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Review

Optimal plant water economy

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ABSTRACT

It was shown over 40 years ago that plants maximize carbon gain for a given rate of water loss if stomatal conductance, g_s, varies in response to external and internal conditions such that the marginal carbon revenue of water, $\partial A/\partial E$, remains constant over time. This theory has long held promise for understanding the physiological ecology of water use and for informing models of plant-atmosphere interactions. Full realization of this potential hinges on three questions: (i) Are analytical approximations adequate for applying the theory at diurnal time scales? (ii) At what time scale is it realistic and appropriate to apply the theory? (iii) How should g_s vary to maximize growth over long time scales? We review the current state of understanding for each of these questions and describe future research frontiers. In particular, we show that analytical solutions represent the theory quite poorly, especially when boundary layer or mesophyll resistances are significant; that diurnal variations in hydraulic conductance may help or hinder maintenance of $\partial A/\partial E$, and the matter requires further study; and that optimal diurnal responses are distinct from optimal long-term variations in g_s, which emerge from optimal shifts in carbon partitioning at the whole-plant scale.

Key-words: CO2; drought; optimisation; stomata; transpiration.

INTRODUCTION

Stomata regulate the terrestrial water cycle, balancing leaf water loss with photosynthetic carbon gain. Although it has long been understood that water supply and transport influence stomatal behaviour, there is no consensus regarding how to interpret and formally model this linkage from the perspective of adaptation or carbon economy. Farquhar (1973) and Cowan & Farquhar (1977) (collectively referred to as CF here) suggested that stomatal behaviour could be predicted from the hypothesis that stomatal responses to the environment tend to maximize photosynthetic carbon gain for a given water supply. The two most obvious approaches to identifying 'optimal' stomatal conductance, g_s, lead to trivial solutions: maximizing net CO_2 assimilation rate, A, with respect to g_s leads to the trivial solution $g_s \rightarrow \infty$ because A generally increases monotonically with g_s, and maximizing the

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ratio of A to transpiration rate, E, (i.e., A/E), with respect to g_s generally leads to the trivial solution $g_s \rightarrow 0$, because the curvature of A versus E is typically negative ($\partial^2 A/\partial E^2 < 0$), so that A/E decreases with increasing g_s. (A list of symbols is given in Table 1.) CF therefore approached the problem differently, by asking how g_s should vary so as to maximize the total carbon gain over some time interval during which external and perhaps internal conditions vary, $\int A(g_s)dt$, for a given total water loss over the same interval, $\int E(g_s)dt$. The integrals are over an arbitrary time span, which is typically interpreted as one day but is not specified in the theory. The solution to this problem uses the method of Lagrange multipliers (see Supporting Information File S1 for more details), and leads to the solution

$$\frac{\partial A/\partial g_s}{\partial E/\partial g_s} \equiv \frac{\partial A}{\partial E} = \mu_w, \qquad (1)$$

where μ_w is a constant with respect to variation in g_s, formally known as a Lagrange multiplier. This solution assumes that the curvature of A versus E is negative. The superscripted lowercase letter 'o' next to the equation number indicates that this equation does not represent either a definition of μ_w or a biophysical constraint linking μ_w and $\partial A/\partial E$, but rather a statement of the optimal coordination between two distinct quantities: the marginal carbon revenue of water, $\partial A/\partial E$ and an unspecified constant, μ_w.

The CF solution, Equation 1, says that the optimal pattern of g_s is one in which the marginal carbon revenue of water is invariant over time, and is equal to the arbitrary constant μ_w. The left-hand side of Equation 1, $\partial A/\partial E$, is a function of g_s and many other plant and environmental parameters, so the CF solution is an implicit function for g_s; to make the solution more explicit and generate predictions for stomatal behaviour, one must derive a detailed expression for $\partial A/\partial E$ from biophysical models of photosynthesis and gas exchange. The resulting predictions include familiar stomatal responses such as a positive response to photosynthetic photon flux density, PPFD and a negative response to evaporative demand, Δw. Many tests have broadly supported most qualitative predictions of Equation 1 (Ball & Farquhar 1984; Mäkelä et al. 1996; Buckley et al. 2014; Farquhar et al. 1980; Fites & Teskey 1988; Guehl & Aussenac 1987; Hari et al. 1999; Küppers 1984; Meinzer 1982; Sandford & Jarvis 1986; Schymanski et al. 2008; Thomas et al. 1999; Way et al. 2011; Williams 1983; Wong et al. 1979), with the exception of short-term stomatal responses to c_a, which are typically negative under a far wider range of conditions than predicted by

Table 1. List of symbols used in this paper, with definitions and units.

Symbol	Description	Units
A	net CO_2 assimilation rate	$\mu mol\,m^{-2}\,s^{-1}$
A_c	RuBP carboxylation-limited value of A	$\mu mol\,m^{-2}\,s^{-1}$
A_j	RuBP regeneration-limited value of A	$\mu mol\,m^{-2}\,s^{-1}$
a	Δw sensitivity parameter in Equation 5	$mol\,mmol^{-1}$
a'	unit cost of E, per Prentice et al. (2014)	$\mu mol\,mmol^{-1}$
a_1	photosynthetic parameter in Equation 8	$\mu mol\,m^{-2}\,s^{-1}$
a_2	photosynthetic parameter in Equation 8	$\mu mol\,mol^{-1}$
A_t	whole plant net CO_2 assimilation rate	$mol\,yr^{-1}$
b	slope parameter in USO model	unitless
b'	unit cost of V_{cmax}, per Prentice et al. (2014)	unitless
β_j	unit respiratory and senescence cost of carbon pool j	yr^{-1}
c_a	ambient CO_2 mole fraction	$\mu mol\,mol^{-1}$
c_i	intercellular CO_2 mole fraction	$\mu mol\,mol^{-1}$
c_c	chloroplastic CO_2 mole fraction	$\mu mol\,mol^{-1}$
C_j	carbon content of pool j	mol
χ_w	marginal carbon cost of water, per Givnish (1986)	$mmol\,\mu mol^{-1}$
$\partial A/\partial E$	marginal carbon revenue of water	$\mu mol\,mmol^{-1}$
Δw	leaf to air water vapour mole fraction difference	$mmol\,mol^{-1}$
E	leaf transpiration rate	$mmol\,m^{-2}\,s^{-1}$
E_t	whole plant transpiration rate	$mol\,yr^{-1}$
f_g	growth respiration fraction	unitless
G_{plant}	whole plant growth rate	$mol\,yr^{-1}$
G	gravitational constant	$m^2\,s^{-1}$
Γ	CO_2 compensation point	$\mu mol\,mol^{-1}$
Γ'	$(W\Gamma_* + R_d M)/(W - R_d)$	$\mu mol\,mol^{-1}$
Γ_*	photorespiratory CO_2 compensation point	$\mu mol\,mol^{-1}$
g	total conductance to CO_2	$mol\,m^{-2}\,s^{-1}$
g_1	parameter involving μ_w and Γ_* in Equation 11a	$(mmol\,mol^{-1})^{0.5}$
g_0	intercept in Equations 10 and 11a	$mol\,m^{-2}\,s^{-1}$
g_b	boundary layer conductance to CO_2	$mol\,m^{-2}\,s^{-1}$
g_m	mesophyll conductance to CO_2	$mol\,m^{-2}\,s^{-1}$
g_s	stomatal conductance to CO_2	$mol\,m^{-2}\,s^{-1}$
g_{tw}	total conductance to H_2O	$mol\,m^{-2}\,s^{-1}$
h	relative humidity	unitless
I	photosynthetic photon flux density	$\mu mol\,m^{-2}\,s^{-1}$
J	potential electron transport rate	$\mu mol\,m^{-2}\,s^{-1}$
k	slope of the photosynthetic CO_2 demand curve	$mol\,m^{-2}\,s^{-1}$
K_c	Michaelis constant for RuBP carboxylation	$\mu mol\,mol^{-1}$
K_o	Michaelis constant for RuBP oxygenation	$\mu mol\,mol^{-1}$
K_{plant}	whole-plant hydraulic conductance per unit leaf area	$mmol\,m^{-2}\,s^{-1}\,MPa^{-1}$
λ	marginal water cost of leaf carbon gain ($=1/\mu_w$)	$mmol\,\mu mol^{-1}$
m	slope parameter in Equation 10	$mol\,m^{-2}\,s^{-1}$
M	$K_c(1 + O/K_o)$ or $2\Gamma_*$	$\mu mol\,mol^{-1}$
μ_w	setpoint for $\partial A/\partial E$	$\mu mol\,mol^{-1}$
$\mu_{w,initial}$	value of μ_w in saturated soil, per Mäkelä et al. (1996)	$\mu mol\,mol^{-1}$
O	oxygen concentration	$\mu mol\,mol^{-1}$
PPFD	photosynthetic photon flux density	$\mu mol\,m^{-2}\,s^{-1}$
θ_a	curvature parameter for A versus A_c and A_j (Equation 13)	unitless
R_d	non-photorespiratory CO_2 release in the light	$\mu mol\,m^{-2}\,s^{-1}$
s	sensitivity to c_a in denominator of Equation 8	unitless
S	derivative of saturated H_2O mole fraction with respect to T	$mmol\,mol^{-1}\,{}^oC^{-1}$
t	time after rain event, per Mäkelä et al. (1996)	days
τ	mean rainfall interval, per Mäkelä et al. (1996)	days
T	temperature	^oC
T'	dT/dE (derivative of T with respect to E)	$^oC\,[mmol\,m^{-2}\,s^{-1}]^{-1}$
V_{cmax}	maximum RuBP carboxylation rate	$\mu mol\,m^{-2}\,s^{-1}$
Ω	setpoint for carbon profit	yr^{-1}
W	V_{cmax} or J_{max}	$\mu mol\,m^{-2}\,s^{-1}$
W'	$W - R_d$	$\mu mol\,m^{-2}\,s^{-1}$
ω_j	marginal carbon revenue of carbon for pool j	yr^{-1}
ω_l	leaf carbon profit	yr^{-1}

(Continues)

Table 1. (Continued)

Symbol	Description	Units
ω_r	root carbon profit	yr^{-1}
ω_s	stem-carbon profit	yr^{-1}
ξ	partial derivative of A with respect to T at constant c_c	$\mu mol\,m^{-2}\,s^{-1}\,°C^{-1}$
X_k	photosynthetic input k	varies
ψ_{leaf}	leaf water potential	MPa
ψ_{soil}	soil water potential	MPa

Equation 1 (e.g., Messinger et al. 2006; Morison 1998; Mott 1988). 'Stomatal optimisation theory' is thus thought to have great potential as a tool for predicting and interpreting stomatal function (Buckley & Schymanski 2014; Duursma et al. 2013; Medlyn et al. 2013; Vico et al. 2013).

To fully realize this potential requires clarity on three major questions: (i) How should the theory be applied, in practice, for predicting and interpreting diurnal variations in g_s? (ii) At what time scales is it appropriate to apply the CF solution? This is equivalent to asking, when is it reasonable to treat the constraint $\int E(g_s(t))dt$ and the associated Lagrange multiplier μ_w as constants, and when should they instead be treated as variables? (iii) What is the optimal long-term response of water loss and μ_w to sustained changes in environmental conditions or plant growth? Question #1 arises because when $\partial A/\partial E$ is expanded using rigorous biophysical models of leaf photosynthesis and stomatal diffusion, g_s can only be found by iterative numerical solution. To derive analytical solutions for optimal g_s, it is necessary to adopt potentially unrealistic assumptions, such as assuming important parameters to be arbitrarily constant (e.g., the slope of the biochemical A versus c_i 'demand' curve) or negligible (e.g., boundary layer and mesophyll resistance and/or day respiration). Consequently, it remains unclear whether the many analytical approximations of optimal g_s that have emerged over the years faithfully represent the optimal diurnal dynamics of g_s represented by Equation 1. Question #2, that is, at what time scale should the CF solution be applied, arises because the time interval for the constraint on total water loss in the CF problem is arbitrary. Resolving this question requires a careful exploration of $\partial A/\partial E$, μ_w and the relation between the two, as well as factors that control the amount of water loss that a plant can tolerate. Question #3, that is, the response of water loss to sustained changes in environmental or plant parameters, hinges on how structural changes at the whole-plant scale influence water supply to the leaf, and how leaf carbon-water economy is linked to whole-plant carbon economy; addressing this question links stomatal optimisation to a broader theory (e.g., Bloom et al. 1985; Buckley & Roberts 2006a; Givnish 1986). Our objective is to summarize current understanding concerning these three major questions and to suggest directions for continuing research on optimal expenditure of water by plants.

QUESTION #1: HOW SHOULD THE THEORY BE APPLIED FOR PREDICTING DIURNAL VARIATIONS IN g_s?

A challenge in applying Equation 1 is that it requires iterative numerical solution when applied to rigorous biophysical

models for photosynthesis and gas exchange, which allow analysis or testing of the theory against measurements. Problems arise because when using models of realistic complexity, the resulting expression for $\partial A/\partial E$ cannot be solved for g_s. A complete expression for $\partial A/\partial E$ (derived in Supporting Information File S1) is

$$\frac{\partial A/\partial g_s}{\partial E/\partial g_s} = \left(1.6\frac{g}{g_{tw}}\right)^2 \left(\frac{c_a - c_c}{1.6\Delta w}\right) \left(\frac{k}{k+g}\right)(1 - T'g_{tw}S) + T'\xi,$$

(2)

where g is total conductance to CO_2 (including stomatal, boundary layer and mesophyll components), g_{tw} is total conductance to H_2O, c_a and c_c are ambient and chloroplastic CO_2 mole fractions, respectively, k is the slope of the biochemical A versus c_c demand curve at constant temperature, or $(\partial A/\partial c_c)_T$, S is the slope of the response of saturation vapour pressure to temperature, $\xi = (\partial A/\partial T)_{cc}$ where T is leaf temperature and $T' = dT/dE$. Although g_s appears implicitly on the RHS of Equation 2 (g and g_{tw} both depend on g_s), Equation 2 cannot be solved analytically for g_s because c_c, k, T', S and ξ also all depend implicitly on g_s, often in complicated fashion. It is thus common to adopt simplifying assumptions that lead to an analytical solution (Table 2). A common assumption is that the leaf and air are perfectly aerodynamically coupled; that is, boundary layer conductance (g_b) is infinite, which implies $T' \rightarrow 0$, so that Equation 2 simplifies to

$$\left(\frac{\partial A/\partial g_s}{\partial E/\partial g_s}\right)_{g_b \rightarrow \infty} = \left(1.6\frac{g}{g_{tw}}\right)^2 \frac{c_a - c_c}{1.6\Delta w}\left(\frac{k}{k+g}\right).$$

(3)

If one assumes further that mesophyll conductance to CO_2 (g_m) is so large as to have a negligible impact, then g/g_{tw} approaches the ratio of stomatal conductances to CO_2 and H_2O (1/1.6), c_c approaches the intercellular CO_2 mole fraction, c_i, and g approaches g_s, so that Equation 3 simplifies further to

$$\left(\frac{\partial A/\partial g_s}{\partial E/\partial g_s}\right)_{g_b,g_m \rightarrow \infty} = \left(\frac{c_a - c_i}{1.6\Delta w}\right)\left(\frac{k}{k+g_s}\right) = \frac{A}{E}\left(\frac{k}{k+g_s}\right),$$

(4)

At first glance this appears easy to solve for g_s; doing so and applying the condition of optimality from Equation 1 ($\partial A/\partial E = \mu_w$) gives

$$g_s = k\left(\frac{c_a - c_i}{1.6\Delta w\mu_w} - 1\right).$$

(5)

However, c_i depends on g_s and k in turn depends on c_i, so Equation 5 is still not actually a closed-form solution for g_s. To obtain a simple solution, some authors have assumed that

Table 2. List of analytical approximations to the CF solution for optimal stomatal behaviour, including the assumptions used to derive and/or to apply each model. g_b, boundary layer conductance; g_m, mesophyll conductance to CO_2; R_d, non-photorespiratory CO_2 release in the light; k, slope of the biochemical A versus c_c demand curve. Under 'k', 'constant' means 'unaffected by stomatal conductance'.

	Assumptions				
Analytical solution	g_b	g_m	R_d	k	other
Equation 6 (Hari et al. 1986)	∞	∞	0	constant (*)	*k varies with PPFD
Equation 7 (Lloyd 1991)	∞	∞	varies via Γ	constant	—
Equation 8 (Katul et al. 2009)	∞	∞	0	constant (**)	**k varies inverse-hyperbolically with c_a
Equation 9 (de Pury 1995; Lloyd et al. 1995)	∞	∞	varies via Γ	constant (***)	***k assumed constant in derivation but varies if applied using a biochemical model for A
Equation 11 (Medlyn et al. 2011)	∞	∞	0	free	assumes stomata optimize as if A were always regeneration-limited
Arneth et al. (2002)	∞	∞	0	free	—
Equation 12 (this study)	∞	∞	free	free	—

k is independent of c_i. For example, Hari et al. (1986) derived the relation

$$g_s = f(I)\left(\left(\frac{c_a}{1.6\Delta w \cdot \mu_w}\right)^{0.5} - 1\right), \tag{6}$$

where $f(I)$ is the functional response of CO_2 assimilation rate to PPFD (I). Lloyd (1991) derived a nearly identical expression,

$$g_s = k\left(\left(\frac{c_a - \Gamma}{1.6\Delta w \cdot \mu_w}\right)^{0.5} - 1\right), \tag{7}$$

in which Γ is the CO_2 compensation point (c_i at which $A = 0$) (note that $\Gamma \neq \Gamma_*$, the photorespiratory CO_2 compensation point; that is, the value of Γ in the absence of non-photorespiratory CO_2 release). Equations 6 and 7 do not correctly predict observed stomatal responses to increasing c_a: the observed response is generally negative, whereas Equations 6 and 7 predict positive responses. Katul et al. (2009, 2010) derived a result similar to Equations 6 and 7:

$$g_s = \left(\frac{a_1}{a_2 + sc_a}\right)\left(\left(\frac{c_a}{1.6\Delta w \cdot \mu_w}\right)^{0.5} - 1\right), \tag{8}$$

in which a_1 and a_2 are photosynthetic parameters and s is the long-term mean value of the ratio c_i/c_a. Although the appearance of c_a in the denominator of Equation 8 enhances the negative response to c_a, Equation 8 still predicts a positive response of g_s to c_a under carboxylation limited conditions. Katul et al. (2010) overcame this by assuming that μ_w is proportional to c_a itself, based on the observation that μ_w was apparently greater in plants grown at elevated c_a. This assumption cancels the effect of c_a in the radical in Equation 8 and creates a negative response to c_a (Fig. 1). Another solution to the c_a problem, originally derived by de Pury (1995) and published by Lloyd et al. (1995), was to replace k in Equation 7 with $A/(c_i - \Gamma)$, which leads to

$$g_s = A\left(\frac{1.6}{(c_a - \Gamma) \cdot \Delta w \cdot \mu_w}\right)^{0.5}. \tag{9}$$

Although the derivation of Equation 9 assumes constant k, as for Equations 6 and 7, its predictions differ from those of Equations 6 and 7 if Equation 9 is applied using a biochemical model for A that allows k to vary. Regardless, Equation 9 still predicts a positive response of g_s to c_a under carboxylation-limited conditions (Fig. 1).

Unlike the approximate optimal solutions described earlier, the widely used Ball-Berry (BB) model (Ball et al. 1987) predicts a negative response to c_a under most conditions (Fig. 1, Equation 10). The BB model is

$$g_s = g_o + mh\frac{A}{c_a}, \tag{10}$$

where g_o and m are fitted parameters and h is relative humidity. Medlyn et al. (2011) showed that the CF optimum (Equation 4) leads to an equation similar to Equation 10, in which g_s is proportional to the ratio of A/c_a, and in which g_s declines as c_a increases – provided one assumes that g_s 'acts as if it is optimizing for RuBP regeneration-limited photosynthesis', whether photosynthesis is in fact carboxylation-limited or regeneration-limited. Arneth et al. (2002) had earlier derived an analytical solution for optimal c_i under regeneration-limited conditions by applying Equation 4 to the photosynthesis model of Farquhar et al. (1980), combined with an expression for CO_2 diffusion ($A = g_s(c_a - c_i)/1.6$). By recombining the Arneth optimal c_i expression with the diffusion expression, Medlyn et al. (2011) created the USO ('unified stomatal optimisation') model:

$$g_s = \left(\left(\frac{3\Gamma_*}{1.6\Delta w \cdot \mu_w}\right)^{0.5} + 1\right)\frac{A}{c_a}. \tag{11}$$

Medlyn et al. (2011) further simplified Equation 11 to permit the term involving μ_w to be estimated empirically, and added another coefficient, g_o, to permit nonzero g_s in darkness:
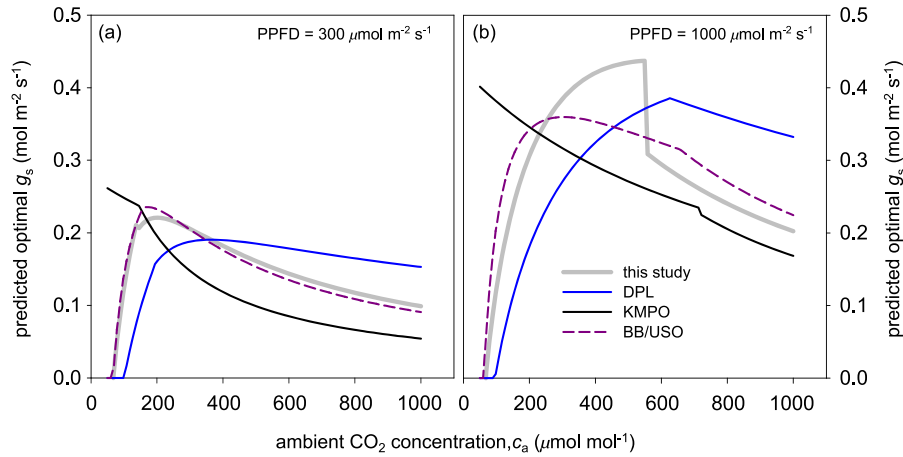
Figure 1. Optimal stomatal responses to atmospheric CO_2 concentration (c_a) predicted by analytical approximations to the CF solution assuming infinite boundary layer and mesophyll conductances. Grey line: exact solution under these conditions (this study, Equation 12); blue line: the solution of de Pury (1995) and Lloyd et al. (1995) ('DPL', Equation 9); black line: the solution of Katul et al. (2009, 2010) ('KMPO', Equation 8); dashed purple line, the solution of Medlyn et al. (2011), which is identical to the Ball-Berry model in these simulations ('BB/USO', Equations 10 and 11) The solution for KMPO shown here assumes, as per Katul et al. (2010), that $\mu_w \propto c_a$, nullifying the dependence of the radical in Equation 8 on c_a.

$$g_s = g_o + \left(\frac{g_1}{\Delta w^{0.5}} + 1 \right) \frac{A}{c_a}. \qquad (11a)$$

Thus, the parameter g_1 in the USO model should scale inversely with μ_w.

Each of the approximations described above assumes that the slope of the demand curve (k) is unaffected by changes in g_s (Equations 6–9), and/or that the leaf respiration rate in the light is zero (6, 8 and 11), but Equation 4 can actually be solved for g_s without either of these assumptions (as shown in Supporting Information File S1), to give

$$g_s = \frac{W'(c_i - \Gamma')}{(c_a - c_i)(c_i + M)}, \qquad (12)$$

where $c_i = (-q_1 - (q_1^2 - 4q_2 q_0)^{0.5})/(2q_2)$, and the parameters q_2, q_1 and q_0 are

$$q_2 \equiv 1 - 1.6 \Delta w \mu_w W'/(W(M + \Gamma_*))$$
$$q_1 \equiv 1.6 \Delta w \mu_w - 2c_a - (M - \Gamma') 1.6 \Delta w \mu_w W'/(W(M + \Gamma_*))$$
$$q_0 \equiv c_a^2 - 1.6 \Delta w \mu_w c_a + M \Gamma' 1.6 \Delta w \mu_w W'/(W(M + \Gamma_*)), \qquad (12a)$$

where $W' = W - R_d$ and $\Gamma' = (W\Gamma_* + R_d M)/(W - R_d)$; W and M depend on whether photosynthesis is carboxylation-limited or regeneration-limited; in the former case, $W = V_{cmax}$ and $M = K_c(1 + O/K_o)$, and in the latter case, $W = 0.25 \cdot J$ and $M = 2\Gamma_*$.

An important strength of all the analytical approximations described above is that they can be applied directly, whereas Equation 2 requires numerical inversion. Their potential weakness is that they are based on assumptions that differ across models and contradict known properties of leaf biophysics ($g_b = g_m = \infty$ and either constant k or $R_d = 0$) (Table 2 lists these analytical approximations and their assumptions). As a consequence, these solutions diverge substantially from one another (Fig. 2a) and predict wide diurnal variation in $\partial A/\partial E$ despite

ostensibly being derived from the assumption of constant $\partial A/\partial E$ (Fig. 2b). Interestingly, the empirical BB model is as good a proxy for the exact optimal solution as most of the putatively optimal approximations; this was in fact the central message of Medlyn et al. (2011) – that is, that BB is approximately consistent with optimal stomatal behaviour. Indeed, the BB model itself was partly motivated by the data of Wong et al. (1979), who showed the c_i/c_a ratio was conserved and argued that this behaviour was approximately optimal. However, these approximations are not particularly faithful proxies for optimal g_s when mesophyll conductance is allowed to take on realistic finite values (Fig. 2c), even assuming perfect aerodynamic coupling. Perhaps most importantly, the assumption of perfect aerodynamic coupling is quite incorrect under many conditions (Buckley et al. 2014; Daudet et al. 1998; Daudet et al. 1999; Grantz & Vaughn 1999; Jones et al. 2002; Schymanski & Or 2015), so that Equation 4 probably diverges substantially from Equation 2 in nature. Furthermore, because Δw can be very sensitive to evaporative cooling under decoupled conditions, decoupling can lead to positive curvature in the relationship between A and E at low conductances, that is, $\partial^2 A/\partial E^2 > 0$ (Buckley et al. 1999; Buckley et al. 2014; Cowan 1977; Cowan 1986; Cowan & Farquhar 1977), violating the CF solution's premise that $\partial^2 A/\partial E^2 < 0$.

One might remark that the optimal time courses of g_s predicted without analytical simplifications are not biologically realistic, because they require a discrete 'jump' in g_s at the point corresponding to the transition between carboxylation-limited and regeneration-limited photosynthesis (e.g., grey lines in Figs. 1b, 2a and 2c). However, this jump is an artefact of assuming that the carbon and photochemical reactions of photosynthesis are perfectly coupled (Farquhar et al. 1980); anything less than perfect coupling would, in effect, 'smooth' the transition, eliminating the apparent discontinuities in optimal stomatal behaviour. Moreover, even if photosynthesis were perfectly coupled in any given chloroplast, averaging across paradermal
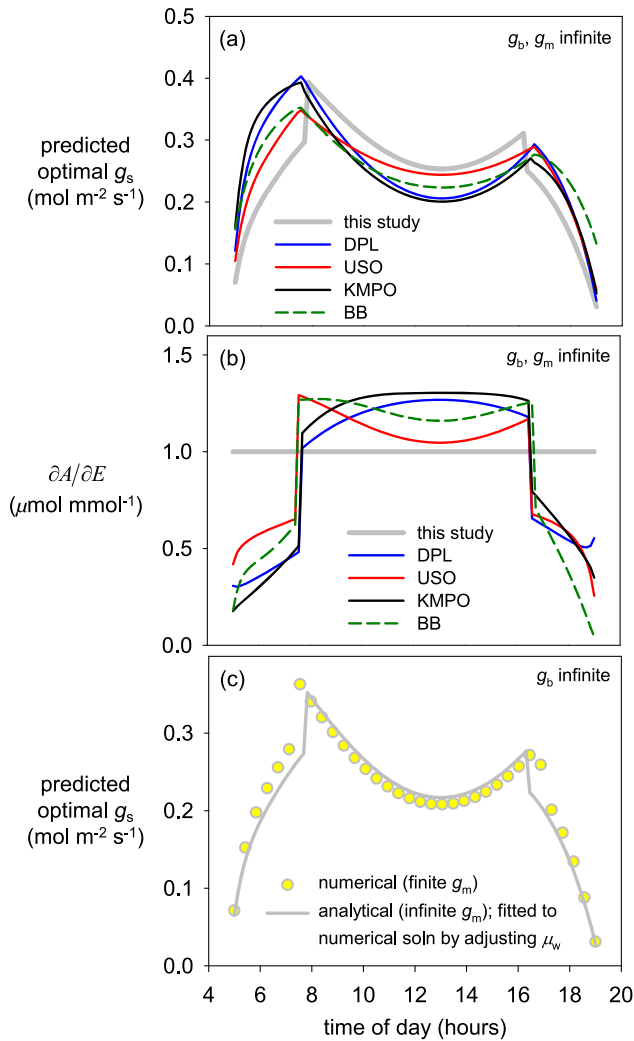
Figure 2. (a) Optimal variations in stomatal conductance over a simulated diurnal time course predicted by Equations 8–11, 11a, 12 under conditions of perfect aerodynamic coupling ($g_b \rightarrow \infty$) and infinite mesophyll conductance ($g_m \rightarrow \infty$), with free parameters in each model adjusted to maximize fit to the exact solution (Equation 12, this study). Lines as in Fig. 1. (b) Variations in the marginal carbon revenue of water ($\partial A / \partial E$) for each g_s response from (a). (c) Optimal variations in g_s predicted by a numerical solution of Equation 3 in which mesophyll conductance at 25 °C is 0.5 mol m^{-2} s^{-1} (yellow circles), compared with the exact solution assuming infinite boundary layer and mesophyll conductances (grey line, Equation 12), but with μ_w in the latter solution adjusted to maximize fit to the numerical solution. The simulations assumed PPFD varies sinusoidally between 50 and 1000 μmol m^{-2} s^{-1}, peaking at noon, and temperature varies sinusoidally between 15 and 35 °C, peaking at 1 pm. Evaporative demand (Δw) varies passively with temperature assuming an ambient humidity of 15 mmol mol^{-1}.

chloroplast layers would likely lead to a 'smoothed' relationship at the leaf level, because it would be extremely rare for every layer within the leaf to be at the transition point simultaneously for a given incident PPFD (Buckley & Farquhar 2004; Farquhar 1989). Slight apparent decoupling can be represented empirically by modelling net assimilation rate in relation to RuBP-saturated and RuBP-limited rates (A_c and A_j, respectively) using a non-rectangular hyperbola

with a curvature factor, θ_A, less than unity, in the same way that potential electron transport rate is commonly modelled (e.g., Farquhar & Wong 1984):

$$\theta_A A^2 - (A_c + A_j)A + A_c A_j = 0, \qquad (13)$$

where A is taken as the lesser root of Equation 13, and $\theta_A \leq 1.0$. Figure 3a shows an A versus c_i curve modelled in three ways: assuming $\theta_A = 1.0$ (no smoothing), assuming $\theta_A = 0.998$ (slight smoothing) and again assuming $\theta_A = 1.0$ but fitting the model to a sample of points from the smoothed curve by adjusting V_{cmax}, J and R_d. The un-smoothed model still fits extremely well even if the true underlying relationship is slightly decoupled. If the slightly smoothed A versus c_i relationship is then used to predict optimal g_s, the discontinuities disappear (Fig. 3b). Thus, the discontinuities are an artefact of a common simplification in the way photosynthesis is commonly modelled, and they do not imply that the true optimum is biologically unachievable.

Conclusions (Question #1)

Because of the concerns outlined earlier, we argue that simple analytical approximations for optimal g_s are not suitable for inferring whether observed patterns in g_s are in fact optimal – refined comparison between the theory and observations demand refined models to identify the optimum, and this precludes analytical solution. An important open question in this regard is whether and how analytical approaches might be improved to accommodate finite and variable mesophyll and boundary layer conductances. However, the approximations described earlier have all been validated experimentally to some degree, and are therefore potentially useful for predicting g_s. Although other models exist for g_s that have also been widely validated and applied (e.g., Ball et al. 1987; Jarvis 1976), optimisation-based models are distinct in that they include an economic parameter, μ_w. This parameter explicitly links stomatal function to resource economy and ecology at higher scales, and thus represents an important advance over models that are not framed in economic terms. It is important to recognize, however, that none of the models for optimal g_s described earlier, including the CF solution itself, offers any insight about how μ_w should change at longer time scales or how to estimate its numerical value (Givnish 1986), other than by fitting the equations above to measurements of g_s and other parameters, which is not useful for predictive purposes. We explore those questions in the next two sections.

QUESTION #2: AT WHAT TIME SCALES IS IT APPROPRIATE TO TREAT μ_W AS A CONSTANT OR AS A VARIABLE?

A conceptual difficulty with the CF approach is that it requires one to specify an arbitrary, finite time period over which total water loss is constant. This makes it difficult to understand how to apply the theory at different time scales. Katul et al. (2009, 2010) attempted to circumvent this difficulty by reframing the problem as an *instantaneous* maximization, not
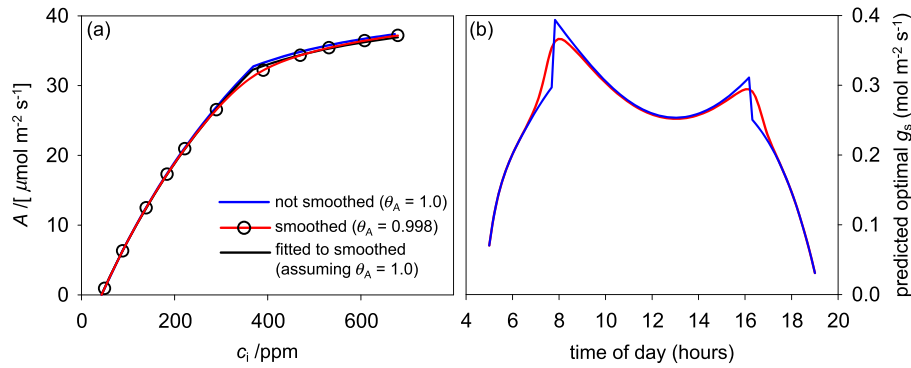
Figure 3. Effect of accounting for slight decoupling between the carbon reactions and the photochemical reactions of photosynthesis. (a) Three relationships between net CO_2 assimilation rate (A) and intercellular CO_2 concentration (c_i): that used to generate Fig. 2, which assumes perfect coupling (blue line, 'not smoothed'), the same relationship but using Equation 13 with the curvature parameter $\theta_A = 0.998$ to simulate slight decoupling (red line and open circles, 'smoothed'), and the model fitted to a sample of points (open circles) from the smoothed relationship, but assuming $\theta_A = 1.0$ ('fitted to smoothed'). The three relationships are nearly indistinguishable, suggesting that experimental data and model-fitting likely could not distinguish between $\theta_A = 0.998$ and $\theta_A = 1.0$. (b) Comparison of predicted optimal time courses of g_s using the un-smoothed A versus c_i relationship from (a) (blue line), or using the smoothed relationship (red line), with all other conditions as for Fig. 2.

of A, but of the quantity $A - \mu_w E$, with respect to g_s, which leads to

$$\frac{\partial(A - \mu_w E)}{\partial g_s} = 0 \rightarrow \frac{\partial A}{\partial g_s} = E\mu_w\left(\frac{\partial \ln\mu_w}{\partial g_s} + \frac{\partial \ln E}{\partial g_s}\right). \quad (14)$$

Katul et al. (2009, 2010) then asserted that

$$\frac{\partial \ln\mu_w}{\partial g_s} << \frac{\partial \ln E}{\partial g_s}, \quad (15)$$

which reduces Equation 14 to Equation 1. However, the identity of μ_w in the goal function ($A - \mu_w E$) of Equation 14 is critically important. Katul et al. (2009, 2010) explicitly identified μ_w as $(\partial A/\partial g_s)/(\partial E/\partial g_s)$, that is, $\partial A/\partial E$, in framing the problem. Unfortunately, this cannot lead to an informative solution. For example, if we accept Equation 15 and then apply μ_w as $(\partial A/\partial g_s)/(\partial E/\partial g_s)$ to Equation 14, the result is merely an identity:

$$\frac{\partial A}{\partial g_s} = E\left(\frac{\partial A/\partial g_s}{\partial E/\partial g_s}\right)\left(\frac{\partial \ln E}{\partial g_s}\right) = \left(\frac{\partial A/\partial g_s}{\partial E/\partial g_s}\right)\frac{\partial E}{\partial g_s} = \frac{\partial A}{\partial g_s}. \quad (16)$$

However, Equation 15 is easily disproven (as shown in Supporting Information File S1); but if Equation 15 is rejected and μ_w in Equation 14 is replaced with the expression for $\partial A/\partial E$ from Equation 4, we have

$$\frac{\partial\left(A - \left(\frac{\partial A}{\partial E}\right)E\right)}{\partial g_s} = \frac{\partial\left(A - \frac{A}{E}\left(\frac{k}{k+g_s}\right)E\right)}{\partial g_s} = \frac{\partial\left(A\frac{g_s}{k+g_s}\right)}{\partial g_s} = 0, \quad (17)$$

which leads to the trivial solution $g_s \rightarrow \infty$ because the quantity $A \cdot g_s/(k + g_s)$ increases monotonically with g_s. As noted by Buckley & Schymanski (2014), to use $\partial A/\partial E$ as part of the goal function (i.e., maximizing $A - (\partial A/\partial E)\cdot E$ rather than $A - \mu_w E$) is to misinterpret Equation 1 as a definition or a biophysical constraint, when in fact Equation 1 only applies in the optimum. Failing to distinguish μ_w from $\partial A/\partial E$ leads to the perception that μ_w, like $\partial A/\partial E$, is solely a function of leaf gas exchange properties. We suggest that the proper interpretation of μ_w is as the *setpoint* or *target* for $\partial A/\partial E$: whereas $\partial A/\partial E$ is a dynamic

property of leaf gas exchange, μ_w reflects constraints largely external to the leaf.

What, then, is the appropriate time scale at which to treat μ_w as a constant, as required to apply the CF solution? Although that solution is mathematically valid at any time scale, it is well known that daily water loss changes systematically over time, both during soil drought and during structural acclimation at the whole plant scale, and this in turn suggests that μ_w must also change at such time scales. Cowan & Farquhar (1977) noted that 'the greater the expenditure [of water] *that can be tolerated* the greater λ should be' (where $\lambda = 1/\mu_w$; emphasis added). Thus, to understand when μ_w should be treated as a variable rather than as a constant, we must understand what determines the tolerable rate of water loss. Firstly, a plant can only tolerate water loss if it does not carry the risk of mortality or inefficient carbon gain because of overly profligate water use, and likewise, the plant cannot tolerate overly conservative water use that results in uncompetitive performance. Secondly, water loss from the leaf must not exceed water supply to the leaf from the soil. These considerations depend on both internal and external factors, many of which operate at longer than diurnal time scales, and we discuss them further below.

Optimal dynamic response of water loss during intermittent soil drought

External factors that determine how much water loss can be tolerated include not only soil water potential (ψ_{soil}) but also the timing of soil water depletion and recharge. Ignoring competition, a given finite supply of soil moisture should not be used more rapidly than necessary, because this would reduce total carbon gain even if $\partial A/\partial E$ were held constant; this is a consequence of the fact that the average carbon revenue of water (A/E or $\int A\,dt/\int E\,dt$) generally decreases as average E increases, which in turn is a consequence of negative curvature in A versus E ($\partial^2 A/\partial E^2 < 0$). An optimal strategy would therefore choose the highest value of μ_w (generally, the lowest

average E) that used all the available water, causing soil water to run out just as the next recharge event occurred (Manzoni et al. 2013). However, this is not biologically realistic: at best, natural selection might preserve a statistical 'memory' about the probability distribution of soil water potential, but not the duration of a specific future drought. In the context of intermittent soil drought, the 'tolerability' of a given amount of water loss is therefore essentially a problem in game theory: to determine what is the optimal stomatal closure response during intermittent soil drought, one must consider factors involving uncertainty and risk, such as the expected duration of the drought, the risk of mortality from running out of water and the impact of competition for water with other plants.

Mäkelä et al. (1996) showed that if rainfall intervals are randomly and normally distributed, the response that maximizes expected carbon gain over many such droughts is for μ_w to increase exponentially over time with a time constant inversely proportional to the mean rainfall interval (τ): $\mu_w = \mu_{w,initial} \cdot \exp(t/\tau)$. However, the optimal strategy becomes more complex if one considers other factors not included in the treatment by Mäkelä et al., such as the impact of competition with other plants and/or the risks of mortality or catastrophic loss of hydraulic conductance in the soil or plant tissue. Cowan (1982) showed that these scenarios led to a sigmoidal time course for gas exchange rates: decreasing slowly at first, then rapidly and finally more slowly again towards the end of the drought. More recently, Lu et al. (2016) analysed the problem using a stochastic model that allowed for the effects of a plant's strategy during one drought to influence the outcome of its strategy during the next event. Their simulations predicted that g_s should vary sigmoidally in relation to soil relative water content, declining more rapidly as soil moisture approaches total depletion. Interestingly, they also predicted that the general magnitude of g_s (and thus transpiration) should depend on the average frequency of rainfall events, even for a given total mean annual precipitation, with g_s being greater if rainfall is more frequent; this was partly because a greater proportion of rainfall is lost to surface runoff in environments with low-frequency rainfall, when controlling for total annual precipitation.

The role of cyclical variations in hydraulic conductance

Internal factors that determine how much water loss can be tolerated include plant hydraulic structure and physiology and biomass partitioning. To prevent leaf desiccation, on average transpiration cannot exceed water flow into the leaf, which in turn is determined by the water potential gradient between leaf and soil and by the whole-plant hydraulic conductance per unit leaf area (K_{plant}), which depends on hydraulic conductances throughout the plant and declines in response to dehydration. A plant can directly increase the tolerable expenditure of water by modifying K_{plant} by investing more carbon into roots or stems to increase water uptake and transport capacity relative to leaf area, and/or by changing the intrinsic transport properties of new conductive tissue (e.g., Givnish & Vermeij 1976; we discuss this issue in addressing Question #3 below). Plant hydraulic

conductance may also vary cyclically at diurnal time scales – often declining during the daytime as a result of stem, root and/or leaf dehydration before recovering overnight (e.g., Brodribb & Holbrook 2004; Clarkson et al. 2000; Zwieniecki & Holbrook 1998). Because K_{plant} influences the tolerable rate of water loss, it is reasonable to wonder whether μ_w should shift dynamically in response to diurnal changes in K_{plant}. However, such changes are somewhat predictable, in the sense that since the earliest vascular plants evolved, plants would have experienced diurnal cycles of decline and recovery in K_{plant}. Given that any diurnal variation in $\partial A/\partial E$ will reduce daily carbon gain when controlling for total daily water loss (Cowan & Farquhar 1977), natural selection will favour stomatal response mechanisms that can distinguish diurnal cycles of K_{plant} from more sustained shifts, and that can set μ_w high enough to prevent excessive or damaging declines in K_{plant} on a diurnal basis. For example, if the plant 'chose' a value of μ_w that were too low, the resulting optimal time course for g_s would be too high, causing leaf water potential to drop below the threshold causing excessive loss of K_{plant} during some part of the day; in order to prevent this, g_s would then have to divert below the optimal time course during part of the day, leading to diurnal variation in $\partial A/\partial E$, which is inherently suboptimal (Buckley 2005).

Diurnal variation in K_{plant} may in fact be an important component of the mechanisms of stomatal regulation that have presumably evolved to optimize diurnal variation in g_s. This is because invariance in $\partial A/\partial E$ often requires E to plateau or even decline at high Δw (Buckley 2005), which in turn requires very high sensitivity of g_s to Δw; one way to achieve such high sensitivity, given evidence that stomata respond to ψ_{leaf} (Buckley 2005; McAdam & Brodribb 2016; McAdam et al. 2015; Rodriguez-Dominguez et al. in press), is through an enhanced decline in ψ_{leaf} with increasing Δw, driven by loss of hydraulic conductance (Buckley & Mott 2002; Oren et al. 1999). Together, these considerations suggest that μ_w should not necessarily change during diurnal cycles of loss and recovery in K_{plant}.

Conclusions (Question #2)

The time scale and predictability of changes in K_{plant} and ψ_{soil} are central to determining when to treat μ_w as a constant or as a variable: generally, it may be reasonable to treat μ_w as a constant with respect to predictable, cyclical variations in the factors that determine the tolerable rate of water loss, but not with respect to unpredictable changes in those factors that take the plant out of its 'comfort zone'. This could be tested experimentally by computing $\partial A/\partial E$ during observed diurnal cycles of variation in K_{plant}. In the next section, we discuss how μ_w should change in relation to sustained changes in the environmental and structural determinants of whole plant carbon-water economy.

QUESTION #3: WHAT IS THE OPTIMAL LONG-TERM RESPONSE OF WATER LOSS TO SUSTAINED CHANGES IN ENVIRONMENTAL CONDITIONS OR PLANT GROWTH?

At longer time scales, large changes in biomass partitioning associated with growth or sustained environmental change

increasingly influence the tolerable rate of water loss. In the CF framework, that rate of water loss is viewed as a 'cost', which is converted into carbon units by the Lagrange multiplier μ_w. Three important studies in the 1970s and 1980s set the stage for how μ_w might be more explicitly understood and even calculated as a cost. Ironically, although these earlier works have been heavily cited, their implications for the meaning of μ_w have gone largely unnoticed in most of the literature on stomatal optimisation until very recently.

Givnish & Vermeij (1976) suggested that the multiplier could be interpreted as 'the cost of arranging for the supply of a unit flow rate of water,' and attempted to calculate that cost from measurements of tree growth rate and structure. Their calculations, although tentative, suggested these costs were on the same order of magnitude as the associated photosynthetic gains. Givnish (1986) later built on the same principle – that is, that optimal g_s should depend on the costs of acquiring water – and used a simple model in which leaf transpiration was balanced by root water uptake and photosynthetic metabolism was directly affected by leaf water potential, ψ_{leaf}, to show that the marginal carbon revenue of water should be equal to the marginal carbon *cost* of water, χ_w. This differed from CF, which did not explicitly consider direct effects of ψ_{leaf} on photosynthetic metabolism. In Givnish's model, χ_w is given by

$$\chi_w = -\left(\frac{\partial A}{\partial \psi_{leaf}}\right)\left(\frac{\partial \psi_{leaf}}{\partial E}\right). \tag{18}$$

Because at steady state $E = K_{plant}(\psi_{soil} - \psi_{leaf})$, $\partial \psi_{leaf}/\partial E = -1/K_{plant}$, so that $\partial A/\partial E = \chi_w$, combined with the CF solution, Equation 1 ($\partial A/\partial E = \mu_w$), implies

$$\mu_w = \frac{1}{K_{plant}}\left(\frac{\partial A}{\partial \psi_{leaf}}\right). \tag{19}$$

The Givnish solution (Equation 19) was the first to express μ_w in terms of measurable physiological properties. This solution suggests that μ_w depends on coordination of biomass partitioning among roots, stems and leaves (via K_{plant}), because the tolerable expenditure of water is determined by how much carbon must be invested in roots and stems to ameliorate suppression of photosynthetic metabolism by low ψ_{leaf}. Equation 19 predicts that if a plant has either a surfeit of leaf area or a deficit of water uptake and transport capacity (so that K_{plant} is small) or photosynthetic metabolism that is particularly sensitive to ψ_{leaf} (so that $\partial A/\partial \psi_{leaf}$ is large), it should 'choose' a greater value of μ_w and thus a lower E and g_s. That is, drier soils, lower humidity, lower photosynthetic capacity, lower root hydraulic conductance and greater direct sensitivity of A to reduced ψ_{leaf} all favour lower g_s. Some important considerations are not addressed by this solution. Firstly, Equation 18 predicts $\mu_w = 0$ unless the direct effect of water stress on photosynthetic metabolism, independent of reduced g_s, is substantial, but most experimental evidence suggests such effects are typically negligible under normal diurnal ranges of ψ_{leaf}, and even under moderate soil water stress in many cases (Kaiser 1987; Downton et al. 1988; Sharkey & Seemann 1989; Quick et al. 1992; Centritto et al. 2003; Koch et al. 2004; Chaves et al. 2009, but see Lawlor & Tezara 2009). Secondly, the

marginal carbon cost of water should also include indirect impacts of carbon investments for water transport on photosynthesis – for example, from changes in light capture (which is affected by leaf area and vertical growth) and nitrogen uptake (which is affected by investment in fine roots). A more integrative theory is thus required to predict μ_w in more general circumstances.

Around the same time as Givnish's work, Bloom et al. (1985) presented a synthesis of microeconomic and production theory as applied to plant resource economy that laid the groundwork for a more integrative view. A recurring theme in that paper was the concept of equimarginality – the notion that resources are used to greatest effect if the marginal revenue from investment of a resource (the infinitesimal increase in income or production rate that is realized from an infinitesimal increase in resource investment) is equal among sites of investment. One powerful notion that arises from production theory as applied to photosynthesis is that of 'resource substitution' – the idea that a given photosynthetic rate can be achieved by many combinations of various 'inputs', which are chiefly water, nitrogen and light. For example, a given photosynthetic rate can be achieved using either high photosynthetic capacity (high N) and low g_s (low water loss) or low N and high water loss (Farquhar et al. 2002; Miller 2002; Wright et al. 2003). Prentice et al. (2014) suggested that the trade-off implicit in resource substitution might be optimized by adjusting the ratio of c_i to c_a so as to minimize the summed costs of transpiration and carboxylation (a weighted sum of E and V_{cmax}), expressed relative to assimilation rate:

$$\frac{\partial}{\partial (c_i/c_a)}\left[\frac{a'E + b'V_{cmax}}{A}\right] = 0, \tag{20}$$

which leads to an expression for an optimal ratio of c_i/c_a. However, by treating the unit costs of E and V_{cmax} (a' and b', respectively) as invariant with respect to c_i/c_a, this solution ignores the trade-off between water (E) and nitrogen (V_{cmax}) acquisition: the plant cannot simply choose to arbitrarily increase E and/or V_{cmax}, but instead must adjust the balance between nitrogen capture and water capture and transport by partitioning a finite supply of carbon among roots, stems and leaves. For example, increasing V_{cmax} would require a preferential shift in carbon partitioning to roots, and therefore away from stems and leaves, which would in turn influence E. In other words, the products $a'E$ and $b'V_{cmax}$ in Equation 20 appear to be functions of gas exchange parameters (E and V_{cmax}), but in fact they are functions of the magnitude of investments in various carbon pools. Prentice et al. (2014) did recognize that the water cost (a') involves whole-plant hydraulic architecture, but they did not attempt to formally express the tradeoff between hydraulics and nitrogen uptake. Equation 20 also omits the fundamental role of light in photosynthesis, and of carbon investment in leaves for light capture.

To capture all of these interacting tradeoffs, we must account for the limitation of canopy net photosynthesis, A_t (mol C yr^{-1}), by multiple inputs (denoted X_k; at a minimum, these include water, nitrogen and light, although this can be expanded to include other resources that limit photosynthesis), and for

the dependence of those inputs, in turn, on the amount of carbon invested in various functional carbon pools (denoted C_j; e.g., fine root carbon or leaf carbon; mol C). Each carbon pool incurs operating costs (denoted β_j; mol C mol^{-1}C yr^{-1}), which represent maintenance respiration as well as the carbon inputs needed to offset senescence. We define growth, G_{plant} (mol C yr^{-1}), as the net rate of carbon accumulation, or $G_{plant} = (1 - f_g) \cdot (A_t - \Sigma_j \beta_j \cdot C_j)$, where f_g (dimensionless) is a growth respiration fraction that is independent of the C_j. We also define the *marginal carbon revenue of carbon* for pool C_j, or more succinctly the *carbon profit*, as $\omega_j = \partial G_{plant}/\partial C_j$ (mol C mol^{-1}C yr^{-1}), or

$$\omega_j = \left(1 - f_g\right)\left(\sum_k \frac{\partial A_t}{\partial X_k}\frac{\partial X_k}{\partial C_j} - \beta_j\right). \tag{21}$$

Buckley & Roberts (2006b) showed that G_{plant} is maximized with respect to variations in carbon partitioning when ω_j is equal for all carbon pools:

$$\sum_k \left(\frac{\partial A_t/\partial X_k}{\partial C_j/\partial X_k}\right) - \beta_j = \Omega \, for \, all \, j, \tag{22}$$

where Ω is invariant among carbon pools, just as μ_w is invariant over time in Equation 1. Below we provide discussion of several examples that illustrate the implications of Equation 22 for optimal changes in water loss and μ_w during acclimation of whole plant structure to sustained changes in environmental conditions, or during growth.

## Optimal response of water loss to a sustained increase in c_a

To see how Equation 22 can be used to understand optimal changes in g_s, μ_w and water transport capacity at different time scales, consider the sequence of 'events' that would take place following a sustained increase in atmospheric CO_2 concentration, c_a, in a plant adhering to the theory expressed by Equation 22. The reader may find it helpful to refer to Fig. 4, which shows inter-relationships among key parameters of gas exchange, water transport and economics, relevant to this example. The first event following a step increase in c_a is a decline in $\partial A/\partial E$ (Figure S2). What is the optimal response for this plant, both in the immediate time frame of dynamic stomatal responses and in the longer time frame of structural acclimation? Firstly, the plant should reduce g_s to return $\partial A/\partial E$ to its initial value. This reduces E, thereby increasing ψ_{leaf} and decreasing the soil-to-leaf water potential gradient. The reduced gradient for water transport increases the marginal carbon cost of water, $\partial C_j/\partial E_t$, for all carbon pools that are involved in water uptake and transport (Equations S16–S18, Supporting Information File S1), which in turn reduces the carbon profit for each pool, by Equation 21. However, the size of the resulting changes in the marginal C cost of water, and thus in carbon profit, will generally differ among carbon pools, because that cost scales with different factors for each pool (cf. Equations S16–S18). Therefore, a sustained increase in c_a will likely create a divergence in carbon profit among
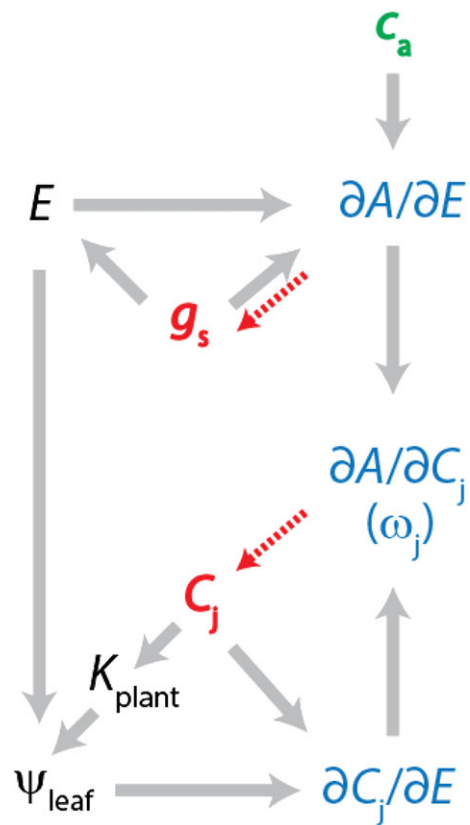
Figure 4. Diagram illustrating inter-relationships among parameters involved in the optimal response of water loss to a sustained increase in atmospheric CO_2 (c_a). Grey arrows show the directions of direct dependencies among parameters, and dashed red arrows show 'economic feedback' from economic parameters to control parameters (stomatal conductance, g_s; the carbon content of a particular pool, C_j). $\partial A/\partial E$ is the marginal carbon revenue of water, $\partial A/\partial C_j$ (ω_j) is the marginal carbon profit of carbon for pool C_j, and $\partial C_j/\partial E$ is the marginal carbon cost of water for pool C_j. Other symbols are defined in Table 1.

pools. This violates the condition of optimality specified by Equation 22. To re-establish invariance of carbon profit, shifts in carbon partitioning are required.

The resulting changes in plant structure, and in the balance between the supplies of water and other photosynthetic inputs to the canopy, may lead to long-term changes in μ_w, but the direction and magnitude of such changes are difficult to deduce without the aid of a model. Figure 5 shows a prediction of the optimal long-term responses to elevated c_a, using a numerical model of tree growth (DESPOT, Buckley & Roberts 2006a) in which carbon partitioning is continuously adjusted during growth to maximize growth rate – that is, DESPOT implements Equation 22 numerically. (The model is described in greater detail in Supporting Information File S1, and we merely summarize its structure here. DESPOT computes the amount of each photosynthetic input [transpired water, photosynthetic N and absorbed light] available in the canopy using process descriptions for capture and delivery of this inputs, which depend on the amounts of C invested in each of various functional pools [fine roots, leaves, etc.], and then uses a biochemical photosynthesis model to compute carbon gain. C is partitioned
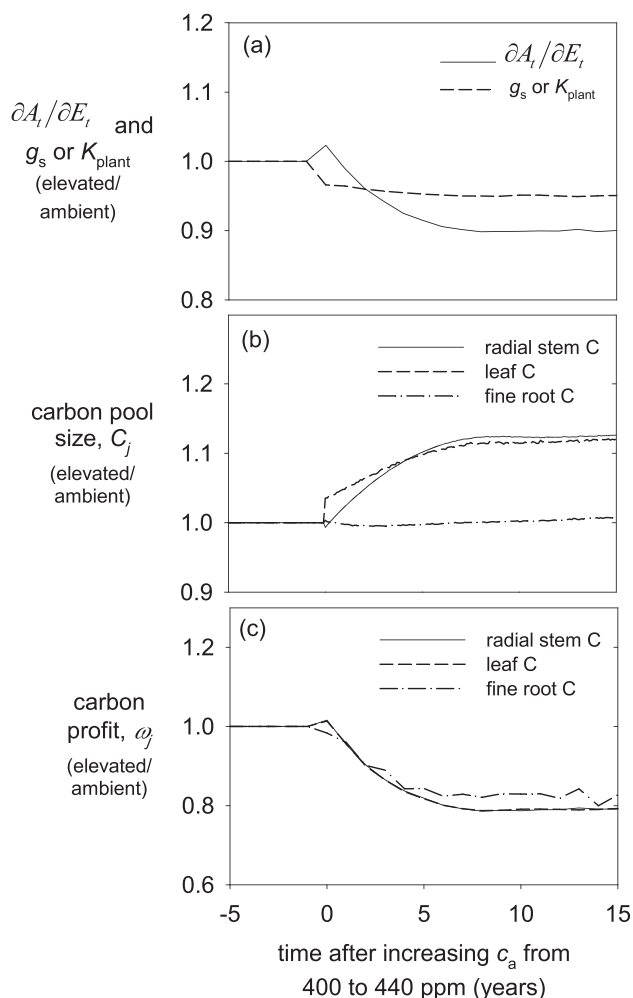
Figure 5. Predictions from the DESPOT model for relative shifts in parameters of plant carbon economy, structure and gas exchange following an increase in c_a from 400 to 440 ppm, for a mature 100-year-old *Pinus contorta* tree. (a) Changes in marginal carbon revenue of water ($\partial A_t/\partial E_t$, solid line) and either stomatal conductance or plant hydraulic conductance per unit leaf area (g_s or K_{plant}, respectively; dashed line), expressed relative to their values in a control simulation in which c_a was unchanged; relative changes in g_s and K_{plant} are identical because the soil-to leaf water potential gradient and evaporative demand were assumed constant. (b) Changes in carbon partitioning among carbon pools: stem carbon (solid line), leaf carbon (dashed line) or fine root carbon (dash-dot line), expressed relative to a control simulation. (c) Changes in carbon profit (ω_j) among carbon pools, expressed relative to the control simulation.

among the C pools in each step so as to maximize C gain in the next time step. Because μ_w is a function of leaf-level gas exchange parameters, which in turn are defined by quantities of photosynthetic input use, μ_w can be computed directly from DESPOT's predictions.) DESPOT predicts a sustained decrease in $\partial A/\partial E$ and in both g_s and leaf-specific hydraulic conductance in the years after a step increase in c_a (Fig. 5a); these changes result from a shift in carbon partitioning at the whole plant scale (Fig. 5b), which in turn is driven by changes in carbon profit (Fig. 5c). Thus, the often-reported decline in g_s in CO_2 enrichment experiments (e.g., Ainsworth & Long

2005; Ainsworth & Rogers 2007) is in fact an emergent property of optimal changes in carbon partitioning at the whole-plant scale, distinct from the optimal diurnal response predicted by the CF theory (Buckley 2008).

Effects of sustained changes in other environmental parameters

As a second example to show how μ_w should vary depending on shifts in biomass allocation in response to the environment, we used Equation 22 (as implemented by the DESPOT model) to predict long-term changes in water loss, hydraulic conductance and the target value μ_w for the marginal carbon revenue of water, in response to sustained, long-term changes in several other environmental parameters (Fig. 6). In each of these simulations, the environmental change was imposed at an age of 100 years, after the tree had stopped growing vertically, and parameters were recorded immediately before the change and again 10 years after the change. To facilitate comparison between simulations, we chose the direction of change for each environmental parameter that led to a long-term increase in water loss. The model predicted a sustained increase in water loss with decreasing c_a and soil N availability, or with increasing evaporative demand, light availability or water availability. The model also predicted an increase in K_{plant} in most cases, although when water supply was increased or evaporative
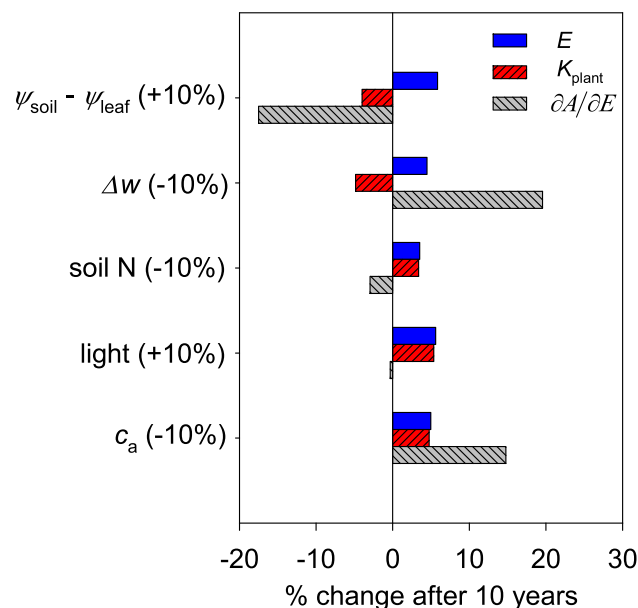
Figure 6. Predicted long-term shifts in whole-plant water loss (E) and hydraulic conductance per unit leaf area (K_{plant}), and the marginal carbon revenue of water ($\partial A/\partial E$), 10 years after changes in environmental variables are imposed on a mature 100-year-old *Pinus contorta* tree simulated by the DESPOT model. The direction of each environmental change was chosen to produce an increase in plant water loss: 10% increase in the soil to leaf water potential gradient ($\psi_{soil} - \psi_{leaf}$), a 10% decrease in evaporative demand (Δw) or soil nitrogen availability, a 10% increase in the intensity of light above the canopy, or a 10% decrease in atmospheric CO_2 concentration (c_a).

demand was decreased, the model predicted a decrease in K_{plant}. Finally, μ_w was predicted to change in the opposite direction from water loss in some cases (decreased soil and increased light availability or water availability), but in the same direction in others (decreased c_a or increased Δw).

One important implication of these results is that the optimal rate of water loss depends strongly on many environmental conditions – not only those that are obviously and directly linked to water transport or transpiration. For example, the optimal structural acclimation to a decline in soil nitrogen availability includes increased water transport capacity and increased water loss. This is an example of resource substitution (Bloom et al. 1985; Buckley & Roberts 2006b; Field et al. 1983; Miller et al. 2001; Schulze et al. 1998; Smith & Huston 1989), of water for nitrogen in this case. Another implication is that $\partial A / \partial E$ and water loss are not always inversely associated with one another. These predictions also depend on the initial state of the plant and its surroundings (in the example here, they apply to a mature tree), so they may differ in other circumstances. The most important message is that optimal water loss is not determined solely by leaf-level gas exchange properties, but also by features of the whole plant, including the interacting effects of carbon partitioning on the supplies of each photosynthetic input to the canopy.

Effect of height growth

Height growth is different from sustained changes in environmental parameters in that its initial, direct manifestation is in plant structure rather than in leaf gas exchange. While it is true that height growth is typically accompanied by some decline in ψ_{leaf} and g_s, and that the latter may, mechanistically, be a response to the former, the empirical fact of a decline in either g_s or ψ_{leaf} with height cannot be taken as a given when attempting to predict what stomata *should* do, and how ψ_{leaf} should vary in consequence. Therefore, we must first ask the question, how does height growth change plant carbon economy if we assume there is no change in g_s? The answer is that the marginal stem-carbon cost of water increases and the marginal root and leaf-carbon costs are unaffected (see Equations S17 and S18 in Supporting Information File S1); therefore, the stem-carbon profit (ω_s) decreases and the root and leaf carbon profits (ω_r and ω_l, respectively) are unchanged (cf. Equation 21). To interpret this, we must consider the implications of the underlying assumption, namely that g_s and ψ_{leaf} are unchanged. To achieve constant ψ_{leaf} as height increases, given constant g_s and thus water loss, requires a compensatory increase in K_{plant}, which in turn requires a large investment of carbon into stems to increase the ratio of sapwood area to leaf area. However, this leads to a decrease in the marginal profit for stem carbon relative to the other pools, which means *this pattern is not optimal*. To prevent ω_s from decreasing, it is necessary instead to permit either g_s or ψ_{leaf}, or both, to decline during height growth.

This contrasts with the classical 'pipe model theory', which holds that the decline in hydraulic conductance caused by increasing transport distance during height growth must be counteracted by increased cross-sectional area of conducting stem xylem in order to prevent decline in g_s (Shinozaki et al. 1964). However, Buckley & Roberts (2006b) showed that declining g_s with height may in fact be optimal, as a collateral effect of profitable investments in leaf production; in other words, it would be inefficient to enhance sapwood production in this case, because other uses of carbon, such as capturing more light or nitrogen via increased leaf or fine root production, would be more effective for sustaining canopy photosynthesis. To understand the rationale behind this idea, consider that the pipe-model view ignores the main reason that plants grow vertically, which is to access more light. If height growth is indeed accompanied by greater light availability, this will decrease the marginal carbon cost of light, increasing the profitability of investment in leaves. Greater leaf production, in turn, will increase total water loss for a given stomatal conductance; in order to prevent this increase in total water loss and the resulting decline in ψ_{leaf}, a decline in g_s is required. Declining g_s is in fact commonly observed during height growth, but is usually interpreted as a detrimental impact of hydraulic limitations (e.g., Delzon et al. 2004; McDowell et al. 2002; Phillips et al. 2003; Ryan & Yoder 1997; Ryan et al. 2006). Thus, if the economic pressure driving rapid height growth is strong enough and the suppression of stem-carbon profit by height growth is strong enough, then leaf area production may outpace sapwood production. This is consistent with the fact that many observations of decreasing ratios of sapwood area to leaf area with height have been in shade-intolerant pioneer taxa such as *Eucalyptus delegatensis* (e.g., Mokany et al., 2003), *Pinus contorta* (Dean & Long 1986) and *E. regnans* (Dunn & Connor 1993; Vertessy et al. 1995; Watson et al. 1999).

Conclusions (Question #3)

When the optimisation principle is extended to the whole plant (Equation 22), long-term variations in μ_w and g_s are seen to emerge from biophysical constraints that link whole-plant carbon partitioning with the capture and delivery of photosynthetic inputs. This contrasts with the perception that μ_w has no biophysical basis and therefore cannot be independently inferred (Katul et al. 2010) – μ_w can in fact be inferred, but only by considering issues far beyond the leaf-scale scope of the original stomatal optimisation problem (Buckley & Schymanski 2014; Medlyn et al. 2013). The framework outlined above predicts, for example, that observed declines in g_s and transpiration rate during height growth or during long-term acclimation to elevated CO_2 are in fact emergent properties of optimal carbon allocation, rather than detrimental physiological constraints. It also predicts resource substitution – that is, an increase in water loss – as an acclimatory response to soil nitrogen depletion, consistent with many field data (e.g., Field et al. 1983; Miller et al. 2001; Prentice et al. 2014).

SUMMARY: DIRECTIONS FOR FUTURE RESEARCH

Identifying optimal plant water loss requires thoughtful attention to scale – stomatal responses, intermittent soil drought and structural acclimation all occur at very different temporal

and organization scales. We therefore reviewed work on three key questions: how to apply the Cowan-Farquhar solution at diurnal time scales, when should μ_w be treated as a constant or as a variable, and how optimal water loss should vary during sustained environmental change and growth. Based on our analysis of the current state of understanding in these areas, we suggest that continuing work on optimal water loss should focus on four challenges (Table 3). Firstly, application of the CF solution usually relies on analytical approximations that assume infinite mesophyll and boundary layer conductances (g_m and g_b, respectively), whereas it is now well understood that g_m is often on the same order as g_s and may respond dynamically to environmental conditions, and it has long been known that g_b is often small in natural conditions. Thus, we recommend detailed assessment of the impact of finite g_m and g_b on the relationship between optimal g_s and that predicted by commonly used analytical solutions. This is particularly important in light of evidence that wind speeds are declining globally (McVicar et al. 2012; Roderick et al. 2007). Secondly, the equimarginal concept as developed earlier by Givnish (1986) and Bloom et al. (1985) and formalized here as Equation 22 offers promise as a rigorous, integrative theoretical framework for predicting long-term dynamics and species differences in optimal water loss. Continuing work should expand predictions from this framework and focus on experimental or observational tests of those predictions. Thirdly, as discussed below, the predictions from Equation 22 for optimal long-term trends in water loss depend on the acclimatory plasticity of diurnal minimum leaf water potential. Research should focus on understanding the mechanistic basis of that plasticity and characterizing its variation across species. Fourthly, the optimisation framework as discussed here focuses exclusively on maximizing carbon gain – both at the leaf level and at the whole-plant level. Theoretical and modelling work should focus on extending this framework to encompass delayed costs and benefits. The latter two challenges arise from considerations discussed below.

Acclimatory plasticity of leaf water potential

Predicting the optimal response of g_s and ψ_{leaf} to variations in carbon partitioning is difficult, because C partitioning directly affects K_{plant}, not g_s or ψ_{leaf}, and a given change in K_{plant} can be matched either by a change in g_s but not ψ_{leaf}, a change in ψ_{leaf} but not g_s, or a change in both g_s and ψ_{leaf}. In other words, there is an additional degree of freedom in the system that is not constrained by C partitioning. However, there is a physical limit to ψ_{leaf} (the threshold causing runaway conductivity loss), but not for g_s (although g_s is theoretically constrained below a 'packing limit' in which every epidermal cell is a guard cell, that limit is rarely approached in nature). Carbon invested in water transport would always be used most efficiently if ψ_{leaf} were as negative as the plant could tolerate without necessarily reducing g_s, because a given C investment yields the greatest flow rate when ψ_{leaf} is most negative (i.e., when the water transport gradient is largest). Thus, setting ψ_{leaf} at the cavitation threshold represents a 'global' optimum. This is the rationale behind DESPOT's assumption that the prevailing diurnal range of ψ_{leaf} is unchanged during long-term changes in plant structure. A counter-argument to this rationale is that many plants in fact permit the diurnal range of ψ_{leaf} to shift during drought or during height growth. However, there is no evidence that such behaviour is adaptively beneficial; instead, it probably arises at least partly from mechanistic constraints on stomatal regulation. To account for such a constraint within optimisation theory, one would need to understand the underlying mechanism well enough to represent it mechanistically within the process framework to which the equimarginal principle (Equation 22) is applied. Buckley (2008) showed how this might be accomplished, in principle, using a feedback-based model for g_s to permit concurrent changes in both g_s and ψ_{leaf} during simulated acclimation to elevated CO_2. Properly accounting for acclimatory plasticity in ψ_{leaf} may be important for understanding and predicting how plants acclimate to sustained changes in environmental conditions or structure. For example, most experiments have found reduced g_s following sustained elevation of c_a (e.g., Ainsworth & Long 2005; Leakey et al. 2009; Medlyn et al. 2001), but the resulting changes in ψ_{leaf} vary widely, and a literature review by Wullschleger et al. (2002) found an increase in ψ_{leaf} in just over half of reported studies (19 of 32), and no change in ψ_{leaf} in nearly a third of cases (10 of 32). The mechanistic and optimal approaches to modelling g_s therefore converge in this context

Table 3. List of conclusions and hypotheses concerning optimal stomatal conductance, and suggested actions or tests. CF = Cowan-Farquhar; g_s = stomatal conductance; $\partial A/\partial E$ = marginal carbon revenue of water; ψ = water potential; PPFD = photosynthetic photon flux density.

Conclusion/hypothesis	Action/test
1. analytical solutions do not accurately represent the CF theory	seek analytical solutions that permit finite and variable boundary layer and mesophyll conductances
2. cyclical diurnal variation in hydraulic conductance may help or hinder maintenance of constant $\partial A/\partial E$	quantify diurnal dynamics of $\partial A/\partial E$ in relation to natural dynamics of hydraulic conductance
3. decline in g_s during height growth may arise due to the influence of optimal acclimation of carbon partitioning to leaves, stems and roots on stomatal optimization	quantify the relationships between g_s decline during height growth, biomass allocation and carbon economy
4. long-term change in g_s under CO_2 enrichment emerges from optimal structural acclimation, not the diurnal response	compare magnitude of long-term and diurnal responses to enrichment
5. optimal g_s increases following sustained increases in soil-leaf ψ gradient or PPFD, or sustained decreases in evaporative demand or soil N	quantify long-term changes in g_s following sustained changes in these environmental conditions

because neither can fully explain long-term behaviour until mechanistic constraints on acclimation of ψ_{leaf} are better understood. Likewise, continued plant growth may lead to soil water depletion and thus decline in ψ_{soil}, particularly in systems such as field crops that do not experience self-thinning, so proper treatment of optimal acclimation of water transport and g_s in such systems may require a formal model of soil water balance as well.

On the need for an even broader view

The link between the marginal revenue and cost of water predicted by Equation 22 arises from assuming that carbon should be partitioned so as to maximize growth rate continuously during growth. However, several insights from the work of Bloom et al. (1985) suggest that an even broader perspective may be required to understand optimal water loss in many cases. Firstly, resources other than carbon, notably nitrogen and water, also influence processes other than photosynthesis, such as seed production and competition with neighbours. Such benefits should be explicitly included by expanding the goal function, which for Equation 22 is simply whole plant net carbon accumulation. Secondly, it is unlikely that maximizing instantaneous growth rate is always an optimal strategy: for example, this predicts that it is inherently preferable for a tree to focus on vertical growth early in its life to compete for light, yet many successful tree species do not behave in this way. Long-run growth may be maximized by a strategy that includes investments that are not immediately profitable, in plants just as in business. Indeed, when the light-capture incentive that drives for height growth is artificially suppressed in the DES-POT model, stand-level productivity actually increases (Buckley & Roberts 2006b). It may be possible to accommodate such delayed returns, as well as non-photosynthetic benefits of N, water and other resources, within the growth-maximizing perspective, but to our knowledge this has not yet been attempted. Accounting for benefits that do not accrue instantaneously may benefit from a game-theory perspective, analogous to those applied by Cowan (1982); Mäkelä et al. (1996) and Lu et al. (2016) to the problem of variations in μ_{w} during intermittent drought.

In closing, we suggest that the most fruitful path forward in the field of 'stomatal optimization theory' necessarily entails difficult approaches such as the game-theory and whole-plant ideas mentioned earlier. Plant life is inordinately complex, and there is more to be gained by embracing that complexity with the full power of modern computing than by continuing the age-old attempt to reduce plant function in a changing environment to a few simple analytical equations – however strongly such equations may appeal to our intuition.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Supporting Information File S1
Figure S1. Simulated variation in the ratio of the relative sensitivities of $\partial A/\partial E$ and E to g_s, or $(\partial \ln(\partial A/\partial E)/g_s)/(\partial \ln E/\partial g_s)$, in relation to intercellular CO_2.
Figure S2. Response of $\partial A/\partial E$ to atmospheric CO_2.