Optimizing stomatal conductance for maximum carbon gain under water stress: a meta-analysis across plant functional types and climates

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Summary

1. Quantification of stomatal responses to environmental variables, in particular to soil water status, is needed to model carbon and water exchange rates between plants and the atmosphere.

2. Models based on stomatal optimality theory successfully describe leaf gas exchange under different environmental conditions, but the effects of water availability on the key optimization parameter [the marginal water use efficiency (WUE), $\lambda = \partial A / \partial E$] has resisted complete theoretical treatment. Building on previous optimal leaf gas exchange models, we developed an analytical equation to estimate λ from gas exchange observations along gradients of soil water availability. This expression was then used in a meta-analysis of about 50 species to investigate patterns of variation in λ.

3. Assuming that cuticular water losses are negligible λ increases under mild water stress but decreases when severe water stress limits photosynthesis. When cuticular conductance is considered, however, λ increases monotonically with increasing water stress, in agreement with previous theoretical predictions. Moreover, the shape of these response curves to soil water availability changes with plant functional type and climatic conditions. In general, λ is lower in species grown in dry climates, indicating lower marginal WUE.

4. The proposed parameterization provides a framework to assess the responses of leaf gas exchange to changes in water availability. Moreover, the model can be extended to scale leaf-level fluxes to the canopy and ecosystem level.

Key-words: elevated atmospheric CO_2, optimal water use, photosynthesis model, stomatal conductance, water stress, water use efficiency

Introduction

Stomatal adjustment allows plants to occupy habitats with fluctuating environmental states by permitting dynamic regulation of CO_2 and H_2O diffusion into and out of leaves. Open stomata facilitate the diffusion of CO_2 from the atmosphere into the substomatal cavity. Simultaneously, water is lost by transpiration, which is driven by the leaf-to-air gradient in water vapour concentration. If soil water becomes scarce, plant water status worsens, leading to cascading effects that can be severely adverse at both the leaf and plant scales (Hsiao 1973; Porporato *et al.* 2001). To reduce these risks, plants under water deficit reduce transpirational water losses by reducing stomatal conductance. Moreover, water stress hinders leaf internal transport of CO_2, enzyme activity and hence photosynthetic capacity (Ghannoum 2009; Lawlor & Tezara 2009). These so-called internal (metabolic and diffusive) limitations become predominant relative to stomatal limitation as water stress further increases (Du *et al.* 1996; Galmes, Medrano & Flexas 2007; Tezara, Driscoll & Lawlor 2008; Ghannoum 2009; Lawlor & Tezara 2009).

The complexity of these stomatal controls and feedbacks on leaf carbon (C) assimilation and water loss poses a major challenge to modelling stomatal and internal limitations on photosynthesis under water stress. Empirical relationships have been used to link photosynthetic rate, stomatal conductance and leaf (or soil) water status (Collatz *et al.* 1991;

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Friend 1995; Leuning 1995; Tuzet, Perrier & Leuning 2003; Daly, Porporato & Rodriguez-Iturbe 2004; Siqueira, Katul & Porporato 2008; Vico & Porporato 2008). More mechanistic models link stomatal aperture to changes in the pressure gradient along the soil–plant–atmosphere continuum or to drought-related chemical signals from roots (Sperry 2000; Dewar 2002; Gao *et al.* 2002; Buckley, Mott & Farquhar 2003). These models, however, require several parameters that are not easily quantified across species.

The stomatal optimality hypothesis represents a viable alternative to detailed mechanistic or empirical models of stomatal responses to the environment. According to optimality theory, stomatal conductance to CO_2 (g_C) is regulated by the plant so as to maximize C assimilation during a finite time interval, subject to a given water availability (Cowan 1977; Farquhar & Sharkey 1982; Hari *et al.* 1986). Over a single day, soil moisture (i.e. the water availability constraint) varies much less than plant-atmosphere fluxes, which fluctuate in response to high frequency environmental variability. As a consequence, the optimization problem is simplified to the condition that the Lagrange multiplier is a constant during a given day (Cowan & Farquhar 1977). Here, we denote this Lagrange multiplier, representing the marginal water use efficiency (WUE; i.e. the change in photosynthesis per unit of change in transpiration), as λ, thus adopting the notation by Hari *et al.* (1986). Note that this notation is the inverse of the definition used by Cowan & Farquhar (1977). Once λ is defined, the functional relationship between stomatal conductance and environmental conditions is readily obtained.

Several studies have confirmed that λ is nearly constant during a day (Hall & Schulze 1980; Fites & Teskey 1988; Berninger & Hari 1993; Hari, Mäkelä & Pohja 2000; but see Thomas, Eamus & Bell 1999), whereas optimality can only be achieved at time-scales of soil water changes if the Lagrange multiplier λ evolves in time (Cowan 1982, 1986; Mäkelä, Berninger & Hari 1996; Schymanski *et al.* 2009). During a single dry-down event, changes in λ can be calculated by using the initial amount of available soil water and expected duration of the dry period. Both initial soil water and duration of the dry-down are, however, highly variable quantities, depending on the stochasticity of rainfall (Laio *et al.* 2001; Porporato, Daly & Rodriguez-Iturbe 2004). This makes the full analytical treatment of the optimization problem extremely complex (Cowan 1986). When the stochasticity of rainfall depths is neglected (Cowan 1982; Mäkelä, Berninger & Hari 1996), λ can be analytically shown to increase in time during a dry-down because of decreasing soil and plant water availability (solid line in Fig. 1). As higher λ implies decreased stomatal conductance (when other environmental parameters are held constant), the predicted pattern is consistent with the commonly observed decrease in stomatal conductance at low soil moisture. Contrary to these expectations, empirical estimates of λ often show a decrease under severe water stress (Farquhar, Schulze & Kuppers 1980b; Grieu, Guehl & Aussenac 1988) (data points and dashed lines in Fig. 1).
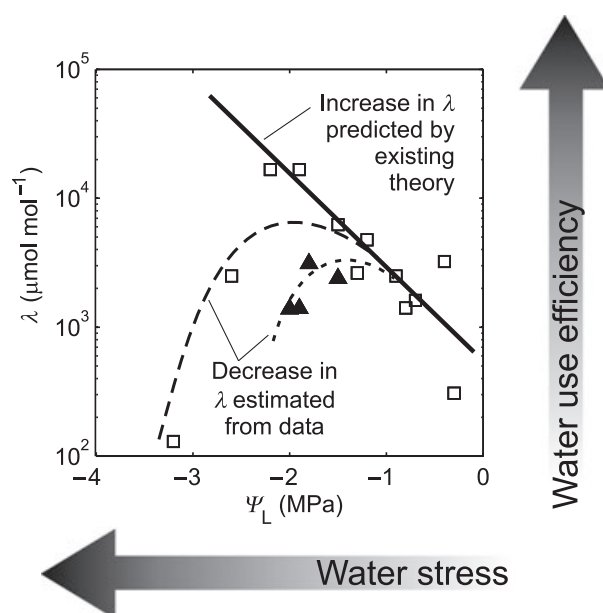

Fig. 1. Qualitative dependence of the marginal water use efficiency, λ, on leaf water potential, ψ_L. Theoretical considerations suggest a monotonic increase of λ with water stress (solid line), while a decrease is apparent from some estimates under severe stress (▲ and dotted line, estimates for *Corylus avellana* from Farquhar, Schulze & Kuppers 1980b; □ and dashed line, estimates for *Pseudotzuga* species from Grieu, Guehl & Aussenac 1988). Lines are qualitatively drawn to guide the eye.

The objective of this work is to assess how λ changes as a function of water availability for the plant, and discuss inconsistencies between current optimization theories and experimental evidence. To study the effects of water stress on λ, an existing simplified model of leaf gas exchange that optimizes stomatal conductance to achieve maximal C gain is revised. Values of λ are estimated from gas exchange data for nearly 50 species exposed to different levels of water stress and atmospheric CO_2. Using this data base, a meta-analysis is performed on the response patterns of leaf gas exchange to combined water stress and CO_2 availability.

Materials and methods

An analytical leaf gas exchange model is described and used to express λ as a function of measurable quantities (Section 'Theory'). This expression is then used to estimate λ from data available in the literature for a variety of species under worsening water conditions (Section 'Data analysis').

THEORY

The leaf gas exchange model we employ is based on previous studies (Hari *et al.* 1986; Lloyd & Farquhar 1994; Katul, Palmroth & Oren 2009; Katul, Manzoni, Palmroth and Oren, 2010) and thus will be only briefly reviewed here, highlighting the approximations necessary to address the effects of plant water status on leaf gas exchange. The model accounts for water vapour and CO_2 fluxes between a leaf and

the atmosphere based on molecular diffusion (Section 'Leaf-scale transpiration'), coupled with a photosynthesis model defining CO_2 demand by the photosynthetic biochemical machinery (Section 'Leaf-scale CO_2 fluxes'). In this framework, an equation relating stomatal conductance to environmental and leaf biochemical parameters is derived based on the C gain maximization principle (Section 'Optimal leaf gas exchange equations'). Finally, the stomatal model is inverted to estimate the value of λ from gas exchange measurements (Section 'Estimation of the marginal water use efficiency'). Symbols are explained in Table 1.

Leaf-scale transpiration

The mass transfer of water vapour (E) between the leaf and the atmosphere is described by Fickian diffusion (Farquhar & Sharkey 1982)

$$E = g_W D, \qquad \text{eqn 1}$$

where D is the vapour pressure deficit (expressed as molar ratio) that approximates the external driving force for transpiration (i.e. the difference between water vapour concentration in the bulk atmosphere and that of the stomatal cavity), and g_W is the leaf conductance to water vapour, resulting from a leaf-controlled stomatal component and the residual permeability that cannot be directly regulated by the plant (Larcher 2003). Accordingly, $g_W = a g_C + g_{W,0}$, where $a = 1.6$ is the ratio between the diffusivities of water vapour and CO_2 in air, g_C is the stomatal conductance to CO_2 and $g_{W,0}$ is the cuticular conductance to water vapour. This water loss mainly occurs through the leaf cuticle, which discriminates against CO_2 diffusion (Boyer, Wong & Farquhar 1997; Larcher 2003). The leaf boundary layer conductance is assumed to be much larger than g_W, as is typically the case in the gas exchange measurements used in this study.

Leaf-scale CO_2 fluxes

The net mass transfer of CO_2 (A) across stomata is also described by Fickian diffusion (as in eqn 1),

$$A = g_C(c_a - c_i), \qquad \text{eqn 2}$$

where c_a and c_i are the atmospheric and internal CO_2 concentrations, respectively. Note that CO_2 exchange is not impacted by cuticular conductance (which we assume to be negligible for CO_2). Equation (2) sets the rate of CO_2 supplied by the environment for a given c_i. In turn, c_i results from the balance of the CO_2 supplied by the atmosphere (i.e. eqn 2) and the CO_2 demand by the photosynthetic machinery. The CO_2 demand function is modelled after Katul, Palmroth & Oren (2009) using a linearized form of the Farquhar biochemical model (Farquhar, Caemmerer & Berry 1980a):

$$A = \frac{a_1(\eta c_i - \Gamma^*)}{\eta r c_a + a_2} - R_d, \qquad \text{eqn 3}$$

where a_1 and a_2 can be interpreted as 'macroscopic' kinetic constants (accounting in a lumped way for metabolic capacity and mesophyll conductance, both of which are impacted by water status), R_d is the mitochondrial respiration, and Γ^* the compensation point. In the denominator, c_i is approximated by $r c_a$, where r is the long-term average c_i/c_a ratio. This approximation allows simpler mathematical expressions while retaining the main characteristics of the biochemical demand function, namely the nonlinear dependence of A on c_a, and a variable c_i determined by combining eqns (2) and (3). The kinetic constants a_1 and a_2 are here expressed as generic parameters that, depending on the environmental conditions, might represent C-limited or light-limited kinetics. However,

Table 1. List of symbols

Symbol	Explanation	Units
a	Ratio of H_2O to CO_2 diffusivities, $a = 1.6$	–
a_1	Kinetic constant for photosynthesis (eqn 3)	$\mu mol\ m^{-2}\ s^{-1}$
a_2	Half saturation constant for photosynthesis (eqn 3)	$\mu mol\ mol^{-1}$
β, β_0	Parameters for $\lambda(\psi_L)$ (eqns 9 and 10; Table S1, Supporting information)	MPa^{-2}, MPa^{-1}
c_i	Internal CO_2 concentration	$\mu mol\ mol^{-1}$
c_a, c_a^*	Atmospheric (actual and reference) CO_2 concentrations	$\mu mol\ mol^{-1}$
$g_C, g_{C,WW}$	Stomatal conductance to CO_2	$mol\ m^{-2}\ s^{-1}$
$g_W, g_{W,WW}$	Total conductance to H_2O	$mol\ m^{-2}\ s^{-1}$
$g_{W,0}$	Cuticular conductance to H_2O	$mol\ m^{-2}\ s^{-1}$
k	Carboxylation efficiency	$mol\ m^{-2}\ s^{-1}$
r	Long-term mean c_i/c_a ratio (eqn 3)	–
R_d	Mitochondrial respiration	$\mu mol\ m^{-2}\ s^{-1}$
A, A_{WW}	Net CO_2 assimilation	$\mu mol\ m^{-2}\ s^{-1}$
D	Vapour pressure deficit	$mol\ mol^{-1}$
E	Rate of transpiration	$mol\ m^{-2}\ s^{-1}$
η	Efficiency of the CO_2 pump in C4 species	–
Γ^*	Compensation point	$\mu mol\ mol^{-1}$
λ, λ^*	Marginal water use efficiency (actual and at $c_a = c_a^*$)	$\mu mol\ mol^{-1}$
λ_{WW}^*	Marginal water use efficiency in well-watered conditions at $c_a = c_a^*$ (eqn 10, Table S1)	$\mu mol\ mol^{-1}$
λ_{max}^*	Maximum marginal water use efficiency at $c_a = c_a^*$ (eqn 9, Table S1)	$\mu mol\ mol^{-1}$
ψ_L	Leaf water potential	MPa
$\psi_{L,max}$	Leaf water potential at maximum λ (eqn 9, Table S1)	MPa
ψ_S	Soil water potential	MPa

WW, well-watered conditions.

in what follows, $a_1 = V_{c,\max}$ and a_2 is the half saturation constant for carboxylation, because all the gas exchange data used in the meta-analysis have been acquired under saturating light. For the sake of simplicity, we apply the same biochemical model to both C3 and C4 species, with different values of Γ^* and η. The latter represents the effect of the CO_2 concentration mechanism: in C3 species $\eta = 1$ (i.e. no CO_2 pump), while in C4 species $\eta > 1$. For notational simplicity, we also group the kinetic parameters in eqn (3) and obtain $A = k(\eta c_i - \Gamma^*) - R_d$, where $k = a_1(\eta r c_a + a_2)^{-1}$ is the carboxylation efficiency (Lloyd 1991), which may decrease under low water potentials due to reduced metabolic rates.

Optimal leaf gas exchange equations

We can now derive expressions of optimal stomatal conductance and photosynthesis under varying water availability. For simplicity, we assume that changes in soil water are the only source of variability in gas exchange (i.e. light and vapour pressure deficit are maintained nearly constant during the soil dry-down), and that g_C is the main control variable for gas exchange (i.e. we neglect direct effects of cavitation on g_C). As explained in Appendix S1 (Supporting information), these assumptions enable us to treat ψ_L (which is often measured) as a proxy of the root-zone integrated ψ_S (seldom measured), and to use equations that are mathematically equivalent to ones used under well-watered conditions.

Based on these assumptions, the condition for maximizing C uptake for a given water availability can be expressed through the control variable g_C as (Hari *et al.* 1986; Katul, Palmroth & Oren 2009),

$$\frac{\partial A}{\partial g_C} - \lambda \frac{\partial E}{\partial g_C} = 0, \qquad \text{eqn 4}$$

where λ is the Lagrangian multiplier of the problem, mathematically equivalent to the marginal WUE,

$$\lambda = (\partial A/\partial g_C)/(\partial E/\partial g_C)$$

[we again stress that this definition of λ is consistent with the notation of Hari *et al.* (1986) and Katul, Palmroth & Oren (2009), but is the inverse of the definition used in Cowan & Farquhar (1977)].

To compute the optimal gas exchange rates, we first express A as a function of g_C by combining eqns (2) and (3),

$$A = \frac{g_C[k(\eta c_a - \Gamma^*) - R_d]}{g_C + \eta k}. \qquad \text{eqn 5}$$

Differentiating eqns (1) and (5) the partial derivatives $\partial E/\partial g_C$ and $\partial A/\partial g_C$ are obtained, so that, for a given λ, eqn (4) yields a simple analytical expression for the optimal stomatal conductance as a function of environmental variables (i.e. D and c_a), carboxylation efficiency k, and respiration terms,

$$g_C = k\eta \left(\sqrt{\frac{\eta c_a - \Gamma^* - R_d/k}{\eta a D \lambda}} - 1 \right). \qquad \text{eqn 6}$$

When the dark respiration is neglected and $\eta = 1$ (C3 species), the expression derived by Lloyd (1991) is recovered. When $R_d > 0$ and $\Gamma^* \approx 0$, eqn (6) leads to the formulation obtained by Hari & Mäkelä (2003). If all respiration terms can be neglected, eqn (6) simplifies to the expression reported by Katul, Palmroth & Oren (2009). Combining eqns (5) and (6) also yields

$$A = k\left[\eta c_a - \Gamma^* - \sqrt{\eta a \lambda D(\eta c_a - \Gamma^* - R_d/k)}\right] - R_d. \qquad \text{eqn 7}$$

Our primary goal here is to estimate λ, so eqns (5)–(7) are inverted to provide an analytical expression for λ as a function of measured A and g_C, as explained next.

Estimation of the marginal water use efficiency

In optimal control theory, λ should change in time whenever the constraint to the optimization (in our case water availability) is dynamic (e.g. Luenberger 1979). However, computing analytically the time trajectory of λ is complicated by the stochastic nature of the soil water balance (Cowan 1982, 1986; Mäkelä, Berninger & Hari 1996). Hence, we use the definition of marginal WUE to estimate λ from available photosynthesis and stomatal conductance measurements, assuming quasi-equilibrium conditions. That is, we consider soil moisture constant over the time period of the gas exchange measurement (generally less than an hour). This assumption allows us to estimate a series of values for λ as the soil dries and leaf water potential decreases.

As before, the partial derivatives $\partial E/\partial g_C$ and $\partial A/\partial g_C$ are obtained from eqns (1) and (5). The carboxylation efficiency is expressed as $k(A, g_C)$ by inverting eqn (5), that is, k is estimated as the slope of the linearized $A - c_i$ curve at the intersection with the supply curve. This approximation allows closing the problem by only employing individual gas exchange measurements. More rigorous methods of approximating the carboxylation efficiency require measured $A - c_i$ curves, which are often not available at different water availabilities. The feasibility of the method we use is discussed in Katul, Manzoni, Palmroth and Oren (2010). Based on the above assumptions, λ is computed from the definition of marginal WUE,

$$\lambda = \frac{(A/g_C)^2(1 + R_d/A)}{aD(c_a + R_d/g_C - \Gamma^*/\eta)}$$
$$= \text{WUE}^2 \frac{D(a + g_{W,0}/g_C)^2(1 + R_d/A)}{a(c_a + R_d/g_C - \Gamma^*/\eta)}. \qquad \text{eqn 8}$$

Lloyd *et al.* (1994) estimated λ from gas exchange measurements using a similar methodology, while an analogous expression for λ (without respiration terms) was employed by Katul *et al.* (2010). Equation (8) shows explicitly the linkage between the marginal WUE and the flux-based instantaneous WUE (WUE = A/E). Note that no *a priori* dependence of λ on leaf water status has been assumed or imposed. Instead, changes in A and g_C induced by water availability (the main source of variability in gas exchange rates in this meta-analysis) completely determine the estimated values of λ. Also, hereafter we do not consider measurements where $A < 0$ because no optimal stomatal control can be achieved when respiration exceeds gross C uptake.

In the following, two assumptions regarding the cuticular conductance $g_{W,0}$ are considered: (i) $g_{W,0} = 0$, so that g_C in eqn (8) is equal to the measured stomatal conductance to CO_2, and (ii) $g_{W,0} > 0$, so that g_C is estimated from measured g_W as $g_C = (g_W - g_{W,0})/a$. Because $g_{W,0}$ does not appear in the first equality in eqn (8), these assumptions only affect the interpretation of the gas exchange data, but not the expression used to estimate λ.

DATA ANALYSIS

Equation (8) was used to estimate λ from two sets of gas exchange experiments regarding (i) a single dry-down period (published data)

and (ii) several dry-down periods under field conditions along an atmospheric CO_2 gradient (unpublished data from the Lysimeter CO_2 Gradient experiment, LYCOG; see Fay *et al.* 2009 for details on the experimental setup). We collected data sets including photosynthesis, stomatal conductance and leaf water potential under well-watered to severely water-stressed conditions in about 50 species from a variety of environmental conditions (details and data are provided in Appendix S2, Supporting information).

To estimate λ, eqn (8), other than measured A and g_C, requires estimates for dark respiration and CO_2 compensation point, which were obtained from Givnish (1988) and Leuning (1995), and Lloyd & Farquhar (1994), respectively, and were assumed not to vary with ψ_L. For C4 species, we assumed a fixed efficiency of the CO_2 concentration mechanism, mathematically represented by a factor $\eta \approx 25$ that increases c_i to values occurring at the location of CO_2 fixation (Osmond, Winter & Ziegler 1975; Von Caemmerer 2000). Also, in C4 species, we assumed $\Gamma^* \approx 0$ (Lloyd *et al.* 1994).

Values of water vapour conductance obtained through gas exchange measurements were interpreted according to two contrasting assumptions on cuticular conductance [i.e. negligible cuticular conductance (i) $g_{W,0} = 0$ and (ii) $g_{W,0} > 0$], resulting in two different patterns of estimated $\lambda(\psi_L)$ relationships. Assuming negligible $g_{W,0}$, λ scales as the square of WUE (eqn 8), which in turn generally increases under moderate water stress, but then decreases due to metabolic limitations (Larcher 2003). It is then reasonable to describe this dependence by means of a bell-shaped curve (equivalent to a parabola in the semi-logarithmic plane) for $\lambda(\psi_L)$,

$$\lambda(\psi_L) = \lambda_{max}^* \frac{c_a}{c_a^*} \exp\left[-\beta\left(\psi_L - \psi_{L,max}\right)^2\right], \qquad \text{eqn 9}$$

where $\psi_{L,max}$ (the leaf water potential at maximum λ), λ_{max}^* (the maximum marginal WUE at a reference $c_a = c_a^* = 400$ μmol mol^{-1}) and β [a measure of the peakedness of $\lambda(\psi_L)$] are fitting parameters. Conversely, data sets where the expected decrease in WUE at very negative ψ_L was not apparent (resulting in $\beta < 0$ in eqn 9) were fitted by an exponential function - formally equivalent to the one discussed below.

The second assumption on cuticular conductance (i.e. $g_{W,0} > 0$, resulting in $g_W = ag_C + g_{W,0}$) implies that values of stomatal conductance that are calculated from gas exchange measurements overestimate the conductance to CO_2 (Boyer, Wong & Farquhar 1997). To account for this effect, we set $g_{W,0}$ to 90% of the minimum measured g_W and accordingly corrected g_C in eqn (8). Under this assumption the curvature of $\lambda(\psi_L)$ disappears. A suitable empirical relationship to fit the corrected gas exchange data is

$$\lambda_0(\psi_L) = \lambda_{WW}^* \frac{c_a}{c_a^*} \exp[\beta_0 \psi_L], \qquad \text{eqn 10}$$

where λ_{WW}^* (the marginal WUE in well-watered conditions at $c_a = c_a^* = 400$ μmol mol^{-1}), and the slope parameter β_0 are fitted to the data.

The effect of c_a on λ was assumed linear (Katul *et al.*, 2010) in both cases (eqns 9 and 10). This allows us to define a normalized marginal WUE $\lambda^* = \lambda c_a^*/c_a$ that can be used to compare gas exchange data from different atmospheric conditions.

Results

In this section, the overall patterns of the estimated marginal WUE as a function of plant water status and atmospheric

CO_2 concentration are explored across plant functional types and climate conditions. We then show an example of a potential application of the model to describe leaf gas exchange under water stress and contrast these findings with model runs in which λ is assumed to be constant.

VARIABILITY OF λ WITH WATER STATUS AND c_a

An example of the variability of λ with leaf water potential estimated using eqn (8) is shown in Fig. 2. Under the assumption that $g_{W,0} = 0$, λ shows a concave downward pattern with a maximum around $\psi_L = -5$ MPa (filled symbols). In contrast, when data are interpreted assuming $g_{W,0} > 0$, λ increases monotonically with increasing water stress (open symbols). This change is because of the decreased values of stomatal conductance to CO_2 when ψ_L is low and $g_{W,0} > 0$. Because g_C appears in the denominator of eqn (8), this results in increased λ at very negative potentials.

Most of the other analysed data sets follow similar patterns, with a nonlinear dependence of λ on ψ_L if $g_{W,0} = 0$ and a linear increase of λ with water stress if $g_{W,0} > 0$. Overall, eqn (9) (assuming $g_{W,0} = 0$) and eqn (10) ($g_{W,0} > 0$) capture most of the variability in the relationship between λ and ψ_L (Table S1), although the values of the different parameters are highly variable among species. These results are summarized in Fig. 3, where the normalization of both marginal WUE and leaf water potential cause all the data to collapse on a single parabola (Fig. 3a; $g_{W,0} = 0$) and a single line (Fig. 3b; $g_{W,0} > 0$) on the semi-logarithmic plane. Few data sets (mainly those limited to a small range in ψ_L) do not show the full nonlinear pattern in λ assuming $g_{W,0} = 0$. These data can be described by an exponential function
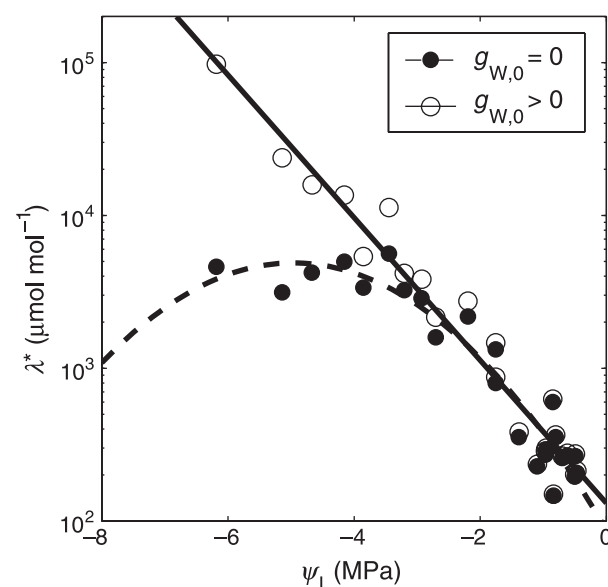

Fig. 2. Example of variations in λ^* (marginal water use efficiency at $c_a^* = 400$ μmol mol^{-1}) with leaf water status when cuticular conductance is neglected ($g_{W,0} = 0$) or accounted for ($g_{W,0} > 0$). Data for *Artemisia tridentata* are from Delucia *et al.* (1989); regression statistics are reported in Table S1 (Supporting information).
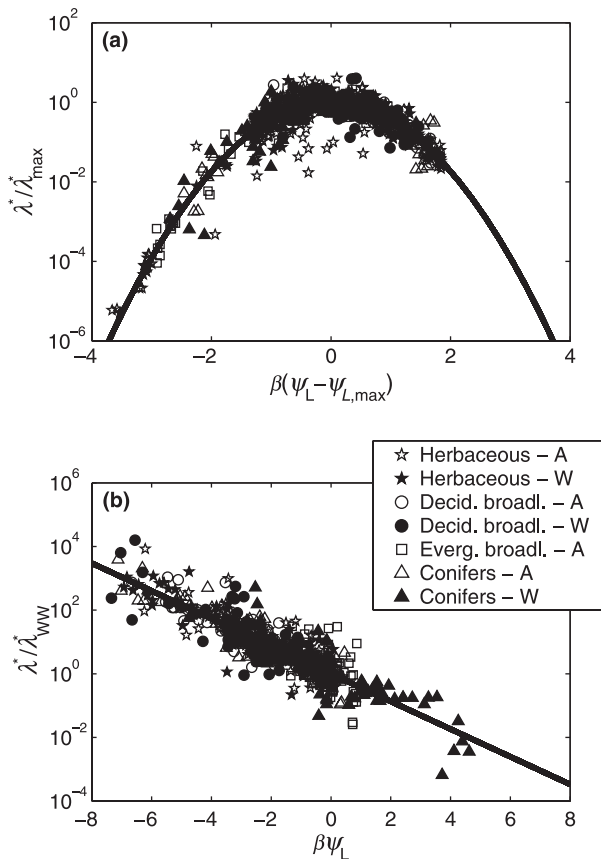
Fig. 3. Normalized marginal water use efficiency as a function of normalized leaf water potential ψ_L, when cuticular conductance is either (a) neglected ($g_{W,0} = 0$, eqn 9) or (b) accounted for ($g_{W,0} > 0$, eqn 10). In (a), data sets where $\beta < 0$ are not shown; in the legend, A indicates arid, semiarid, and Mediterranean climates, W refers to wet and mesic climates.

formally similar to eqn (10) (see details in Table S1, Supporting information). When $g_{W,0} > 0$, few data sets exhibit a moderate decrease in λ with water stress (data points corresponding to $\beta\psi_L > 0$ in Fig. 3b). In one data set (*Pinus contorta*, not considered in the following analyses), the decrease in λ at low ψ_L is strong despite accounting for $g_{W,0}$. Similar patterns in λ are also found when we fit eqns (9) and (10) to ψ_S (for the few data sets that provide it) instead of ψ_L (results not shown).

Figure 4 illustrates the compounded effects of leaf water potential and atmospheric CO_2 concentration on λ for two grassland species grown in the LYCOG experiment, the C3 forb *Solidago canadensis* and the C4 grass *Sorghastrum nutans* (only the case $g_{W,0} > 0$ is shown). Interestingly, leaf water status has no significant effect in either species at any given CO_2 concentration (Table S1, Supporting information; Fig. 4a,b). In contrast, the effects of c_a are significant and seem to be adequately captured by a linear function (eqns 9 and 10; Fig. 4c). Overall, the marginal WUE of the C3 *S. canadensis* is about one-third of that of the C4 *S. nutans* (Table S1, Supporting information).

The regression parameters from the fitting of eqns (9) and (10) were analysed to investigate the occurrence of general patterns in the $\lambda(\psi_L)$ relationship driven by plant functional type and climatic conditions (arid and semiarid vs. mesic and wet conditions). In general, when $g_{W,0} = 0$, λ^*_{max} is highest in conifers and lowest in Mediterranean evergreen species (Table 2). Except for deciduous trees, species from wet climates have higher λ^*_{max} than species in the same functional group from drier environments. The peakedness of the $\lambda(\psi_L)$ response, β, is lower in broadleaved trees than in herbs, grasses and conifers, and in general is lower under dry conditions. Leaf water potential at maximum λ tends to be more negative under dry conditions than under wet conditions. As a result, β and $|\psi_{L,max}|$ are strongly and significantly negatively correlated both at the species and at the functional type level (Fig. 5).

When $g_{W,0} > 0$, some patterns in the values of λ in well-watered conditions, λ^*_{WW}, and the slope of the regression of eqn (10), β_0, are also apparent (Fig. 6). As expected from the analytical relationship between λ and g_C (eqn 8), λ^*_{WW} is negatively correlated with the maximum measured stomatal conductance, $g_{C,WW}$ (Fig. 6a). Also, β_0 tends to be negatively correlated with $g_{C,WW}$, although this relationship is significant for conifers only (Fig. 6b, $P < 0.05$). When looking at the functional type level, clear differences between wet and dry conditions emerge (Table 2; Fig. 6, insets). λ^*_{WW} is higher for plants grown under wet conditions than under dry conditions. The median slope β_0 is negative for all functional types and water availability conditions, suggesting increased marginal WUE with increasing water stress. Except for conifers, β_0 tends to be more negative under wet conditions than under
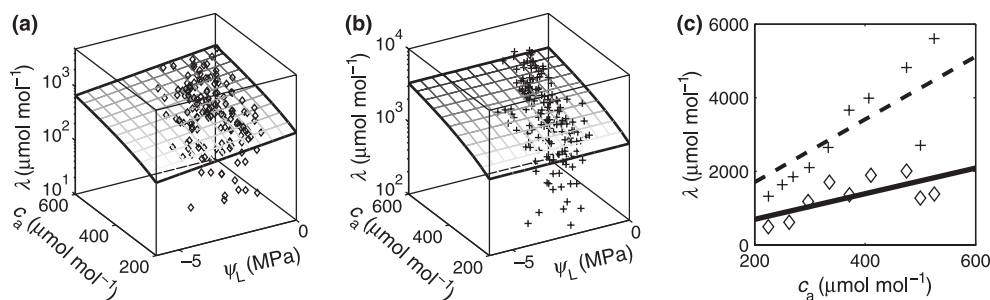

Fig. 4. Interacting effects of leaf water potential ψ_L and atmospheric CO_2 concentration c_a on the marginal water use efficiency λ, in (a) the C3 forb *Solidago canadensis* ($\diamond$) and (b) the C4 grass *Sorghastrum nutans* (+), along with multiple regression of estimated λ using eqn (10) (see also Table S1, Supporting information). (c) Linear regression of λ averaged across leaf water potentials against c_a.

Table 2. Medians of the regression parameters for eqns (9) and (10) grouped by plant functional type and water availability conditions (units as in Table 1)

Plant functional type	Water availability	$g_0 = 0$ (eqn 9)			$g_0 > 0$ (eqn 10)	
		λ^*_{max}	β	$\psi_{L,max}$	λ^*_{WW}	β_0
H	A (☆)	4442	0·37	−1·45	536	−0·97
	W (★)	5970	1·16	−1·32	981	−1·26
D	A (○)	3410	0·17	−3·69	279	−1·06
	W (●)	2912	0·78	−1·85	349	−1·62
E	A (□)	1755	0·12	−2·77	1211	−0·13
C	A (△)	6613	0·32	−2·29	664	−1·56
	W (▲)	6944	0·98	−1·40	4615	−0·56

H, forbs and grasses; D, deciduous trees and shrubs; E, evergreen trees and shrubs; C, conifers; A, arid, semiarid, and Mediterranean climates; W, wet and mesic climates. Symbols in parentheses are used in Figs 3, 5 and 6.
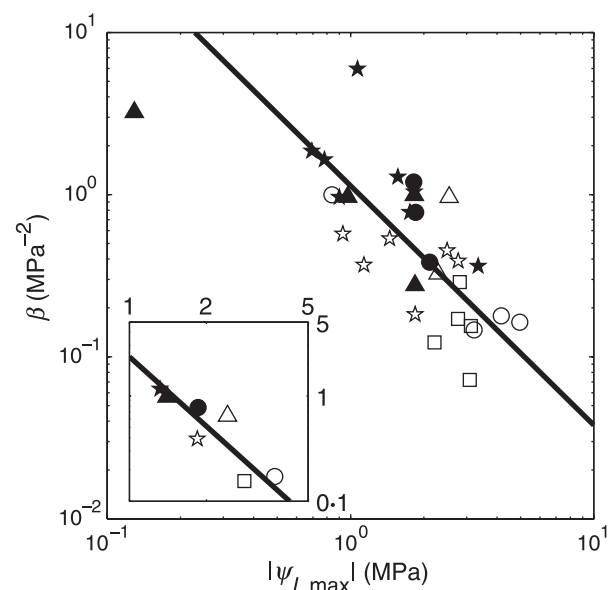

Fig. 5. Relationship between the parameter β and the absolute value of the leaf water potential at maximum λ, $|\psi_{L,max}|$ (eqn 9) for all species (main plot; $R = -0.79$, $P < 0.001$; see also Table S1, Supporting information) or median values for species aggregated in functional types and climatic conditions (inset; $R = -0.87$, $P < 0.05$; see also Table 2). Solid lines are type II linear regressions of the data points. Symbols as in Fig. 3.

dry conditions. Mediterranean evergreen broadleaved trees have less negative β_0 than other functional types.

MODEL APPLICATION TO *HELIANTHUS ANNUUS*

As a case study, the effects of different $\lambda(\psi_L)$ on modelled leaf gas exchange rates are considered. Using the function $\lambda(\psi_L)$ estimated from a single experiment (Tezara, Driscoll & Lawlor 2008), we modelled leaf gas exchange rates as a function of ψ_L, and compared the results with independent data from different *H. annuus* cultivars grown in a range of environmental
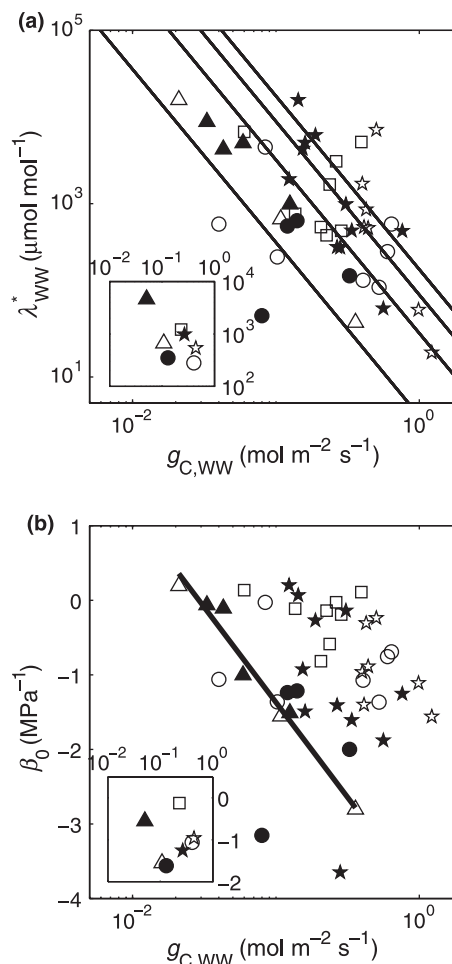

Fig. 6. Correlations between λ^*_{WW} and β_0 (eqn 10; Table S1, Supporting information), and maximum stomatal conductance, $g_{C,ww}$, for all species (main panels), or median values for species aggregated in functional types and climatic conditions (insets; see also Table 2). In panel (a), lines indicate the theoretical relationship $\lambda^*_{WW}(g_{C,ww})$ (eqn 8), for A_{WW} increasing from 5 to 35 μmol m^{-2} s^{-1} (from bottom to top); in panel (b), the solid line is the linear least square regressions for conifers. Symbols as in Fig. 3.

conditions (Fig. 7). Photosynthesis, stomatal conductance and c_i/c_a computed from eqns (6) and (7) (without any further adjustment) are shown as solid lines in Fig. 7b,c. The models based on the two different parameterizations of $\lambda(\psi_L)$ (eqns 9 and 10) correctly describe the decrease of A and g_C, as well as the decrease and subsequent increase of internal CO_2 as the leaf undergoes increasingly stressful conditions. In contrast, when λ is assumed to be constant and equal to the marginal WUE in well-watered conditions (λ_{WW}, dashed line), A and g_C were strongly overestimated, and the observed nonlinear pattern of c_i/c_a with ψ_L was not captured.

Discussion

VARIABILITY OF MARGINAL WATER USE EFFICIENCY

Cowan & Farquhar (1977, p. 486) visualize λ as 'a parameter which varies over periods which are significant in terms of the

relative growth and ontogenetic development of a plant, and is affected also by changes in the water relations insofar as they are determined by the state of water in the ground'. Optimal control theory, consistently with the above view, predicts that λ should change in time when the constraint to the optimization (e.g. water availability) changes depending on the control variable itself (e.g. stomatal conductance) (Luenberger 1979). Stomatal optimization theory predicts that λ increases under water stress because of increased risk of tissue damage (Cowan 1982; Mäkelä, Berninger & Hari 1996). These theoretical results were obtained under strong simplifying assumptions regarding the stochastic nature of rainfall (which plays a role in the formulation of the optimization problem) and neglected cuticular conductance and metabolic limitations to photosynthesis because of water stress. More recently, λ was related to soil water availability through plant hydraulic constraints (Konrad, Roth-Nebelsick & Grein 2008), or assuming a power law scaling with ψ_S (Schymanski et al. 2009).

Instead of predicting (or assuming) a priori the changes in λ along water availability gradients, here we estimated a 'macroscopic' marginal WUE from observations, thus implicitly accounting for all physiological effects of water availability on photosynthesis and transpiration. A similar approach has been adopted to explore the variations of λ across biomes (Lloyd et al., 1994), over a range of wet to dry climates (Palmroth et al. 1999), during a growing season (Hari & Mäkelä 2003), and in response to CO_2 enrichment (Katul et al., 2010), and water stress (Grieu, Guehl & Aussenac 1988). Fewer works have focused on changes in λ during ontogeny. Buckley (2008) assumed that the marginal WUE changes at this time-scale in concert with marginal nitrogen and light use efficiencies, thus implying that λ must increase under elevated c_a. Here, we focus on drought-related changes in λ. We cannot investigate long-term changes in λ because of slowly evolving climatic conditions during growth, because most of the available data are obtained from potted plants grown under controlled conditions until the initiation of the experimental drought.

We also neglected the potential contribution of direct hydraulic limitations on g_C (i.e. we assumed that g_C effectively acts as a free control variable). This could be expected to be critical in our analysis, since g_C could be bound to lower-than-optimal values as transpiration reaches the cavitation threshold because of strictly hydraulic limitations (Buckley 2005). Indeed, a model based on the assumption that λ should reflect the maximum transpiration rate allowed by the soil–leaf hydraulic system has been recently proposed (Konrad, Roth-Nebelsick & Grein 2008). Nevertheless, while hydraulic constraints are certainly involved in the regulation of g_C, it should also be taken into account that evolution selected for a coordinated response of both plant tissues and stomata, resulting in simultaneous decrease of plant and stomatal conductances as the soil dries (Sperry 2000; Brodribb et al. 2003). This coordinated change (as opposed to $g_{S \to L}$ driving g_C) allows us to model g_C as a free control variable even in the presence of some degree of cavitation. It is true, however, that

in extreme cases where runaway cavitation occurs g_C cannot be considered anymore a free control variable. In such cases (likely seldom occurring in the data sets used because of mild vapor pressure deficit), our estimates of λ would be overestimated, as they would reflect suboptimal conditions (e.g. g_C lower than the optimal one) (Buckley 2005).

Changes in λ assuming negligible cuticular conductance

In contrast to previous studies on λ, we considered two alternative assumptions for cuticular conductance to water vapour ($g_{W,0}$): (i) a perfectly regulated stomatal conductance (i.e. $g_{W,0} = 0$), and (ii) a non-zero water losses through the cuticle ($g_{W,0} > 0$). When assuming $g_{W,0} = 0$, we found an increase in λ under mild water stress, followed by a decrease under severe water stress (Fig. 3a). The complete nonlinear pattern appears when gas exchange is sampled throughout the dry-down, including severe water limitation. The nonlinear pattern may explain why others have reported different responses of λ to water stress. Some studies estimated an increase in λ with ψ_L or decreasing soil water (Hall & Schulze 1980; Berninger & Hari 1993; Thomas, Eamus & Bell 1999; Kolari, Nikinmaa & Hari 2008), and others a decrease (Farquhar, Schulze & Kuppers 1980b), or no significant response (Xu & Baldocchi 2003). In one case, a nonlinear, concave response similar to the results in Fig. 3a was found (Grieu, Guehl & Aussenac 1988). In addition, modelling studies have shown that λ must increase when soil water decreases to capture observed trends in gas exchange (Schymanski et al. 2008, 2009) and wood isotopic composition (Arneth et al. 2002).

Recalling that $\lambda \sim WUE^2$ (eqn 8), the observed decline under severe stress is not surprising. A decrease in WUE is in fact expected because internal (metabolic and diffusive) limitations on photosynthesis become predominant over stomatal control (Tezara et al. 1999; Lawlor et al., 2009). The result is the frequently observed concave relationship between WUE and leaf water potential (Delucia & Heckathorn 1989; Ni & Pallardy 1991; Kalapos, Van Den Boogaard & Lambers 1996; Medrano, Flexas & Galmes 2009). Analogously, the nonlinear shape of $\lambda(\psi_L)$ shown in Fig. 3a suggests that a transition from stomatal control on photosynthesis (increasing λ) to internal controls (decreasing λ) occurs around $\psi_{L,max}$. The parameter λ_{max} can thus be interpreted as a normalized measure of the maximum WUE that is achieved during the transition from well-watered to water-stressed conditions. It should be noted, however, that gas exchange measurements have traditionally been interpreted assuming $g_{W,0} = 0$ and the estimated WUE are affected by this assumption.

Changes in λ assuming non-negligible cuticular conductance

Observations suggest that in general $g_{W,0}$ is different from zero (Kerstiens 1996; Boyer, Wong & Farquhar 1997; Larcher 2003), resulting in patterns in λ that are different from the case $g_{W,0} = 0$. Specifically, the concavity observed when
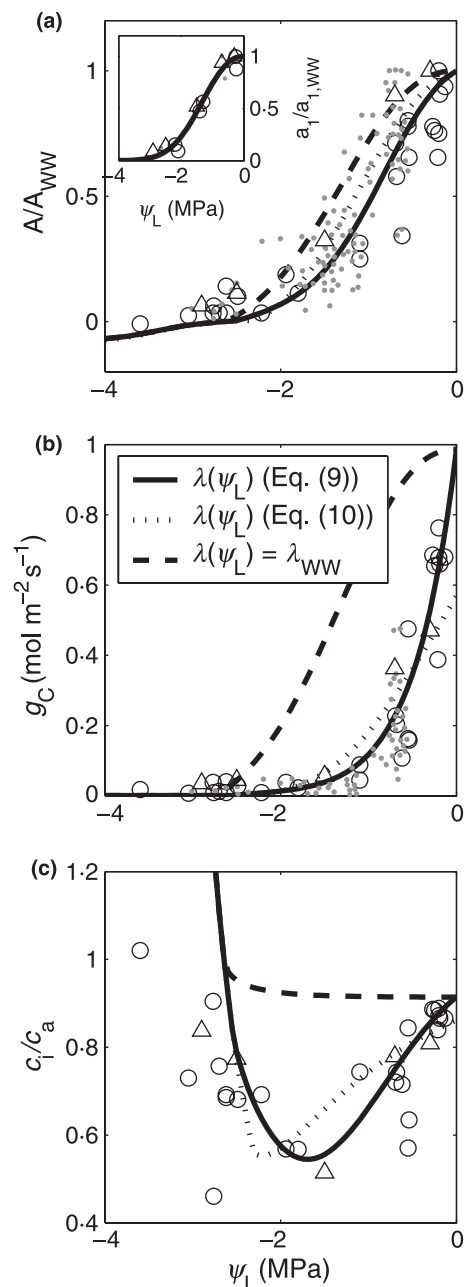
Fig. 7. Leaf biochemical and physiological responses to water stress (in terms of ψ_L) in *Helianthus annuus* under light-saturated conditions (i.e. $a_1 = V_{c,max}$). (a) Rate of photosynthesis and maximum carboxylation rate (inset), both expressed as a fraction of maximum ($V_{c,max}$ data are estimated from published $A - c_i$ curves, $V_{c,max} = 90 \times \exp{(\psi_L/1.7)^{2.3}}$, $R^2 = 0.96$); (b) stomatal conductance; (c) ratio of internal to atmospheric CO_2 concentrations (data points computed using eqn 7 from Farquhar *et al.*, 1982). Model results are obtained using eqns (6) and (7) (and setting a minimum g_C where $A < 0$), the empirical function $a_1 = V_{c,max}(\psi_L)$ from panel (a), and different formulations for $\lambda(\psi_L)$, as detailed in the legend. Gas exchange data are from Gimenez, Mitchell & Lawlor (1992), Tezara *et al.* (1999) and Tezara, Driscoll & Lawlor (2008) ($\bullet$, $\triangle$ and $\bigcirc$, respectively).

assuming $g_{W,0} = 0$ disappears and λ increases monotonically with more negative ψ_L. The monotonic increase of λ found under this assumption is consistent with previous theoretical

predictions based on perfect stomatal control. Hence, correcting the gas exchange data with $g_{W,0} > 0$ to obtain an estimate of the 'true' stomatal conductance allows recovering the shape of $\lambda(\psi_L)$ predicted by optimization theory. The specific assumption on $g_{W,0}$ is thus critical to test the optimality hypothesis along gradients of water availability. Both formulations, however, yield realistic simulations of water status effects on leaf gas exchange (Fig. 7).

Atmospheric CO₂ and other sources of variability

The marginal WUE was found to increase along a gradient of c_a from $\sim$250 to $\sim$500 µmol mol^{-1} (Fig. 4), in agreement with previous studies (Arneth *et al.* 2002; Buckley 2008; Katul *et al.*, 2010). This pattern is also consistent with the higher WUE often found under elevated CO_2 (Anderson *et al.* 2001; Polley, Johnson & Derner 2002).

Part of the variability in λ is caused by other factors in addition to ψ_L and c_a. Natural fluctuations in leaf physiology and biochemistry, light environment, and nutrient availability during canopy development may contribute to leaf-to-leaf and temporal variability in λ (the coefficient of variation of λ for a given ψ_L is in the range 0.2–0.9 for the LYCOG data). As a consequence, hypothesized effects of atmospheric CO_2 and leaf water status on λ emerge only in an ensemble average sense under field conditions. That is, leaf-scale dynamics on average follow the patterns described by eqns (9) and (10), but λ of individual leaves may vary widely (Fig. 4). Similar results have been found in a pine forest at ambient and elevated CO_2 concentrations, where the coefficient of variation of λ was about 0.4 (Katul *et al.*, 2010).

EFFECTS OF PLANT FUNCTIONAL TYPE AND CLIMATE ON $\lambda(\psi_L)$

The parameters of the $\lambda(\psi_L)$ curves are highly species-specific (Table S1, Supporting information); however, when grouped by plant functional type and climate, some patterns do emerge (Figs 5 and 6; Table 2). In general, conifers have higher λ^*_{max} and λ^*_{WW} than other functional types in any given climate. At the opposite end of the spectrum, deciduous broadleaved trees have lower λ^*_{max} and λ^*_{WW} than most functional types, excluding evergreen Mediterranean broadleaved species that have even lower λ^*_{max}. Moreover, for a given functional type, λ^*_{max} and λ^*_{WW} tend to be higher for plants grown under wet conditions than under dry conditions. Our results can be compared with some of the biome-level estimates of λ reported by Lloyd & Farquhar (1994), in particular for biomes characterized by relatively uniform plant functional types. For conifers and grasses (in mesic or wet climates), we found median $\lambda^*_{WW} \approx 4600$ and 1000 µmol mol^{-1}, respectively (Table 2), which compare relatively well with $\lambda \approx 4000$ and 1430 µmol mol^{-1} calculated by Lloyd & Farquhar (1994). In contrast, our estimates of λ^*_{WW} for rice and broadleaved deciduous trees (Table S1, Supporting information) are, respectively, higher and lower than the values reported by Lloyd & Farquhar (1994), likely because of intraspecific

and climatic differences. Lower λ under dry conditions is also consistent with the less conservative water use strategies (low WUE) employed by annuals and shrubs from arid ecosystems to achieve competitive advantages in face of limited water availability (DeLucia & Heckathorn 1989, Delucia & Schlesinger 1991) as opposed to more conservative water use (high WUE) in drought-sensitive species from mesic ecosystems. The variability of the parameters in the $\lambda(\psi_L)$ curves across species can thus be interpreted as the long-term result of competition strategies, while the shape of $\lambda(\psi_L)$ describes the responses of a single species to short-term changes in water availability.

The median of $\psi_{L,max}$ for a given functional type is more negative under dry than under wet growing conditions (Fig. 5; Table 2). This suggests that the maximum WUE in dry climates is achieved at relatively lower water availabilities than in wet climates, possibly because of higher drought resistance and delayed effects of water stress on the photosynthetic machinery in drought-adapted species. In contrast, conifers and deciduous broadleaved trees growing in mesic ecosystems tend to transition from stomatal to internal limitations at less negative leaf water potential (Larcher 2003). The $\psi_{L,max}$ in herbaceous species from dry climates is less negative than in other functional groups, suggesting low drought resistance, while drought avoidance (e.g. dormancy or short growing period coincident with the rainy season) could be a more effective strategy under such conditions (Snyman, Venter & Vanrensburg 1997; Larcher 2003). It is also interesting to note that, in all functional types, species from dry ecosystems are characterized by lower curvature of $\lambda(\psi_L)$ (i.e. lower β; see Fig. 5) than species from wet ecosystems, indicating relatively small changes in WUE under water stress. The resulting negative correlation between $|\psi_{L,max}|$ and β thus hints at a consistent pattern of high drought resistance and small changes in WUE in species growing under dry conditions, as opposed to low resistance and large changes in WUE in species adapted to more mesic conditions.

Conclusions

We analysed leaf gas exchange observations under well-watered to water-limited conditions to test the hypothesis that the marginal WUE, λ, increases with water stress. We first assumed that plants can perfectly control water losses (i.e. we neglected cuticular water losses). Under this assumption, λ was found to increase under mild water stress as previously hypothesized, while it decreased under severe water stress due to stronger internal limitations to photosynthesis, differently from predictions by previous theoretical work. In contrast, when considering cuticular conductance, λ increases monotonically with decreasing water availability, consistently with optimization theory. The parameters that describe the empirical $\lambda(\psi_L)$ relationships are species-specific, but significant patterns emerge when species are grouped according to functional type and climate, allowing us to interpret changes in marginal WUE in terms of plant strategies to cope with water stress.

Our results highlight the need to revise optimality models of gas exchange by accounting for leaf internal limitations under water stress and the presence of water loss pathways that cannot be controlled by stomata. As a next step, leaf water potential is being included in a dynamic manner, by coupling transpiration to soil moisture and atmospheric conditions (Manzoni *et al.* 2010). By adding the indirect effects of stomatal conductance on leaf (and canopy) gas exchange and energy balance the optimization problem becomes more complicated (as also explained in Appendix S1, Supporting information), precluding explicit analytical solutions (Buckley, Miller & Farquhar 2002; Schymanski *et al.* 2009). Despite this increased complexity, this coupled model allows testing the optimality hypothesis at the canopy scale and describing vegetation dynamics under field conditions.

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Supporting Information

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

Appendix S1. Optimal gas exchange under varying water status.

Appendix S2. Data set selection and characteristics.

Table S1. Summary statistics for the function $\lambda(\psi_L)$.

Table S2. Leaf gas exchange and water potential data base.

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