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# Effects of stomatal delays on the economics of leaf gas exchange under intermittent light regimes

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## Summary

• Understory plants are subjected to highly intermittent light availability and their leaf gas exchanges are mediated by delayed responses of stomata and leaf biochemistry to light fluctuations. In this article, the patterns in stomatal delays across biomes and plant functional types were studied and their effects on leaf carbon gains and water losses were quantified.

• A database of more than 60 published datasets on stomatal responses to light fluctuations was assembled. To interpret these experimental observations, a leaf gas exchange model was developed and coupled to a novel formulation of stomatal movement energetics. The model was used to test whether stomatal delays optimize light capture for photosynthesis, whilst limiting transpiration and carbon costs for stomatal movement.

• The data analysis showed that stomatal opening and closing delays occurred over a limited range of values and were strongly correlated. Plant functional type and climate were the most important drivers of stomatal delays, with faster responses in graminoids and species from dry climates.

• Although perfectly tracking stomata would maximize photosynthesis and minimize transpiration at the expense of large opening costs, the observed combinations of opening and closure times appeared to be consistent with a near-optimal balance of carbon gain, water loss and movement costs.

## Introduction

When incident light levels drop below *c.* 20% of full sun, light availability becomes the most limiting resource for photosynthesis (Chazdon, 1988). Light limitation is particularly relevant in understory environments (Pearcy, 1990), shallow rivers partly shaded by riparian vegetation (Davies-Colley & Quinn, 1998) and sites with frequent occurrence of intermittent clouds (Knapp & Smith, 1988). These environments are characterized by sunflecks, defined as periods of relatively high light irradiance followed by periods of background low diffuse light. Although each sunfleck may last only seconds to minutes, sunflecks can contribute up to 80% of the total solar energy flux to the understory (Chazdon, 1988), thus being primary drivers for photosynthesis in these environments (Pearcy, 1990).

Stomatal movement mechanisms are key to the quantification of photosynthetic responses to variable light (e.g. Kirschbaum *et al.*, 1988; Pfitsch & Pearcy, 1989; Ooba & Takahashi, 2003). The transport of osmoticum associated with stomatal opening is an active, energy-requiring mechanism (Zeiger, 1983; Assmann *et al.*, 1985; Hanstein & Felle, 2002), and thus bioenergetic considerations are necessary to assess the 'optimality' of stomatal response times to sunflecks. A large number of experiments on leaf-level responses to step changes in light irradiance have been conducted over the past 30 yr with the aim of exploring stomatal delays in response to light changes. These experiments have shown large variations in stomatal delays (here denoted by the characteristic time scales of opening and closing, $\tau_{op}$ and $\tau_{cl}$) across species and environmental conditions (Chazdon, 1988; Pearcy, 1990; Ooba & Takahashi, 2003). Despite such variability, there is general agreement that, when considering fully induced leaves, delays in stomatal response to variable light are the most relevant driver for leaf gas exchange, with biochemical

delays occurring at much shorter time scales (Weber *et al.*, 1985; Knapp & Smith, 1987, 1988). Furthermore, in most species, delays in stomatal opening appear to be shorter than delays in closing, that is $\tau_{op} < \tau_{cl}$ (Ooba & Takahashi, 2003). Finally, the time scales associated with stomatal movements have been shown to be commensurate with sunfleck durations (Cardon *et al.*, 1994; Naumburg *et al.*, 2001), and to depend on the time of day and history of sunfleck occurrence (Kaiser & Kappen, 1997). The range of scales and environmental drivers involved complicate the quantification of the dynamic response of photosynthesis to light availability. Partly because of these complexities, the evolutionary causes of the variation in $\tau_{op}$ and $\tau_{cl}$ across species and growth conditions have not been fully addressed, despite the fact that delays in stomatal opening and closing have well-documented implications in terms of cumulative $CO_2$ assimilation and transpiration (Naumburg *et al.*, 2001) and, hence, leaf water use efficiency (WUE) (Knapp & Smith, 1989).

Ideally, perfectly tracking stomata ($\tau_{op} = \tau_{cl} = 0$ in Fig. 1) can fully exploit the available light during sunflecks, whilst minimizing the transpiration losses not associated with carbon gain by immediately closing the stomata when light decreases. However, delays between the change in light conditions and stomatal movement are inevitable because of inherent physical and biochemical limitations. When exploring various combinations of $\tau_{op}$ and $\tau_{cl}$ (Fig. 1), two other 'end-member' cases are worth considering: (1) fast opening stomata ($\tau_{op} \cong 0$) with a significant lag in closing (high $\tau_{cl}$; points along the abscissa in the delay space of Fig. 1); and (2) fast closing stomata with a significant lag in opening (high $\tau_{op}$ and $\tau_{cl} \cong 0$, i.e. points on the ordinate in Fig. 1). In (1), the fast opening of stomata guarantees the ideal exploitation of available light during sunflecks, thus maximizing leaf cumulative photosynthesis (unless leaf water status worsens during the sunfleck; for example, Seastedt & Knapp, 1993). However, during the ensuing low light period, higher $\tau_{cl}$ causes more significant water losses through transpiration at times when assimilation is light limited. Conversely, in (2), the fast closure of stomata when light is abruptly reduced ($\tau_{cl} \cong 0$) minimizes the amount of 'wasted' water for transpiration, and the significant lags in stomatal opening when light is restored further contribute to the minimization of the water losses. These water savings have a negative effect on leaf carbon gain, because the delay in stomatal opening reduces assimilation. Hence, the delays in stomatal response affect WUE by altering both assimilation and transpiration. This conceptual exploration suggests the hypothesis that the most feasible combinations of stomatal delays in opening and closure represent a compromise between the need to maximize carbon gain and the need to minimize unproductive water losses (and, hence, the duration of periods under water stress), whilst simultaneously limiting energetic costs for stomatal movements. The case of perfect coordination between stomatal opening and closing (i.e. the 1 : 1 line in Fig. 1) does not necessarily represent the best solution. Rather, the optimal combination of delays will depend on a number of factors, including the plant 'perceived values' of water loss vs carbon gain, the average duration of the periods of light and darkness, and the energetic costs of moving stomata. The exploration of this delay space frames the objectives of this study.

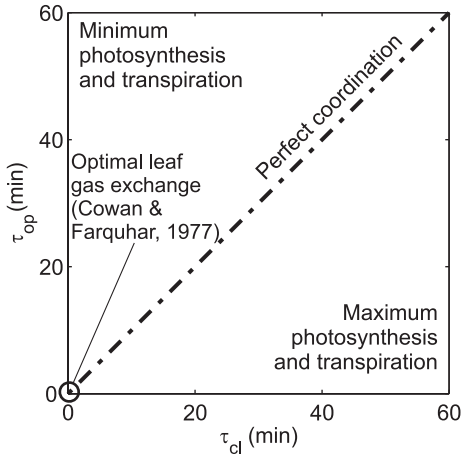


**Fig. 1** Qualitative depiction of the role of the characteristic times of stomatal opening $\tau_{op}$ and closing $\tau_{cl}$ on leaf gas exchange. The experimentally observed combinations of $\tau_{op}$ and $\tau_{cl}$ are hypothesized to be the result of evolutionary pressures to balance carbon gains through photosynthesis, water losses through transpiration and to reduce periods of water stress.

Specifically, two inter-related questions pertinent to stomatal delays are addressed. We first investigate whether the measured delays in stomatal response and the asymmetry in opening/closing times noted in the literature can be broadly related to plant functional types and traits, such as drought and shade tolerance (which are expected to be associated with better light tracking stomata). Second, we assess whether the patterns in the observed delays can be explained by net carbon gain optimization, and how different values of $\tau_{op}$ and $\tau_{cl}$ may affect photosynthetic gains, transpiration losses and, more generally, the economics of leaf gas exchange. To address the first question, an extensive meta-analysis is conducted on stomatal responses to abrupt changes in light irradiance using published datasets. The second question is addressed by developing a dynamic model of leaf stomatal conductance and photosynthesis, coupled to a novel minimalist description of stomatal movement costs. This modeling approach provides a framework for exploring stomatal delays in the context of strategies adopted by plants to cope with light intermittency. We assess the dependence of these strategies on plant features, such as marginal WUE, stomatal movement cost parameters and 'scaling laws' relating stomatal conductance to aperture size.

## Description

### Dynamic stomatal model

A database of published stomatal responses to changing light environment in fully induced leaves across a variety of species was assembled (Supporting Information Methods S1). The goal was to assess whether any plant trait can explain the observed stomatal delays. To describe the temporal evolution of stomatal conductance $g(t)$ in response to an abrupt change in light (from which stomatal delays are estimated), piecewise linear, logistic and exponential models have been frequently employed (Kirschbaum *et al.*, 1988; Zipperlen & Press, 1997; Naumburg *et al.*, 2001). For simplicity, the exponential model (corresponding to a first-order opening/closure equation; for example, Knapp (1993) and Whitehead & Teskey (1995)) was selected:

$$\frac{dg(t)}{dt} = \frac{g^*(\phi) - g(t)}{\tau_g} \qquad \text{Eqn 1}$$

where $g^*(\phi)$ is the asymptotic stomatal conductance achieved under constant light. Solving Eqn 1, and assuming $g(t = 0) = g_0$ as the initial condition for the problem, the exponential time evolution for $g(t)$ is obtained as:

$$g(t) = g^*(\phi) + [g_0 - g^*(\phi)] \exp\left(-\frac{t}{\tau_g}\right) \qquad \text{Eqn 2}$$

where $g^*(\phi) > g_0$ during the stomatal opening phase, and $g^*(\phi) < g_0$ during the closure. In Eqns 1, 2, $\tau_g$ represents the time necessary to cover 63% of the difference between the initial value $g_0$ and the asymptotic value $g^*(\phi)$. As most species exhibit an asymmetric response to light changes, $\tau_g$ may assume different values depending on whether the plant is responding to a sudden increase or decrease in light, that is:

$$\tau_g = \begin{cases} \tau_{op} & g(t) < g^*(\phi) \\ \tau_{cl} & g(t) \geq g^*(\phi) \end{cases} \qquad \text{Eqn 3}$$

### Data analysis

Our first goal was to obtain the characteristic times of stomatal response delay ($\tau_{cl}$ and $\tau_{op}$) by fitting Eqn 2 to the observed stomatal conductance time series reported in the literature. Given the heterogeneity in sampling frequencies and in the durations of periods of light and darkness employed in the experiments, we followed different methods to obtain these characteristic delay times, depending on the available data and their presentation. When the stomatal response allowed the identification of a clear asymptote (i.e. $g^*$), the exponential decay/recovery was fitted directly (Eqn 2). Otherwise, when data just covered the initial phase of stomatal response, a linear fitting was performed, and the stomatal delay was estimated as the time to reach a 63% change in conductance (see details in Methods S1). All regressions were based on least-squares fitting of the digitized data.

The relevance of the species features and growing conditions in the characteristic delay times obtained was investigated through a multi-factor ANOVA for unbalanced design. The relationship between opening and closing delays has been quantified through correlation coefficients and reduced major axes regression (Niklas, 2006). All statistical analyses were performed with MatLab (MathWorks, 2011, Natick, MA, USA).

### Minimalist gas exchange model

Although several models of photosynthesis, accounting for the light response of metabolite pools, enzyme activity and/or stomatal movement delays, have been proposed (Kirschbaum *et al.*, 1988; Pearcy *et al.*, 1997; Ooba & Takahashi, 2003), a minimalist model was employed here to assess the sensitivity of leaf photosynthesis and transpiration rates to the delayed responses of stomatal opening and closure. First, we assumed that leaves were fully induced. Because the induction state over relatively short periods (20–60 min) of low light remains fairly high in most species (e.g. Valladares *et al.*, 1997; Allen & Pearcy, 2000; Naumburg & Ellsworth, 2000; Rijkers *et al.*, 2000), this assumption does not limit significantly the applicability of the model, with the exception of early mornings. Second, only stomatal lags were explicitly considered. The rationale here is that, compared with stomatal movements, biochemical delays are typically much shorter (Weber *et al.*, 1985; Knapp & Smith, 1987, 1988) and, hence, can be assumed to be instantaneous relative to their stomatal counterparts.

**A simplified dynamic photosynthesis model** Instantaneous mass transfer of $CO_2$ and water vapor from the atmosphere to the leaves, and vice versa, may be described by Fickian diffusion as:

$$A = g_{CO_2}(c_a - c_i) \qquad \text{Eqn 4}$$

$$E = gD \qquad \text{Eqn 5}$$

where $A$ is the net $CO_2$ assimilation rate of the leaf, $E$ is the transpiration rate of the leaf, $c_a$ and $c_i$ are the $CO_2$ concentrations in the atmosphere and the intercellular spaces, respectively, $g_{CO_2}$ and $g$ are the stomatal conductances to $CO_2$ and water vapor, respectively (with $g = 1.6g_{CO_2}$), and $D$ is the atmospheric vapor pressure deficit (VPD). The resistance of the leaf boundary layer was neglected as most of the gas exchange data used in the meta-analysis were

obtained in well-mixed leaf chambers. Moreover, the mesophyll conductance was assumed to be much larger than $g_{CO_2}$, so that diffusion through the stomata was the only limiting factor to the $CO_2$ flux. For the sake of analytical tractability, to determine the $CO_2$ assimilation rate, a multiplicative model of photosynthesis was employed (Hari *et al.*, 1986; Berninger & Hari, 1993; Vesala *et al.*, 2000). This model was derived by linearizing the dependence on $c_i$ in the denominator (e.g. Lloyd & Farquhar, 1994; Katul *et al.*, 2010) to obtain:

$$A = a_1(\phi)\frac{c_i - \Gamma^*}{a_2 + R_c c_a} - R_d \qquad \text{Eqn 6}$$

where Γ* is the compensation point (assumed to be negligible in the following), $a_2$ is the half-saturation constant of the $CO_2$ response, $R_c$ is the long-term ratio of internal to atmospheric $CO_2$ concentrations, resulting from the linearization, and $R_d$ is the day respiration. The function $a_1(\phi)$ captures the instantaneous light dependence of photosynthesis (e.g. Givnish, 1988; Mäkelä *et al.*, 1996) as:

$$a_1(\phi) = V_{c\max}\frac{\phi}{\phi + \phi_0} \qquad \text{Eqn 7}$$

In Eqn 7, $\phi$ represents the incident photosynthetically active radiation and $\phi_0$ denotes the half-saturation constant.

To close the problem, an estimate of stomatal conductance is needed. We assume that the temporal evolution of $g_{CO_2}$ is controlled by the first-order delay (Eqn 1), where the asymptotic limit $g^*(\phi)$ corresponds to the optimal stomatal conductance allowing plants to maximize photosynthesis for a given water availability (Cowan & Farquhar, 1977; Hari *et al.*, 1986). This optimization problem is mathematically equivalent to instantaneously maximizing the quantity $A - \lambda E$, where the Lagrange multiplier $\lambda$ represents the marginal WUE, that is $\lambda = (\partial A/\partial g_c)/(\partial E/\partial g_c)$. This definition of $\lambda$ is consistent with the notation of Hari *et al.* (1986) and Katul *et al.* (2009), but is the inverse of the definition used by Cowan & Farquhar (1977). The resulting optimal stomatal conductance as a function of environmental conditions ($D$, $\phi$) is given as (Katul *et al.*, 2009, 2010; Manzoni *et al.*, 2011):

$$g_{\text{opt}} = \frac{a_1(\phi)}{a_2 + R_c c_a}\left[\sqrt{\frac{c_a - R_d(a_2 + R_c c_a)\,a_1(\phi)^{-1}}{1.6D\lambda}} - 1\right] \qquad \text{Eqn 8}$$

It should be emphasized here that, by assuming $g^*(\phi) = g_{\text{opt}}$, no formal optimization of plant assimilation under variable light conditions is attempted. The use of the steady-state optimal stomatal conductance for $g^*(\phi)$ is based on the need to set an asymptotic value for stomatal conductance when light remains steady. Other models for stomatal conductance (e.g. Jarvis, 1976; Norman, 1982; Leuning, 1995; Buckley *et al.*, 2003) could also be used to define a steady state $g$, should these alternative formulations be deemed more appropriate. Compared with other approaches, Eqn 8 has the advantage of analytical tractability and requires only one parameter (i.e. $\lambda$).

Clearly, this model does not explicitly describe the mechanisms and signaling pathways involved in stomatal movements. However, despite its simplicity, it does capture the main features of stomatal response to an abrupt change in light and the resulting interplay among intercellular $CO_2$ concentration, the degree of opening of the stomatal aperture (and hence conductance $g$) and $CO_2$ assimilation rate.

**Application to intermittent light regimes** In the following analyses, the model is forced by an artificial light regime consisting of alternating periods of high light and low light (or darkness) of given durations. For a high light/low light cycle of duration $T_c$, $\alpha T_c$ represents the duration of the period of high light and $(1 - \alpha)T_c$ denotes the duration of the period of low light, with $0 \le \alpha \le 1$, where the equalities correspond to the case of no intermittency. This artificial light regime differs from natural light experienced by understory plants or under passing clouds, but closely resembles the artificial light conditions under which the experiments are typically performed. Furthermore, such a primitive light regime facilitates the assessment of how the light intermittency impacts on the economics of leaf gas exchange. We neglect the initial transient (lasting, at most, a few light cycles) and focus on the phase in which leaf responses are periodic, that is, $g(t + T_c) = g(t)$ and $A(t + T_c) = A(t)$. Under such conditions, the temporal evolution of $g$ can be computed analytically, as presented in Methods S2. Based on the analytical function $g(t)$, the temporal evolutions of photosynthesis $A(t)$ and transpiration $E(t)$ can also be obtained.

## Leaf gas exchange economics

To interpret the observed stomatal response delays, we couple the gas exchange model with a mathematical description of guard cell energetics. This is necessary because more responsive guard cells (i.e. faster stomatal movements) come at a higher energetic cost of operation, which, in turn, has an impact on the net carbon gain.

**Stomatal movement costs** Stomatal movements in response to variable environmental conditions are triggered by changes in guard cell osmotic potential, which, in turn, drive changes in water content and hence guard cell volume. The transport of osmoticum (chiefly $K^+$ and sucrose)

through the ion channels of the guard cell membrane is responsible for changes in osmotic potential (Zeiger, 1983; Vavasseur & Raghavendra, 2005; Shimazaki *et al.*, 2007). During stomatal opening, the extrusion of $H^+$ by a proton pump and malate$^{2-}$ synthesis inside the guard cell (Assmann *et al.*, 1985; Assmann, 1999; Hanstein & Felle, 2002; Shimazaki *et al.*, 2007) cause the entrance of osmoticum through the activated inward channels (Roelfsema & Hedrich, 2005; Vavasseur & Raghavendra, 2005). When stomata close, outward ion channels are activated and, presumably, the activity of the proton pump is reduced (Hanstein & Felle, 2002). Thus, the proton pump operation and malate synthesis require energy (as ATP) to proceed, whereas, in a first-order analysis, stomatal closing may be regarded as a comparatively passive mechanism (Assmann & Zeiger, 1987; Roelfsema & Hedrich, 2005). The current limited understanding of the precise mechanism driving the signaling and stomatal movements prevents a mechanistic description of all energetic costs of stomatal response to fluctuating light. Hence, in the following, a simplified representation of stomatal opening mechanics and energetics is employed to estimate the stomatal movement cost $C(t)$. This modeling approach combines the limited experimental evidence available on stomatal movement energetics with existing theories and experimental results relative to gas diffusion through stomata. This approach is the first dynamic model of stomatal movement energetics, as previous attempts were restricted to the total energetic cost of a single opening (Assmann & Zeiger, 1987) or only considered steady-state conditions (Dewar, 2002; Buckley *et al.*, 2003).

The opening cost per unit time $C(t)$ (in units consistent with gas exchange measurements, i.e. μmol $CO_2$ $m^{-2}$ $s^{-1}$) equals the marginal cost $dc$ for a stomatal conductance change $dg$ multiplied by the corresponding change in $g$ per unit time, that is, $C = dc/dg \times dg/dt$. The first term in the product accounts for the costs involved at different levels of stomatal aperture (i.e. $c$ is expressed as μmol $m^{-2}$), and the second term represents the speed of stomatal changes (see Eqn 1). To link these theoretical developments with measurable quantities and existing theories, the term $dc/dg$ is further decomposed as $dc/d\mu \times d\mu/dg$, where $\mu$ is the mean stomatal aperture and $c(\mu)$ is the cost to achieve a given $\mu$.

Because of the 1 : 1 stoichiometry of ion uptake and proton extrusion (Raschke, 1975), the shape of the relationship $c(\mu)$ can, in principle, be inferred from experimental data linking stomatal aperture and guard cell cation concentration, and hence total ion uptake (Hsiao, 1976). Such dependence suggests that $c(\mu)$ can be described as:

$$c(\mu) = \gamma\left(\frac{\mu}{\mu_{max}}\right)^{v} \qquad \text{Eqn 9}$$

where $\gamma$ is the cost per unit leaf area needed to fully open the stomata and $v$ is a shape factor (Fig. 2a). To estimate $\gamma$,

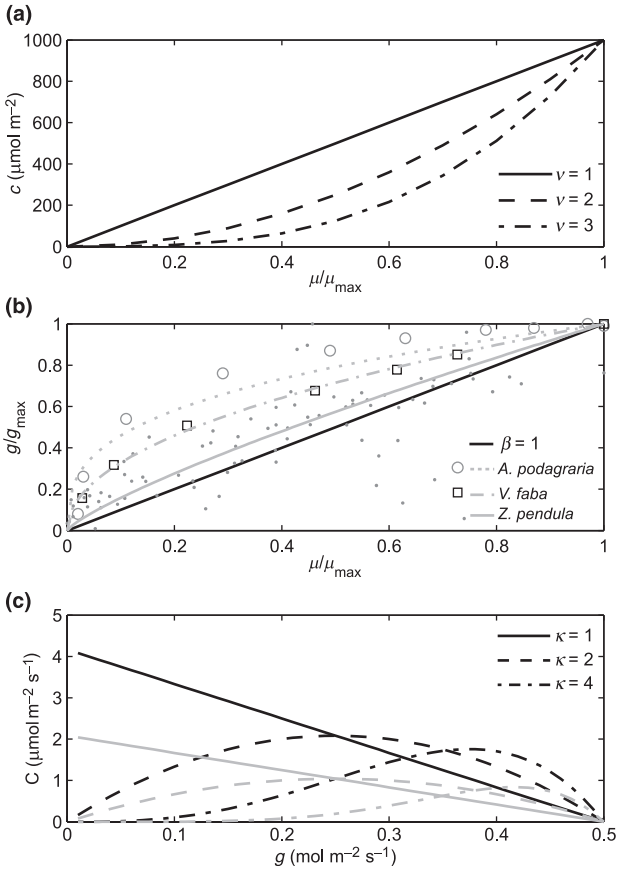


**Fig. 2** (a) Relationship between aperture cost and stomatal aperture (Eqn 9). (b) Nonlinear effect of aperture on stomatal conductance (Eqn 11). (c) Carbon cost per unit time of stomatal opening, as a function of stomatal conductance (Eqn 12), for two stomatal opening response times (black lines, 4 min; gray lines, 8 min). For illustration, (b) also shows data for *Aegopodium podagraria* (circles; Kaiser & Kappen, 2000), *Vicia faba* (squares; Kaiser, 2009) and *Zebrina pendula* (dots; Bange, 1953), collected in well-ventilated chambers. Fitting Eqn 12 to the presented data leads to exponents $\beta$ = 0.34 (dotted gray line), 0.48 (dash-dotted gray line) and 0.80 (solid gray line) for *A. podagraria*, *V. faba* and *Z. pendula*, respectively. The 1 : 1 line (solid black line) can be interpreted as Stefan's diameter law (Brown & Escombe, 1900). In (c), $g_{max} = g_{opt}$ = 0.5 mol $m^{-2}$ $s^{-1}$ (i.e. saturating light conditions during sunflecks).

we consider the energetic costs of proton extrusion and malate synthesis for an individual stomata (following the rationale of Assmann & Zeiger, 1987), and scale the cost up to the leaf level, consistently with the other flux calculations. To proceed, we start from proton extrusion rates per unit stomatal aperture, which range between 0.2 and 5.14 μmol $H^+$ $\mu m^{-1}$ per stomata (data for *Vicia faba* and *Commellina communis* after Raschke & Humble, 1973; Gepstein *et al.*, 1982; Inoue & Katoh, 1987). If 1 mol ATP is assumed to be necessary to extrude 2 mol $H^+$ (Assmann & Zeiger, 1987) and the efficiency of ATP production (either through respiration or photophosphorylation) is *c.* 5 mol ATP per

mol $CO_2$ (Taiz & Zeiger, 2006), a range of opening costs of $c$. (0.64–5.58) × $10^{-7}$ μmol $CO_2$ $\mu m^{-1}$ per stomata are obtained. In the absence of more precise data, this range is assumed to account for most of the variability in guard cell metabolism when no water stress is present. To scale up the costs to the leaf level, these costs need to be multiplied by the species-specific maximum stomatal aperture and stomatal density. Using the values reported by Larcher (2003), a range of $\gamma \approx$ 13–3350 μmol $CO_2$ $m^{-2}$ of leaf area for the full aperture of all stomata is obtained. When considering a particular species with specific values for the maximum stomatal aperture and stomatal density, a narrower range may be found.

The shape factor $v$ in Eqn 9 can be set to unity when the cost increases linearly with aperture, or can be greater than unity when the extrusion of protons becomes increasingly costly as the aperture reaches its maximum and a stronger proton gradient across the guard cell wall has to be overcome, as suggested by Assmann & Zeiger (1987). In the following analyses, it is conservatively assumed that a moderate nonlinearity prevails (i.e. $v$ = 2).

The relationship between stomatal conductance and aperture, $g(\mu)$, can be inferred from experimental data (Bange, 1953; Ting & Loomis, 1965; van Gardingen *et al.*, 1989; Kaiser & Kappen, 2000, 2001; Kaiser, 2009) or a number of theories (Brown & Escombe, 1900; Patlak, 1959; Cooke, 1967; Parlange & Waggoner, 1970; Troyer, 1980; Lushnikov *et al.*, 1994; Vesala *et al.*, 1995). In general, these theories and experimental studies (Fig. 2b) predict that stomatal conductance and aperture scale as:

$$g \sim \mu^{\beta} \qquad \text{Eqn 10}$$

where the exponent $\beta > 0$ depends on the geometry of the stomata (its shape and depth) and the wind velocity (Bange, 1953; Lee & Gates, 1964; Ting & Loomis, 1965; Waggoner & Zelitch, 1965; Parlange & Waggoner, 1970; van Gardingen *et al.*, 1989; Kaiser, 2009). This expression recovers the classical Brown & Escombe's (1900) result when $\beta$ = 1 (often referred to as Stefan's diameter law), although interferences between adjacent stomata, elongation of the stomatal pore and depth of the diffusive pathway may yield $\beta < 1$ even for high boundary layer conductance (Ting & Loomis, 1965; Parlange & Waggoner, 1970; van Gardingen *et al.*, 1989; Kaiser, 2009). For a generic $\beta$, the relative aperture is then computed as:

$$\frac{\mu(g)}{\mu_{max}} = \left(\frac{g}{g_{max}}\right)^{1/\beta} \qquad \text{Eqn 11}$$

where $g_{max}$ is the maximum stomatal conductance. A direct consequence of the nonlinearity in $\mu(g)$ is that a small change in aperture when stomata are closed results in a relatively large conductance gain. At high apertures, the gain in conductance decreases.

Finally, obtaining $dg/dt$ from Eqn 1, an analytical expression for the total instantaneous costs of stomatal opening can be derived as:

$$C(t) = \frac{dc}{d\mu}\frac{d\mu}{dg}\frac{dg}{dt} = \frac{\gamma\kappa}{g(t)}\left[\frac{g(t)}{g_{max}}\right]^{\kappa}\frac{g_{opt} - g(t)}{\tau_{op}} \qquad \frac{dg}{dt} > 0 \qquad \text{Eqn 12}$$

where $C(t)$ is set to zero when $dg/dt \le 0$. In Eqn 12, the parameter $\kappa = v\beta^{-1}$ is in the range 2–6 based on realistic values of $\beta$ and $v$. Here, we consider an intermediate value $\kappa$ = 4 that accounts for mild nonlinearities in both $c(\mu)$ and $\mu(g)$ relationships (Eqns 9, 11; Fig. 2c), and $g_{max}$ can be estimated from gas exchange data. It should be noted that, despite $\tau_{op}$ being the only time constant explicitly included in the above formulation of $C(t)$ (because stomatal closure is assumed to be passive), because of the relevance of the previous history of stomatal conductance, the integrated cost over a certain time period depends on both time constants of the stomatal response. Indeed, rapidly closing stomata increases the total costs of stomatal movements, because low $g$ at the end of the dark period causes higher opening costs at the beginning of the subsequent light period.

**Inclusion of stomatal movement costs in leaf economics** The modeling framework described above was employed to explore the impact of stomatal delays on the mean net carbon gain $\overline{G}$, including the mean costs of stomatal movements: $\overline{C}$,

$$\overline{G} = T_C^{-1}\int_0^T [A(t) - \lambda E(t) - C(t)]dt = \overline{A} - \lambda\overline{E} - \overline{C} \qquad \text{Eqn 13}$$

where the overbar denotes mean fluxes. The ideal case of perfectly tracking stomata and no cost of stomatal movements is considered as a reference, resulting in a net carbon gain of $\overline{G_{opt}} = T_C^{-1}\int_0^T \left[A_{opt}(t) - \lambda E_{opt}(t)\right]dt$. Here, no formal optimization of assimilation under variable light is implemented, that is, no maximization of the cumulative value of $A(t) - \lambda E(t) - C(t)$ is attempted for stochastic light levels. Rather, the metric $A(t) - \lambda E(t) - C(t)$ is employed to account for assimilation, transpiration losses and stomatal movement costs in a common framework, to search for combinations of stomatal delays that maximize such a metric.

## Results and Discussion

### Meta-analysis

Fig. 3(a) shows the temporal evolution of the normalized stomatal conductance, $g_n = (g(t) - g_0)\,(g^*(\phi) - g_0)^{-1}$, in response to an abrupt change in light irradiance, according to the experimental data available in the literature (Table S1). Time is normalized as $t_n = t\,\tau_g^{-1}$, where $\tau_g$ is $\tau_{cl}$ during stomatal closure and $\tau_{op}$ during stomatal opening (black and gray symbols, respectively). The corresponding exponential decay and recovery are reported for comparison (solid and dashed lines). Overall, the data collected are well described by the exponential model, particularly in the case of stomatal closure. In a few cases, patterns not captured by the proposed model are superimposed on the exponential behavior. Examples are the delayed initiation of stomatal aperture after a sudden light increase (Grantz & Zeiger, 1986; Kirschbaum & Pearcy, 1988) and oscillations (Cardon *et al.*, 1994; Zipperlen & Press, 1997 and references therein).

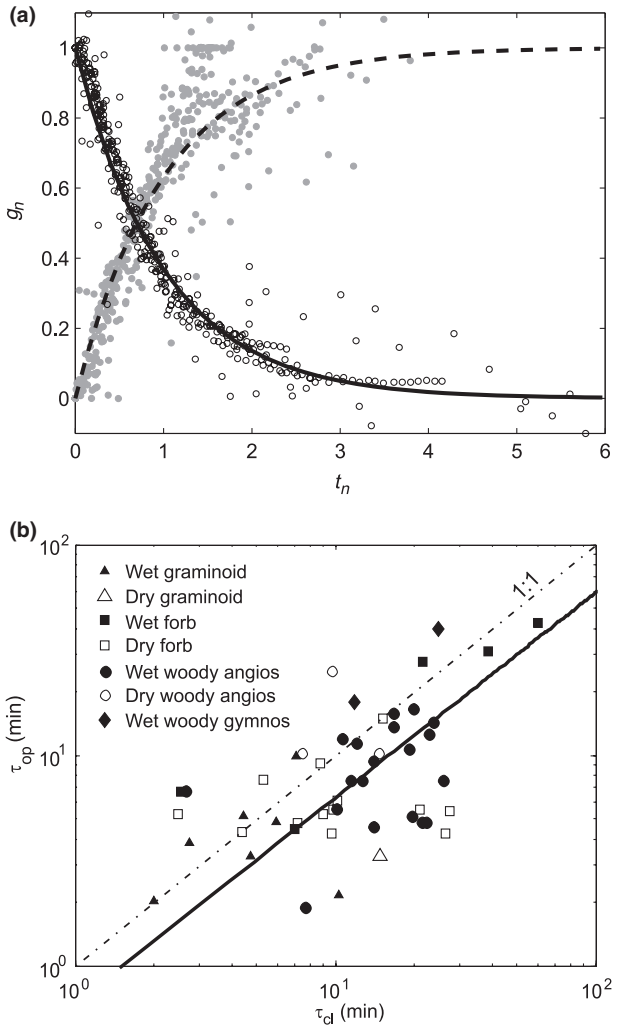


**Fig. 3** (a) Normalized stomatal conductance time series $g_n(t_n)$ for stomatal closing (open black symbols) and opening (gray symbols). The corresponding theoretical exponential decay ($g_n(t_n) = \exp(-t_n)$, $R^2 = 0.95$; solid line) and increase ($g_n(t) = 1 - \exp(-t_n)$, $R^2 = 0.87$; dashed line) are plotted for reference. (b) Logarithmic scatter plot of observed delays in stomatal response to abrupt changes in light irradiance as a function of plant functional type and typical climate. The black solid line represents the allometric relationship between opening and closing times, $\tau_{op} = b\tau_{cl}^a$, obtained through reduced major axis regression of the data (curve parameters and 95% confidence intervals: $a = 0.97 \pm 0.21$, $b = 0.67 \pm 0.17$).

The characteristic response times of stomatal movements for each species are reported in Methods S1, together with some plant traits, and shown in Fig. 3(b) (where data are grouped by functional type and climate). In all species, stomatal delays in response to changing light last 5–30 min. The allometric relationship between stomatal opening and closing delays, $\tau_{op} = b\tau_{cl}^a$, obtained through reduced major axis regression of the data (Niklas, 2006), suggests an asymmetric response (estimated curve parameter and 95% confidence interval, $b = 0.67 \pm 0.17$), with the majority of species exhibiting longer $\tau_{cl}$ than $\tau_{op}$, but no significant difference from a linear relationship ($a = 0.97 \pm 0.21$, solid line in Fig. 3b). From a mechanistic perspective, this asymmetric response may be determined by different inward and outward ion channels controlling the diffusion of $K^+$ in the guard cells. Despite this asymmetry, the two characteristic response times are significantly correlated ($R = 0.44$, $P = 0.0012$), with species exhibiting long $\tau_{op}$ also showing relatively long $\tau_{cl}$.

Experimental evidence suggests that the stomatal response times to fluctuating light depend not only on species and growth form (Knapp & Smith, 1989), but also on air temperature (Pepin & Livingston, 1997), air humidity (Tinoco-Ojanguren & Pearcy, 1993; Kaiser & Kappen, 2000, 2001), leaf water status (Davies & Kozlowski, 1975; Knapp & Smith, 1990; Barradas *et al.*, 1994), $CO_2$ concentration (Knapp *et al.*, 1994), magnitude of the light change (Woods & Turner, 1971) and length of the period of darkness, even when these intervals are short enough to presumably avoid induction loss (Cardon *et al.*, 1994; Whitehead & Teskey, 1995; Kaiser & Kappen, 2000). Because, in the meta-analysis, these factors might be confounded (and are often poorly characterized in the individual studies), no plant trait or environmental condition alone is able to explain the observed pattern of stomatal delays (Fig. 3b). Nevertheless, a multi-factor ANOVA showed that, in both $\tau_{op}$ and $\tau_{cl}$, the most significant factors are plant functional type and climate ($P < 0.05$), with shorter response times in graminoids and in species adapted to dry conditions (Table 1). The former result is in agreement with previous observations of the capacity for rapid stomatal movements in grasses, which has

**Table 1** Mean (± standard deviation) of stomatal closing ($\tau_{cl}$) and opening ($\tau_{op}$) delays for different plant functional types and climates

| | $\tau_{cl}$ (min) | $\tau_{op}$ (min) |
|---|---|---|
| Plant functional type | | |
| Graminoids | 5.9 (± 4.4) | 3.9 (± 2.6) |
| Forbs | 16.0 (± 14.9) | 10.8 (± 11.2) |
| Angiosperm woody | 15.2 (± 5.9) | 10.3 (± 5.2) |
| Gymnosperm woody | 18.4 (± 9.3) | 29.0 (± 15.6) |
| Climate | | |
| Wet | 15.0 (± 11.4) | 11.2 (± 10.0) |
| Dry | 12.0 (± 7.3) | 7.7 (± 5.4) |

been explained by the dumb-bell guard cell design typical of this group (Hetherington & Woodward, 2003; Franks & Farquhar, 2007). Conversely, no significant differences ($P > 0.1$) emerge when data are grouped by shade and drought tolerance. This seems to be in contrast to the most logical expectations that shade- and drought-tolerant species tend to track more closely light changes to utilize the limited light availability and to limit water losses, respectively. The absence of correlation between stomatal delays and the shade tolerance has been observed previously by other authors, even in *ad hoc* experiments considering species grown under contrasting conditions (e.g. Pereira & Kozlowski, 1977; Naumburg & Ellsworth, 2000; Montgomery & Givnish, 2008), and other adaptive strategies for understory plants have been proposed (e.g. Kaiser & Kappen, 2000; Valladares & Niinemets, 2008).

Because groups of data based on plant traits or environmental conditions are not clearly separated, we proceed by exploring whether the maximization of the carbon gain is consistent with: $\tau_g \sim$ 5–30 min to balance carbon gains and costs; strong coordination between $\tau_{op}$ and $\tau_{cl}$; and sensitivity of $\tau_{op}$ and $\tau_{cl}$ to environmental factors that depend on the experimental set-up. We address these questions by means of the mathematical model described previously.

## Role of stomatal delays in leaf economics

**Periodic temporal evolution of stomatal conductance and assimilation rate** Fig. 4 shows the temporal evolution of the stomatal conductance, assimilation rate and stomatal movement costs, as forced by a periodic light cycle for three combinations of response times. After the light is set to high, stomatal conductance increases, reaching a maximum that depends on the response time (and the duration of high light; not shown), with higher values corresponding to faster stomatal responses (dotted line in Fig. 4a). After the light drops to low irradiances, stomatal conductance is progressively reduced. The assimilation rate exhibits more abrupt changes when the light is modified (Fig. 4b) because of its dependence on light availability through the kinetic coefficient $a_1(\phi)$ (assumed to respond instantaneously to light changes; Eqn 7). The subsequent more progressive decrease in assimilation is a direct consequence of changes in stomatal conductance, mediated by $c_i$. These predicted couplings between $A$ and $g$ are typical of leaf responses to intermittent light and have been observed in several studies (e.g. Whitehead & Teskey, 1995; Fay & Knapp, 1996;

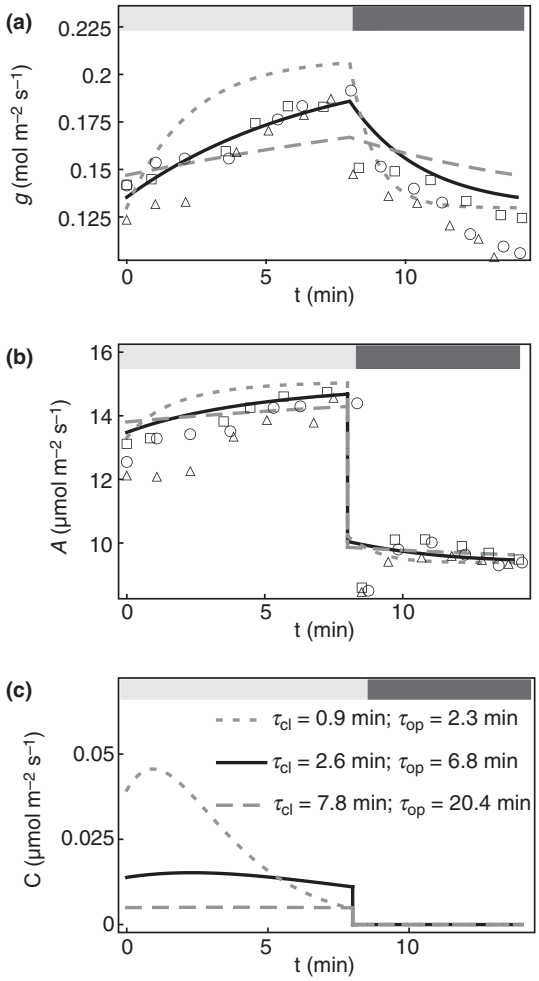


**Fig. 4** Example of modeled stomatal conductance (a), assimilation rate (b) and stomatal movement costs (c) (solid lines) during a light : dark cycle (light and dark bars, respectively) and comparison with measurements on *Quercus macrocarpa* (Knapp, 1992). The different symbols refer to three subsequent light cycles, here superimposed for comparison. Dotted and dashed lines refer to stomata responding three times more rapidly and three times more slowly, respectively, than those of *Q. macrocarpa* (maintaining a constant $\tau_{op}/\tau_{cl}$). In agreement with the experimental set-up, the light : dark cycle has the duration $T_c$ = 14 min, with a fraction of light duration $\alpha$ = 4/7, and high and low irradiances $\phi_h$ = 315 W m$^{-2}$ and $\phi_l$ = 74 W m$^{-2}$. Other parameters are $c_a$ = 330 ppm, $D$ = 0.019 mol mol$^{-1}$, $a_2$ = 228 μmol mol$^{-1}$, $R_d$ = 2 μmol m$^{-2}$ s$^{-1}$, $CO_2$ assimilation rate and stomatal conductance under constant light 14.5 μmol m$^{-2}$ s$^{-1}$ and 0.188 mol m$^{-2}$ s$^{-1}$, respectively, from which $\lambda$ = 630 μmol mol$^{-1}$ follows. Finally, by observing the dependence of $A$ on $\phi$ of the above data, we estimated $V_{cmax}$ = 33 μmol m$^{-2}$ s$^{-1}$ and $\phi_0$ = 72 W m$^{-2}$. The stomatal movement cost parameters are set to $\gamma$ = 300 μmol m$^{-2}$ and $\kappa$ = 4.

Greenway & Lieffers, 1997; Peek *et al.*, 2004). As an example, the modeled trajectories are compared in Fig. 4 with stomatal conductance and $CO_2$ assimilation rate time series observed in *Quercus macrocarpa* (Knapp, 1992). When parameterized according to the experimental conditions and stomatal characteristic response times, density and aperture (solid lines), the model captures the main features of the observed temporal evolution of both variables, without further calibration. During the light cycle, energy is used to open the stomata (Fig. 4c). These costs are nonlinearly dependent on $g$ during opening (Eqn 12), resulting in higher energetic expenses when aperture movement is faster, whereas they are set to zero during closure. It should be noted that opening costs are much lower than photosynthesis in this example, but they play a role when exploring the effect of low opening delays on the net gain defined by Eqn 13, as discussed later.

**Stomatal delays and carbon fluxes** The impact of stomatal delays on the mean photosynthesis, transpiration costs, stomatal opening costs and net gain is plotted in Fig. 5. As expected (Fig. 1), both $A$ and $E$ increase from low $\tau_{cl}$ and high $\tau_{op}$ to high $\tau_{cl}$ and low $\tau_{op}$ (Fig. 5a,b). Opening costs rapidly decrease with increasing $\tau_{op}$; because of the relevance of the stomatal conductance history on opening costs, they are also impacted by $\tau_{cl}$, although marginally (Fig. 5c). Despite being small when compared with mean photosynthesis, $\overline{C}$ becomes relevant when the net carbon gain, $\overline{G} = \overline{A} - \lambda\overline{E} - \overline{C}$, is considered. $\overline{G}$ is highest when $\tau_{cl}$ and $\tau_{op}$ are correlated (Fig. 5d), in agreement with the results of the meta-analysis (solid line; Fig. 3b). Nevertheless, the dependence of $\overline{G}$ on the stomatal delays is not strong, with a relatively broad plateau that would encompass the majority of the delay combinations exhibited by available data (Fig. 3b). In Fig. 5(d), the absolute maximum for $\overline{G}$ is found at $\tau_{cl} \approx 0$, a limiting case that is not realistic because of intrinsic time delays in the biochemical signaling, guard cell membrane depolarization time, and actual water efflux from the guard cells (Roelfsema & Hedrich, 2005). A realistic lower limit for the stomatal closing response time is the membrane depolarization characteristic time, which is estimated to be on the order of 2–4 min (Felle *et al.*, 2000) (the vertical dot-dashed lines in Figs 5, 6). Should the stomatal costs not be considered, the maximum net carbon gain would be attained with perfectly tracking stomata and, the longer the stomatal delays, the further away from the ideal case the system would be in terms of net carbon gains (not shown).

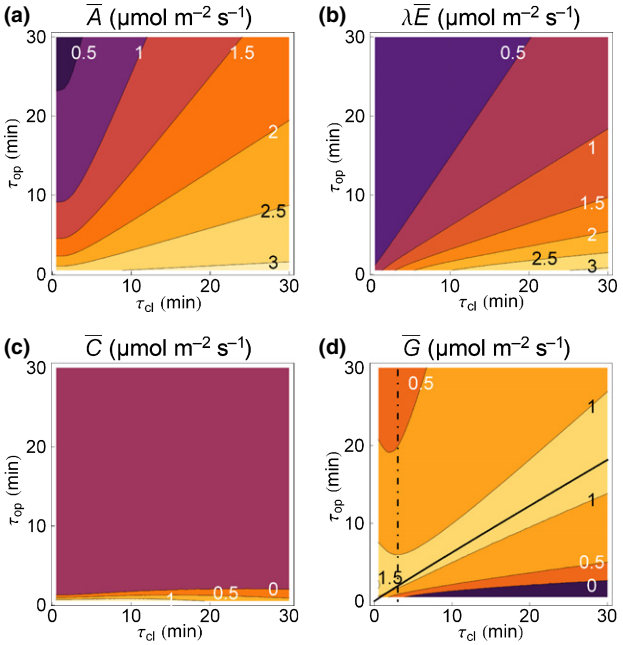


**Fig. 5** Impact of stomatal time constants on mean leaf assimilation (a), transpiration costs (b), stomatal opening costs (c) and net gain (Eqn 13) (d) for $\phi_h$ = 400 W m$^{-2}$, $\phi_l$ = 0 W m$^{-2}$, $\alpha$ = 0.2, $T_c$ = 10 min, $\lambda = 10^3$ μmol mol$^{-1}$, $D$ = 0.01 mol mol$^{-1}$ and $\gamma$ = 1000 μmol m$^{-2}$. All the other parameters are as in Fig. 4. In all panels, lighter tones refer to higher values while darker tones refer to lower values. The dot-dashed line in (d) indicates the time scale of membrane depolymerization, below which stomatal closure is unrealistically fast; the solid line is the reduced major axis regression of the data (see Fig. 3b).

When the light pattern and plant parameters are allowed to vary, the model still predicts higher $\overline{G}/\overline{G_{opt}}$ (where $\overline{G_{opt}}$ is the net carbon gain for perfectly tracking stomata) for coordinated delays, mostly for $\tau_{op}$ comparable with or slightly smaller than $\tau_{cl}$, in agreement with the results of the meta-analysis, summarized here by the reduced major axis regression of the data (solid lines in Fig. 6). Because explicit accounting for intrinsic limitations caused by depolarization and water efflux dynamics was not considered, some choices of the parameters cause the absolute maximum of $\overline{G}/\overline{G_{opt}}$ to occur for unrealistically low stomatal delays. In the following, we thus focus on the combinations of stomatal delays beyond this 'unrealistic' range, even though they do not necessarily correspond to the absolute maximum of $\overline{G}/\overline{G_{opt}}$.

With regard to the role of the light regime on the net carbon gain, the most relevant parameter is the fraction of time with high light availability during the light : dark cycle (i.e. $\alpha$). Low $\alpha$ results in overall lower $\overline{G}/\overline{G_{opt}}$, with optimal stomatal delay combinations corresponding to relatively low $\tau_{cl}$ and $\tau_{op}$ (Fig. 6a). Under these circumstances, light is in limited supply and must be harvested, even if this may result in significant opening costs. By contrast, for higher $\alpha$ (i.e. higher light availability; Fig. 6b), long response times reduce the opening costs and allow generally higher net carbon gain. It should be noted that intermediate $\alpha$ maximizes cumulated stomatal movement costs (not shown), because, for this light pattern, intermediate stomatal conductances (corresponding to the highest movement costs if $\kappa > 1$) are frequently experienced. Conversely, the duration of the

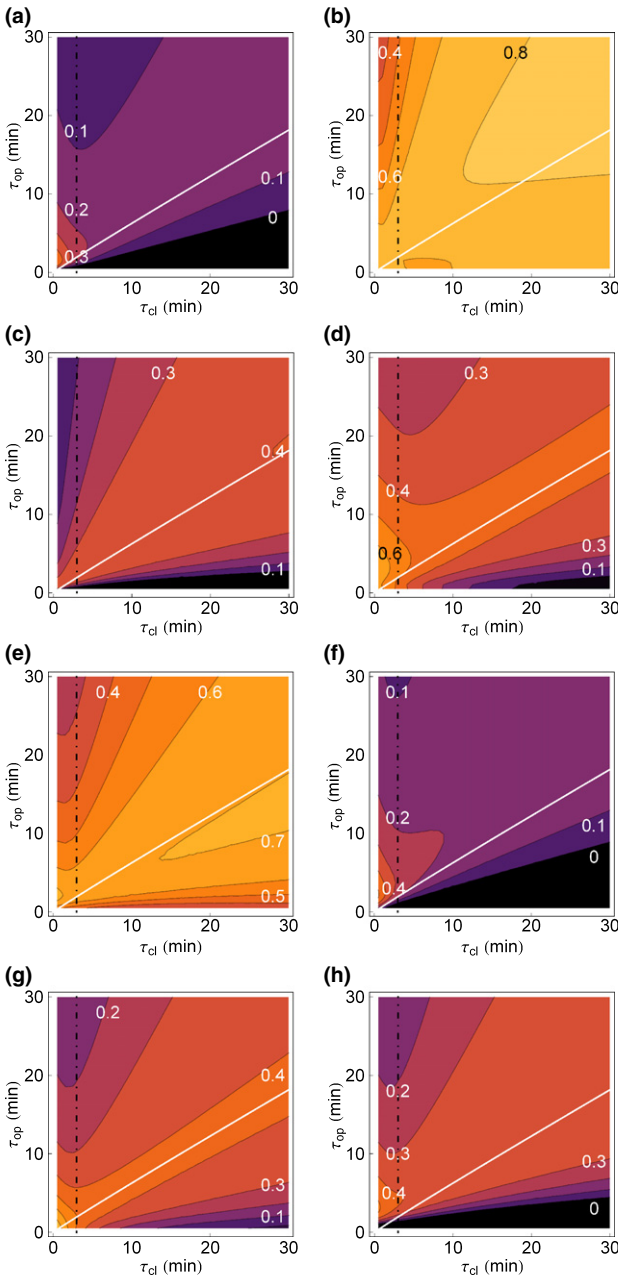


**Fig. 6** Effect of $\tau_{cl}$ and $\tau_{op}$ on $\overline{G}/\overline{G_{opt}}$ for different choices of light regime and plant parameters: (a,b) $\alpha$ = 0.1 and 0.6; (c,d) $T_C$ = 2 and 20 min; (e,f) $\lambda$ = 200 and 2000 μmol mol$^{-1}$; (g,h) $\gamma$ = 100 and 3000 μmol m$^{-2}$. All the other parameters are as in Fig. 5. The reference conditions depicted in Fig. 5(d) are intermediate between each pair of parameter values above. In all panels, lighter tones refer to higher $\overline{G}/\overline{G_{opt}}$ (with a maximum of ≈ 0.8 in (b)), and darker tones refer to lower $\overline{G}/\overline{G_{opt}}$ (≈ 0); the dot-dashed lines indicate the time scale of membrane depolymerization; the solid white line shows the reduced major axis regression of the data (see Fig. 3b).

light : dark cycle $T_c$ and the incident radiations, $\phi_h$ and $\phi_l$, play a secondary role on the dependence of the net carbon gain on stomatal delays. This relatively weak dependence lends support to the simplified light regime employed here. Specifically, short $T_c$ makes it more advantageous for the leaf not to track light changes (high optimal $\tau_{cl}$ and $\tau_{op}$; Fig. 6c); such dependence on $T_c$ would be more pronounced should $\alpha$ near 1/2. Finally, higher $\phi_l/\phi_h$ ratios result in slightly higher $\overline{G}/\overline{G_{opt}}$, with the extreme case of $\phi_l/\phi_h = 1$ corresponding to the absence of light intermittency, and hence $\overline{G}/\overline{G_{opt}} = 1$ (not shown).

With regard to the role of plant features, the impact of $\lambda$ on $\overline{G}/\overline{G_{opt}}$ (Fig. 6e,f) can be explained by considering $\lambda$ as a measure of the plant 'perceived value' of water loss vs carbon gain. For a fixed light regime, increasing $\lambda$ causes a shift in the combination of delays that maximizes net carbon gain towards lower $\tau_{cl}$ to limit unproductive (and costly) water losses through transpiration. The absolute maximum becomes more marked and shifts towards lower $\tau_{op}$, further limiting total transpiration losses, and the optimal ratio $\tau_{op}/\tau_{cl}$ shifts towards higher values. This shift in the optimal combination of delay times with increasing $\lambda$ is in agreement with the few data on gymnosperms presented in Table S1, characterized by $\tau_{op}/\tau_{cl} > 1$ (Table 1) and by high $\lambda$ (see Manzoni *et al.* (2011) and Lloyd & Farquhar (1994)). Furthermore, there is experimental evidence that $\tau_{cl}$ decreases with worsening plant water status (Davies & Kozlowski, 1975; Knapp & Smith, 1990; Barradas *et al.*, 1994), possibly as a consequence of decreased epidermal counter-pressure. By contrast, changes in $\tau_{op}$ are less consistent, with *Phaseolus vulgaris* showing strong decreases (Barradas *et al.*, 1994), whereas several tree species show a lengthening in the opening times (Davies & Kozlowski, 1975). In other words, plant water stress increases both $\lambda$ (Manzoni *et al.*, 2011) and the optimal $\tau_{op}/\tau_{cl}$ (at least in the case of trees). The effect of large $\lambda$ (Fig. 6f) is similar to the effect of high VPD, which drives stomatal closure and favors rapid responses to light changes (not shown). Finally, the cost per unit leaf area to open the stomata appears to play a secondary effect on $\overline{G}/\overline{G_{opt}}$, with higher costs causing an overall decrease in $\overline{G}/\overline{G_{opt}}$ and a shift in the optimal combinations of delays towards slightly higher $\tau_{op}$ (Fig. 6g,h).

The large variability exhibited by the results of the meta-analysis (Fig. 3b) suggests that several factors contribute to the evolution or adaptation towards a given combination of opening and closure delay times. Probably, these factors have contrasting effects, leading to a variety of quasi-optimal combinations of $\tau_{op}$ and $\tau_{cl}$, for example, for shade- (and drought-) tolerant vs intolerant species. The model results allow the quantification of the effects of these factors. For example, a light environment with prolonged sunflecks (large $\alpha$) would favor plants with high $\tau_{op}$ and $\tau_{cl}$ (Fig. 6b), but dry conditions (high $D$ and $\lambda$) would favor quicker stomatal responses (Fig. 6f). As a consequence of these contrasting effects, species living in dry climates with generally long sunflecks might exhibit different near-optimal combinations of $\tau_{op}$ and $\tau_{cl}$, corresponding to alternative

strategies. Specifically, light-tracking (short $\tau_{op}$ and $\tau_{cl}$, incomplete sunfleck use, but high WUE in the shade) and nontracking (long $\tau_{op}$ and $\tau_{cl}$, full sunfleck exploitation, but low WUE in the shade) leaves may equally well lead to successful growth in relatively arid environments (Knapp & Smith, 1989).

Finally, the acclimation of individuals to their growing conditions can be a further confounding factor not assessed by means of standard light : dark experiments. Indeed, most of the experiments reported here were performed on potted plants grown under controlled conditions, for example, in glasshouses. It is reasonable to assume that these plants were subjected to constant light availability during the day and well-watered conditions from their emergence to the moment in which the experiment was performed. As such, the results of our meta-analysis, when combined with the modeling sensitivity analysis, suggest the need for further experiments to assess the role of instantaneous light conditions vs the natural light patterns on leaf net carbon gain and plant activity in general.

### Conclusions

A large dataset of response times of stomata to changes in light was assembled and analyzed. Plant functional type and climate were found to control stomatal response times (faster in graminoids and dry environments) more than other plant traits (e.g. shade or drought tolerance). Despite the large scatter in the response times, we found significant correlation between $\tau_{op}$ and $\tau_{cl}$, with $\tau_{op}$, on average, shorter than $\tau_{cl}$. A modeling framework describing leaf economics (including stomatal opening costs) was employed to assess the optimality of such combinations of stomatal response times, leading to two main conclusions. First, the broad correlations between the opening and closing times scale with each other, and the overall scaling found here, $\tau_{op} = 0.67\tau_{cl}^{0.97}$, appears to be consistent with the maximization of photosynthetic gain, whilst minimizing water losses and stomatal opening expenses. Second, there is no unique combination of opening and closing time scales that determines an absolute maximum in net carbon gain. Instead, combinations of environmental and biochemical factors contribute to determine the region in the stomatal delay space in which the net carbon gain is maximized. In some cases, a clear maximum appears (e.g. at high marginal WUE), whereas most cases exhibit a relatively weak dependence of the net carbon gain on stomatal delays. The lack of a clear maximum is consistent with the variety of delay time combinations shown in the meta-analysis and, possibly, the inability of individual plant traits to explain the measured delays.

The analysis here is primarily diagnostic. Moving towards a prognostic framework will necessitate precise estimates of $\lambda$ and the parameters of the stomatal movement cost function. Although the former can often be estimated from the available data and information on the experimental set-up, the latter would require datasets measuring the bioenergetics of guard cell movements.

## Acknowledgements

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## Supporting Information

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

**Table S1** Summary of the meta-analysis of stomatal response delays

**Methods S1** Meta-analysis: dataset selection and data analysis.

**Methods S2** Periodic solution of stomatal conduction.

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