaf *R* can lead to large overestimates of daily ecosystem respiration (Wohlfahrt *et al.*, 2005; Mercado *et al.*, 2007), to date no study has attempted to quantified *in vivo* rates of R_{light} over a wide environmental envelope that encompasses past and future climate scenarios. Light inhibition of leaf *R* can also have important implications for our understanding of the processes controlling A_{net}, where assuming that R_{light} and R_{dark} occur at similar rates may generate substantial errors in estimating carboxylase (v_c) and oxygenase (v_o) rates of Rubisco. Here, we found that atmospheric [CO_2] and growth temperature had relatively little impact on rates of leaf R_{light} and R_{dark} measured at a common temperature. However, drought was found to have strong and asynchronous effects on R_{light} and R_{dark}, with drought-mediated changes in R_{light} and *A* being closely coupled, whereas the inhibitory effect of drought on R_{dark} was less marked. If widespread in other species, these findings have important implications for our understanding of how drought events will impact on the carbon economy forests – past, current and future.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Representative plot of net CO_2 exchange rate (A_{net}; μmol CO_2 m^{-2} s^{-1}) vs irradiance (μmol photons m^{-2} s^{-1}) to illustrate the Kok effect.

Fig. S2 Effect of decreasing irradiance on estimated concentrations of CO_2 at the site of carboxylation (c_c) and in the internal airspace (c_i).

Fig. S3 Plot of light-saturated photosynthesis against growth [CO_2] during the recovery phase.

Fig. S4 Modelled impacts of assumed internal conductance (g_i) values on the predicted rate of respiration in the light (R_{light}) and the R_{light} : R_{dark} ratio.

Fig. S5 Effect of growth [CO_2], temperature and water availability treatments on leaf respiration rates, when calculating respiration in the light (R_{light}) values assuming $g_i = 0.012A_{sat}$ (g_i, internal conductance; A_{sat}, net CO_2 assimilation in the light).

Table S1 Results of three-way ANOVA of leaf gas exchange traits of *Eucalyptus saligna* grown under two growth temperatures and three growth CO_2 concentrations for the initial drought phase

Table S2 Results of three-way ANOVA of leaf gas exchange traits of *Eucalyptus saligna* grown under two growth temperatures and three growth CO_2 concentrations for the sustained drought phase and the recovery phase

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