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Conserved stomatal behaviour under elevated CO_2 and varying water availability in a mature woodland

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Summary

1. Rising levels of atmospheric CO_2 concentration (C_a) and simultaneous climate change profoundly affect plant physiological performance while challenging our ability to estimate vegetation–atmosphere fluxes. To predict rates of water and carbon exchange between vegetation and the atmosphere, we require a formulation for stomatal conductance (g_s) that captures the multidimensional response of stomata to changing environmental conditions. The unified stomatal optimization (USO) theory provides a formulation for g_s with the ability to predict the response of g_s to novel environmental conditions such as elevated C_a (eC_a), warmer temperatures and/or changing water availability.

2. We tested for the effect of eC_a and seasonally varying climate on stomatal behaviour, as defined by the USO theory, during the first year of free-air CO_2 enrichment in a native eucalypt woodland (the EucFACE experiment). We hypothesized that under eC_a, g_s would decrease and photosynthesis (A_{net}) would increase, but fundamental stomatal behaviour described in the USO model would remain unchanged. We also predicted that the USO slope parameter g_1 would increase with temperature and water availability. Over 20 months, we performed quarterly gas exchange campaigns encompassing a wide range of temperatures and water availabilities. We measured g_s, A_{net} and leaf water potential (Ψ) at mid-morning, midday and pre-dawn (Ψ only) under ambient and eC_a and prevailing climatic conditions, at the tree tops (20 m height).

3. We found that eC_a induced a 20% reduction in stomatal conductance under non-limiting water availability, enhanced mid-morning A_{net} by 24% in three out of five measurement campaigns and had no significant effect on Ψ. The parameter g_1 was conserved under eC_a, weakly increased with temperature and did not respond to increasing water availability.

4. Our results suggest that under eC_a and variable rainfall, mature eucalypt trees exhibit a conservative water-use strategy, but this strategy may be modified by growth temperature. We show that the USO theory successfully predicts coupling of carbon uptake and water loss in future atmospheric conditions in a native woodland and thus could be incorporated into ecosystem-scale and global vegetation models.

Key-words: drought, *Eucalyptus tereticornis*, instantaneous transpiration efficiency, photosynthesis, rising CO_2, stomatal conductance, water-use efficiency

Introduction

Stomata regulate water and carbon exchange between the vegetation and the atmosphere by their opening and closing (Sellers *et al.* 1997; Hetherington & Woodward 2003; Buckley & Mott 2013). They provide a means for balancing water loss while allowing access to CO_2 into the sub-stomatal cavity for photosynthesis (Brodribb *et al.* 2009). Stomata respond in a complex and nonlinear way to multiple environmental factors such as temperature (T), vapour

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*Correspondence author. E-mail: teresa.gimeno@bordeaux.inra.fr pressure deficit between the leaf and the atmosphere (D), CO_2 concentration in the atmosphere (C_a) and photosynthetically active radiation (Jarvis 1976; Cowan 1982; Damour *et al.* 2010). Ecosystem and land surface models require reliable modules to describe stomatal behaviour in response to these climatic variables (Sellers *et al.* 1997; Kleidon 2004; Damour *et al.* 2010), particularly under current global change scenarios with rising levels of C_a (De Kauwe *et al.* 2013) and simultaneous climate change (IPCC 2014). Despite this, some stomatal models still have difficulty predicting stomatal responses under future global change scenarios (Buckley & Mott 2013; Medlyn *et al.* 2013; De Kauwe *et al.* 2014).

Plants adjust their physiological performance to directional changes in environmental conditions to maintain or improve their growth and survival under the novel conditions. While stomata typically close in response to rising C_a, there is variation in the magnitude of their response (Saxe, Ellsworth & Heath 1998; Franks *et al.* 2013). Stomatal responses to elevated C_a may involve overall reductions in stomatal conductance as has been frequently observed (Ainsworth & Rogers 2007; Franks *et al.* 2012; but see Leakey *et al.* 2006). However, the response to elevated C_a could involve a change in the magnitude of the intrinsic response of g_s to a strong environmental stimulus such as D (Heath 1998; Maherali, Johnson & Jackson 2003). Despite understanding the mechanisms of the stomatal closure response (Buckley, Mott & Farquhar 2003), an intrinsic change in stomatal behaviour in elevated C_a has rarely been specifically assessed (although see Berryman, Eamus & Duff 1994).

Stomatal optimization theory is based on the idea that stomatal opening to allow CO_2 uptake inevitably comes at the expense of H_2O loss (Cowan & Farquhar 1977; Orians & Solbrig 1977; Cowan 1982). Thus, stomata should maximize photosynthetic uptake (A) minus the carbon cost of water used in transpiration (λE, where λ is the marginal carbon cost of water, in mol CO_2 mol^{-1} H_2O). This optimization theory has been applied many times to successfully predict stomatal responses to environmental conditions, including D (e.g. Arneth *et al.* 2002; Hari *et al.* 1986; Thomas, Eamus & Bell 1999; Kleidon 2004; Katul *et al.* 2010; Vico *et al.* 2013; Buckley & Schymanski 2014), but it appeared that the theory predicted incorrect responses to C_a (Lloyd & Farquhar 1994). Following optimization theory, Medlyn *et al.* (2011) proposed a formulation, called the unified stomatal optimization theory (USO hereafter) that correctly predicts the direction of stomatal response to C_a and has been shown to fit data from a number of high C_a experiments (e.g. Barton *et al.* 2012; De Kauwe *et al.* 2013; Duursma *et al.* 2013).

It stands to reason that stomatal behaviour should vary according to the water available to the plant (Cowan 1982; Makela, Berninger & Hari 1996; Manzoni *et al.* 2011, 2013). Optimization theory predicts that λ should increase with decreasing water availability (Cowan & Farquhar 1977; Zhou *et al.* 2013). The USO parameter g_1 (proportional to $1/\sqrt{\lambda}$) has been shown to decrease with decreasing water availability in grasses (Manzoni *et al.* 2011; Zhou *et al.* 2013) and fast-growing plantation trees (Héroult *et al.* 2013). Nevertheless, in slow-growing trees from drier origins, g_1 was less responsive to changes in water availability than fast-growing trees from mesic provenances (Héroult *et al.* 2013). Furthermore, Eamus *et al.* (2013) found that their proxy for λ, calculated at the whole ecosystem level, increased from the wet to the dry season in an arid savanna. A second prediction from optimization theory is that λ (and g_1) shows a weak dependency on temperature (Duursma *et al.* 2013). This latter prediction emerges from the dependency of λ on the temperature-sensitive non-photorespiratory CO_2 compensation point (Γ^*, Brooks & Farquhar 1985; Bernacchi *et al.* 2001), and other temperature-sensitive considerations like water viscosity (Prentice *et al.* 2014; Lin *et al.* 2015).

While measurements of the variation in g_s in response to environmental variability in the field can be achieved with seasonal measurements of gas exchange, it is more difficult to test how different C_a levels affect stomatal behaviour in natural conditions. Here, we utilized a free-air CO_2 enrichment (FACE) experiment to test the effect of rising C_a and seasonal climate on stomatal behaviour. We quantified the effect of rising C_a on g_s during the first year of exposure to elevated C_a in a mature (>80 year old), native *Eucalyptus* woodland under seasonally varying water availability and temperature. Measurements during the first year of the *Eucalyptus* FACE experiment ('EucFACE') allow testing for the effect of elevated C_a on g_s and its coupling with A_{net} while assessing interactions with a variable climate. The EucFACE experiment is the first of its kind established in a native woodland in a seasonally water-limited climate. This, together with the uniqueness of the facility (Figure S4, Supporting information), provides the opportunity to test whether the USO theory can predict stomatal regulation under rising C_a in native old tall trees (>20 m). We specifically hypothesized that (i) in mature *Eucalyptus tereticornis* Sm. trees in natural conditions, elevated C_a would enhance A_{net} and reduce g_s, across a range of climatic conditions, (ii) despite the occurrence of stomatal closure in elevated C_a, the coupling of A_{net} and g_s would not be affected by elevated C_a in these mature trees over an annual time frame, that is the USO parameter g_1 would remain unchanged in response to elevated C_a, and (iii) g_1 would increase with warmer temperatures and greater water availability.

Materials and methods

MODEL AND THEORY

We evaluated the unified stomatal optimization model (USO) of Medlyn *et al.* (2011) for estimating stomatal conductance as a function of environmental and photosynthetic parameters. The USO is based on optimization theory following Cowan & Farquhar (1977). In the USO model, g_s is approximated as follows:

$$g_s \approx g_0 + 1\cdot6\left(1 + \frac{g_1}{\sqrt{D}}\right)\frac{A_{\mathrm{net}}}{C_a} \qquad \text{eqn 1}$$

where the term g_0 (in mol $\mathrm{m^{-2}\,s^{-1}}$) accounts for cuticular conductance and residual stomatal conductance when photosynthesis (A_{net} in μmol $\mathrm{m^{-2}\,s^{-1}}$) approaches zero, D is the leaf-air vapour pressure deficit (in kPa), and g_1 is the slope parameter (in $\mathrm{kPa^{0\cdot5}}$). Fitting the USO model allows to test for differences in g_0 and g_1 between C_a treatments and among measurement campaigns conducted across seasons.

One further implication of the USO is that the increase in instantaneous transpiration (E) efficiency (ITE) in response to elevated C_a (Saxe, Ellsworth & Heath 1998; Ainsworth & Rogers 2007) should be proportional to the increase in C_a (Barton *et al.* 2012; Duursma *et al.* 2013). ITE is the ratio of A_{net} to E, which can also be written as (eqn 14 in Medlyn *et al.* 2011):

$$\mathrm{ITE} = \frac{A_{\mathrm{net}}}{E} = \frac{C_a P_a}{1\cdot6(D + g_1\sqrt{D})} \qquad \text{eqn 2}$$

where P_a is the atmospheric pressure in kPa. Under common prevailing environmental conditions (P_a and D), if g_1 remained unchanged from ambient to elevated C_a, then the ratio of ITE between elevated and ambient C_a should be proportional to the elevated to ambient C_a ratio. In our case, we expected the ITE to increase from ambient (≈390 μmol $\mathrm{CO_2\,mol^{-1}}$) to eC_a (ambient + 150 = 540 μmol $\mathrm{CO_2\,mol^{-1}}$) by a ratio of 540/390 = 1·38.

SITE DESCRIPTION AND EXPERIMENTAL DESIGN

The EucFACE experiment is located on an ancient alluvial floodplain about 5 km from the Hawkesbury River in western Sydney (NSW, Australia) at 30 m a.s.l. elevation (33°37′S, 150°44′E). It is located within a 170-ha remnant of native Cumberland Plain woodland. The vegetation, maintained in this location since the 1880s, retains some old-growth trees and some re-growth vegetation, which has been undisturbed for >80 years. The site mean annual temperature is 17 °C, and mean annual precipitation is 810 mm (Bureau of Meteorology, station 067105 in Richmond, NSW Australia; http://www.bom.gov.au).

At the site, total incoming precipitation (P), air temperature (T) and relative humidity (H_R) were monitored on a continuous basis and 15-min total (P) and 1-min averages (T and H_R) were recorded on data loggers (CR3000, Campbell Scientific Australia, Townsville, Australia). There were six humidity and temperature sensors (HUMICAP ® HMP 155 Vaisala, Vantaa, Finland) on the top of the central tower of each study plot. P was monitored with four automated tipping buckets (Tipping Bucket Rain gauge TB4, Hydrological Services Pty Ltd, Liverpool, NSW, Australia), three on top of the central tower at 23·5 m height and one in an open space 100 m away. Atmospheric water vapour pressure deficit (D) was calculated from temperature and humidity measurements.

The soil at the site is a Clarendon loamy sand, a slightly acid, low-organic C, well-drained soil. The loamy sand surface has >75% sand content, and from ca. 50–300 cm depth, the soil is a sandy clay loam with >30% silt and clay. Groundwater depth is 12 m below the soil surface. The vegetation consists of an open woodland with a density from 600 to 1000 trees $\mathrm{ha^{-1}}$, a vegetation area index (including leaves and woody stems) ≤2 $\mathrm{m^2\,m^{-2}}$ and basal area: 27·6 ± 2·7 $\mathrm{m^2\,ha^{-1}}$, n = 6 plots (Figure S4). There is a single major canopy-forming tree (*Eucalyptus tereticornis* Sm.) and a minor component of *E. amplifolia* Naudin. Further description of the site and particularly its nutrient status is found in Crous, Ósvaldsson & Ellsworth (2015).

EucFACE consists of six 25-m-diameter circular plots (designated hereafter as 'rings'). Each ring comprises 32 vent pipes in a cylindrical frame extending above the tree canopy to 28 m high (Figure S4). Each vertical pipe is subtended by a control valve and is connected to a circular plenum (Hendrey *et al.* 1999) through which a computer-controlled system modulates the release of dilute $\mathrm{CO_2}$ into the vegetation according to a proportional–integral–differential type of algorithm (Lewin *et al.* 2009). Within each ring, there are 39 ± 3 canopy trees (mean ± SE, n = 6 rings, max = 48, min = 27), with ~17 dominant and co-dominant canopy-forming trees. The vegetation within three rings was exposed to a $\mathrm{CO_2}$ mole fraction (C_a) higher than ambient starting in September 2012, whereas the three ambient rings were identical to the $\mathrm{CO_2}$ treatment rings in infrastructure and instrumentation but received ambient C_a, and are used as control plots. At full operation, the target diurnal C_a for the rings exposed to elevated $\mathrm{CO_2}$ (henceforth 'eC_a') was 150 μmol $\mathrm{CO_2\,mol^{-1}}$ above ambient C_a. However, C_a was increased gradually for 6 months to this full operation mode. C_a was increased at a rate of ca. 30 μmol $\mathrm{CO_2\,mol^{-1}\,month^{-1}}$ until mid-summer (February 2013; Figure S1).

LEAF GAS EXCHANGE AND WATER RELATIONS MEASUREMENTS

We performed seven campaigns of leaf gas exchange and water potential measurements from April 2012 to November 2013, inclusive. Five of the campaigns coincided with the period when C_a was enriched above ambient level according to Figure S1, and four campaigns corresponded with the full treatment of ambient +150 μmol $\mathrm{CO_2\,mol^{-1}}$. Each campaign was carried out over the course of two sunny days, representative of the season (Table S1). Leaf-level $\mathrm{CO_2}$ and $\mathrm{H_2O}$ exchange measurements were performed with four open-flow portable photosynthesis systems (Li-6400, Li-Cor, Inc., Lincoln, NE, USA). We measured photosynthetic $\mathrm{CO_2}$ assimilation (A_{net}) and stomatal conductance to water (g_s) under 1800 μmol $\mathrm{m^{-2}\,s^{-1}}$ photon flux density (provided by the in-built Li-6400 red-blue LED lamp). The C_a in the cuvette varied between $\mathrm{CO_2}$ treatments and among campaigns as follows: cuvette C_a was set to ~390 μmol $\mathrm{CO_2\,mol^{-1}}$ in all rings in April and May 2012 (pre-treatment) and in all ambient rings thereafter. For eC_a rings, C_a in cuvettes was set to 450 μmol $\mathrm{mol^{-1}}$ (60 μmol $\mathrm{CO_2\,mol^{-1}}$ above ambient) in October 2012, and 540 μmol $\mathrm{CO_2\,mol^{-1}}$ (150 μmol $\mathrm{CO_2\,mol^{-1}}$ above ambient) in February, May, September and November 2013. Gas exchange measurements were taken when maximum and minimum diurnal g_s were expected: at mid-morning (9·30–11·10 AEST) and at midday (13·00–15·00), respectively (Barton *et al.* 2012; Héroult *et al.* 2013). At each campaign, we measured one single cohort of mature fully expanded leaves. During campaigns when two or more leaf cohorts coexisted, we measured the cohort aged 3–8 months. For the four 2013 campaigns (when the eC_a treatment was 150 μmol $\mathrm{CO_2\,mol^{-1}}$ above ambient in the eC_a rings), we followed the leaf cohort that had flushed in October 2012 and hence had developed under the eC_a treatment. In each ring, canopy access was achieved with permanent 43-m tall freestanding tower cranes equipped with a 35-m-long jib and working gondola (J4010, Jaso Cranes, Idiazábal, Guipuzkoa, Spain). This access allowed measurements to be conducted right at the treetops at a mean height of 20·1 ± 0·2 m (mean ± SE for n = 3–4 trees per n = 3 rings per C_a treatment, per campaign). Once the elevated C_a treatment in FACE started, measurements were taken simultaneously in pairs of rings comprising one ambient and one elevated $\mathrm{CO_2}$ ring. We measured two leaves per tree in three (April 2012-May 2013) or four (September and November 2013) dominant or co-dominant *E. tereticornis* trees per ring. Prior to measurements within each ring (or pair or rings), conditions within the chamber were adjusted to the prevailing environment. Chamber humidity and temperature were maintained as close as possible to prevailing conditions during each measurement period.

In each campaign, except April 2012, we measured leaf water potential (Ψ) at pre-dawn (Ψ_{pd}) and midday (Ψ_{md}) and from February 2013 also at mid-morning (Ψ_{mm}). In April 2012, there had recently been standing water owing to heavy rainfall prior to measurements; hence, no Ψ measurements were taken. We took additional measurements of Ψ in mid-summer (January 2013), when soil water availability reached its minimum within the measurement period (October 2013, only Ψ_{pd}), and in the middle of the following summer (January 2014, Fig. 1). Ψ_{pd} was measured on two random leaves from three random trees in each ring. Ψ_{mm} and Ψ_{md} were measured in the same leaves used for gas exchange. Water potentials were measured with a Scholander-type pressure chamber (Model 600l; PMS Instrument Company, Corvallis, OR, USA).

DATA ANALYSIS

We fit the USO model (eqn 1) to our measurements of gas exchange in different trees measured at different times of day and campaigns: that is, for each tree, we averaged simultaneous measurements from two leaves. In order to test for significant differences ($P < 0{\cdot}05$) in g_1 (eqn 1) between C_a and among sampling dates, we used two-way analysis of variance conducted on the residuals from the USO model fits. This analysis follows the principle that deviation from the overall USO model fit would represent either C_a or campaign effects analogous to a repeated-measures mixed-model ANOVA. We also tested for the correlation between the ITE in ambient vs. eC_a. To do so, we fitted a linear regression between mean ITE in pairs of ambient and eC_a rings measured simultaneously (i.e. same D and P_a). This approach rendered 24 pairs of rings measured simultaneously.

For each campaign during which FACE was operating, we tested for differences between ambient and elevated C_a in gas exchange measurements (A_{net}, g_s and internal to atmospheric CO_2 mole fraction, C_i/C_a) and water potential with one-way ANOVA. For these analyses, we considered measurements from different campaigns at different times of the day from each ring as the experimental unit ($n = 3$ for each treatment level); that is, we averaged simultaneous measurements across leaves in each tree and then averaged measurements across trees in each ring. Significance level was set to $P = 0{\cdot}05$ with findings with $0{\cdot}05 < P < 0{\cdot}10$ considered marginally significant. All analyses were performed in R (R Development Core Team 2014) v 3.1.

Results

CLIMATE, PLANT WATER RELATIONS ACROSS SEASONS AND CO_2 TREATMENTS

The first two measurement campaigns were preceded by a wet summer (‘La Niña’ event), with heavy rainfall (>100 mm, Fig. 1) falling about 3 weeks prior to the first set of measurements (Table S1). This period was followed by a relatively dry (with respect to long-term trends) autumn, winter and beginning of the spring, when we took the first round of gas exchange measurements under eC_a (October 2012, with eC_a 60 μmol CO_2 mol^{-1} above ambient). Subsequent campaigns, when eC_a was 150 μmol CO_2 mol^{-1} above ambient, occurred during a wet summer (February 2013), a temperate autumn (May 2013) and

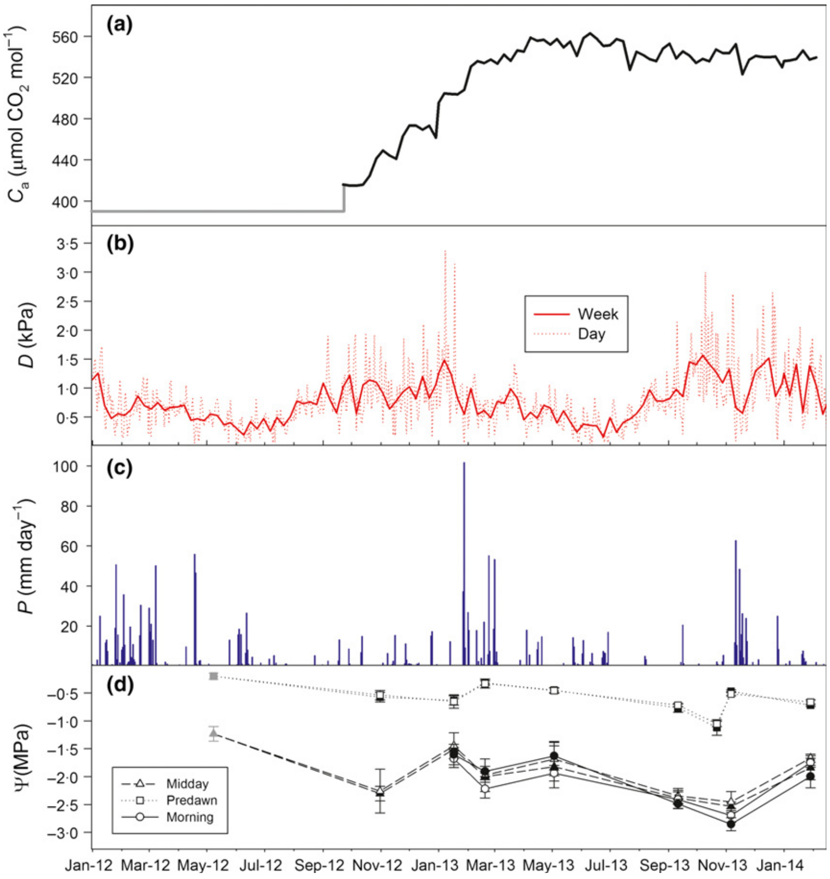

Fig. 1. (a) Step-up increase of CO_2 mole fraction (C_a) in elevated C_a rings (mean weekly C_a, black line) and approximate ambient C_a during pre-treatment (grey line), (b) mean daily (dotted line) and weekly (continuous line) air water vapour pressure deficit (D), (c) daily precipitation (P) and (d) mean (±SE, $n = 3$) pre-dawn, morning and midday water potential (Ψ) in ambient C_a (open symbols), elevated C_a (closed symbols) and pre-treatment (grey symbols).

after abnormally warm and dry conditions in both winter and beginning of spring (Fig. 1, September and November 2013, Table S1).

Leaf water potential reflected the rainfall pattern over the year (Fig. 1). The lowest pre-dawn leaf water potential (Ψ_{pd}) was measured at the driest point reached after the longest sustained period without substantial rain (October 2013). A series of four small rain events (10·5 mm of precipitation in total) prior to the following measurement of Ψ_{pd} (November 2013) was sufficient to boost mean Ψ_{pd} from $-1{\cdot}09 \pm 0{\cdot}07$ to $-0{\cdot}49 \pm 0{\cdot}02$ MPa (mean ± SE). Mean Ψ_{pd}, Ψ_{mm} and Ψ_{md} did not differ between ambient and eC_a in any campaign (Fig. 1).

LEAF STOMATAL CONDUCTANCE AND OTHER GAS EXCHANGE PARAMETERS UNDER ELEVATED C_A

Leaf stomatal conductance to water vapour (g_s) varied seasonally with prevailing climatic conditions (Figs 2 and 3). Maximum g_s was measured in February 2013 (high water availability and warm temperatures) and minimum in November 2013 (low water availability). In all campaigns, g_s decreased from mid-morning to midday (Fig. 2) and the magnitude of this drop was largest in spring (in both 2012 and 2013) when Ψ_{pd} reached its minimum and midday D was 2·5 and 4·4 kPa (in 2012 and 2013, respectively).

We found that an increase of 60 μmol CO_2 mol^{-1} above ambient (October 2012) did not have a statistically significant effect on g_s. In February 2013, when eC_a reached 150 μmol CO_2 mol^{-1} above ambient, mean g_s was 20% lower in eC_a (morning: $0{\cdot}43 \pm 0{\cdot}02$, midday: $0{\cdot}31 \pm 0{\cdot}05$ mol H_2O m^{-2} s^{-1}) than in ambient C_a (morning: $0{\cdot}53 \pm 0{\cdot}06$, midday: $0{\cdot}43 \pm 0{\cdot}03$ mol H_2O m^{-2} s^{-1}), although this difference was only marginally significant for midday g_s ($F = 5{\cdot}3$, $P = 0{\cdot}084$).

Photosynthesis (A_{net}) followed a similar seasonal pattern to g_s. A_{net} peaked in February 2013, when temperatures were warm and water availability was high, and was minimal in May 2012, when it was coldest. A_{net} always declined with g_s from mid-morning to midday, and this drop was most pronounced in October 2012 and November 2013. There was significant stimulation of photosynthesis in elevated C_a ($P < 0{\cdot}05$) for three out of five sampling dates when FACE was operating (Fig. 2). First, in October 2012, when eC_a was 60 μmol mol^{-1} above ambient, A_{net} in the morning was $13{\cdot}3 \pm 2{\cdot}7$ and $19{\cdot}1 \pm 1{\cdot}2$ μmol CO_2 m^{-2} s^{-1} in ambient and eC_a, respectively, although the difference between treatments was not statistically significant ($F = 3{\cdot}8$, $P = 0{\cdot}124$, Fig. 2). In February 2013, an increase of 150 μmol mol^{-1} above ambient C_a significantly increased A_{net} in the morning ($F = 16{\cdot}0$, $P = 0{\cdot}016$). In May 2013, A_{net} was not significantly different between C_a treatments. In the following campaign (September 2013), we found that A_{net} was enhanced under eC_a both in the morning and at midday ($F = 11{\cdot}4$ and $9{\cdot}9$, $P = 0{\cdot}032$ and $0{\cdot}005$). Finally, in November 2013, we found significantly higher A_{net} under eC_a in the morning ($F = 26{\cdot}1$, $P < 0{\cdot}001$).

Leaf internal CO_2 mole fraction (C_i) in ambient C_a was conserved across the seasons and was always higher in the morning (285 ± 3 μmol CO_2 mol^{-1}) than at midday (248 ± 5 μmol CO_2 mol^{-1}, $n = 7$ campaigns). In eC_a, C_i was 390 ± 8 (morning) and 340 ± 13 μmol CO_2 mol^{-1} (midday, $n = 4$ campaigns with eC_a = 150 μmol CO_2 mol^{-1} above ambient). The ratio of leaf internal to atmospheric CO_2 mole fraction (C_i/C_a) did not differ significantly between C_a treatments, neither in the morning ($F = 0{\cdot}1$, $P = 0{\cdot}717$) nor at midday ($F = 2$, $P = 0{\cdot}156$) in any campaign. C_i/C_a was always significantly higher ($F = 55{\cdot}8$, $P < 0{\cdot}001$) in the morning ($0{\cdot}72 \pm 0{\cdot}01$) than at midday ($0{\cdot}63 \pm 0{\cdot}01$), when D was higher. There was some seasonal variation in C_i/C_a (Table S2).

STOMATAL RESPONSE TO ENVIRONMENTAL PARAMETERS AND COUPLING WITH PHOTOSYNTHESIS

A_{net} and g_s were broadly correlated, but there was wider scatter in this relationship under eC_a compared to ambient C_a (Fig. 3). Stomatal conductance (g_s) was coupled with D (Fig. 3) for all campaigns and C_a levels. C_i/C_a decreased with D (Table S2).

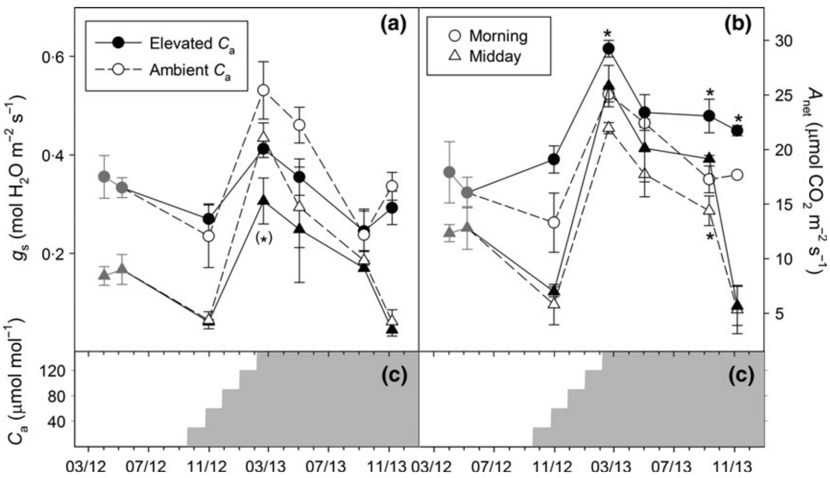

Fig. 2. (a) Mean (±SE, $n = 3$) stomatal conductance to water (g_s) and (b) photosynthesis (A_{net}) in the morning (circles) and at midday (triangles) for different C_a (CO_2 mole fraction) levels: ambient C_a (open symbols), elevated C_a (closed symbols) and pre-treatment (grey symbols). Asterisks * denote significant ($P < 0{\cdot}05$) or marginally significant (*) ($0{\cdot}1 < P < 0{\cdot}05$) differences between ambient and elevated C_a. (c) Schematic representation of the step-up increase in the elevated C_a treatment over time.

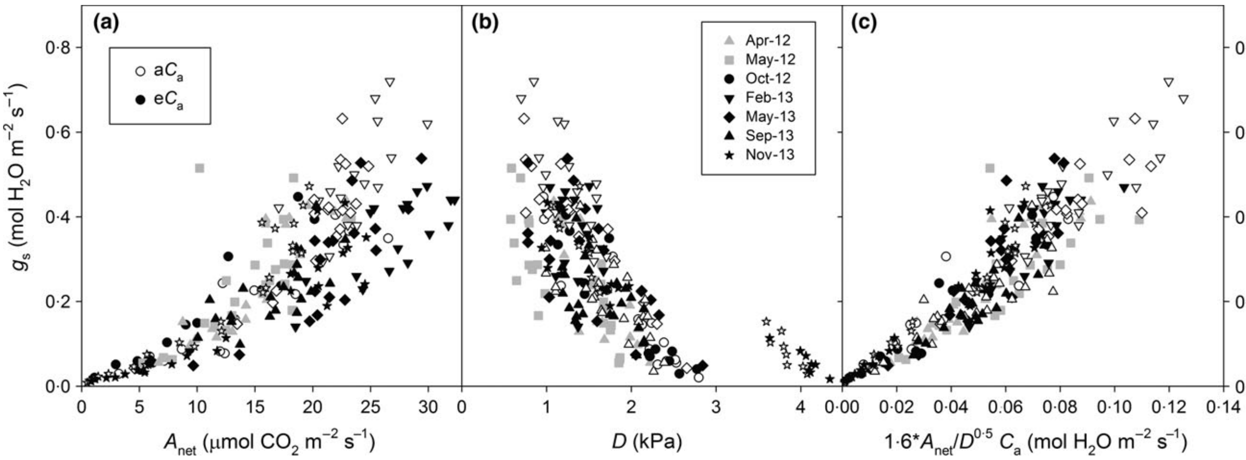

Fig. 3. Relationship between stomatal conductance to water (g_s) with (a) net photosynthesis (A_{net}) (b) leaf-air vapour pressure deficit (D) and (c) an index based on the USO formulation calculated from D, A_{net} and CO_2 mole fraction (C_a) for different campaigns and C_a levels: ambient C_a (open symbols), elevated C_a (closed symbols) and pre-treatment (grey symbols). Each point is the mean of two leaves in one tree either in the morning or at midday.

The fitted g_0 parameter, representing the model intercept, was not significantly different from zero for the USO model, in any campaign (Table 1), although the intercept of the overall fit came out as significantly lower than zero, which has no meaningful biological explanation. Given that the g_0 parameter was not part of the theoretical derivation of the USO and that it was not different from zero, it was dropped from subsequent analyses.

A two-way ANOVA of the residuals of the USO fit showed that there were differences among campaigns in the fitted intercept term (Table 1) and that there were no significant differences in the USO fit neither among campaigns nor between C_a levels in any campaign (Table 1, Fig. 4). While a slight tendency towards higher g_1 is shown between ambient and eC_a (Fig. 4), there was no significant difference in g_1 between C_a levels in any of the four sampling dates during full elevated C_a fumigation.

The g_1 parameter (eqn 1) showed some seasonal variation across campaigns (Table 1). Hence, we tested for correlations between g_1 and prevailing climatic conditions of the different campaigns: Ψ_{pd} and Ψ_{md}, cumulative precipitation of the 30 days prior to the measurements; minimum and maximum temperatures of the measurement days; monthly mean, maximum and minimum daily temperatures. Among those, we only found that there was a marginally significant positive correlation between g_1 and monthly mean daily temperatures ($F = 2{\cdot}4$, $P = 0{\cdot}083$, $R^2 = 0{\cdot}19$). Contrary to our expectations, we did not find a significant correlation between campaign-based g_1 neither with cumulative precipitation prior to the campaign nor with Ψ_{pd} (Figure S2).

Finally, we tested for the ability of the USO to predict the effect of eC_a on instantaneous transpiration efficiency (ITE) by fitting a linear correlation between mean ITE in ambient and eC_a measured simultaneously (same D and P_a). There was a significant correlation ($F = 684{\cdot}5$, $P < 0{\cdot}001$, $n = 24$ pairs of ITE measured under ambient and eC_a rings simultaneously) with a slope of 1·36. The theoretical value of 1·38 (see Model and theory in the methods section) was well within the 95% confidence interval (CI) of 1·25–1·47 (Fig. 5). We followed a similar approach for the October 2012 campaign (with eC_a =

Table 1. Results of the two-way ANOVA of the residuals to test for differences between C_a (CO_2 mole fraction) levels, among measurement campaigns and their interaction on the nonlinear fit of the USO formulation. USO model fits (parameter estimates ± SE: g_0 in mol H_2O m^{-2} s^{-1} and g_1 in kPa$^{0.5}$) for the overall data set ('All') and for each campaign. Parameter values in bold are significantly ($P < 0{\cdot}05$) different from 0

		Results			Parameters	
Campaign	Effect	DF	F	P	g_0	g_1
All	Campaign	1	7·4	<0·001	**−0·038 ± 0·007**	**4·275 ± 0·132**
	C_a	6	0·2	0·672		
	Campaign × C_a	6	1·5	0·186		
Apr-12	Pre-treatment				−0·057 ± 0·03	**4·637 ± 0·538**
May-12	Pre-treatment				−0·017 ± 0·043	**3·564 ± 0·692**
Oct-12	C_a	1	0·6	0·444	−0·015 ± 0·014	**4·049 ± 0·334**
Feb-13	C_a	1	2·2	0·145	−0·058 ± 0·047	**4·841 ± 0·564**
May-13	C_a	1	1·6	0·209	−0·044 ± 0·032	**4·69 ± 0·456**
Sep-13	C_a	1	<0·1	0·959	−0·023 ± 0·021	**3·413 ± 0·406**
Nov-13	C_a	1	<0·1	0·917	−0·009 ± 0·009	**4·242 ± 0·217**

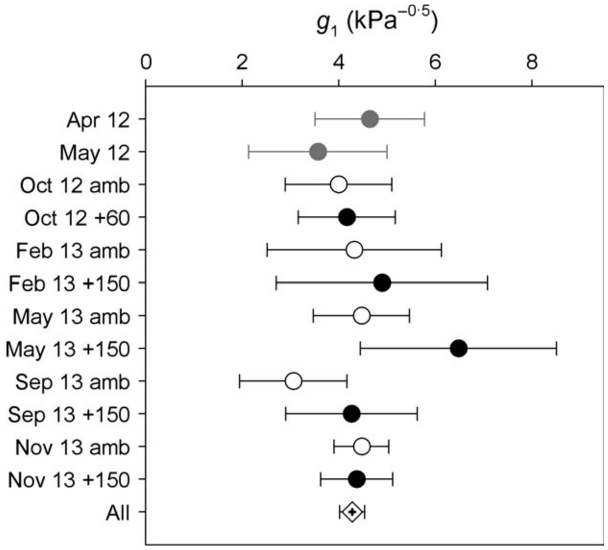

Fig. 4. Fitted parameter with 95% confidence intervals for the unified stomatal optimization model (g_1) for different campaigns and C_a (CO_2 mole fraction) levels: ambient C_a (open symbols), elevated C_a (closed symbols: 60 and 150 μmol CO_2 mol^{-1} above ambient) and pre-treatment (grey). Overall fit for all campaigns and C_a levels is shown with a different symbol (diamond shape).

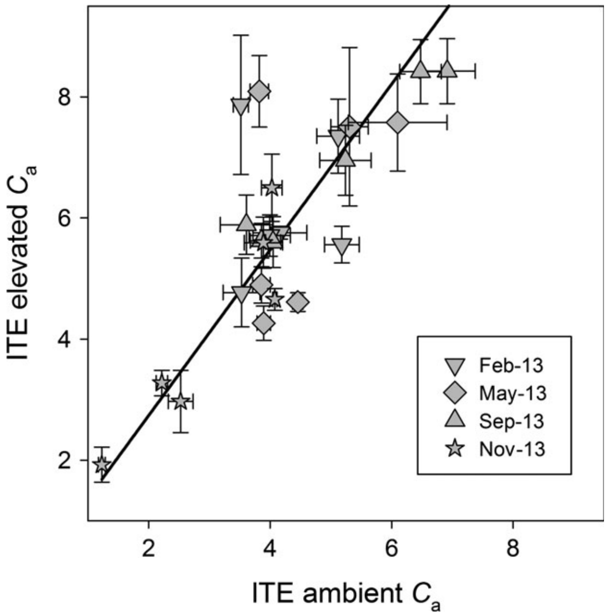

Fig. 5. Correlation between mean (±SE, n = 3–4 trees) instantaneous transpiration efficiency (ITE in μmol CO_2 mmol^{-1} H_2O) from leaf gas exchange in ambient and elevated C_a rings measured simultaneously in the four campaigns when eC_a was 150 μmol CO_2 mol^{-1} above ambient. Line denotes the fitted correlation with a slope of 1·36 ± 0·05 (SE).

60 μmol CO_2 mol^{-1} above ambient) a significant correlation (F = 76·5, P < 0·001, n = 5 pairs of rings) with a slope of 1·26 (95% CI: 0·86–1·67 including the expected theoretical value: 450/390 = 1·15). Hence, ITE was correlated proportionally to the amount of CO_2 enhancement.

Discussion

COUPLING BETWEEN PHOTOSYNTHESIS AND CONDUCTANCE UNDER ELEVATED C_A

We had predicted that stomatal control in ambient and elevated C_a (eC_a) would behave according to optimization theory, in particular with the formulation of the unified stomatal optimization model (USO, Medlyn *et al.* 2011) under a range of conditions, including eC_a. The USO formulation successfully predicted g_s under both ambient and eC_a and parameter g_1 (proportional to the square root of the marginal water cost of carbon gain, $\sqrt{\lambda}$) did not change in response to eC_a. In agreement with our first hypothesis, eC_a increased photosynthesis (A_{net}) and marginally decreased stomatal conductance (g_s). Elevated C_a profoundly affects physiological performance at different scales, at the leaf scale, and in the short term, eC_a increases CO_2 supply to the chloroplasts and reduces stomatal conductance. In the longer term, eC_a could affect leaf and xylem properties related to water supply, but also carbon investments in different organs, which in turn influence photosynthetic function (Domec *et al.* 2009; Buckley & Schymanski 2014; Tor-ngern *et al.* 2015), and properties linked to the control of stomata opening (Jones 1998; Katul *et al.* 2010).

Previously, a possible change in stomatal optimization on forest trees in response to eC_a had only been evaluated a posteriori, with contradictory findings for different optimization formulations applied to the same experimental data set (Katul *et al.* 2010; Medlyn *et al.* 2011; but see Medlyn *et al.* 2013) and with similar results to ours with a second experimental data set (De Kauwe *et al.* 2013). Our study is the first that explicitly tests for the effect of eC_a on optimal stomatal behaviour, in mature trees in natural conditions. Within our study time frame, the USO parameter g_1 was conserved under rising C_a, as predicted by the USO theory (Medlyn *et al.* 2011). The USO formulation assumes that stomatal aperture is optimally regulated as if photosynthesis was limited by RuBP regeneration (Medlyn *et al.* 2013); in our experiment, the eC_a treatment achieved C_i > 350 μmol mol^{-1} (except at high D), which is consistent with RuBP-regeneration-limited photosynthesis in *Eucalyptus* (Lin *et al.* 2013; Ellsworth *et al.* 2015). We measured A_{net} and g_s along the complete life span of the first leaf cohort developed under eC_a and yet our results did not show any feedback effects of eC_a on the coupling between A_{net} and g_s, resulting from anatomical or physiological changes due to eC_a (Tor-ngern *et al.* 2015). Finally, we observed the expected effects of elevated C_a on leaf-level physiology that underlie the theoretical derivation of the original optimization model: A_{net} increased while the ratio C_i/C_a did not change between C_a levels, providing further evidence of coupling of A_{net} and g_s under different C_a levels. It remains to be seen whether this persists in the experiment over many years under eC_a.

One further advantage of the USO theory over previous formulations is that it predicts the theoretical effect of elevated C_a on instantaneous transpiration efficiency (ITE) from the increase in C_a. Our analyses showed that the enhancement of ITE under eC_a agreed with the theoretical value predicted by the USO. These results agree with previous studies where the USO successfully predicted the enhancement of ITE in response to a 60% increase in C_a in *E. saligna* (Barton *et al.* 2012) and cotton plants (Duursma *et al.* 2013). The ability to predict ITE enhancement under elevated C_a relies on the assumption that the key USO parameter (g_1) and the C_i/C_a do not change from ambient to elevated C_a, both of which we observed in this study. However, this might not always be the case, at least in conifers (Uddling & Wallin 2012). On the other hand, reduced g_s in response to elevated C_a is not uncommon among broadleaved species (Ainsworth & Rogers 2007; but see Keel *et al.* 2007).

Optimization theory predicts that g_s should be zero when A_{net} is zero (Cowan & Farquhar 1977). Nevertheless, Medlyn *et al.* (2011) added an intercept term to their formulation, arguing that when A_{net} is zero, total conductance to water can be greater than zero due to cuticular transpiration and positive night time g_s (Barnard & Bauerle 2013). We found that the intercept of the USO was not distinguishable from zero in any campaign. Moreover, when measured under high D (>4 kPa), both A_{net} and g_s neared zero, which suggests that the USO successfully predicts day time g_s without the need of an additional intercept term.

OPTIMAL STOMATAL BEHAVIOUR UNDER CHANGING CLIMATIC CONDITIONS

The theoretical derivation of the USO model suggests a relationship between g_1 and temperature, through the temperature sensitivity of Γ^* (Medlyn *et al.* 2011). Assuming the temperature dependence of Γ^* in *Eucalyptus* reported by Crous *et al.* (2013), for a 7 °C range in temperature, similar in magnitude to the difference between campaigns with the two most different g_1 values, a $\approx$ 22% change in Γ^* can be expected. The g_1 parameter differed by a similar magnitude (24%) between these dates. Across all measurement campaigns, g_1 only weakly co-varied with mean temperature. Under a warmer climate, temperature sensitivity of g_1 could have important implications for water and CO_2 fluxes, since small changes in g_1 influence physiological processes such as temperature and D sensitivity of photosynthesis and canopy transpiration (Lin, Medlyn & Ellsworth 2012; Duursma *et al.* 2014).

The original optimization theory predicts that the marginal water cost of carbon gain decreases with increasing water availability (Cowan 1982; Makela, Berninger & Hari 1996; Manzoni *et al.* 2013). Analogously and based on the USO theory, we had hypothesized that the g_1 parameter of the USO model would be related to water availability (Zhou *et al.* 2013). In contrast to these expectations, our results did not show a discernible effect of water availability on g_1. Our measurements encapsulated a range of water availability spanning from extremely wet conditions when soil water storage was at a maximum across a 4·5-m vertical profile, to a dry period after an unusually warm and dry winter and beginning of the spring when total soil water storage was ca. 25% lower across the same vertical profile (T. E. Gimeno, unpublished data). Nevertheless, we should acknowledge that our study in natural conditions does not allow us to completely disentangle the effects of temperature and water availability on stomatal behaviour. For example, the lowest fitted g_1 corresponded to the coldest campaign (May 2012) and the largest g_1 to the warmest (February 2013), but the latter was also the wettest. In contrast, the driest campaign (September 2013) had an intermediate value for g_1. Overall, in our study, we did not find a statistically significant trend of g_1 in response to changing water availability, represented by either precipitation of the previous month or pre-dawn water potential. In line with our results, Héroult *et al.* (2013) and Zhou *et al.* (2013) showed that in shrubs and trees from subhumid and semi-arid origins, g_1 barely changed with increasing water stress. Regarding the effect of future atmospheric conditions on stomatal control, we propose that in mature trees, the marginal water cost of carbon gain will be conserved under increasing $C_{a.}$, whereas we speculate, based on our data, that g_1 could increase with warmer mean temperatures.

Conclusions

Stomatal control of simultaneous carbon gain and water loss in *Eucalyptus* is consistent with the unified stomatal optimization formulation of Medlyn *et al.* (2011) under seasonally varying climate and more importantly under ambient and future projected C_a. In agreement with the USO theory, the enhancement of ITE under elevated C_a was proportional to the increase in C_a. The key USO parameter g_1 (related to the marginal carbon cost per unit water used), in this mature woodland, was conserved under rising C_a, which allows for predictions of carbon and water exchange under current and future C_a levels. Also, we did not find evidence for a response of g_1 to changes in water availability, which could suggest that stomatal control of *E. tereticornis* may have been selected for optimization of C-gain under a drought-prone and extremely variable climate. Nevertheless, this broadly distributed species is likely to be exposed to longer and more severe periods of water scarcity than in this study (Drake *et al.* 2015). Therefore, we should not generalize this lack of effect of water availability on g_1 to the whole distribution range of this species. Our results suggest that the USO formulation with only a slope term, and no additional intercept, is useful for further incorporation into large-scale models of forest functioning (De Kauwe *et al.* 2014). Finally, our findings suggest that g_1 could increase with temperature. We venture that this could entail a greater marginal water cost of carbon gain under a warmer climate.

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Data accessibility

All data used in the manuscript and its supporting information are stored in our institutional archive and publicly available here: http://dx.doi.org/10.4225/35/55b6e313444ff.

References

Ainsworth, E.A. & Rogers, A. (2007) The response of photosynthesis and stomatal conductance to rising [CO_2]: mechanisms and environmental interactions. *Plant, Cell and Environment*, **30**, 258–270.

Arneth, A., Lloyd, J., Santruckova, H., Bird, M., Grigoryev, S., Kalaschnikov, Y.N. *et al.* (2002) Response of central Siberian Scots pine to soil water deficit and long-term trends in atmospheric CO2 concentration. *Global Biogeochemical Cycles*, **16**, Art. 1005.

Barnard, D.M. & Bauerle, W.L. (2013) The implications of minimum stomatal conductance on modeling water flux in forest canopies. *Journal of Geophysical Research-Biogeosciences*, **118**, 1322–1333.

Barton, C.V.M., Duursma, R.A., Medlyn, B.E., Ellsworth, D.S., Eamus, D., Tissue, D.T. *et al.* (2012) Effects of elevated atmospheric CO_2 on instantaneous transpiration efficiency at leaf and canopy scales in *Eucalyptus saligna*. *Global Change Biology*, **18**, 585–595.

Bernacchi, C.J., Singsaas, E.L., Pimentel, C., Portis, A.R. & Long, S.P. (2001) Improved temperature response functions for models of Rubisco-limited photosynthesis. *Plant Cell and Environment*, **24**, 253–259.

Berryman, C.A., Eamus, D. & Duff, G.A. (1994) Stomatal responses to a range of variables in 2 tropical tree species grown with CO_2 enrichment. *Journal of Experimental Botany*, **45**, 539–546.

Brodribb, T.J., McAdam, S.A.M., Jordan, G.J. & Feild, T.S. (2009) Evolution of stomatal responsiveness to CO_2 and optimization of water-use efficiency among land plants. *New Phytologist*, **183**, 839–847.

Brooks, A. & Farquhar, G.D. (1985) Effect of temperature on the CO2/O2 specificity of Ribuluse-1,5, Bisphophate Carboxylase Oxygenase and the rate of respiration in the light- Estimates from gas-exchange measurements on spinach. *Planta*, **165**, 397–406.

Buckley, T.N. & Mott, K.A. (2013) Modelling stomatal conductance in response to environmental factors. *Plant Cell and Environment*, **36**, 1691–1699.

Buckley, T.N., Mott, K.A. & Farquhar, G.D. (2003) A hydromechanical and biochemical model of stomatal conductance. *Plant Cell and Environment*, **26**, 1767–1785.

Buckley, T.N. & Schymanski, S.J. (2014) Stomatal optimisation in relation to atmospheric CO_2. *New Phytologist*, **201**, 372–377.

Cowan, I.R. (1982) Regulation of water use in relation to carbon gain in higher plants. *Physiological Plant Ecology Volume: II* (eds O.L. Lange, P.S. Nobel, C.B. Osmond & H. Zeigler), pp. 589–613. Springer, Berlin Heidelberg, Berlin.

Cowan, I.R. & Farquhar, G.D. (1977) Stomatal function in relation to leaf metabolism and environment. *Symposia of the Society for Experimental Biology*, **31**, 471–505.

Crous, K.Y., Ósvaldsson, A. & Ellsworth, D.S. (2015) Is phosphorus limiting in a mature *Eucalyptus* woodland? Phosphorus fertilisation stimulates stem growth. *Plant and Soil*, **391**, 293–305.

Crous, K.Y., Quentin, A.G., Lin, Y.S., Medlyn, B.E., Williams, D.G., Barton, C.V.M. *et al.* (2013) Photosynthesis of temperate *Eucalyptus globulus* trees outside their native range has limited adjustment to elevated CO_2 and climate warming. *Global Change Biology*, **19**, 3790–3807.

Damour, G., Simonneau, T., Cochard, H. & Urban, L. (2010) An overview of models of stomatal conductance at the leaf level. *Plant Cell and Environment*, **33**, 1419–1438.

De Kauwe, M.G., Medlyn, B.E., Zaehle, S., Walker, A.P., Dietze, M.C., Hickler, T. *et al.* (2013) Forest water use and water use efficiency at elevated CO_2: a model-data intercomparison at two contrasting temperate forest FACE sites. *Global Change Biology*, **19**, 1759–1779.

De Kauwe, M.G., Kala, J., Lin, Y.S., Pitman, A.J., Medlyn, B.E., Duursma, R.A. *et al.* (2014) A test of an optimal stomatal conductance scheme within the CABLE Land Surface Model. *Geoscientific Model Development Discussion*, **7**, 6845–6891.

Domec, J.C., Palmroth, S., Ward, E., Maier, C.A., Therezien, M. & Oren, R. (2009) Acclimation of leaf hydraulic conductance and stomatal conductance of Pinus taeda (loblolly pine) to long-term growth in elevated CO_2 (free-air CO_2 enrichment) and N-fertilization. *Plant Cell and Environment*, **32**, 1500–1512.

Drake, J.E., Aspinwall, M.A., Pfautsch, S., Rymer, P.D., Reich, P.B., Smith, R.A. *et al.* (2015) The capacity to cope with climate warming declines from temperate to tropical latitudes in two widely distributed *Eucalyptus* species. *Global Change Biology*, **21**, 459–472.

Duursma, R.A., Payton, P., Bange, M.P., Broughton, K.J., Smith, R.A., Medlyn, B.E. *et al.* (2013) Near-optimal response of instantaneous transpiration efficiency to vapour pressure deficit, temperature and CO_2 in cotton (*Gossypium hirsutum* L.). *Agricultural and Forest Meteorology*, **168**, 168–176.

Duursma, R.A., Barton, C.V.M., Lin, Y.S., Medlyn, B.E., Eamus, D., Tissue, D.T. *et al.* (2014) The peaked response of transpiration rate to vapour pressure deficit in field conditions can be explained by the temperature optimum of photosynthesis. *Agricultural and Forest Meteorology*, **189**, 2–10.

Eamus, D., Cleverly, J., Boulain, N., Grant, N., Faux, R. & Villalobos-Vega, R. (2013) Carbon and water fluxes in an arid-zone Acacia savanna woodland: an analyses of seasonal patterns and responses to rainfall events. *Agricultural and Forest Meteorology*, **182**, 225–238.

Ellsworth, D.S., Crous, K.Y., Lambers, H. & Cooke, J. (2015) Phosphorus recycling in photorespiration maintains high photosynthetic capacity in woody species. *Plant, Cell and Environment*, **38**, 1142–1156.

Franks, P.J., Leitch, I.J., Ruszala, E.M., Hetherington, A.M. & Beerling, D.J. (2012) Physiological framework for adaptation of stomata to CO_2 from glacial to future concentrations. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **367**, 537–546.

Franks, P.J., Adams, M.A., Amthor, J.S., Barbour, M.M., Berry, J.A., Ellsworth, D.S. *et al.* (2013) Sensitivity of plants to changing atmospheric CO_2 concentration: from the geological past to the next century. *New Phytologist*, **197**, 1077–1094.

Hari, P., Makela, A., Korpilahti, E. & Holmberg, M. (1986) Optimal control of gas exchange. *Tree Physiology*, **2**, 169–175.

Heath, J. (1998) Stomata of trees growing in CO_2-enriched air show reduced sensitivity to vapour pressure deficit and drought. *Plant Cell and Environment*, **21**, 1077–1088.

Hendrey, G.R., Ellsworth, D.S., Lewin, K.F. & Nagy, J. (1999) A free-air enrichment system for exposing tall forest vegetation to elevated atmospheric CO_2. *Global Change Biology*, **5**, 293–309.

Héroult, A., Lin, Y.S., Bourne, A., Medlyn, B.E. & Ellsworth, D.S. (2013) Optimal stomatal conductance in relation to photosynthesis in climatically contrasting Eucalyptus species under drought. *Plant Cell and Environment*, **36**, 262–274.

Hetherington, A.M. & Woodward, F.I. (2003) The role of stomata in sensing and driving environmental change. *Nature*, **424**, 901–908.

IPCC (2014) Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. *IPCC, 2013: Climate Change 2013,* (eds T.F. Stocker, D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex & P.M. Midgley), pp. 1535. Intergovernmental Panel on Climate Change, Cambridge, UK and New York, NY, USA.

Jarvis, P.G. (1976) Interpretation of variations in leaf water potential and stomatal conductance found in canopies in field. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, **273**, 593–610.

Jones, H.G. (1998) Stomatal control of photosynthesis and transpiration. *Journal of Experimental Botany*, **49**, 387–398.

Katul, G., Manzoni, S., Palmroth, S. & Oren, R. (2010) A stomatal optimization theory to describe the effects of atmospheric CO_2 on leaf photosynthesis and transpiration. *Annals of Botany*, **105**, 431–442.

Keel, S.G., Pepin, S., Leuzinger, S. & Korner, C. (2007) Stomatal conductance in mature deciduous forest trees exposed to elevated CO_2. *Trees-Structure and Function*, **21**, 151–159.

Kleidon, A. (2004) Optimized stomatal conductance of vegetated land surfaces and its effects on simulated productivity and climate. *Geophysical Research Letters*, **31**, L21203.

Leakey, A.D.B., Bernacchi, C.J., Ort, D.R. & Long, S.P. (2006) Long-term growth of soybean at elevated CO_2 does not cause acclimation of stomatal conductance under fully open-air conditions. *Plant Cell and Environment*, **29**, 1794–1800.

Lewin, K.F., Nagy, J., Nettles, W.R., Cooley, D.M. & Rogers, A. (2009) Comparison of gas use efficiency and treatment uniformity in a forest ecosystem exposed to elevated CO_2 using pure and prediluted free-air CO_2 enrichment technology. *Global Change Biology*, **15**, 388–395.

Lin, Y.S., Medlyn, B.E. & Ellsworth, D.S. (2012) Temperature responses of leaf net photosynthesis: the role of component processes. *Tree Physiology*, **32**, 219–231.

Lin, Y.S., Medlyn, B.E., De Kauwe, M.G. & Ellsworth, D.S. (2013) Biochemical photosynthetic responses to temperature: how do interspecific differences compare with seasonal shifts? *Tree Physiology*, **33**, 793–806.

Lin, Y.-S., Medlyn, B.E., Duursma, R.A., Prentice, I.C., Wang, H., Baig, S. *et al.* (2015) Optimal stomatal behaviour around the world. *Nature Climate Change*, **5**, 459–464.

Lloyd, J. & Farquhar, G.P. (1994) ^{13}C discrimination during CO_2 assimilation by the terrestrial biosphere. *Oecologia*, **99**, 201–215.

Maherali, H., Johnson, H.B. & Jackson, R.B. (2003) Stomatal sensitivity to vapour pressure difference over a subambient to elevated CO_2 gradient in a C-3/C-4 grassland. *Plant Cell and Environment*, **26**, 1297–1306.

Makela, A., Berninger, F. & Hari, P. (1996) Optimal control of gas exchange during drought: theoretical analysis. *Annals of Botany*, **77**, 461–467.

Manzoni, S., Vico, G., Katul, G., Fay, P.A., Polley, W., Palmroth, S. *et al.* (2011) Optimizing stomatal conductance for maximum carbon gain under water stress: a meta-analysis across plant functional types and climates. *Functional Ecology*, **25**, 456–467.

Manzoni, S., Vico, G., Palmroth, S., Porporato, A. & Katul, G. (2013) Optimization of stomatal conductance for maximum carbon gain under dynamic soil moisture. *Advances in Water Resources*, **62**, 90–105.

Medlyn, B.E., Duursma, R., Eamus, D., Ellsworth, D.S., Prentice, C., Barton, C.V.M. *et al.* (2011) Reconciling the optimal and empirical approaches to modelling stomatal conductance. *Global Change Biology*, **17**, 2134–2144.

Medlyn, B.E., Duursma, R.A., De Kauwe, M.G. & Prentice, I.C. (2013) The optimal stomatal response to atmospheric CO_2 concentration: alternative solutions, alternative interpretations. *Agricultural and Forest Meteorology*, **182**, 200–203.

Orians, G.H. & Solbrig, O.T. (1977) Cost-Income model of leaves and roots with special reference to arid and semiarid areas. *American Naturalist*, **111**, 677–690.

Prentice, I.C., Dong, N., Gleason, S.M., Maire, V. & Wright, I.J. (2014) Balancing the costs of carbon gain and water transport: testing a new theoretical framework for plant functional ecology. *Ecology Letters*, **17**, 82–91.

R Development Core Team, R. (2014) *R: A Language and environment for statistical computing*. R Development Core Team, Vienna.

Saxe, H., Ellsworth, D.S. & Heath, J. (1998) Tree and forest functioning in an enriched CO_2 atmosphere. *New Phytologist*, **139**, 395–436.

Sellers, P.J., Dickinson, R.E., Randall, D.A., Betts, A.K., Hall, F.G., Berry, J.A. *et al.* (1997) Modeling the exchanges of energy, water, and carbon between continents and the atmosphere. *Science*, **275**, 502–509.

Thomas, D.S., Eamus, D. & Bell, D. (1999) Optimization theory of stomatal behaviour - II. Stomatal responses of several tree species of north Australia to changes in light, soil and atmospheric water content and temperature. *Journal of Experimental Botany*, **50**, 393–400.

Tor-ngern, P., Oren, R., Ward, E.J., Palmroth, S., McCarthy, H.R. & Domec, J.C. (2015) Increases in atmospheric CO_2 have little influence on transpiration of a temperate forest canopy. *New Phytologist*, **205**, 518–525.

Uddling, J. & Wallin, G. (2012) Interacting effects of elevated CO_2 and weather variability on photosynthesis of mature boreal Norway spruce agree with biochemical model predictions. *Tree Physiology*, **32**, 1509–1521.

Vico, G., Manzoni, S., Palmroth, S., Weih, M. & Katul, G. (2013) A perspective on optimal leaf stomatal conductance under CO_2 and light co-limitations. *Agricultural and Forest Meteorology*, **182**, 191–199.

Zhou, S.X., Duursma, R.A., Medlyn, B.E., Kelly, J.W.G. & Prentice, I.C. (2013) How should we model plant responses to drought? An analysis of stomatal and non-stomatal responses to water stress. *Agricultural and Forest Meteorology*, **182**, 204–214.

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Supporting Information

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

Fig. S1. Mean (n = 3 rings) hourly CO_2 mole fraction (C_a, black line).

Fig. S2. Values of the fitted parameter of the USO ($g_1 \pm$ SE) for each gas-exchange campaign plotted against their corresponding mean (±SE, n = 6 rings) pre-dawn leaf water potential (Ψ_{pd}).

Fig. S3. Mean (±SE, n = 3 pairs of rings measured simultaneously) relative effect of the elevated C_a (CO_2 mole fraction) treatment on photosynthesis (A_{net}) and transpiration in a well-stirred chamber (E_s) in the morning (red symbols) and in the afternoon (black symbols), with respect to the ambient treatment.

Fig. S4. (A) overview of the EucFACE site, (B) standing water next to one of the study rings in February 2012, (C) *Eucalyptus tereticornis* woodland with dry understorey in September 2013.

Fig. S5. Overview of one of the study rings at the EucFACE experiment during measurements at the tree tops in October 2012.

Table S1. Summary of the seven gas-exchange campaigns with the exact measurement dates, plots ('rings') measured simultaneously each day, the CO_2 mole fraction (C_a) of each ring given as ambient ('Amb'), 60 and 150 μmol CO_2 mol^{-1} above ambient C_a (+60 and +150, respectively), the mean maximum and minimum temperature of the exact measurement days (Max T and Min T), the monthly mean maximum and minimum daily temperatures (Daily Max T and Daily Min T) and cumulative precipitation (P) of the previous 30 days.

Table S2. Mean (±SE, n = 6 rings) values of internal to atmospheric CO_2 mole fraction ratio (C_i/C_a) in the morning and midday for different campaigns.