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Exposure to preindustrial, current and future atmospheric CO_2 and temperature differentially affects growth and photosynthesis in *Eucalyptus*

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Abstract

To investigate if *Eucalyptus* species have responded to industrial-age climate change, and how they may respond to a future climate, we measured growth and physiology of fast- (*E. saligna*) and slow-growing (*E. sideroxylon*) seedlings exposed to preindustrial (290), current (400) or projected (650 μL L^{-1}) CO_2 concentration ($[CO_2]$) and to current or projected (current + 4 °C) temperature. To evaluate maximum potential treatment responses, plants were grown with nonlimiting soil moisture. We found that: (1) *E. sideroxylon* responded more strongly to elevated $[CO_2]$ than to elevated temperature, while *E. saligna* responded similarly to elevated $[CO_2]$ and elevated temperature; (2) the transition from preindustrial to current $[CO_2]$ did not enhance eucalypt plant growth under ambient temperature, despite enhancing photosynthesis; (3) the transition from current to future $[CO_2]$ stimulated both photosynthesis and growth of eucalypts, independent of temperature; and (4) warming enhanced eucalypt growth, independent of future $[CO_2]$, despite not affecting photosynthesis. These results suggest large potential carbon sequestration by eucalypts in a future world, and highlight the need to evaluate how future water availability may affect such responses.

Keywords: *Eucalyptus*, growth, high temperature, photosynthesis, subambient and elevated CO_2

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Introduction

Worldwide, *Eucalyptus* species are used for reforestation of degraded lands, provision of high-quality wood, and carbon sequestration (Varmola & Carle, 2002). In Australia, *Eucalyptus* is an iconic genus comprising more than 700 species, with significant commercial and ecological value. In the past two centuries, rapid fossil fuel consumption and deforestation have raised atmospheric concentrations of carbon dioxide ($[CO_2]$) from 280 to the current 389 μL L^{-1}. Within this century, atmospheric $[CO_2]$ is predicted to exceed 550 μL L^{-1}, and in turn generate a global mean surface temperature warming of 1.9–4.4 °C (Solomon *et al*., 2007). In Australia, the average air temperature has risen by 0.9 °C since 1950, while a mean warming of 0.3–3.4 °C is expected by 2050 for areas within 800 km of the Australian coast (Hennessy *et al*., 2007). Coping with this rapid climate change will require plants, including *Eucalyptus*, to possess sufficient physiological plasticity in their current genomes (Saxe *et al*., 1998).

Tree responses to elevated $[CO_2]$ are well documented (Ceulemans & Mousseau, 1994; Saxe *et al*., 1998; Norby *et al*., 1999), but less is known about tree responses to high temperature (Saxe *et al*., 2001) and the interactive effects of elevated $[CO_2]$ and temperature (Lloyd & Farquhar, 2008). Surprisingly, very few studies have addressed the response of *Eucalyptus* species to climate change factors. In general, these studies examined specific aspects (e.g., stomatal conductance, cyanogenesis, frost-tolerance or hydraulic

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conductivity) of *Eucalyptus* responses to [CO_2] enrichment (Berryman *et al*., 1994; Gleadow *et al*., 1998; Loveys *et al*., 2006; Atwell *et al*., 2007), while fewer studies characterized more general responses such as photosynthesis and whole-plant growth in elevated [CO_2] (Conroy *et al*., 1992; Wong *et al*., 1992; Duff *et al*., 1994; Roden & Ball, 1996a, b; Roden *et al*., 1999). Furthermore, the few studies that have considered the impact of elevated [CO_2] and high temperature have imposed short-term high temperature stress rather than long-term growth at elevated temperature (Roden & Ball, 1996a, b). Importantly, previous studies have addressed *plant*, but not tree response to subambient [CO_2] (Baker *et al*., 1990; Polley *et al*., 1992; Dippery *et al*., 1995; Tissue *et al*., 1995; Cowling & Sage, 1998; Ward *et al*., 1999; Gill *et al*., 2002).

Photosynthetic rates generally increase linearly upon short-term increases in [CO_2] up to $\sim 300\,\mu L\,L^{-1}$, above which the increase is less steep (Farquhar *et al*., 1980; Farquhar & von Caemmerer, 1982). Furthermore, when plants are grown at elevated [CO_2], the degree of photosynthetic enhancement by [CO_2] decreases in a process termed acclimation (Drake *et al*., 1997). Nevertheless, the initial and/or residual photosynthetic stimulation is generally large enough to enhance plant growth. Thus, the growth of C_3 plants is usually stimulated when they are grown at elevated [CO_2] and inhibited when they are grown at subambient [CO_2], compared with growth at ambient [CO_2] (Ceulemans & Mousseau, 1994; Idso & Idso, 1994; Ward, 2005). We hypothesize that eucalypts will exhibit a greater growth response to the transition in atmospheric [CO_2] from subambient [CO_2] to ambient [CO_2] compared with the transition from ambient [CO_2] to elevated [CO_2].

Photosynthesis in C_3 plants generally achieves a temperature optimum in the upper middle portion of an operating range of *ca*. 7–40 °C (Long, 1991; Sage & Kubien, 2007). Whole-plant responses to temperature can be more complex because the majority of plant processes are affected by temperature. The long-term response of plants to different growth temperatures will depend on whether those temperatures move various processes towards or away from their temperature optimum, as well as the thermal plasticity of temperature-sensitive processes (Berry & Björkman, 1980). With respect to photosynthesis, interactions between growth temperature and growth [CO_2] may be expected because the rate of photorespiration is positively correlated with temperature and negatively correlated with [CO_2] (Long, 1991; Sage & Kubien, 2007). Hence, we hypothesize that the growth response to increasing [CO_2] is greater for eucalypts grown at high temperature compared with counterparts grown at ambient temperature.

In this study, we investigated the growth and physiological responses of two *Eucalyptus* species, representing two different growth habits (i.e. slower-growing *E. sideroxylon* and faster-growing *E. saligna*), to past and future climates. Specifically, we assessed the main and interactive effects of air temperature (ambient and ambient + 4 °C) and atmospheric [CO_2] (subambient, ambient, and elevated) on biomass production and photosynthesis. Our choice of species was guided by findings indicating that the strength of growth sinks for photosynthate tends to correlate positively with the magnitude of growth and photosynthetic responses to [CO_2] (e.g., Poorter *et al*., 1996; Poorter, 1998; Atkin *et al*., 1999). This led us to hypothesize that the faster-growing *E. saligna* will be more responsive to elevated [CO_2] and elevated temperature than the slower-growing *E. sideroxylon*.

Materials and methods

Growth conditions

Soil was collected from the A horizon (top 50 cm) of an experimental site located on the grounds of the University of Western Sydney, Richmond, NSW, Australia. The soil is a loamy-sand with low organic matter content (0.7%), fertility [pH 5.5, N ($<1\,mg\,kg^{-1}$), P ($8\,mg\,kg^{-1}$), K (0.23 mEq/100 g), Ca (1.2 mEq/100 g), Mg (0.34 mEq/100 g), S ($5\,mg\,kg^{-1}$), B ($0.2\,mg\,kg^{-1}$), Zn ($0.9\,mg\,kg^{-1}$), Cu ($0.2\,mg\,kg^{-1}$), Fe ($24\,mg\,kg^{-1}$), Mn ($9.1\,mg\,kg^{-1}$), Al (0.14 mEq/100 g), Na (0.1 mEq/100 g) and Cl ($13\,mg\,kg^{-1}$)] and low water holding capacity. The soil was air dried, and then 9 kg of dry soil was added to each of 324 cylindrical pots (PVC pipes, 15 cm diameter × 40 cm length), which were adjusted to the same mass by the addition of pebbles. Pots were then transferred to six adjacent, naturally lit and temperature-controlled glasshouse compartments ($3.0 \times 5.0 \times 3.5\,m^3$, w × l × h each).

Three glasshouse compartments were programmed to simulate the daily temperature of a 30-year average of a local (Richmond, NSW) day for the months of November to May (i.e. ambient temperature treatment). Three glasshouse compartments were programmed to simulate a constant 4 °C step increase in temperature (i.e. high temperature treatment) relative to the ambient temperature treatment. Air temperature was continually adjusted by the temperature-control system and monitored using thermocouples; Tinytag® data loggers (TinyView, Gemini Data Loggers Ltd., Chichester, UK) were used to additionally assess temperature in the glasshouse. The average growing season temperatures for the ambient and high temperature treatments were 26/18 and 30/22 °C (day/night), respectively.

Within each temperature treatment, plants were grown at 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}). Subambient [CO_2] was achieved by continuously passing compartment air over trays filled with calcium hydroxide (Schaefer Kalk GmbH & Co KG, Diez, Germany) within metal boxes fitted with fans (Thermoline Scientific, Sydney, Australia); calcium hydroxide was stirred daily and exchanged twice a week. Elevated [CO_2] was achieved by injecting CO_2 gas (Food grade, AirLiquide, Australia) from pressurized cylinders through solenoid valves connected to a CO_2 monitor/controller (Lambda T, ADC BioScientific Ltd., Hoddesdon, Herts, UK). CO_2 was first passed through a Purafil® column to eliminate possible ethylene contamination. [CO_2] was continuously monitored in all growth compartments by logging the voltage output of the CO_2 monitors/controllers using a data logger (DL2e, Delta-T Devices Ltd, Cambridge, UK). The CO_2 monitors/controllers were calibrated at regular intervals with pure N_2 and two CO_2 calibration gases (406 ± 12 and 714 ± 16 μL L^{-1}) (AirLiquide, Australia). The average daytime [CO_2] during the experimental period for the subambient, ambient and elevated treatments was 290, 400, and 650 μL L^{-1}, respectively. Monitored using Tinytag® data loggers (TinyView, Gemini Data Loggers Ltd., Chichester, UK), relative humidity averaged 70% over the growing season and was not significantly different between the [CO_2] and temperature treatments. Peak mid-day photosynthetic active radiation (PAR) was measured by a nearby (1 km away) weather station and averaged 1250 μmol m^{-2} s^{-1} over the growing period, reaching a maximum of 2360 μmol m^{-2} s^{-1}; glasshouse structure attenuated direct sunlight ca. 10–15%.

Plant culture and growth measurements

Seeds of Sydney blue gum (*Eucalyptus saligna* Sm.) and red ironbark (*Eucalyptus sideroxylon* A. Cunn. ex Woolls) were obtained from Ensis (Australian Tree Seed Centre, ACT, Australia). *E. saligna* and *E. sideroxylon* seeds were collected from NSW at latitudes of 30°34′03″S and 32°59′07″S, longitudes of 152°08′46″E and 147°53′07″E and altitudes of 1012 and 300 m, respectively. The seeds were sown (mid-October 2007) in trays filled with seed raising mix (Plugger Custom, Debco Pty Ltd., Berkshire Park, NSW, Australia) and placed in temperature-controlled germination cabinets maintained at ambient [CO_2]. A month later, seedlings were transplanted by placing a plug, containing two to six seedlings (each with two to four unfolded leaves) in the middle of each pot. There were 27 pots of each species in each of the six [CO_2] and temperature treatment combinations. In early January 2008, seedlings were thinned to one seedling per pot for all treatments; at this stage, seedlings were 3–10 cm in height, depending on the species and treatments. Tree seedlings were watered on a daily basis. On three occasions, 30, 120 and 135 days after planting (DAP), pots were irrigated with a nutrient solution containing a commercial fertilizer (General Purpose, Thrive Professional, Yates Australia) at a concentration of 0.2 g N L^{-1} (N : P : K : S : Fe : Mn : B 25 : 4.1 : 17.3 : 1.6 : 0.06 : 0.003 : 0.022%). Pots were routinely moved within the glasshouses during the experimental period.

Two destructive harvests were conducted 80 and 150 DAP; nine trees at 80 DAP and 18 trees at 150 DAP of each species were harvested within each [CO_2] and temperature treatment combination. During each harvest, we measured the length of the main stem, diameter at stem base, and separated the seedling into stems (including branches and petioles) and leaf blades; shoot tips were removed separately and roots were washed free of soil. Total leaf area was determined using a portable leaf area meter (LI-3100A, LI-COR, Lincoln, NE, USA). The number of leaves and branches were counted at 150 DAP harvest only. Harvested samples were oven-dried at 80°C for 48 h, then weighed. For each plant, mean leaf mass per area (LMA) and leaf area ratio (LAR) were calculated as total leaf dry mass/total leaf area and total leaf area/total plant dry mass, respectively. Relative growth rate (RGR, unit dry mass increase per unit dry mass) and net assimilation rate (NAR, unit dry mass increase per unit leaf area) were calculated as $(\ln W_2 - \ln W_1)/(T_2 - T_1)$ and $(\ln A_2 - \ln A_1)(W_2 - W_1)/(A_2 - A_1)(T_2 - T_1)$, where A and W represent whole plant leaf area and dry mass, respectively. T_1 and T_2 were taken as DAP at the first and second harvests, respectively. To determine RGR and NAR, trees from the two harvests were paired according to their size. Averages of RGR and NAR using this method were similar to those obtained when we used total treatment averages.

Leaf gas exchange measurements

Net photosynthesis at saturating light (A_{sat}), stomatal conductance (g_s) and ratio of intercellular to ambient [CO_2] (C_i/C_a) were measured at 110 DAP on attached, recently fully expanded leaves using a portable open gas exchange system (LI-6400, LI-COR). Measurements were conducted at saturating light (photosynthetic photon flux density of 1200 μmol m^{-2} s^{-1} supplied by an in-built red/blue light-emitting diode source), target growth [CO_2] (280, 400 or 640 μL L^{-1}), mid-day growth temperature (28 or 32 °C), and ambient leaf-to-air vapour pressure deficit (varied between 0.9 and

2.0 kPa). Each leaf was allowed 5–10 min to equilibrate before A_{sat}, g_s and C_i/C_a were measured. There were five replicate measurements per species and treatment.

Leaf N analysis

Oven-dried plant fractions were ground to a powder in a ball mill. Subsamples were analysed for N concentration using a CN analyser (LECO TruSpec, LECO Corporation, St Joseph, MI, USA).

Statistical analyses

Data were analysed using a general linear model, factorial analysis of variance (ANOVA) (Statistica, StatSoft Inc., Tulsa, OK, USA) with species, growth [CO_2] and growth temperature as independent factors. Means were compared using Newman–Keuls *post-hoc* test. A logarithmic transformation was applied before ANOVA if data distribution was not normal.

Results

Two harvests were carried out at 80 and 150 DAP. Similar trends were obtained for both harvests. Consequently, only results from harvest at 150 DAP are discussed below. Results from 80 DAP are shown in two supplementary tables (supporting information Tables S1 and S2).

Whole plant dry mass and leaf area

At ambient temperature, whole plant dry mass and leaf area were similar at subambient and ambient [CO_2] for both species at 150 DAP (Fig. 1, Tables 1 and 2). Whole plant dry mass was 44% higher in *E. saligna* and 74% higher in *E. sideroxylon* at elevated [CO_2] than at ambi-

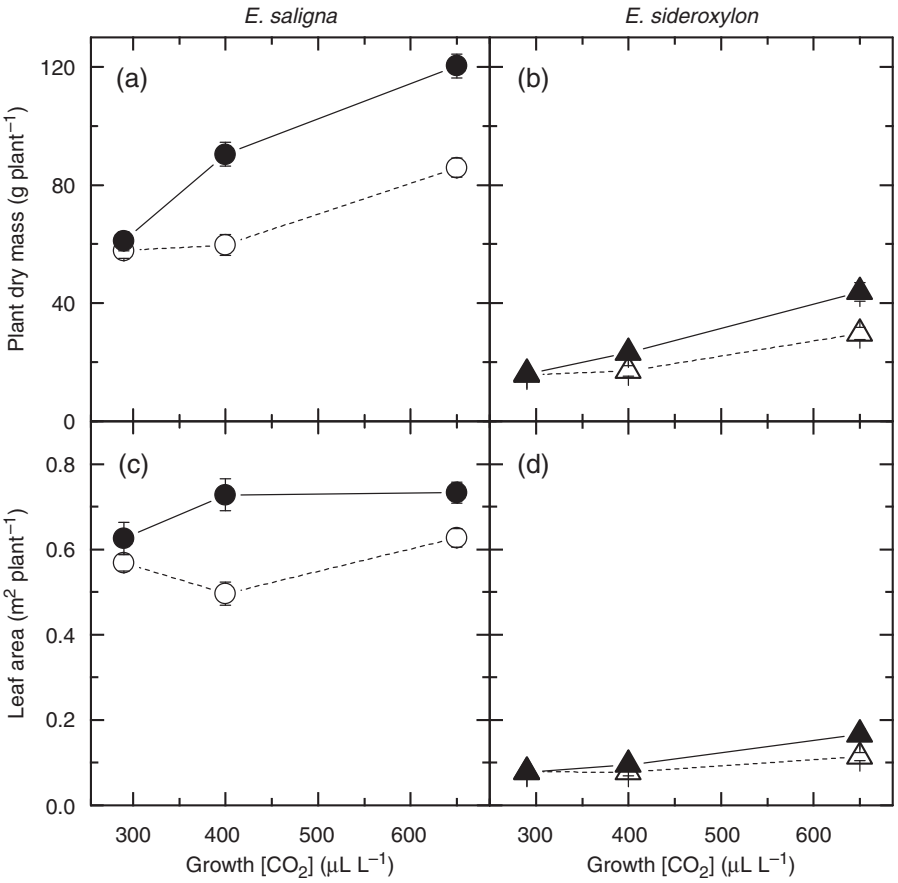

Fig. 1 Total plant dry mass (a and b) and leaf area (c and d) of *E. saligna* (a and c, ○ ●) and *E. sideroxylon* (b and d, △▲) grown at three atmospheric [CO_2] with day averages of 290, 400 or 650 μL L^{-1}, and two air temperatures [ambient (△ ○) or high (ambient + 4 °C, ● ▲)]. Plants were harvested 150 days after planting. Values represent means ± SE.

Table 1 Summary of the three-way ANOVA testing for the effects of species, [CO_2] and temperature on various parameters of *Eucalyptus saligna* and *Eucalyptus sideroxylon* grown at three [CO_2] and two temperatures and harvested 150 DAP

	Main effects			Interactions			
Parameter	Species	CO_2	Temperature	Species × CO_2	Species × temperature	CO_2 × temperature	Species × CO_2 × temperature
Dry mass accumulation							
Plant DM (g)	***	***	***	***	***	***	***
Leaf area (m^2)	***	***	***	ns	***	*	ns
Average leaf size (cm^2)	***	ns	***	ns	***	ns	ns
Leaf number ($plant^{-1}$)	***	***	***	*	*	***	ns
Total branch number ($plant^{-1}$)	***	***	***	ns	**	ns	ns
Main stem height (m)	***	***	***	ns	ns	**	ns
Basal stem diameter (mm)	***	***	***	***	ns	ns	ns
Dry mass partitioning							
Leaf mass fraction (%)	***	ns	ns	*	ns	ns	**
Stem and Branch mass fraction (%)	***	ns	***	ns	ns	ns	*
Root mass fraction (%)	ns	*	*	ns	ns	ns	*
Relative growth analysis							
LMA ($g\,m^{-2}$)	***	***	ns	ns	ns	ns	ns
LAR ($m^2\,kg^{-1}$)	***	***	*	***	ns	ns	ns
NAR ($g\,m^{-2}\,day^{-1}$)	***	***	***	ns	ns	ns	ns
RGR ($mg\,g\,day^{-1}$)	***	*	***	**	*	***	ns
Tissue [N] and whole plant NUE							
Leaf [N] (%)	***	***	*	ns	ns	ns	ns
Stem and Branch [N] (%)	***	***	**	ns	*	*	ns
Root [N] (%)	ns	***	ns	ns	ns	**	ns
NUE [$g\,DM\,(g\,leaf\,N)^{-1}$]	***	***	ns	ns	ns	ns	*
Leaf gas exchange							
A_{sat} ($\mu mol\,m^{-2}\,s^{-1}$)	***	***	ns	ns	ns	ns	ns
g_s ($mol\,m^{-2}\,s^{-1}$)	***	ns	ns	ns	ns	ns	ns
C_i/C_a	ns	ns	ns	ns	ns	ns	ns

Gas exchange measurements were made 110 DAP.
Significance levels are: ns = not significant ($P > 0.05$); *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

ent [CO_2]. Similarly, leaf area was higher at elevated [CO_2] than at ambient [CO_2] for both species (Fig. 1, Tables 1 and 2).

At high temperature, whole plant dry mass was about 30% lower in subambient [CO_2] than in ambient [CO_2] for both *Eucalyptus* species. Leaf area was similar at subambient and ambient [CO_2] for both eucalypts. Elevated [CO_2] stimulated whole plant dry mass by 33% in *E. saligna* and 88% in *E. sideroxylon*. Leaf area was similar at ambient and elevated [CO_2] for both species at high temperature (Fig. 1, Tables 1 and 2).

At ambient [CO_2], growth at high temperature stimulated whole plant dry mass by 52% in *E. saligna* and 37% in *E. sideroxylon* relative to the ambient temperature treatment. Similarly, leaf area was higher in the high than in the ambient temperature treatment for both species (Fig. 1, Tables 1 and 2).

The interactive effects of [CO_2] and temperature on whole plant dry mass differed between *E. saligna* and *E. sideroxylon*, as described above. However, the interactive effects of [CO_2] and temperature on leaf area were similar between *E. saligna* and *E. sideroxylon* (Tables 1 and 2).

Leaf number and size

At ambient temperature, leaf number was higher at subambient than at ambient [CO_2]; and higher at elevated than at ambient [CO_2] for both species.

At high temperature, leaf number was similar at subambient and ambient [CO_2]; and higher at elevated [CO_2] relative to ambient [CO_2] in both species. Growth [CO_2] had no effect on average leaf size in either eucalypt or temperature treatment (Fig. 2, Tables 1 and 2).

Table 2 Summary of means at 150 DAP for *Eucalyptus saligna* and *Eucalyptus sideroxylon* grown at three [CO_2] and two temperatures, as described in the Materials and Methods

		E. saligna			*E. sideroxylon*		
Parameter	[CO_2] ($\mu L\,L^{-1}$)	290	400	650	290	400	650
Dry mass accumulation	Temperature						
Plant DM (g)	Ambient	57.7e	59.7e	85.9f	15.6a	17.0a	29.7c
	High	61.1e	90.4f	120.3g	16.1a	23.2b	43.7d
Leaf area (m^2)	Ambient	0.57	0.50	0.63	0.079	0.077	0.11
	High	0.63	0.73	0.73	0.078	0.095	0.17
Average leaf size (cm^2)	Ambient	11.2	12.6	12.5	2.9	3.1	3.2
	High	9.1	9.5	8.1	2.8	2.8	3.3
Leaf number ($plant^{-1}$)	Ambient	519	410	495	280	249	364
	High	670	790	912	296	359	507
Total branch number ($plant^{-1}$)	Ambient	54	52	66	25	23	35
	High	89	85	117	26	29	46
Main stem height (m)	Ambient	1.07	1.09	1.25	0.74	0.68	0.89
	High	1.30	1.45	1.48	0.84	0.97	1.17
Basal stem diam. (mm)	Ambient	9.8	11.2	13.3	5.2	5.4	6.5
	High	10.6	11.9	13.8	5.2	6.9	7.1
Dry mass partitioning							
Leaf mass fraction (%)	Ambient	36bc	31ab	33abc	33abc	38c	35bc
	High	33abc	33abc	29a	37c	35bc	35bc
Stem and Branch mass fraction (%)	Ambient	31abcd	33bcde	34cde	31abc	27a	29ab
	High	37e	36de	32bcde	33bcde	33bcde	33bcde
Root mass fraction (%)	Ambient	32abc	34abc	32abc	35abc	34abc	36bc
	High	28a	30ab	38c	30ab	32abc	32abc
Relative growth analysis							
LMA ($g\,m^{-2}$)	Ambient	36	37	45	67	83	91
	High	33	41	47	77	86	93
LAR ($m^2\,kg^{-1}$)	Ambient	10.1	8.5	7.4	5.1	4.6	3.9
	High	10.3	8.0d	6.2	4.9	4.2	3.8
NAR ($g\,m^{-2}\,day^{-1}$)	Ambient	4.1	4.9	5.0	5.4	6.8	7.6
	High	3.0	3.7	4.1	4.7	5.7	5.9
RGR ($mg\,g\,day^{-1}$)	Ambient	4.4	5.0	4.3	2.7	3.6	3.5
	High	3.7	3.4	3.2	2.7	2.8	2.7
Leaf gas exchange and [N]							
A_{sat} ($\mu mol\,m^{-2}\,s^{-1}$)	Ambient	11.2	16.6	21.5	18.8	27.9	39.1
	High	11.1	14.7	15.1	18.0	27.9	38.3
g_s ($mol\,m^{-2}\,s^{-1}$)	Ambient	0.33	0.35	0.35	0.52	0.47	0.50
	High	0.48	0.35	0.25	0.48	0.56	0.50
C_i/C_a	Ambient	0.72	0.56	0.77	0.76	0.71	0.75
	High	0.81	0.76	0.74	0.75	0.76	0.76
Leaf [N] (%)	Ambient	3.2	2.7	2.3	2.7	2.6	1.9
	High	3.4	2.7	1.9	2.6	2.1	1.6
NUE [g DM ($g\,leaf\,N)^{-1}$]	Ambient	0.09a	0.13ab	0.14bc	0.11ab	0.11ab	0.19d
	High	0.09a	0.12ab	0.17cd	0.11ab	0.15bc	0.19d

Within parameters, different letters indicate means that were significantly different at $P < 0.05$ based on Newman–Keuls test for pairwise comparisons (shown only when three-way interactions were significant).

At ambient [CO_2], leaf number was higher and leaf size was lower at high relative to ambient temperature for both species (Fig. 2, Tables 1 and 2).

The interactive effects of [CO_2] and temperature on leaf number were similar between the two *Eucalyptus* species (Tables 1 and 2).

Woody tissue production

At ambient temperature, branch number and main stem height were similar while basal stem diameter was lower at subambient relative to ambient [CO_2] for both species. Branch number, main stem height, and basal

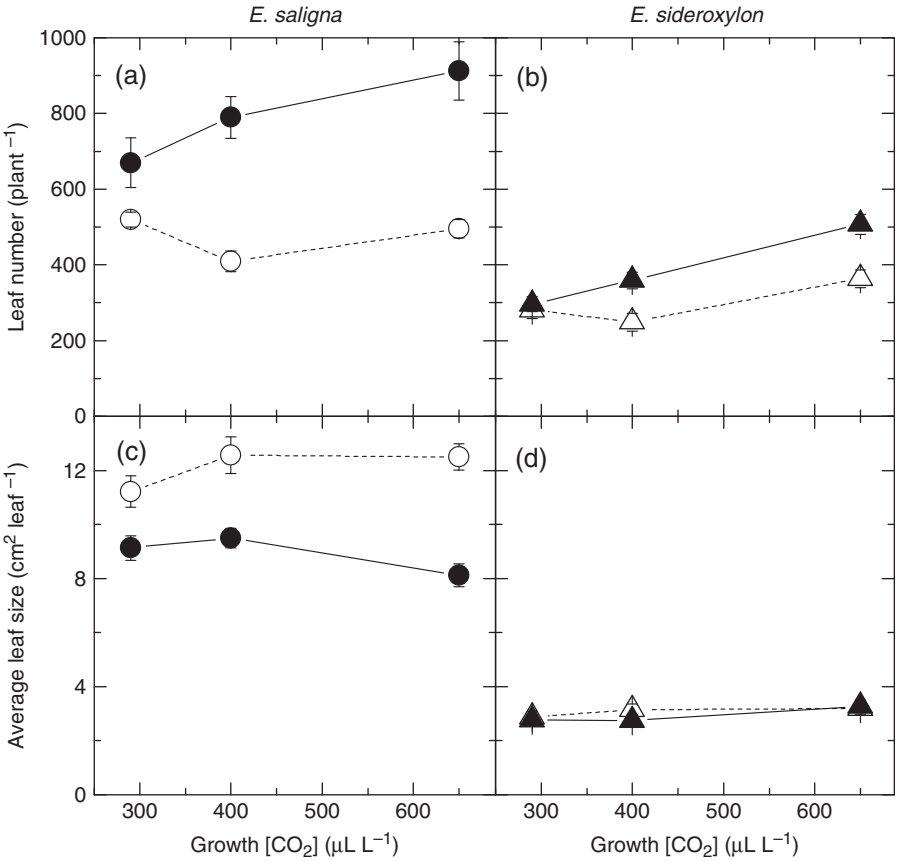

Fig. 2 Total leaf number (a and b) and average leaf size (c and d) of *E. saligna* (a and c) and *E. sideroxylon* (b and d) grown at three atmospheric [CO_2] and two air temperatures. Other details are as described for Fig. 1.

stem diameter were higher at elevated [CO_2] than at ambient [CO_2] for both species (Fig. 3, Tables 1 and 2).

At high temperature, branch number was similar while main stem height and diameter were lower in subambient [CO_2] relative to ambient [CO_2] for both species. Elevated [CO_2] stimulated branch number, stem height, and basal stem diameter relative to ambient [CO_2] for both species (Fig. 3, Tables 1 and 2).

At ambient [CO_2], branch number, main stem height and main stem diameter were higher in the high temperature treatment than in the ambient temperature treatment for both species (Fig. 3, Tables 1 and 2).

The interactive effects of [CO_2] and temperature on main stem height, the effect of [CO_2] on branch number, and the effect of temperature on basal stem diameter were similar between *E. saligna* and *E. sideroxylon* (Tables 1 and 2).

Dry mass partitioning

The interactive effects of increasing growth [CO_2] and temperature on relative allocation to leaves, stems and roots differed between *E. saligna* and *E. sideroxylon* (Tables 1 and 2). In particular, increasing [CO_2] was associated with increased allocation to roots in *E. saligna*, but only in the high temperature treatment; and allocation to roots in *E. sideroxylon* did not vary among the [CO_2] and temperature treatments. The effects of [CO_2] and temperature on dry mass allocation to leaves and stems showed no clear patterns in either species.

Growth analyses (LMA, LAR, NAR and RGR)

At ambient temperature, increasing [CO_2] reduced LAR in both species. With increasing [CO_2], LMA and NAR increased in *E. saligna* while LMA, NAR, and RGR increased in *E. sideroxylon* (Fig. 4, Tables 1 and 2).

At high temperature, LAR decreased and LMA and NAR increased in both species with increasing [CO_2]. RGR was not affected by growth [CO_2] at high temperature in either species (Fig. 4, Tables 1 and 2).

At ambient [CO_2], growth in high temperature did not affect LMA in either species, while LAR, NAR and RGR decreased in both species when compared with ambient temperature (Fig. 4, Tables 1 and 2).

Interactions. LMA and NAR were lower in *E. saligna* relative to *E. sideroxylon* seedlings, and the differences

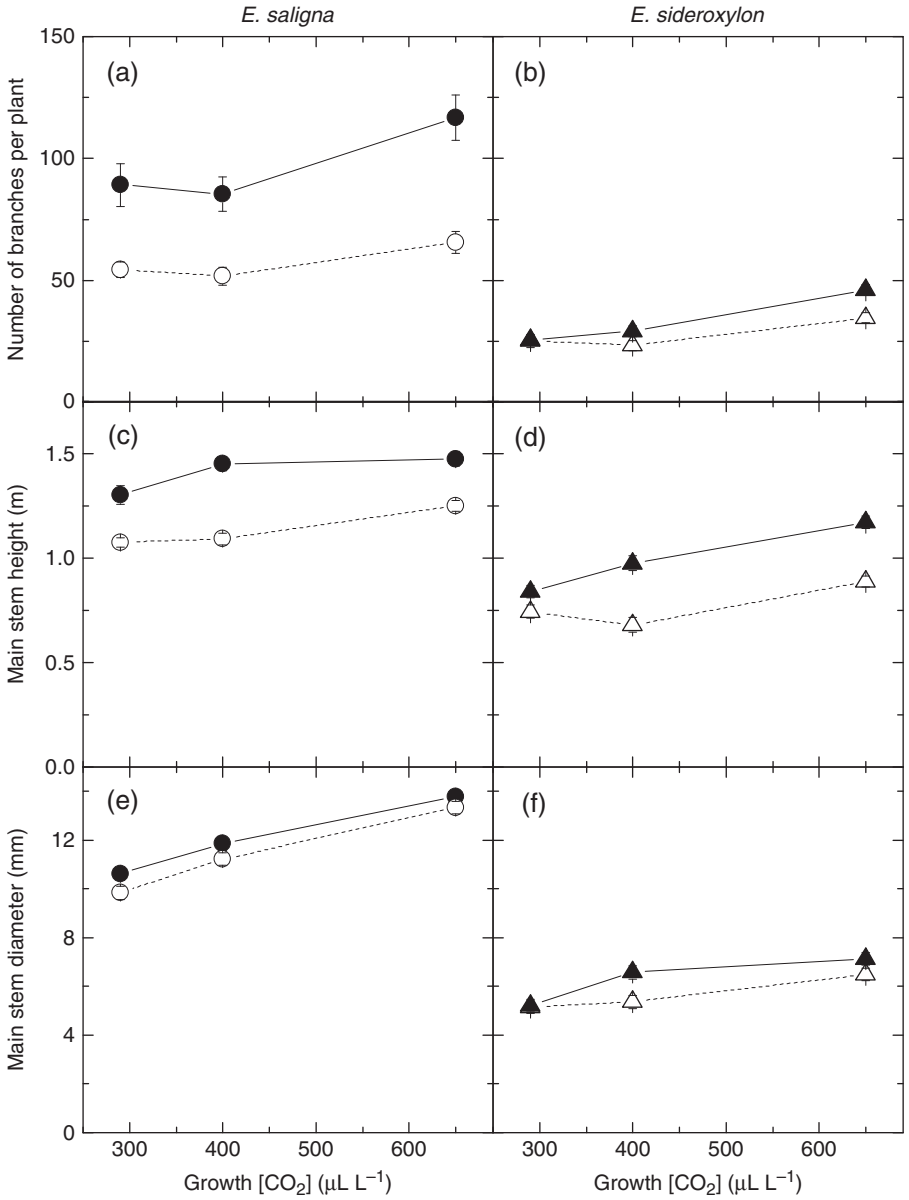

Fig. 3 Total number of branches (a and b), main stem height (c and d) and main stem basal diameter (e and f) of *E. saligna* (a, c and e) and *E. sideroxylon* (b, d and f) grown at three atmospheric [CO_2] and two air temperatures. Other details are as described for Fig. 1.

did not vary among [CO_2] and temperature treatments. The differential effects of [CO_2] treatment on LAR and RGR in *E. saligna* and *E. sideroxylon* were similar between temperature treatments. The differential effect of temperature treatment on RGR in *E. saligna* and *E. sideroxylon* was similar among [CO_2] treatments (Fig. 4, Tables 1 and 2).

Leaf gas exchange (A_{sat}, g_s and C_i/C_a)

At ambient temperature, A_{sat} was 33% lower at subambient compared with ambient [CO_2] for both species (Fig. 5). At elevated [CO_2], A_{sat} was 30% higher in *E. saligna* and 40% higher in *E. sideroxylon* compared with ambient [CO_2]. Growth [CO_2] had no effects on g_s or C_i/C_a (Fig. 5, Tables 1 and 2).

At high temperature, A_{sat} was 24% lower in *E. saligna* and 35% lower in *E. sideroxylon* at subambient compared with ambient [CO_2]. At elevated [CO_2], A_{sat} was 3% higher in *E. saligna* and 38% higher in *E. sideroxylon* compared with ambient [CO_2]. There were no [CO_2] treatment effects on g_s or C_i/C_a (Fig. 5, Tables 1 and 2).

At ambient [CO_2], there were no temperature treatment effects on A_{sat}, g_s or C_i/C_a (Fig. 5, Tables 1 and 2).

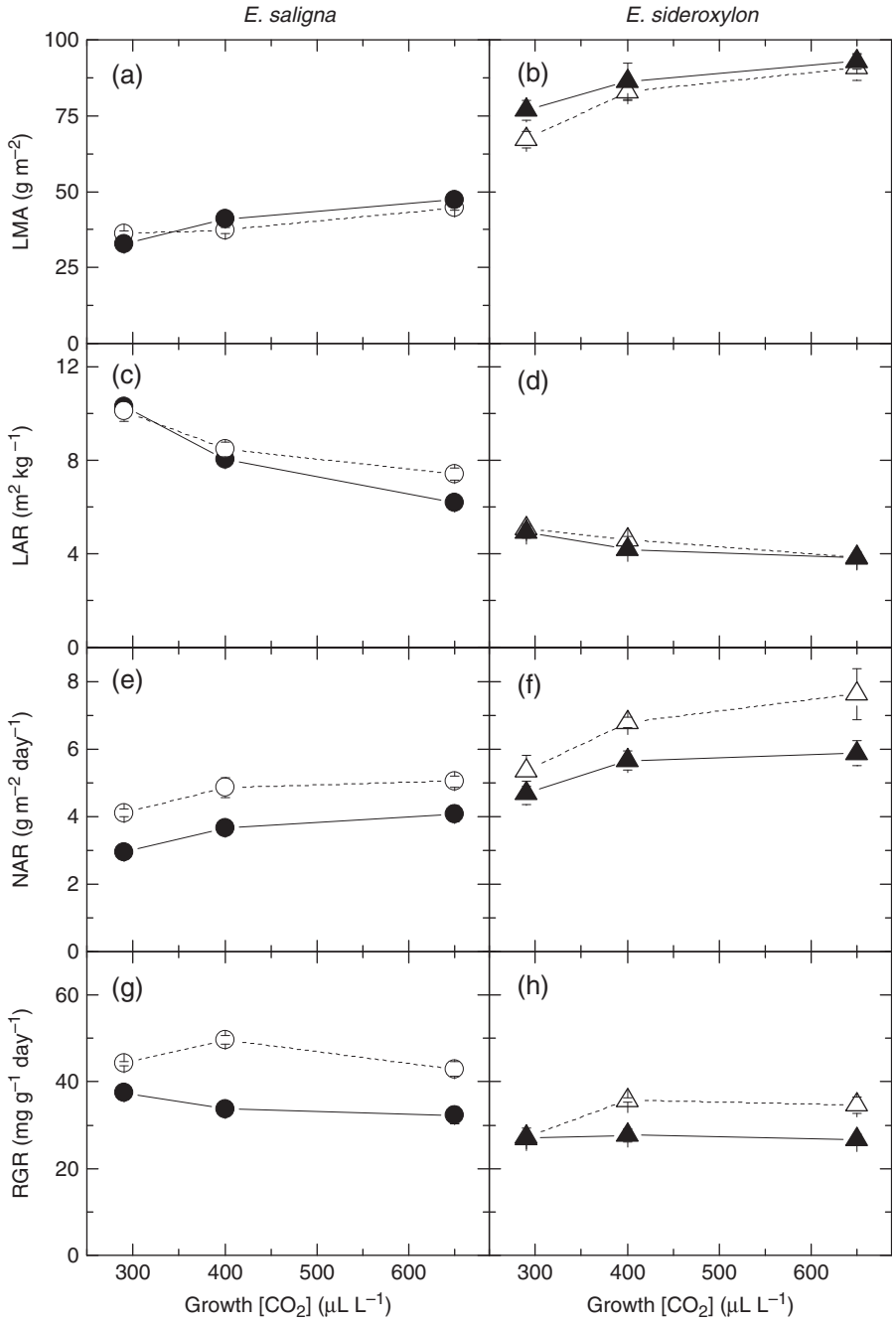

Fig. 4 Leaf mass per area (LMA, a and b), leaf area ratio (LAR, c and d), net assimilation rates (NAR, e and f) and relative growth rates (RGR, g and h) of *E. saligna* (a, c, e and g) and *E. sideroxylon* (b, d, f and h) grown at three atmospheric [CO_2] and two air temperatures. Other details are as described for Fig. 1. [Correction added after online publication 7 September 2009: in Fig. 4g-h, the error in scale was corrected].

At ambient temperature and [CO_2], A_{sat} was 68% higher and g_s was 34% higher in *E. sideroxylon* compared with *E. saligna*; C_i/C_a was similar between the two species. The magnitudes of the species effects on A_{sat} and g_s were similar across [CO_2] and temperature treatments (Table 1). Further, the magnitude of the [CO_2] treatment effect on A_{sat} did not differ between species or temperature treatments (Table 1).

Tissue [N] and whole plant nitrogen use efficiency (NUE)

At ambient temperature, leaf [N] decreased with increasing [CO_2]. Relative to the ambient [CO_2] treatment, growth in subambient and elevated [CO_2] did not affect stem or root [N]. In both species, whole plant nitrogen use efficiency (NUE) was similar at subambient and ambient [CO_2], but NUE in *E. sideroxylon* was 72%

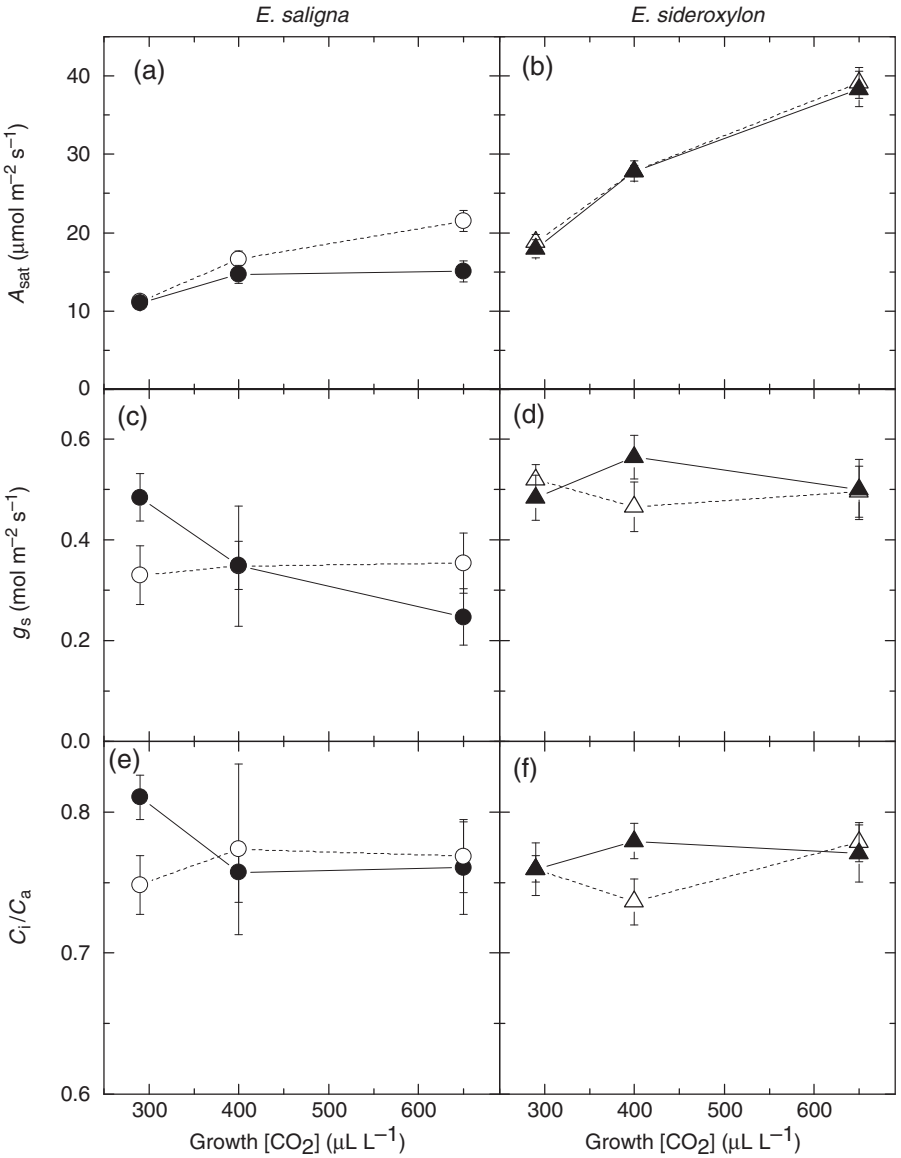

Fig. 5 Light-saturated, CO_2 assimilation rates, A_{sat} (a and b), stomatal conductance, g_s (c and d) and intercellular to ambient [CO_2] ratio, C_i/C_a (e and f) of *E. saligna* (a, c and e) and *E. sideroxylon* (b, d and f) grown at three atmospheric [CO_2] and two air temperatures. Gas exchange measurements (a–d) were made 110 DAP at 1200 μmol quanta m^{-2} s^{-1} and the respective growth [CO_2] and midday temperature (28 or 32 °C). Other details are as described in Fig. 1.

higher in elevated [CO_2] compared with ambient [CO_2] at ambient temperature (Fig. 6, Tables 1 and 2).

At high temperature, leaf [N] decreased with increasing [CO_2]. Stem and root [N] were lower in subambient [CO_2] than in ambient [CO_2], but were similar at ambient and elevated [CO_2]. Growth in elevated [CO_2] increased NUE by 30–40% in both species compared with ambient [CO_2], while NUE was similar between subambient and ambient [CO_2] (Fig. 6, Tables 1 and 2).

At ambient [CO_2], high temperature led to small reductions in leaf and stem [N] across both species. However, root [N] and NUE were similar between ambient and high temperature treatments for both species (Fig. 6, Tables 1 and 2).

Interactions. *E. saligna* had higher leaf [N] than *E. sideroxylon*, and the difference did not vary among [CO_2] and temperature treatments (Fig. 6, Table 1). Further, the [CO_2] treatment effect on leaf [N] did not vary between species or temperature treatments, and the temperature treatment effect on leaf [N] did not vary between species or among [CO_2] treatments (Table 1). Stem [N], root [N] and NUE were similar in both

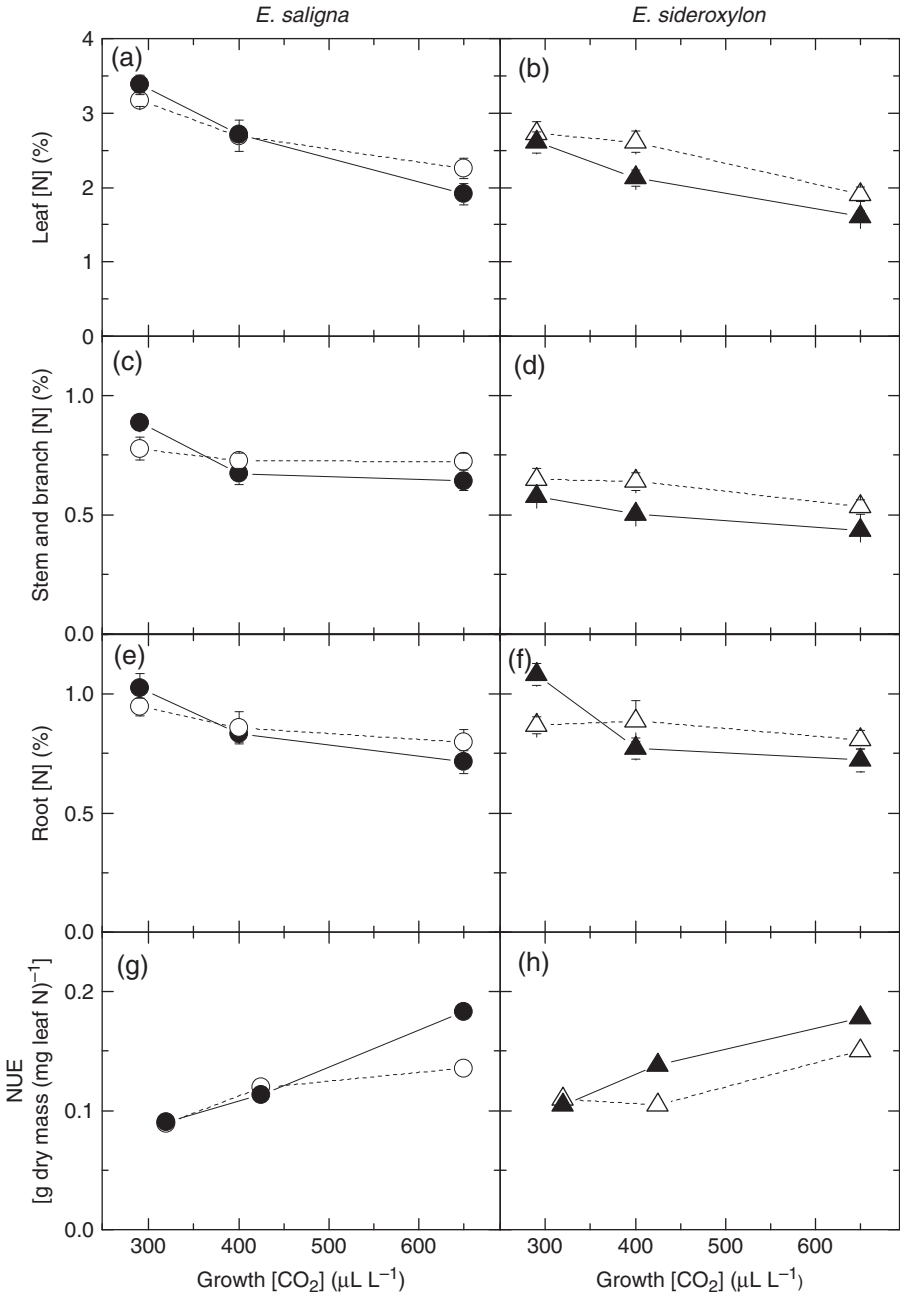

Fig. 6 Nitrogen concentration in the leaves (a and b), stems and branches (c and d) and roots (e and f) and whole plant N use efficiency, NUE (g and h) in *E. saligna* (a, c, e and g) and *E. sideroxylon* (b, d, f and h) grown at three atmospheric [CO_2] and two air temperatures. Other details are as described in Fig. 1.

species at ambient [CO_2] and temperature (Fig. 6, Tables 1 and 2). The interactive effects of [CO_2] and temperature on stem [N] and root [N] were similar between the two species (Table 1). Although the effect of high temperature on stem [N] differed between the two species (Table 1), the magnitude of the effect generally was similar between temperature treatments (Fig. 6, Table 2).

Discussion

In this study, we investigated the interactive effects of [CO_2] and temperature on the growth and photosynthesis of two *Eucalyptus* species (Fig. 7). Contrary to our hypotheses, the growth response of eucalypt tree seedlings to increasing [CO_2]: (i) was not greater in response to the transition from subambient to ambient [CO_2]

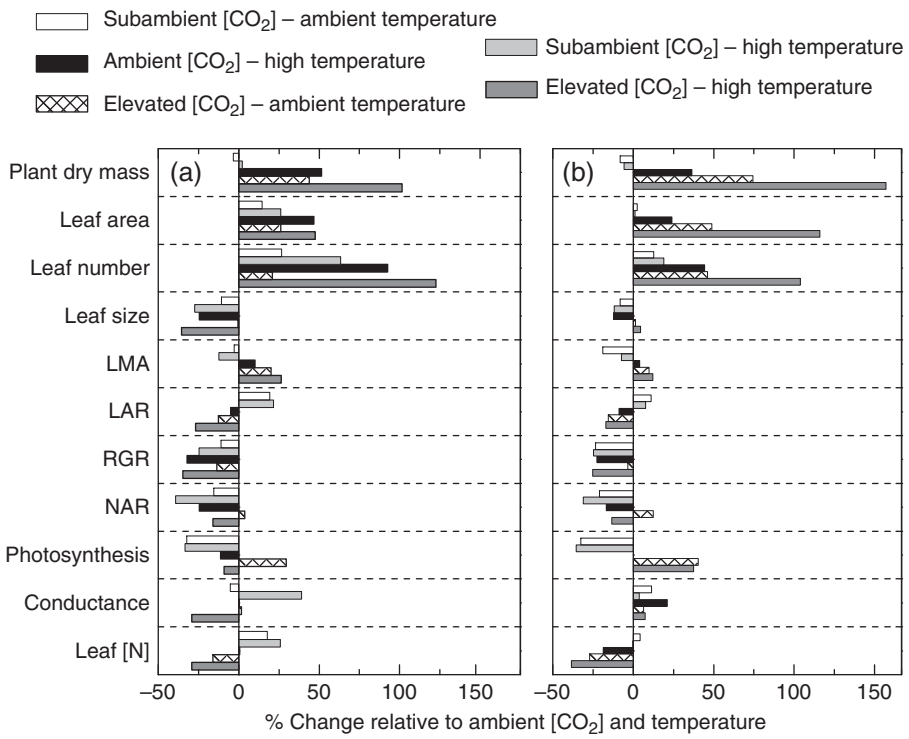

Fig. 7 Percentage change of key measured physiological parameters of *E. saligna* (a) and *E. sideroxylon* (b) relative to current ambient conditions of [CO_2] and temperature. Plants were exposed to five different climatic scenarios: subambient [CO_2] and ambient temperature (white bars); subambient [CO_2] and high temperature (light grey bars); ambient [CO_2] and high temperature (black bars); elevated [CO_2] and ambient temperature (checked bars); and elevated [CO_2] and high temperature (dark grey bars).

compared with the transition from ambient to elevated [CO_2]; (ii) increased at high temperature only for the transition from subambient to ambient [CO_2]; and (iii) was greater in the slower-growing *E. sideroxylon* compared with the faster-growing *E. saligna*. In addition, *E. sideroxylon* responded more strongly to elevated [CO_2] than to high temperature, while *E. saligna* responded similarly to elevated [CO_2] and high temperature (Fig. 7). These results suggest that the two *Eucalyptus* species may differ in their responses to future climate.

Growth and photosynthesis at elevated temperature

A 4°C warming stimulated whole plant dry mass production in *E. sideroxylon* (37%) and *E. saligna* (52%) at ambient [CO_2] (Fig. 7), consistent with biomass stimulation in Norway spruce (*ca.* 57%) exposed to a similar degree of warming (Saxe *et al.*, 2001). Higher air temperature enhanced leaf initiation, generating greater whole tree leaf area and subsequently greater whole tree biomass. High temperature also stimulated main stem elongation, without altering stem thickness, in both eucalypts as has been reported for many tree species (Saxe *et al.*, 2001). The increase in leaf area and stem height at high temperature was not different between *E. saligna* and *E. sideroxylon*. Taken together, these results do not support our hypothesis that faster-growing *E. saligna* would be more responsive to growth at high temperature. For both *E. saligna* and *E. sideroxylon*, plant dry mass was not stimulated by high temperature in the subambient [CO_2] treatment (Fig. 7). Similarly, Ward *et al.* (2008) found that neither photosynthesis nor plant dry mass responded to an 8 °C increase in temperature in a C_3 annual plant grown at 200 μL CO_2 L^{-1}. The lack of a growth response may be explained by the strong CO_2-limitation of photosynthesis at low temperature (Berry & Björkman, 1980; Sage & Kubien, 2007).

Although high temperature increased biomass production in both *Eucalyptus* species grown at ambient [CO_2], RGR was lower at high temperature primarily due to lower NAR, while LMA and LAR were not affected within each [CO_2] treatment (Fig. 7). Similarly, when growth temperature was raised from 23 to 28 °C, Loveys *et al.* (2002) found that RGR and NAR decreased, and LMA was generally unchanged for 16 plant species, including trees. In a meta-analysis of a large number of studies, Poorter *et al.* (2009) observed that LMA is marginally affected by small changes at

moderate temperatures. In their study, Loveys *et al*. (2002) also found that high temperature increased the percentage of daily fixed carbon lost by respiration, which could partly explain reductions in NAR. In our study, the reductions in NAR at high temperature are likely to have been caused by decreases in A_{sat}. In both *Eucalyptus* species, photosynthesis underwent complete thermal acclimation, such that A_{sat} was similar for trees grown at ambient and high temperature, within each $[CO_2]$ treatment. Photosynthetic acclimation to high temperature is commonly observed in a wide variety of plants (Berry & Björkman, 1980; Baker & Allen, 1993; Cowling & Sage, 1998; Teskey & Will, 1999; Dwyer *et al*., 2007), although not always (Lewis *et al*., 2001).

Interestingly, the response of A_{sat} to temperature was independent of growth $[CO_2]$ for both *Eucalyptus* species (Fig. 7). During short-term measurements, the response of A_{sat} to temperature is predicted to increase with increasing $[CO_2]$ because photosynthesis is CO_2-limited at low temperature (Berry & Björkman, 1980; Sage & Kubien, 2007). In the long-term, these responses depend on the extent to which photosynthesis acclimates to the new growth conditions. In our study, the strong thermal acclimation of A_{sat} in the two *Eucalyptus* species precluded any possible temperature $\times$ $[CO_2]$ interactions. In *E. saligna* and *E. sideroxylon*, thermal acclimation of A_{sat} was associated with lower leaf [N] in plants grown at high temperature. This is in line with predicted decreases in Rubsico and electron transport activities, and in turn, reduced sensitivity to $[CO_2]$, in plants acclimated to higher temperatures (Berry & Björkman, 1980; Sage & Kubien, 2007).

Growth and photosynthesis at subambient [CO_2]

At ambient temperature, whole plant biomass production was not inhibited by growth in subambient $[CO_2]$ when compared with ambient $[CO_2]$ in either *Eucalyptus* species (Fig. 7). This contrasts with an average 25% reduction in plant dry mass production at subambient $[CO_2]$ for a range of species grown at various temperatures (Sage & Coleman, 2001). Nevertheless, our results are supported by a number of studies which reported no growth inhibition in response to decreasing $[CO_2]$ from current ambient to preindustrial levels in C_3 plants such as winter wheat (Rogers *et al*., 1998) and barley (Cunniff *et al*., 2008). Particularly significant to our study was the report by Rogers *et al*. (1998) that the growth responsiveness to subambient $[CO_2]$ depended on the wheat cultivar. Taken together, these findings indicate that we cannot extrapolate the plant growth response to recent increases in atmospheric $[CO_2]$ based upon the short-term responses of leaf photosynthesis. In both *Eucalyptus* species, A_{sat} was lower at subambient $[CO_2]$ compared with ambient $[CO_2]$ and ambient temperature (Fig. 7). This reduction in photoassimilate production on an area basis was counterbalanced by increased leaf area production on a total plant dry mass (increased LAR in *E. saligna*) or leaf dry mass (reduced LMA in *E. sideroxylon*) basis. Thus, total dry mass was negligibly affected in both species by growth in subambient $[CO_2]$ at ambient temperature. These results highlight the plasticity of eucalypts and their ability to acclimate to a wide range of environmental variables. A major implication of our study is that at lower temperatures, *Eucalyptus* tree growth may not have responded to the 35% rise in atmospheric $[CO_2]$ from the preindustrial era to the present time.

High temperature enhanced the growth responsiveness of both *Eucalyptus* species to subambient $[CO_2]$ such that plant dry mass production in subambient $[CO_2]$ was reduced by about 30% compared with ambient $[CO_2]$ (Fig. 7). Similarly, Cowling & Sage (1998) reported that high temperature enhanced the growth response of bean plants to increasing $[CO_2]$ from 200 to 380 $\mu L\,L^{-1}$. This was attributed to the markedly decreased leaf area at subambient $[CO_2]$ as photosynthetic rates were similar at both temperatures (Cowling & Sage, 1998). For both *Eucalyptus* species, the reduction in leaf area was smaller than that observed for plant dry mass in subambient $[CO_2]$ at high temperature. This suggests that reduced whole plant photosynthesis and/or increased respiration were also responsible for the growth reductions at subambient $[CO_2]$ and high temperature. Theoretically, the responsiveness of C_3 photosynthesis to elevated $[CO_2]$ is expected to increase with temperature (Long, 1991; von Caemmerer, 2000). In our study, the responsiveness of A_{sat} and NAR to increasing $[CO_2]$ from subambient $[CO_2]$ to ambient $[CO_2]$ was not greater at high temperature in either species. Taken together, these results suggest that whole plant respiration consumed an increasing proportion of fixed carbon at high temperature, which might explain the negative effect of subambient $[CO_2]$ on biomass accumulation (Morison & Lawlor, 1999; Loveys *et al*., 2002).

Growth and photosynthesis at elevated [CO_2]

At ambient temperature, whole plant dry mass increased in response to elevated $[CO_2]$ by 44% in *E. saligna* and 74% in *E. sideroxylon* (Fig. 7), similar to that observed for other *Eucalyptus* (Conroy *et al*., 1992; Wong *et al*., 1992; Duff *et al*., 1994; Roden & Ball, 1996a, b; Gleadow *et al*., 1998; Roden *et al*., 1999) and tree species in general (Ceulemans & Mousseau, 1994; Saxe *et al*., 1998; Norby *et al*., 1999). Biomass enhancement in elevated $[CO_2]$ can be explained by higher leaf area and A_{sat} for both *Eucalyptus* species (Fig. 7).

Interestingly, the slower-growing *E. sideroxylon* exhibited greater biomass enhancement than the faster-growing *E. saligna* when grown in elevated [CO_2] (Fig. 7). Thus, our data do not support our hypothesis that faster-growing *E. saligna* would be more responsive to elevated [CO_2]. In a number of studies, the growth response to elevated [CO_2] was reported to be greater in faster- than slower-growing plants (Poorter *et al*., 1996; Poorter, 1998; Atkin *et al*., 1999; Poorter & Navas, 2002). However, this response pattern was not always observed (Lloyd & Farquhar, 2000). For example, biomass enhancement in four *Eucalyptus* species grown in elevated [CO_2] was greater for the two slow-growing species than for the two fast-growing species (Wong *et al*., 1992). A greater growth response to elevated [CO_2] in slow-growing plants may be due partly to their higher respiratory costs, relative to fast-growing plants, which are better met in elevated [CO_2] (Lloyd & Farquhar, 2000). In addition, a greater degree of self-shading due to higher LAR would reduce radiation use efficiency, and hence growth sensitivity to [CO_2] in *E. saligna* relative to *E. sideroxylon*. This is consistent with the observation that the growth response to elevated [CO_2] decreases with increasing plant size (Conroy *et al*., 1990; Masle *et al*., 1993). However, plant size alone did not explain variation in growth response to elevated [CO_2] in our study because the growth response persisted as plants grew larger. Despite increased biomass production in both *Eucalyptus* species, RGR was not enhanced by elevated [CO_2] in either species. This was related to the fact that elevated [CO_2] reduced LAR without substantially stimulating NAR. These results suggest that increased plant dry mass was generated by stimulation of RGR during early exposure to elevated [CO_2] (Masle *et al*., 1993).

In *Eucalyptus* trees grown at elevated [CO_2] and ambient temperature, the stimulation of A_{sat} (30–40%) (Fig. 7) was similar to that observed in other trees (Roden & Ball, 1996b; Tissue *et al*., 1996, 1997; Li *et al*., 1999; Evans *et al*., 2000; Bernacchi *et al*., 2003; Ainsworth & Rogers, 2007). The strong and persistent stimulation of A_{sat} by elevated [CO_2] in trees may be related to their strong capacity to produce new sinks (e.g., leaves, branches and roots) throughout their development. In contrast, g_s did not respond to growth in elevated [CO_2] in our well-watered *Eucalyptus* species, although it is commonly observed that trees show a moderate reduction in g_s in elevated [CO_2] (Morison, 2001; Ainsworth & Rogers, 2007). In other studies with *Eucalyptus* grown in elevated [CO_2], reductions in g_s were observed in some species (Berryman *et al*., 1994; Roden & Ball, 1996a), but not in others (Roden *et al*., 1999). The nonresponsiveness of g_s to growth [CO_2] we observed in two broad-leaved, evergreen *Eucalyptus* species was similar to the weak or nonsignificant responses of evergreen conifers exposed to elevated [CO_2] (Lewis *et al*., 2002; Tissue *et al*., 1997), rather than to the greater reductions more commonly observed in deciduous trees (Ainsworth & Rogers, 2007).

Relatively few studies have examined the interaction between growth [CO_2] and temperature on growth and photosynthesis of C_3 plants, particularly trees (Morison & Lawlor, 1999). We hypothesized that the growth enhancement brought on by growth at elevated [CO_2] would be relatively greater at elevated growth temperature in comparison with ambient growth temperature. However, our data did not support this hypothesis, since growth temperature did not affect the photosynthetic or biomass responses of either *Eucalyptus* species to growth in elevated [CO_2] (Fig. 7). Similarly in Douglas fir and loblolly pine, A_{sat} responded positively to increasing [CO_2] and temperature, but the relative response to elevated [CO_2] was not affected by growth temperature (Teskey, 1997; Lewis *et al*., 2001). In addition, no clear trend has emerged for the effect of temperature on plant growth response to elevated [CO_2] (Morison & Lawlor, 1999). In temperature studies, it is important to note whether the elevated temperature treatment shifts plants beyond their optimal growth and photosynthetic temperatures. If so, then the impact may be expected to negatively affect plant productivity (Way & Sage, 2008). In our study, $+4\,^{\circ}C$ warming (to a daytime high of $30\,^{\circ}C$) did not negatively affect photosynthesis or growth, suggesting that *E. saligna* and *E. sideroxylon* exhibit higher temperature optima than most other C_3 plants (Baker & Allen, 1993; Cowling & Sage, 1998; Sage & Kubien, 2007), as has been observed for *E. tetrodonta* and *E. grandis* (30–33 °C) (Eamus *et al*., 1995; Thomas *et al*., 2007). Consequently, our results suggest that, when water is not limiting, global warming may not diminish the growth response to elevated [CO_2] of warm temperature-adapted plants, such as *Eucalyptus*.

Conclusions

Growth at temperatures that realistically reflect warming predictions for the coming century enhanced plant biomass production in *E. saligna* and *E. sideroxylon* by stimulating leaf initiation, while A_{sat} underwent full thermal acclimation in both species. Plant growth at subambient and ambient [CO_2] was similar under ambient temperature, despite greater rates of photosynthesis at ambient [CO_2]. Growth and rates of photosynthesis were greater at elevated [CO_2] in comparison with ambient [CO_2] at both growth temperatures. These results suggest that fast- and slow-growing *Eucalyptus*

species may not have responded to the change from preindustrial to present ambient conditions in terms of growth, but that they possess great potential to respond to predicted increases in atmospheric [CO_2] and temperature. This study highlights the need to evaluate how water availability influences responses to high [CO_2] and temperature.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Summary of the 3-way ANOVA testing for the effects of species, [CO_2] and temperature on various parameters of *E. saligna* and *E. sideroxylon* grown at three [CO_2] and two temperatures and harvested 80 days after planting, as described in the Material and Methods. Significance levels are: ns = not significant ($P > 0.05$); * = $P < 0.05$; ** = $P < 0.01$; *** $P < 0.001$.

Table S2. Summary of means at 80 days after planting for *E. saligna* and *E. sideroxylon* grown at three [CO_2] and two temperatures, as described in the Materials and Methods.

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