# *Letters*

## Stomatal optimisation in relation to atmospheric CO₂

### Introduction

Despite the critical role that stomata play in terrestrial carbon and water flux (Hetherington & Woodward, 2003), there remains no consensus theoretical model that can explain and predict variations in stomatal conductance to water vapour ($g_{sw}$) in relation to short- and long-term variations in environmental forcing (Berry *et al.*, 2010; Damour *et al.*, 2010; Buckley & Mott, 2013). One appealing prospect to fill this modelling gap is the hypothesis that plants regulate $g_{sw}$ optimally, that is, they vary $g_{sw}$ so that carbon gain is maximized for a given water loss (Cowan & Farquhar, 1977). Challenges arise when using this hypothesis to predict stomatal behaviour, particularly in relation to elevated atmospheric CO₂ concentration ($c_a$). One challenge is that the optimal short-term response of $g_{sw}$ to $c_a$ is widely perceived to differ from the observed response (Katul *et al.*, 2010; Medlyn *et al.*, 2011, 2013). Another is the apparent lack of a theoretical framework to extend the theory to long-term changes in $c_a$, such as those associated with climate change. Recent work by Katul *et al.* (2009, 2010) and Medlyn *et al.* (2011, 2013) has revitalized the optimization approach by beginning to address these challenges. Our objective in this Letter is to clarify several issues raised by that work, and to offer an alternative perspective based on integration of leaf and whole plant function.

### Overview of stomatal optimisation

The aim of 'stomatal optimization theory' is to find the pattern of $g_{sw}$ that optimizes the tradeoff between carbon gain (net CO₂ assimilation rate, $A$) and water loss (transpiration rate, $E$). There are several ways to formalize this question. One approach that may seem obvious at first glance – maximizing the instantaneous ratio of $A/E$ – actually leads to a trivial solution, because under most conditions $A/E$ is greatest when $g_{sw}$ is zero. Another approach, pioneered by Cowan & Farquhar (1977), uses the method of Lagrange multipliers to maximize the integral of $A$ over some period, $\int A$, subject to the constraint that total water use, $\int E$, is the same among all candidate patterns of $g_{sw}$. That is,

$$\max_{g_{sw}} \left[ \int A - \mu \int E \right], \qquad \text{Eqn 1}$$

where μ is an arbitrary constant. The general solution is simply:

$$\frac{(\partial A/\partial g_{sw})}{(\partial E/\partial g_{sw})} = \mu, \text{ or, more simply,} \frac{\partial A}{\partial E}(t, s) = \mu. \qquad \text{Eqn 2}$$

Thus, the ratio of the marginal sensitivities of $A$ and $E$ to $g_{sw}$ should be invariant over the space/time interval in question. This result is only valid if the relationship between $A$ and $E$ created by varying $g_{sw}$ is convex (i.e. $(\partial A/\partial g_{sw})/(\partial E/\partial g_{sw})$ decreases as $g_s$ increases).

### What happens at elevated CO₂?

At right in Eqn 2, the expression is simplified to $\partial A/\partial E$ and written as a function of time ($t$) and space ($s$) to remind us that $\partial A/\partial E$, the marginal carbon product of water, is a biological variable, whereas μ is an undetermined constant with no a priori biological meaning. Eqn 2 does not tell us how to estimate the numerical value of μ from $c_a$ or other biophysical or environmental data. Its meaning only crystallizes when we take account of processes on longer timescales than that on which the dynamic stomatal response to $c_a$ operates – that is, scales that encompass physiological and developmental acclimation and evolutionary adaptation, which combine to produce changes in μ. The question of how to compute or predict changes in μ therefore requires that we refocus our attention on processes that occur at higher organizational scales (Cowan, 2002).

We can gain some insight by computing $\partial A/\partial E$ from the equations of gas exchange, and asking how the parameters in the resulting expression may be affected by acclimatory responses on longer timescales. It is easily shown (Buckley *et al.*, 2002) that, provided leaf temperature is invariant with $g_{sw}$,

$$\frac{\partial A}{\partial E} = \left( \frac{c_a - c_c}{\Delta w} \right) \left( \frac{k}{k + g_{tc}} \right) \cdot 1.6 \frac{g_{tc}^2}{g_{tw}^2}, \qquad \text{Eqn 3}$$

where $g_{tc}$ and $g_{tw}$ are total conductances to CO₂ and H₂O, respectively, $c_c$ is chloroplastic CO₂ concentration, $\Delta w$ is the leaf to air water vapour mole fraction gradient and $k$ is the slope of the photosynthetic CO₂ demand curve (the slope $\partial A/\partial c_c$ of the $A$ vs $c_c$ relationship obtained by varying $c_a$ while keeping photosynthetic capacity, irradiance, $g_{tc}$ and temperature constant) (see Supporting Information Notes S1 for details). Eqn 3 cannot be solved for $g_{sw}$ without adopting simplifications, such as linearizing the demand curve (assuming $k$ is constant) or ignoring boundary layer and mesophyll resistances (Table 1). Numerical solution predicts a positive response of $g_{sw}$ to $c_a$ at low $c_a$ and a negative response at higher $c_a$ (e.g. Fig. 1). Linearized solutions predict a positive response at all $c_a$ (e.g. Lloyd & Farquhar, 1994). By contrast, most experimental observations show that the short-term dynamic response of $g_{sw}$ to $c_a$ is negative at all $c_a$ (e.g. Morison, 1998).

**Table 1** Parameters used to compute optimal responses of stomatal conductance to atmospheric $CO_2$

| Parameter | Symbol | Units | Value |
|---|---|---|---|
| Carboxylation capacity at 25°C | $V_{m25}$ | µmol m$^{-2}$ s$^{-1}$ | 50 |
| Ratio of electron transport capacity at 25°C to $V_{m25}$ | $J_{m25}/V_{m25}$ | – | 2.3 |
| Ambient $CO_2$ | $c_a$ | µmol mol$^{-1}$ | 400 |
| Leaf to air water vapour mole fraction gradient | $\Delta w$ | mmol mol$^{-1}$ | 15 |
| Incident PPFD | $i$ | µmol m$^{-2}$ s$^{-1}$ | 400 |
| Marginal carbon product of water | $\mu$ | µmol mmol$^{-1}$ | 1.5 |
| Maximum quantum yield of electrons from incident PPFD | $\phi$ | e$^{-}$/hv | 0.35 |
| Curvature parameter for response of potential electron transport rate ($J$) to PPFD and $J_{m25}$ | $\theta_J$ | – | 0.86 |
| Curvature parameter for response of net assimilation rate to carboxylation- and regeneration-limited rates | $\theta_A$ | – | 0.999 |
| Leaf temperature | $T_l$ | degrees C | 25 |
| Boundary layer conductance to H$_2$O | $g_{bw}$ | mol m$^{-2}$ s$^{-1}$ | 2 |
| Mesophyll conductance to $CO_2$ | $g_m$ | mol m$^{-2}$ s$^{-1}$ | 0.2 |



**Fig. 1** Optimal responses of stomatal conductance, $g_{sw}$, to ambient $CO_2$ mole fraction, $c_a$, at different photosynthetic photon flux densities, PPFD (a) and at three values of the marginal carbon product of water, $\mu$ (b). (a) Solid line, optimal; dashed lines, optimal responses under RuBP regeneration-limited conditions; dash-dot line, under carboxylation-limited conditions. $\mu = 1.5$ µmol mmol$^{-1}$ for all curves in (a), and PPFD = 400 µmol m$^{-2}$ s$^{-1}$ for all curves in (b).
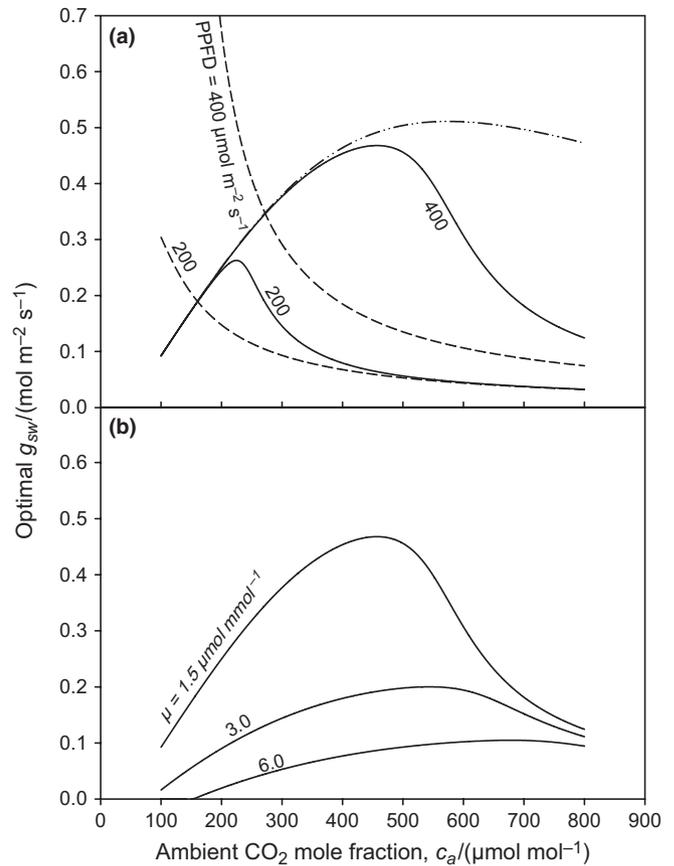
This apparent discrepancy between observed and optimal short-term dynamic responses of $g_{sw}$ to $c_a$ has spawned two recent theoretical developments in an attempt to reconcile the theory with the data. One of these developments, by Medlyn *et al.* (2011), focused on the short-term dynamical response itself. Those authors noted that, because Eqn 3 generally predicts a positive response when photosynthesis is limited by RuBP carboxylation but a negative response when regeneration is limiting (Fig. 1a), it would appear that stomata behave as if regeneration were always limiting. Although such behaviour diverges from the optimal solution under carboxylation-limited conditions, Medlyn *et al.* (2011) suggested that this may reflect a physiological constraint on stomatal function (they noted evidence that stomatal guard cells lack the machinery needed to distinguish these limitations), and that the discrepancy may not be particularly important in practice, because photosynthesis is more often limited by regeneration.

Katul *et al.* (2009, 2010) proposed an alternative resolution. They redefined the optimization problem by positing that stomata maximize the *instantaneous* difference between $A$ and the 'water loss in units of carbon,' which they assumed was proportional to $E$ by a parameter, $\xi$ (Eqn 10 in Katul *et al.*, 2009 or Eqn 6 in Katul *et al.*, 2010, adapted to our notation):

$$\max_{g_{sw}}[A - \xi E]. \qquad \text{Eqn 4}$$

This leads to the solution (modified from Eqn 10 in Katul *et al.*, 2009; see Notes S1 for details):

$$\frac{\partial A}{\partial E}(t, s) = \xi\left(1 + \frac{\partial \log_e \xi/\partial g_{sw}}{\partial \log_e E/\partial g_{sw}}\right). \qquad \text{Eqn 5}$$

Katul *et al.* (2009, 2010) then assumed that $\xi$ is far less sensitive than $E$ to $g_{sw}$, implying that

$$\frac{\partial \log_e \xi/\partial g_{sw}}{\partial \log_e E/\partial g_{sw}} \ll 1, \qquad \text{Eqn 6}$$

and reducing Eqn. 5 to

$$\frac{\partial A}{\partial E}(t, s) = \xi. \qquad \text{Eqn 7}$$

If $\xi$ is constant, this solution is identical to Eqn 2. However, Katul *et al.* (2010) proposed that $\xi$ is not constant, but instead is proportional to $c_a$, that is,

$$\xi = \zeta c_a. \qquad \text{Eqn 8}$$

where $\zeta$ is constant. This assumption transforms the solution to:

$$\frac{\partial A}{\partial E}(t, s) = c_a \zeta. \qquad \text{Eqn 9}$$

When Eqn 9 is applied to Eqn 3, it eliminates the positive response of $g_{sw}$ to $c_a$, thus resolving the discrepancy. It also appears to tell us how $c_a$ affects μ. Eqn 9 has already been adopted in numerous modeling studies (Launiainen *et al.*, 2011; Manzoni *et al.*, 2011a,b; Volpe *et al.*, 2011; Way *et al.*, 2011; Palmroth *et al.*, 2013).

This resolution has several fundamental flaws, however. First, it is unclear why it should be ecologically advantageous to maximize $A - \zeta c_a E$ rather than $\int A - \mu \int E$. In fact it is not: the latter solution leads to greater carbon gain under varying $c_a$ when controlling for water loss (Fig. 2). Second, this resolution is premised on two assumptions (Eqns 6 and 8) about the mathematical and biological properties of the parameter $\xi$. Yet because $\xi$ is never rigorously defined in biophysical terms, Eqns 6 and 8 cannot be justified on biophysical grounds. Katul *et al.* (2009) thus attempted to justify these assertions empirically: they showed that Eqn 8 fitted the data better than assuming that $\xi$ is invariant with $c_a$, and they justified Eqn 6 by attempting to show empirically that $\partial A / \partial E$ is far less sensitive than $E$ to $g_{sw}$. However, the latter empirical comparisons were flawed and circular. The flaw was that Katul *et al.* (2009) estimated $\partial A / \partial E$ from the slope of a relationship between $A$ and $E$ created by changing evaporative demand ($\Delta w$) rather than stomatal conductance ($g_{sw}$). The slope thus computed is actually the ratio of total derivatives of $A$ and $E$ with respect to $\Delta w$ $(dA/d\Delta w)/(dE/d\Delta w)$, which is negative (cf. fig. 6 in Katul *et al.*, 2009), whereas the correct quantity is the ratio of partial derivatives with respect to $g_{sw}$ $((\partial A/\partial g_{sw})/(\partial E/\partial g_{sw}) = \partial A/\partial E)$, which is positive. Thus, Eqn 6 has not in fact been validated empirically. The circularity arose from the attempt to justify Eqn 6 by estimating the sensitivity of $\partial A/\partial E$ to $g_{sw}$. In order to use empirical estimates of $(\partial \ln(\partial A/\partial E)/\partial g_{sw})/(\partial \ln E/\partial g_{sw})$ to validate Eqn 6, Katul *et al.* (2009) had to assume that $\xi = \partial A/\partial E$ (Eqn 7) – that is, they had to adopt Eqn 7 in order to derive Eqn 7, which is circular.
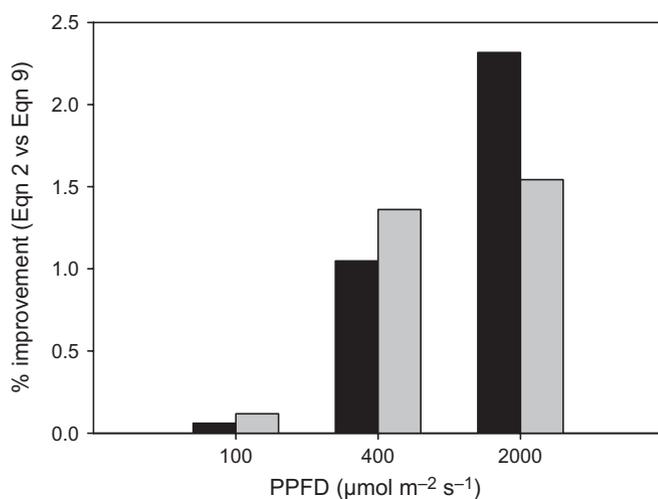


**Fig. 2** Per cent difference in total carbon gain predicted by Eqns 2 and 9. Total carbon gain was calculated by computing optimal solutions at 100 values of $c_a$ from 350 to 700 ppm and summing the results. For Eqn 2, the value of μ was 1.5 μmol mol$^{-1}$ (black bars) or 4.0 μmol mol$^{-1}$ (grey bars); for Eqn 9, the value of $\zeta$ was adjusted to give the same total transpiration rate over the range of $c_a$ as for Eqn 2.

The a priori identification of $\xi$ as $\partial A/\partial E$ may have been motivated by the perception that $\partial A/\partial E$ and μ are the same quantity in the original Cowan–Farquhar problem. This is not correct, despite the impression given by Eqn 2. Eqn 2 is not a definition of the Lagrange multiplier μ, nor of $\partial A/\partial E$; rather, it is the solution to maximizing $\int A - \mu \int E$. It says that stomatal conductance should vary so that the marginal carbon product of water ($\partial A/\partial E$) remains equal to some undefined constant μ. The link between the multiplier μ and the derivative $\partial A/\partial E$ only arises after one has solved the constrained optimization problem (Eqn 1). An alternative perspective is that Katul *et al.* (2009) implicitly adopted $\xi = \partial A/\partial E$ in the problem statement itself. This avoids the circularity but entails a fundamentally different goal function, in which $E$ is multiplied not by an undetermined constant, but by $\partial A/\partial E$ itself. This transforms Eqn 5 and its solution (Eqn 6) to

$$\max_{g_{sw}} \left[ A - \frac{\partial A}{\partial E} E \right], \qquad \text{Eqn 10}$$

and

$$\frac{\partial A}{\partial E} = \frac{\partial A}{\partial E} \left( 1 + \frac{\partial \log_e(\partial A/\partial E)/\partial g_{SW}}{\partial \log_e E/\partial g_{sw}} \right). \qquad \text{Eqn 11}$$

However, the latter solution resolves to

$$\frac{\partial \log_e(\partial A/\partial E)}{\partial g_{sw}} = 0. \qquad \text{Eqn 12}$$

This merely states that $\partial A/\partial E$ should be invariant as $g_{sw}$ changes, which is identical to the original Cowan–Farquhar solution. Unlike the latter solution, however, the instantaneous approach does not specify a timescale at which $\partial A/\partial E$ should be invariant. Furthermore, the goal function in Eqn 10 is of dubious merit. If boundary layer and mesophyll resistance are small, this goal function is equivalent to

$$A - \frac{\partial A}{\partial E} E = \left( \frac{g_{tc}}{k + g_{tc}} \right) A \qquad \text{Eqn 13}$$

(see Notes S1). It is unclear what ecological advantage a leaf would gain by maximising this quantity.

We argue that, although the instantaneous approach *per se* is not inherently flawed, the multiplier for $E$ in the goal function ($\xi$) cannot simply be $\partial A/\partial E$. What, then, does $\xi$ represent, if not $\partial A/\partial E$? If we are to accept $A - \xi E$ as an ecologically meaningful instantaneous goal function, then the product $\xi E$ must represent a carbon cost of water loss (indeed, Katul *et al.* (2010) defined $\xi E$ as the 'water loss in units of carbon'). Thus, $\xi$ is the carbon cost of water loss per unit of transpiration, or the unit carbon cost of water loss. This is a very different quantity from $\partial A/\partial E$. Thus, $\partial A/\partial E$ represents how much carbon the plant *gains* for every additional unit of water that it transpires, whereas $\xi$ represents how much carbon the plant *loses* for every unit of water it transpires. The plant

loses carbon by transpiring because, to replace evaporative losses, it must invest carbon in roots and xylem to capture and transport water (Givnish, 1986). These two quantities are both derivatives, but in very different domains: $\partial A/\partial E$ is the derivative of leaf carbon gain with respect to transpiration rate, whereas $\xi$ is the inverse of the derivative of water supply rate with respect to plant carbon investment in water supply.

## A different perspective

To understand how and why $\xi$ or $\mu$ should change with $c_a$, we must consider the question of timescale. Part of the motivation for assuming $\xi \propto c_a$ (Eqn 9) was the need to reconcile optimal and observed short-term dynamic responses of $g_{sw}$ to $c_a$ (Medlyn *et al.*, 2013; Vico *et al.*, 2013). However, if $\xi$ involves whole-plant carbon costs of water loss, as we argue earlier, then it is clear that the timescale for variations in $\xi$ is much longer than for the short-term dynamic response: namely, it is the timescale at which the carbon costs of water loss change due to changes in soil moisture and root and xylem growth. That timescale ranges from days to decades or even longer. Notably, this is the same as the timescale for changes in total water supply, and thus for the Lagrange multiplier $\mu$ in the constrained optimization problem. The question, then, is how to predict effects of $c_a$ on $\xi$ and $\mu$ over these longer timescales. Katul *et al.* (2009) argued that $\mu$ is nonphysical and therefore cannot be independently inferred. However, $\mu$ has long been understood to involve water supply (Cowan & Farquhar, 1977; Cowan, 1982; Hari *et al.*, 1986; Mäkelä *et al.*, 1996; Schymanski *et al.*, 2008), and Givnish (1986) showed that $\mu$ should also be affected by functional balance between root and shoot function. We argue that $\mu$ is in fact highly constrained by biology and physics, and that, with certain assumptions, its numerical value and response to long-term changes in $c_a$ can be directly computed.

For example, if stomata open too widely, then water loss will reduce leaf water potential below either the turgor loss point or the threshold causing runaway xylem cavitation. This places an upper limit on transpiration rate, $E_{max}$, which can be calculated from biophysical properties, including soil water potential, cavitation threshold water potential, leaf osmotic pressure and plant hydraulic conductance. One possible strategy would be to choose $\mu$ so that $E$ reaches but does not exceed $E_{max}$ during the course of a day (Buckley, 2005). This could be modelled by setting $\mu$ to the largest value that $\partial A/\partial E$ reaches (calculated at $E_{max}$) each day (as illustrated in Fig. 3). Like $E_{max}$, that value of $\partial A/\partial E$ can be computed on a biophysical basis (e.g. Fig. 3b), as shown by Konrad *et al.* (2008). Although other factors might require this strategy to be modified – for example, effects of progressive soil drought (Mäkelä *et al.*, 1996), competition for water (Cowan, 1982), trunk water storage (Scholz *et al.*, 2011; Pfautsch & Adams, 2012) and osmotic adjustment (Bartlett *et al.*, 2012; Sanders & Arndt, 2012) – our point is that for any strategy, the appropriate value of $\partial A/\partial E$ is largely determined by measurable biophysical properties that may be affected by $CO_2$ enrichment. These include leaf and xylem properties and aspects of plant structure that affect water supply, but they also include carbon investments in roots and leaves, which influence photosynthetic function and thus the return from
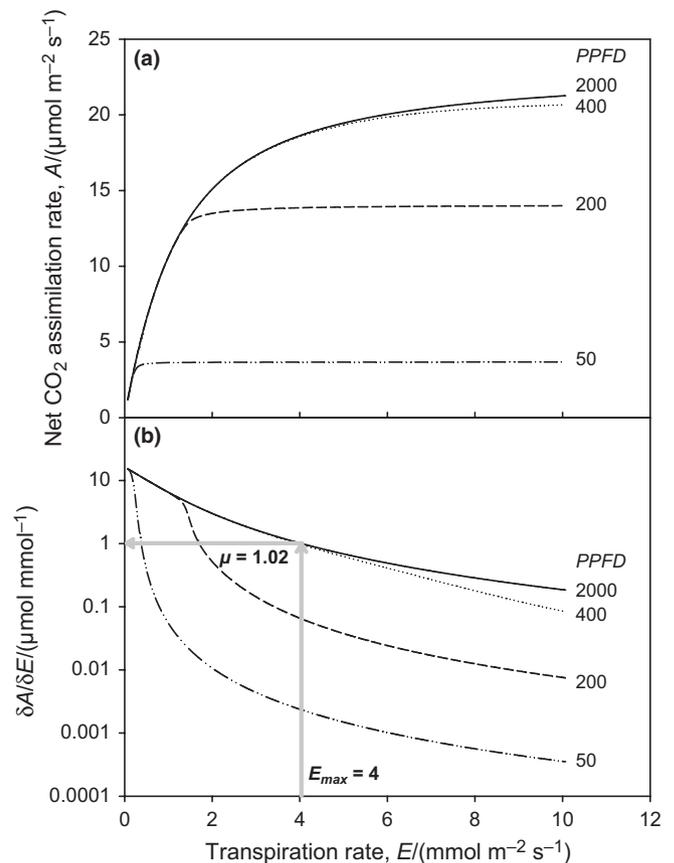


**Fig. 3** Relationships between transpiration rate, $E$, and (a) assimilation rate, $A$, and (b) the marginal carbon product of water, $\partial A/\partial E$, at different photosynthetic photon flux densities (PPFD, $\mu mol\ m^{-2}\ s^{-1}$). In (b), the grey arrows demonstrate inference of the target value for $\partial A/\partial E$ ($\mu$) from the maximum transpiration rate, $E_{max}$ (4.0 mmol $m^{-2}\ s^{-1}$ in this example): $\mu$ is the largest value of $\partial A/\partial E$ that coincides with $E = E_{max}$ during the day (1.02 $\mu mol\ mmol^{-1}$ in this example).

investing carbon to deliver water to leaves. Enrichment may also affect stomatal size and density, which influence how individual guard cell responses translate into changes in $g_{sw}$ (Franks & Beerling, 2009; Lammertsma *et al.*, 2011; Doheny-Adams *et al.*, 2012). It is unlikely that a simple proportionality will emerge between $c_a$ and $\mu$ when effects of enrichment on all these properties are accounted for.

To demonstrate how one might begin to assess these integrated effects of $c_a$ on $\mu$, we used two approaches to estimate changes in $\mu$ following $CO_2$ enrichment. First, we applied to Eqn 3 the common finding from FACE experiments that the ratio of $c_i/c_a$ is unaffected by enrichment (Ainsworth & Long, 2005), which suggests that enrichment increases $\mu$ by c. 17–41% (Fig. 4) (the range reflects differences between regeneration- and carboxylation-limited conditions, and uncertainty about enhancement of dark respiration; details in Supporting Information Notes S1). Second, we present simulations of structural and photosynthetic acclimation of mature trees following a step increase in $c_a$ from 370 to 570 ppm (previously published by Buckley, 2008), based on a tree growth model in which carbon allocation is optimized with respect to whole-plant carbon gain (DESPOT, Buckley & Roberts, 2006). The model operates on an annual time step and assumes the value of
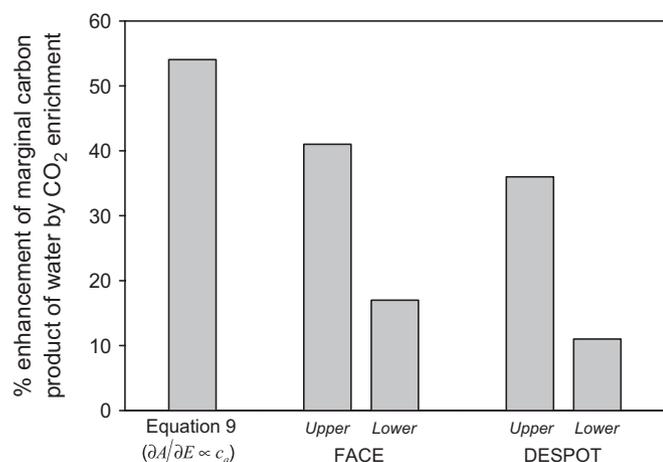
**Fig. 4** Per cent increase in the marginal carbon product of water, $\partial A/\partial E$, resulting from $CO_2$ enrichment from 370 to 570 ppm. The increase in $\partial A/\partial E$ was computed in three ways: using Eqn 8, which assumes that the target value for $\partial A/\partial E$ is simply proportional to $c_a$; by applying observed results from free air $CO_2$ enrichment experiments (Ainsworth & Long, 2005) to Eqn 3; or using the DESPOT tree growth model (Buckley & Roberts, 2006), in which carbon allocation is modulated to maximize carbon gain. FACE data and DESPOT predicted a range of enhancements of $\partial A/\partial E$; upper and lower limits of those ranges are shown.

$\psi_{leaf}$ prevailing during active photosynthesis is constant from year-to-year. Because DESPOT itself is based on optimization, this model is especially suited for the task of predicting long-term structural acclimation in response to $CO_2$ enrichment in relation to the multipliers in stomatal optimization. These simulations predicted an immediate 19% enhancement in μ (relative to a control simulation at constant $c_a$), followed by fluctuations between 36% and 11% over the ensuing years as a result of continuing structural adjustments (Buckley, 2008) (Fig. 4). By contrast, the hypothesis that $\partial A/\partial E$ is simply proportional to $c_a$ (Eqn 8) predicts a much larger increase of 54% in μ following $CO_2$ enrichment (Fig. 4). These results complement the wide-spread finding that enrichment causes rapid direct responses at the leaf scale, but that these effects are often damped by more gradual changes at the plant scale and above (Saxe *et al.*, 1998; Ainsworth & Rogers, 2007; Kirschbaum, 2011; Wang *et al.*, 2012).

## Embracing complexity to move optimisation forward

Medlyn *et al.* (2013) commented that the effects of $c_a$ on μ are unlikely to be understood without considering optimization on a longer timescale. We strongly agree, and we suggest that the appropriate timescale is that at which whole plant photosynthetic resource balance, and therefore carbon allocation, are modulated. The brief analysis earlier shows that it is possible to consider effects of $CO_2$ enrichment on the mysterious Lagrange multiplier, μ, at the heart of stomatal optimization, on a biophysical basis – we simply need to expand our perspective from the leaf to the whole plant. This brings additional complexity and uncertainty, but the benefits of rigorously extending stomatal optimization theory to future climates certainly outweigh the costs.

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## Supporting Information

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

**Notes S1** Details of mathematical derivations and computational methods underlying results presented in the main text.

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