**LETTER**

## Woody plants optimise stomatal behaviour relative to hydraulic risk

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**Abstract**

Stomatal response to environmental conditions forms the backbone of all ecosystem and carbon cycle models, but is largely based on empirical relationships. Evolutionary theories of stomatal behaviour are critical for guarding against prediction errors of empirical models under future climates. Longstanding theory holds that stomata maximise fitness by acting to maintain constant marginal water use efficiency over a given time horizon, but a recent evolutionary theory proposes that stomata instead maximise carbon gain minus carbon costs/risk of hydraulic damage. Using data from 34 species that span global forest biomes, we find that the recent carbon-maximisation optimisation theory is widely supported, revealing that the evolution of stomatal regulation has not been primarily driven by attainment of constant marginal water use efficiency. Optimal control of stomata to manage hydraulic risk is likely to have significant consequences for ecosystem fluxes during drought, which is critical given projected intensification of the global hydrological cycle.

**Keywords**

climate change, extreme events, drought, plant hydraulics, vegetation model.

*Ecology Letters* (2018) **21**: 968–977

### INTRODUCTION

Terrestrial vascular plants take up carbon dioxide from the atmosphere and lose water through small pores called stomata. Terrestrial vegetation absorbs ~ 120 Pg carbon each year through stomata (Beer *et al.* 2010), equivalent to > 10 times human fossil fuel emissions, and transpires more than 30% of terrestrial precipitation through stomata (Jasechko *et al.* 2013). Thus, stomatal response to environmental conditions strongly influences the carbon and water fluxes of terrestrial ecosystems and is critical to global warming projections (Sellers *et al.* 1996; Berry *et al.* 2010; Jasechko *et al.* 2013; Lin *et al.* 2015). Decades of research have shown that stomatal aperture responds to light and photosynthesis (Wong *et al.* 1979; Ball *et al.* 1987), leaf-to-air vapour pressure deficit (VPD) (Leuning 1995), and atmospheric $CO_2$ concentrations (Schulze & Hall 1982) among other drivers, but mechanistic understanding remains elusive. Consequently, all vegetation models use regression-based approaches. Although these stomatal models have strong empirical support under some environmental conditions, predictions under drought are often

problematic (Anderegg *et al.* 2017). Solving this challenge is particularly crucial under future climate regimes, given projected increases in the frequency and severity of droughts in some regions (IPCC 2012). The primary guard against prediction errors has been a successful and long-lived evolutionary theory that predicts how stomata should behave, which we call the water use efficiency (WUE) hypothesis.

The WUE hypothesis posits that natural selection has selected for an optimal stomatal strategy that maximises the integral of net photosynthesis ($A_N$) subject to the constraint of a finite integral of water loss (E) over a given time interval. This occurs when $\partial A_N/\partial E = \lambda$, where $\lambda$, the marginal water use efficiency, is roughly constant over short periods of time (often considered to be daily timescales) (Cowan & Farquhar 1977). The $\lambda$ term depends fundamentally on the 'cost' that stomata experience, which is attributed to E. While this constrained-optimisation theory was initially developed for special circumstances (Cowan & Farquhar 1977), it has empirical support in a wide array of conditions (Küppers 1984; Hari *et al.* 1999; Manzoni *et al.* 2011; Lin *et al.* 2015), despite well-known issues in conditions where leaf water potential ($\psi_L$), a

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metric of plant water stress, varies widely (Cowan 1982; Manzoni *et al.* 2011). In particular, a well-developed literature shows that the WUE hypothesis predicts the approximate functional forms of the empirical stomatal models (Katul *et al.* 2009; Medlyn *et al.* 2011; Lin *et al.* 2015). Thus, WUE hypothesis has become the dominant hypothesis of stomatal regulation for 40 years (Ball *et al.* 1987; Medlyn *et al.* 2011, 2013; Lin *et al.* 2015; Buckley 2017), and is widely thought to help avoid errors in predictions of climate models in future (i.e. out-of-training-sample) conditions (Bonan *et al.* 2014; Kala *et al.* 2015; Xu *et al.* 2016).

Alternate stomatal optimisations have been proposed recently (Prentice *et al.* 2014; Wolf *et al.* 2016) based on a carbon-maximisation (CM) hypothesis, which predict functional responses of stomata to $CO_2$, photosynthesis, and VPD that largely match those in the empirical stomatal models (Wolf *et al.* 2016). But, unlike the WUE hypothesis, the CM hypothesis is also consistent with plant competition for water and directly incorporates the effects of soil and leaf water potential on plant function (Wolf *et al.* 2016). Under a recently proposed CM hypothesis (Wolf *et al.* 2016), plants have been selected evolutionarily to maximise the instantaneous difference between $A_N$ at a given $\psi_L$, and a hydraulic cost/risk term ($\Theta(\psi_L)$) that integrates both short-term and long-term costs of low water potential whereby the risks to the hydraulic continuum increase as water potential declines. The carbon costs of this risk term could include drought-induced impairment of photosynthesis (Flexas & Medrano 2002), feedback inhibition of photosynthesis due to decreased phloem export of sugars (Hölttä *et al.* 2017), lower gas exchange rates due to limited water supply from unrecovered embolism even when wet conditions return (Resco *et al.* 2009), metabolically driven refilling of embolised xylem elements (Brodersen & McElrone 2013), osmotic adjustment and maintenance (Sala *et al.* 2012), production of new conducting tissue (Brodribb *et al.* 2010), damage to fine roots (Cuneo *et al.* 2016), and risk of mortality (Anderegg *et al.* 2016). The $\Theta(\psi_L)$ function will increase monotonically with more negative $\psi_L$ as more xylem is cavitated and the risk of hydraulic failure (at $\frac{\partial E}{\partial \psi_L} = 0$) increases. The $A_N - \Theta(\psi_L)$ difference is maximised when $(\partial A_N / \partial g_s)/(\partial \psi_L / \partial g_s) = (\partial \Theta / \partial g_s)/(\partial \psi_L / \partial g_s)$.

Predictions of stomatal conductance in global vegetation models will depend on the optimisation used, yet while the optimisations have been explored theoretically (Sperry *et al.* 2016, 2017; Wolf *et al.* 2016), rigorous tests comparing the WUE hypothesis and CM hypothesis optimisations against observational data are lacking. Here, we use coordinated gas exchange and water potential datasets from 34 species across a diversity of biomes around the world (Fig. 1b; Tables S1 and S2) to formally test the WUE vs. CM hypotheses in a wide array of species and environmental conditions. We ask: (1) does the WUE or CM hypothesis show higher predictive ability of stomatal conductance when the models are fit at daily time-scales (the most conservative interpretation of WUE hypothesis)? (2) Does the WUE or CM hypothesis show higher predictive ability of stomatal conductance when the models are fit at seasonal-to-annual time-scales (likely to be most relevant for ecosystem models)? (3) Are there biome-specific patterns in the hydraulic cost functions in CM hypothesis?

## METHODS

### Testing the stomatal optimisations

We perform two major tests of the WUE and CM stomatal optimisations at two time-scales – daily (i.e. single set of parameters for each species on each day) and seasons-to-years (i.e. single set of parameters for a given species). Typically, $\lambda$ is considered to be constant over a day in the WUE
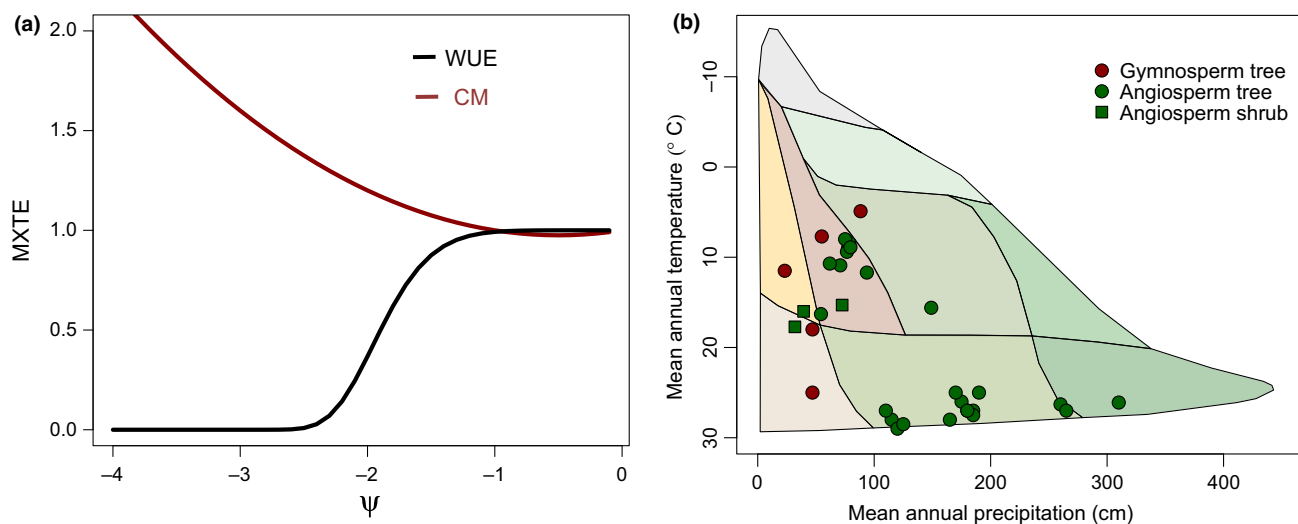


**Figure 1** Study species span most global forest biomes. (a) The predicted marginal xylem tension efficiency (MXTE) for the constant marginal water use efficiency (WUE) hypothesis (black line) and the carbon-maximisation (CM) hypothesis (red line). (b) The 34 species examined here are plotted on the Holdridge diagram of global ecosystems by mean annual precipitation and mean annual temperature. Note that co-located species have been slightly offset to enable visibility.

hypothesis (Cowan & Farquhar 1977; Cowan 1982; Buckley 2017), yet a single, constant λ per species or biome is often used at much longer (e.g. multi-year) time-scales in observational studies (Héroult *et al.* 2013; Lin *et al.* 2015), and modelling studies often modify λ over longer periods as a function of soil water content (De Kauwe *et al.* 2015a; Kala *et al.* 2015). Thus, performing all tests at both a daily and seasonal-to-yearly timescale is a relevant and crucial comparison. We further test an intermediate case of the WUE hypothesis where λ is fit as a linear or nonlinear (negative exponential) function of soil water potential, similar to the approach typically implemented in ecosystem models.

For the first test (Fig. 2), we used well-established equations of leaf gas exchange and plant water transport, along with the given stomatal optimisation, to calculate the values of the key optimisation parameters for the WUE and CM hypotheses that best fit the observed data and compared model fit of stomatal conductance prediction via $R^2$ and Akaike Information Criterion, accounting for the difference in parameters between the hypotheses, on identical datasets for each hypothesis. For the second test (Fig. 3), we use a previously proposed metric to determine whether the observed data best follow the WUE or CM hypotheses. The WUE and CM hypotheses predict qualitatively different trajectories in how optimal $(\partial A_N/\partial g_s)/(\partial \psi_L/\partial g_s)$ (abbreviated for simplicity as

$\partial A_N/\partial \psi_L$) will vary with $\psi_L$. The optimal $\partial A_N/\partial \psi_L$ is the 'marginal xylem tension efficiency' (MXTE), and represents how much photosynthetic carbon gain stomata are willing to forgo to prevent a unit decrease in leaf water potential. We estimated the MXTE from the observed data for each species at each time-scale and examine whether the data-derived MXTE best follows the WUE or CM hypothesis (see *MXTE calculation* below).

### Datasets

We searched the peer reviewed literature extensively to locate all usable datasets that contained concurrent measurements of stomatal conductance, plant water potential, and five driving environmental variables (incident photosynthetically active radiation (PAR), $[CO_2]$ at the leaf surface, leaf temperature, leaf-to-air vapour pressure deficit and soil water potential (i.e. predawn leaf water potential)) (Table S3). Furthermore, for a species to be included in the analysis, the xylem vulnerability to embolism curve needed to be included either in the study itself ($N = 6$ species), or in the Xylem Functional Traits dataset ($N = 28$ species) (Gleason *et al.* 2015). $V_{cmax}$, the species-specific maximum rate of carboxylation, was taken from the study itself ($N = 11$ species), the literature ($N = 13$ species), or estimated using light-saturated maximum photosynthesis per
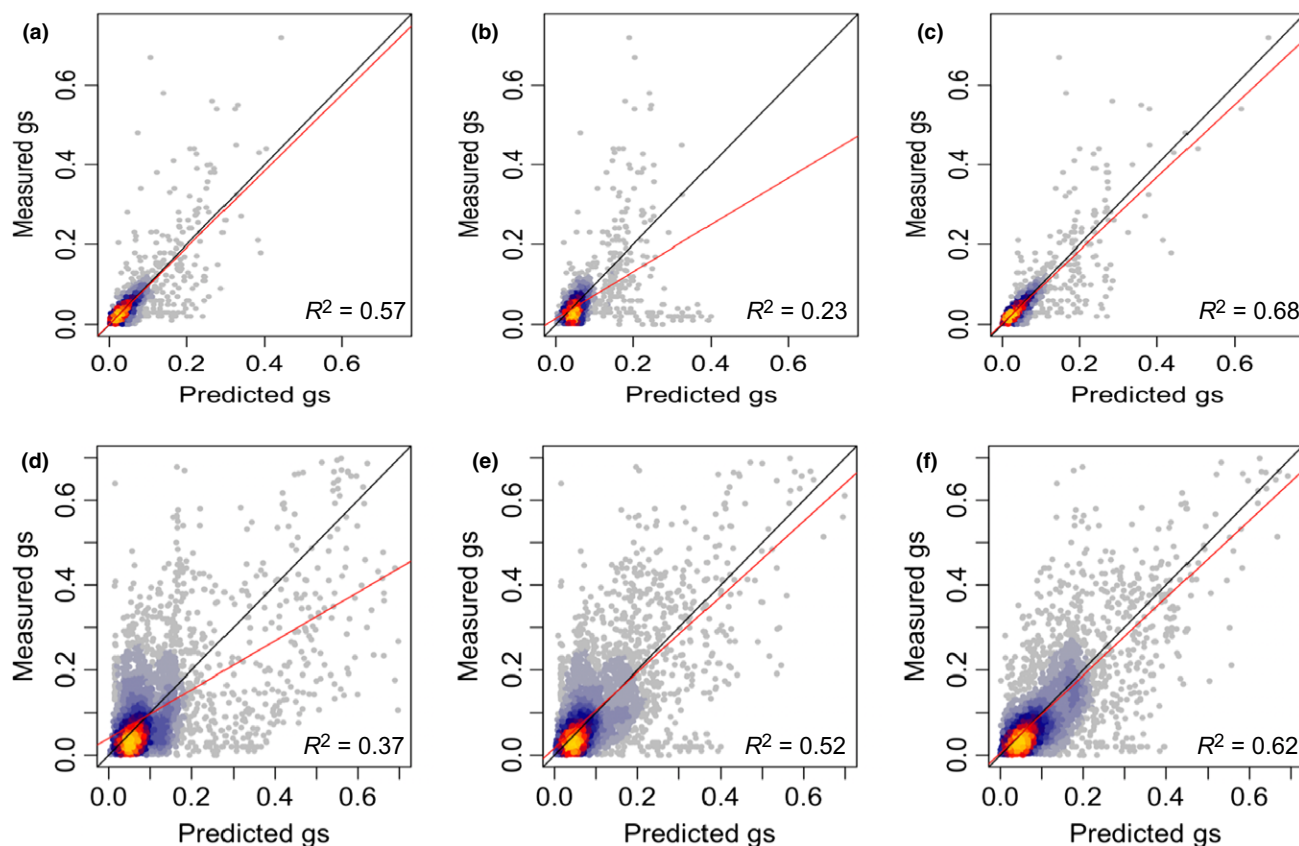


**Figure 2** The predicted vs. measured stomatal conductance ($g_s$; mol m$^{-2}$ s$^{-1}$) for models fit each day for seven species with adequate data for the (a) WUE (b) WUE with λ modified by soil water potential, and (c) CM hypotheses. The predicted vs. measured stomatal conductance ($g_s$) for models fit each of 34 species for the (d) WUE, (e) WUE with λ modified by soil water potential, and (f) CM hypotheses. Black lines represent the 1 : 1 line and red lines are the best fit for ordinary least squares regression. Colours indicate the density of points from highest density (yellow) to lowest (blue to grey).
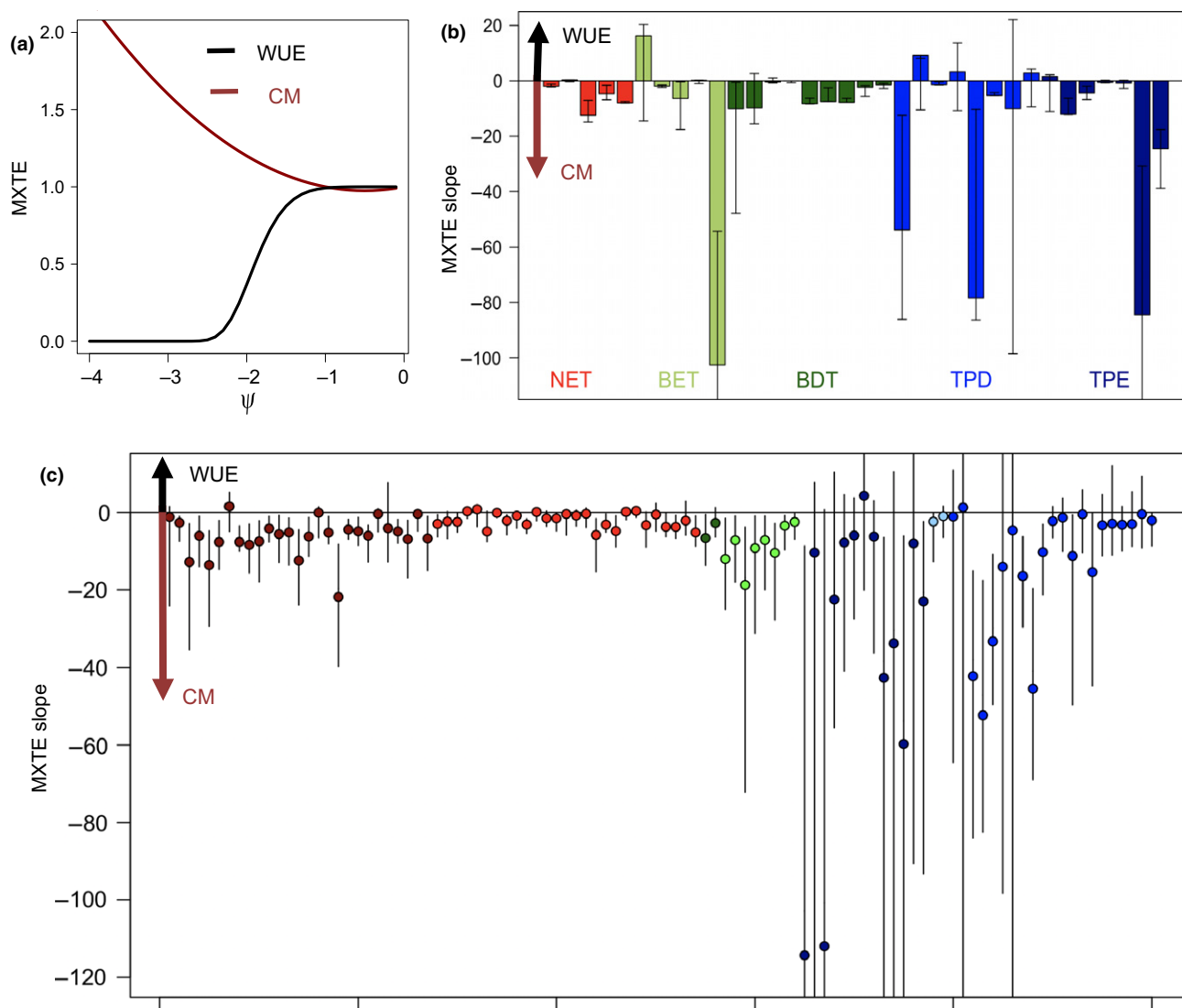
**Figure 3** Stomatal responses to water potential support the carbon maximisation hypothesis. (a) Fig. 1a re-plotted for ease of comparison with marginal xylem tension efficiency (MXTE) slopes (b) The slope of the marginal xylem tension efficiency for 34 species across biomes (NET: needleleaf evergreen temperate; BET: broadleaf evergreen temperate; BDT: broadleaf deciduous temperate; TPD: tropical deciduous; TPE: tropical evergreen). (c) The slope of the marginal xylem tension efficiency for each day for seven species (Dark red: *Pinus edulis*; Light red: *Juniperus monosperma*; Dark green: *Juniperus osteosperma*; Light green: *Quercus douglasii*; Dark blue: *Brachychiton australis*; Light blue: *Tapirira guianensis*; Blue: *Alphitonia excelsa*). Error bars are the 95% confidence intervals.

the method described in De Kauwe *et al*. (2015b) ($N = 10$ species). These trait data are presented in Table S1. This yielded 3698 time-point measurements of stomatal conductance across 34 species that spanned broad taxonomic and geographical coverage of global forest biomes (Fig. 1; Table S1). Measurements in the vast majority of these species spanned wet and dry conditions (Table S2).

**Modelling plant photosynthesis, stomatal conductance and water transport**

Stomatal optimisations cannot be measured directly but instead must be calculated and inferred from observed stomatal response to changing environmental conditions. We use four classic equations that describe the dynamics of photosynthesis, stomatal conductance and hydraulic transport of water

through the plant. First, we use the C3 biochemical photosynthesis model described by Farquhar *et al*. (1980), where assimilation (A) is the smallest of two limiting rates: $w_c$ ($CO_2$/rubisco limitation) and $w_j$ (light limitation):

$$A_N = \min(w_c, w_j) - R_d \tag{1}$$
$$w_c = \frac{V_{cmax}(C_i - \Gamma^*)}{C_i + K_c(1 + O_i/K_o)}$$
$$w_j = \frac{J(C_i - \Gamma^*)}{4(C_i + 2\Gamma^*)}$$

where, $C_i$ is the internal leaf $CO_2$ concentration, $\Gamma^*$ is the $CO_2$ compensation point, $K_c$ and $K_o$ are Michaelis–Menten coefficients of the carboxylation and oxidation reactions performed by rubisco, $O_i$ is the internal partial pressure of oxygen, J is the potential maximum rate of electron transport, calculated as in Medlyn *et al*. (2002) and $R_d$ is the rate of

dark respiration calculated using a Q10 functional form. We used the standard implementation of the photosynthetic model presented in the R package 'plantecophys', which is freely available and well-documented (Duursma 2015). The response of J to incident light was the default equation implemented in (Duursma 2015), based on Medlyn *et al.* (2002). The parameters $K_c$, $K_o$ and $V_{cmax}$ and the equation for $R_d$ depend on leaf temperature, which was an input to the model from measured leaf temperatures, and these equations are the default temperature responses in (Duursma 2015), based on those of Medlyn *et al.* (2002).

The second equation is a simplified version of Fick's Law where the carbon taken up during photosynthesis must be equal to the carbon that diffuses through the stomata:

$$A_N = g_s(C_a - C_i)/1.6 \qquad (2)$$

where $g_s$ is stomatal conductance of the leaf to water vapour (mol m$^{-2}$ s$^{-1}$), 1.6 accounts for the difference in diffusion coefficients between water vapour and $CO_2$, and $C_a$ is the partial pressure of $CO_2$ in the atmosphere. This formulation of the model assumes that cuticular conductance is negligible and boundary layer and mesophyll conductances >> stomatal conductance. We explore the implications of these assumptions in the Supporting Information. We used this model because sufficient data were not available to estimate cuticular, mesophyll, or leaf boundary layer conductances.

The third and fourth equations are the conservation of water lost through evaporation from the leaf and water supplied to the leaf by the hydraulic transport system.

$$E = g_s(e_s - e_a) \qquad (3)$$

where E is transpiration, $e_a$ is the vapour pressure of water in the atmosphere at ambient temperature and relative humidity, and $e_s$ is the vapour pressure of the saturated air space inside the leaf. As is standard in hydraulic models, steady-state E is solved by integrating the conductance function $K(\psi)$ from soil water potential (here, measured plant pre-dawn water potential) to the leaf water potential (Sperry *et al.* 1998):

$$E = \int_{\psi_L}^{\psi_S} K(\psi)d\psi \qquad (4)$$

where $\psi_s$ and $\psi_L$ are the soil and leaf water potentials (MPa), respectively, and $K(\psi)$ is the conductance function of the xylem, treated here as a three-parameter Weibull:

$$K(\psi) = K_{max}e^{-\frac{\psi}{-c}^d} \qquad (5)$$

where $c$ and $d$ are constants that come from values presented in the literature and $K_{max}$ is determined by fitting (see below). We integrated E using the 'integrate' function in the R statistical language. Eqn 4 assumes that the branch xylem vulnerability curve is a reasonable proxy for the whole-plant vulnerability curve, but we consider this assumption in the Supporting Information. Note that by the fundamental theorem of calculus, the $\frac{\partial E}{\partial \psi}$ derivative (e.g. eqn 1) is $K(\psi_L)$.

Eqns 1–4 describe the key components of water transport, water loss and carbon uptake as a function of four species-level parameters ($K_{max}$, $V_{cmax}$, xylem vulnerability curve constants c

and d) and five drivers (incident PAR, $CO_2$ at the leaf surface, leaf temperature, leaf to air vapour pressure deficit and soil water potential). These four equations have five unknowns: $A_N$, $g_s$, $C_i$, $\psi_L$ and E. Thus, a fifth equation – an optimality equation – must be introduced to solve the system of equations.

For the first test, the optimality equations used were:

$$\frac{\partial A_N}{\partial g_s} = \lambda \frac{\partial E}{\partial g_s} \quad \text{for the WUE hypothesis} \qquad (6a)$$

$$\frac{\partial A_N}{\partial g_s} = \frac{\partial \Theta}{\partial g_s} \quad \text{for the CM hypothesis} \qquad (6b)$$

where $(\partial \Theta/\partial g_s)$ was fit as the following function of leaf water potential:

$$\frac{\partial \Theta}{\partial g_s} = \frac{\partial \Theta}{\partial \psi_L}\frac{\partial \psi_L}{\partial g_s} = \frac{\partial \psi_L}{\partial g_s}(a\psi_L + b) \qquad (7)$$

where $a$ and $b$ are constants fit in the same manner that $\lambda$ is fit for the WUE hypothesis. Note that the linear functional form of $\frac{\partial \Theta}{\partial \psi_L}$ (hereafter $\Theta'(\psi_L)$ for simplicity):

$$\frac{\partial \Theta}{\partial \psi_L} = a\psi_L + b \qquad (8)$$

implies that the cost function $\Theta(\psi_L)$ is a parabolic function of the form:

$$\Theta(\psi_L) = \frac{a}{2}\psi_L{}^2 + b\psi_L + c \qquad (9)$$

although we do not solve for $\Theta(\psi_L)$ directly because only its derivative is needed to satisfy the optimality criterion of CM hypothesis.

In the second test (Fig. 3), we solved for the generic MXTE parameters (see below) that best matches the observed stomatal conductance data. If the MXTE slope is significantly different from zero, it allows us to statistically discriminate between the two optimisations per Fig. 1a. We also consider an alternate and more nuanced model formulation for the MXTE of WUE hypothesis in the Supporting Information that allows parsing apart the relative differences in predictive ability due to the optimisation criterion vs. the different costs considered (i.e. costs due to $\psi_L$ vs. E).

To solve eqns 1–4 and 6 with a given value of $\Theta'(\psi_L)$ or $\lambda$, we first used eqns 3–4 and the measured pre-dawn and midday water potentials to solve for the best $K_{max}$ that minimised the root-mean-squared error between predicted and observed midday water potentials for a given species. Midday water potentials were used only for fitting $K_{max}$, as the optimisation model solves for leaf water potential as part of determining $g_s$ for each theory (solving eqns 1–4). When both pre-dawn and midday water potentials were not available (N = 9 species), a $K_{max}$ was assumed that would maximise the difference between pre-dawn and midday water potentials but still provide a solution to eqns 1–4 for all observations, although we tested the influence that $K_{max}$ had on all results in a sensitivity analysis.

Next, for each data point (i.e. concurrent measurement of stomatal conductance, leaf water potential, and the driver variables), an initial guess of $g_s$ of 0.010 mol m$^{-2}$ s$^{-1}$ was made. At this stomatal conductance, $A_N$ was calculated by solving eqns 1–2 for $C_i$ and $\psi_L$ and E were calculated by

solving eqns 3–4 for $\psi_L$. Next, the stomata were opened a tiny fraction (0.001 mol m$^{-2}$ s$^{-1}$) and $A_N$, $\psi_L$ and E were recalculated as above. This allowed estimation of the marginal benefit ($\frac{\partial A_N}{\partial g_s}$) and marginal cost (RHS of eqn 6a or 6b) of opening stomata at that guessed value of stomatal conductance for each hypothesis.

A Newton–Raphson solver was then implemented to find the stomatal conductance where marginal benefit = marginal cost (i.e. eqn 6) for a given stomatal optimisation equation. Then, at a given set of parameters of $\lambda$, $\lambda(\psi_s)$, or $\Theta'(\psi_L)$, the sum of the squared error was calculated between the observed stomatal conductance and that predicted by the model. These errors are then summed over all data points for a given test (either species or day), providing the total error for the given set of chosen parameters for $\lambda$, $\lambda(\psi_s)$, or $\Theta'(\psi_L)$.

## Parameter estimation

Finally, we used a Markov-Chain Monte Carlo (MCMC) algorithm to calculate the stomatal optimisation parameters that minimise the error between the observed and modelled stomatal conductance. We specified prior values of $\lambda = 0.1$ for the WUE hypothesis and $a = 0.1$ and $b = 0$ for the CM hypothesis, selected as the most uninformed prior that still solved the equations for most species (i.e. non-zero cost of opening stomata). For six species, this prior did not solve the equations and a prior of $\lambda = 1$ or $a = 1$ was used. Acceptance probability of a step was based on the Metropolis-Hastings algorithm whereby the probability is the ratio of the likelihoods of step $i + 1$ and step $i$ and a ratio of $> 1$ is set to probability = 1. We assumed a Gaussian distribution of error and fit the standard deviation of error in measurements as part of the MCMC process. To find the best stepsize, we initiated three MCMC chains per species with 5000 steps per chain with stepsizes of 0.2, 0.4 and 0.8. Per standard practice, we iterated with various stepsizes, aiming to find the stepsize for each species where $c$. 20–25% of steps are accepted (median stepsize across all species was $\sim 0.2$ for $\lambda$ and $\sim 0.55$ for $a$ and $b$). No covariance was assumed between $a$ and $b$ for the CM hypothesis.

Once the ideal stepsize was identified for each species, we ran three MCMC chains for 10 000 steps for each species and made sure that we had rapid mixing by ensuring that the ratio of inter-chain to intra-chain variances was close to 1. We then discarded the first 2000 for burn-in for each chain, and calculated the confidence intervals by subsampling every 10 steps to minimise autocorrelation. Species whose CIs overlap zero do not have enough data to reject either null hypothesis – which could be due either to too limited a sample size or the measurements not covering enough environmental variation to enable discrimination among the hypotheses (Table S2). Daily estimates of the parameters were made using the same methods above on the seven species with adequate sample size within a day (average of $> 8$ measurements per day) to estimate $\lambda$, $\lambda(\psi_s)$ or $\Theta'(\psi_L)$ accurately.

## MXTE calculation

The MXTE gets at the heart of the differences between the two optimisations – their response to plant water stress. In the WUE hypothesis, the cost of opening stomata is water loss (E) and thus progressive decline in $\frac{\partial E}{\partial \psi_L}$ caused by loss of hydraulic conductance creates an eventual decline in MXTE (Fig. 3a black line). In the CM hypothesis, the cost is a function of water potentials so the MXTE is constant or increasing with water stress (Fig. 1a red line). For the WUE hypothesis:

$$\text{MXTE}_{\text{WUE}} = \frac{\frac{\partial A_N}{\partial g_s}}{\frac{\partial(-\psi_L)}{\partial g_s}} = \lambda \frac{\partial E}{\partial \psi_L} \tag{10a}$$

As noted above, the $\frac{\partial E}{\partial \psi_L}$ derivative declines to zero with more negative $\psi_L$ because xylem cavitation ultimately drives plant hydraulic conductance to zero. Because $\lambda$ is constant, the WUE hypothesis predicts that MXTE will eventually *decrease* with lower $\psi_L$. For the CM hypothesis:

$$\text{MXTE}_{\text{CM}} = \frac{\frac{\partial A_N}{\partial g_s}}{\frac{\partial(-\psi_L)}{\partial g_s}} = -\frac{\partial \Theta}{\partial \psi_L} \tag{10b}$$

$\Theta$ and $\frac{\partial \Theta}{\partial \psi_L}$ will increase in some manner as $\psi_L$ becomes more negative because the risks to the hydraulic continuum increase as water potential declines. Thus, the CM hypothesis predicts that MXTE will eventually *increase* with lower $\psi_L$ (Fig. 1a).

For the second major test of the stomatal optimisations, we adopt a generic (i.e. agnostic between WUE and CM, see SI) form of the MXTE as:

$$\text{MXTE} = \beta_1 \psi_L + \beta_0 \tag{10}$$

where $\beta_1$ and $\beta_0$ are parameters that are estimated in the same manner as described above. As above, we perform separate fits for the MXTE to each day for seven species that had substantial within-day measurements and one set of parameters for each species for all 34 species. We estimated the MXTE using the same MCMC approach as above and priors of $\beta_1 = 0.1$ and $\beta_0 = 0$.

## RESULTS

We first use the WUE and CM stomatal optimisations to predict stomatal conductance for seven representative species with adequate measurements at diurnal time-scales, the most conservative interpretation of the WUE hypothesis. Both optimisations perform well at the diurnal scale ($P < 0.0001$), but the CM hypothesis ($R^2 = 0.68$) is a large improvement in predictive ability compared to the WUE hypothesis ($R^2 = 0.57$) (Fig. 2a, c). Comparing the two models with the Akaike Information Criterion, which provides quantitative assessment of model likelihoods while accounting for the different number of parameters between models, we find that the WUE hypothesis shows a $\Delta$AIC of $> 400$ compared to CM hypothesis (i.e. a relative likelihood of zero for the WUE hypothesis). This improved predictive ability of the CM hypothesis appears to come in part from an increased ability to account for variation in soil and leaf water potential (Fig. S1). The predictive ability of the intermediate case of the WUE hypothesis where $\lambda$ is fit as a linear function of soil water potential, the typical approach implemented in ecosystem models, on these same seven species performed the poorest ($R^2 = 0.23$) (Fig. 2b).

We then examined the stomatal optimisations' predictive ability at a species level, which is more relevant for parameterising vegetation models to be run over years and decades. Here, we observe an even larger increase in predictive ability in the CM hypothesis compared to the WUE hypothesis ($R^2_{CM} = 0.62$, $R^2_{WUE} = 0.37$; $\Delta AIC > 2000$) (Fig. 2d,f). The intermediate WUE case of $\lambda(\psi_s)$ performed better than a constant $\lambda$ ($R^2 = 0.52$, Fig. 2e for linear; $R^2 = 0.52$, Fig. S2 for nonlinear function), but still substantially poorer than the CM hypothesis ($\Delta AIC > 850$ for both). These analyses reveal that the CM hypothesis provides substantial predictive improvements relative to WUE hypothesis at both diurnal and seasonal-to-yearly time-scales, even when $\lambda$ can vary with soil water potential.

We next estimated the MXTE from observed data and assessed whether its slope with respect to $\psi_L$ supported CM (slope < 0) or WUE hypothesis (slope > 0; Fig 3). At the diurnal timescale, the data unanimously supported the CM over the WUE hypothesis where a significant MXTE slope ($\beta_1$) occurred (CI did not overlap zero for 56% of days; Fig. 3c). Significant slopes that supported the CM hypothesis occurred in both wet and dry periods, with 33% of significant slopes occurring on days where water potentials did not fall below values that would have caused a 5% loss in hydraulic conductance. Fitting the MXTE for each species, the data also strongly supported the CM over the WUE hypothesis where a significant slope occurred (58% of species; Fig. 3b). Considering sensitivity analyses of the key plant traits, the estimate of MXTE slope parameters ($\beta_1$) was quite similar across the sensitivity analyses (Table S4). Thus, while these traits (whose base case values are based on observed measurements) matter slightly in estimating the stomatal cost function, the broad scale patterns and support of the CM hypothesis appear to be robust.

Examining stomatal responses and the hydraulic cost functions across species, a diversity of slopes of MXTE functions was observed in all ecosystems and in both gymnosperms and angiosperms (Fig. 3b). This highlights the diversity of water use strategies present in all biomes and, indeed, biome or clade were not significant predictors of MXTE slope ($P > 0.1$) (Fig. 4). The slope of MXTE was not significantly related to site-level mean annual temperature or precipitation, or maximum carboxylation capacity of species (Linear model: $P > 0.1$ in all cases).

In the preliminary land surface model upscaling simulations, we found that the exchanges of latent energy predicted by the model differed most from the previous stomatal algorithm during the severe drought year (Fig. S3). The hydraulic-stomata water stress scheme reduced latent energy fluxes by up to 15% in the drought year compared to the previous scheme by closing stomata to curtail transpiration.

## DISCUSSION

Several lines of evidence reveal that the data better support the CM hypothesis than the WUE hypothesis at all time scales considered here. First, the CM hypothesis improved predictive ability (Fig. 2), with a standard model selection criterion giving a relative likelihood of zero to all implementations of the WUE hypothesis. Second, in all cases where the
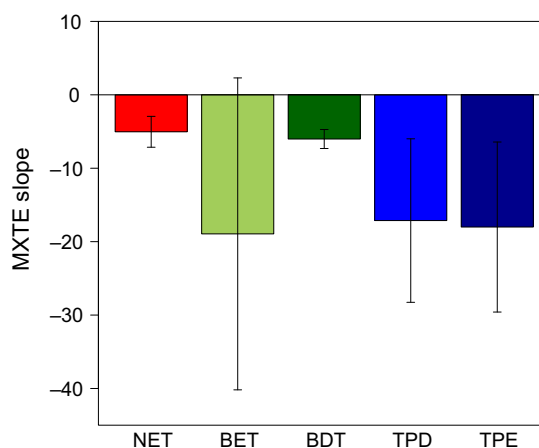


**Figure 4** Plot of the slope of the marginal xylem tension efficiency (MXTE) by biome. Bars show mean ± 1 standard error. Biomes are needleleaf evergreen temperate (NET), broadleaf evergreen temperate (BET), broadleaf deciduous temperate (BDT), tropical deciduous (TPD) and tropical evergreen (TPE).

data could discriminate between optimisations in their response to water potential (i.e. a significant slope of the MXTE), the CM hypothesis is supported (Fig. 3). In other words, the stomata of these species behave as though they are simply maximising net carbon gain – that is, carbon uptake minus a shadow carbon cost of hydraulic risk – at each instant in time, and none behave as though they were pricing water loss or deferring carbon gain to a future time with less evaporative loss.

We note that there are two key differences between CM and WUE hypotheses – (1) the profit maximisation optimisation as opposed to the constrained optimisation and (2) carbon costs attributed to risk of a given water potential as opposed to water loss – and, in theory, the predictive improvements in CM hypothesis may be driven by either or both. In the Supporting Information, we use a more complex approach to separate these two differences and find that the data offer strong support for both the competitive optimisation criterion behind the CM hypothesis and costs driven by risk of hydraulic damage (Figs S4 and S5).

The physiology of the shadow costs of low water potential – how stomata 'price' hydraulic risk – is a critical area for future research. Some direct costs, including the impairment of photosynthesis at low water potentials (Flexas & Medrano 2002) and osmotic regulation (Bartlett *et al.* 2014), which requires energy as a function of water potential, have been identified previously. More likely, however, is that the shadow price of hydraulic risk involves avoiding damage to hydraulic transport, which could incur large carbon and fitness costs in rebuilding (Brodribb *et al.* 2010; Skelton *et al.* 2017), opportunity costs of lost photosynthesis and risk of mortality (Anderegg *et al.* 2015). Furthermore, progressive weakening of xylem after embolism (Hacke *et al.* 2001), even if refilling of embolism occurs, could provide a strong selective force to provide a shadow price of hydraulic risk that closes stomata well before water potentials reach levels that drive embolism.

Based on theoretical and empirical work (Cowan 1982; Hari *et al.* 1986; Manzoni *et al.* 2011), current implementations of

the WUE hypothesis typically include a function that changes $\lambda$ as a function of soil water content or potential (Kala *et al.* 2015). This allows the WUE hypothesis to capture to some degree the longer-term soil moisture declines during drought. We capture this behaviour both through an analysis that varies $\lambda$ as a function of soil water potential (Fig. 2b, e, Fig. S2) and by fitting $\lambda$ every day (Fig. 2a). Even in this best-case-scenario for the WUE hypothesis, the CM hypothesis performs substantially better (Fig. 2a,c). As an additional advantage, the effects of declining soil water potential are mechanistically and directly included in the CM hypothesis, rather than a second fitting step (i.e. fitting $\lambda(\psi)$) prior to the WUE optimisation, and can be successfully parameterised from measured species hydraulic traits (Sperry *et al.* 2017).

Differences between the two stomatal optimisations should matter most during drought conditions (e.g. conditions where plant water potentials reach levels that risk hydraulic damage) when soil water potentials become highly negative or VPD rises. These are precisely the situations in which representation of plant hydraulics and its effects through the cost function on stomatal behaviour would be most critical to capture in vegetation and land surface models. Multiple studies have shown that incorporating plant hydraulics improves land surface model simulation (Bonan *et al.* 2014; Xu *et al.* 2016) and the CM hypothesis provides a quantitative way to link hydraulics with plant stomatal control. Indeed, our preliminary simulations indicated that there were substantial differences in simulations of ecosystem fluxes during dry years (Fig. S3). While more work is needed to test and validate the CM hypothesis at ecosystem scales and in multiple ecosystems, this model-based scaling exercise demonstrates that stomatal strategies can have substantial effects on predicted ecosystem fluxes. More sensitive stomatal closure during drought may even be a mechanism that intensifies drought by reducing land water fluxes and recycling.

We observed a wide diversity of MXTE functions and thus stomatal response strategies within biomes (Fig. 3). This result underscores that multiple strategies that likely involve a suite of plant traits, including xylem vulnerability, leaf area changes, rooting depth and stomatal behaviour among others (Bartlett *et al.* 2016), are possible within a given ecosystem or climate. Furthermore, these results suggest that current plant functional types in global vegetation models may be missing an important element of diversity – coupled hydraulic-stomatal response to low water potential – that would not be captured with a single set of stomatal parameters assumed for each biome (Lin *et al.* 2015) and the currently rare representation of the plant hydraulic continuum in global models.

There are several potential reasons why the CM hypothesis fits the data better than the WUE hypothesis, which should be explored in future research. First, the CM hypothesis is consistent with the idea that plants can steal each others' water, which makes sense given the widespread evidence that plants compete for water in the soil in many ecosystems (Casper & Jackson 1997). In the face of competition for water, the WUE hypothesis assumes individual plants can control their soil water pools like an individual bank account, thereby saving water in the soil so that it can be used during the best conditions for carbon gain. Second, the CM hypothesis appears to predict stomatal

conductance over both short and relatively long periods of time. Our finding that a single $\Theta'(\psi_L)$ parameter set for a species performs quite well indicates that the CM hypothesis is promising for improving prediction of fluxes in novel conditions in future climates in Earth system models. Finally, the fitness costs represented in CM hypothesis through $\psi_L$ are likely much more direct than $\partial E$ in the WUE hypothesis because it is the imbalance between water uptake and water loss resulting in low water potentials, not water loss itself, that damages plants and is related to mortality (Anderegg *et al.* 2016).

We show here that the pure carbon gain optimisation in CM hypothesis is more consistent with empirical data than WUE hypothesis at seasonal timescales and even during single days. Moreover, the new criterion explains stomatal behaviour before, during, and after drought, and provides significant increases in predictive power at the leaf-level. Crucially, our findings emphasise that constant 'marginal water use efficiency' is not the quantity that governs the evolution of stomatal regulation. Rather, observed changes in $\lambda$ between and within single days are simply the result of stomatal behaviour that maximises carbon gain while maintaining hydraulic function. Ultimately, the CM stomatal optimisation linked to plant water transport provides an integrated and internally consistent foundation for more mechanistic stomatal simulation in Earth system models during future climates marked by more climate extremes.

## AUTHOR CONTRIBUTIONS

WRLA and SWP designed the research. AW, AAV, BC, DJC, SJ, TK, SL, FM, PP, VRD, JS and BW contributed data and analytical tools. WRLA performed the analysis and wrote the paper, with all authors contributing comments.

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