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RESEARCH PAPER

Importance of mesophyll diffusion conductance in estimation of plant photosynthesis in the field

Ülo Niinemets[1],*, Antonio Díaz-Espejo[2], Jaume Flexas[3], Jeroni Galmés[3] and Charles R. Warren[4]

[1] Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Kreutzwaldi 1, Tartu 51014, Estonia
[2] Instituto de Recursos Naturales y Agrobiología, CSIC, Apartado 1052, 41080 Sevilla, Spain
[3] Grup de Recerca en Biologia de les Plantes en Condicions Mediterrànies, Universitat de les Illes Balears, Carretera de Valldemossa Km 7.5, 07122 Palma de Mallorca, Spain
[4] School of Biological Sciences, Heydon-Laurence Building A08, The University of Sydney, NSW 2006, Australia

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Abstract

Mesophyll diffusion conductance to CO_2 (g_m) is an important leaf characteristic determining the drawdown of CO_2 from substomatal cavities (C_i) to chloroplasts (C_C). Finite g_m results in modifications in the shape of the net assimilation (A) versus C_i response curves, with the final outcome of reduced maximal carboxylase activity of Rubisco (V_{cmax}), and a greater ratio of the capacity for photosynthetic electron transport to V_{cmax} (J_{max}/V_{cmax}) and alterations in mitochondrial respiration rate (R_d) when estimated from A/C_i responses without considering g_m. The influence of different Farquhar *et al.* model parameterizations on daily photosynthesis under non-stressed (C_i kept constant throughout the day) and stressed conditions (mid-day reduction in C_i) was compared. The model was parameterized on the basis of A/C_C curves and A/C_i curves using both the conventional fitting procedure (V_{cmax} and R_d fitted separately to the linear part of the response curve and J_{max} to the saturating part) and a procedure that fitted all parameters simultaneously. The analyses demonstrated that A/C_i parameterizations overestimated daily assimilation by 6–8% for high g_m values, while they underestimated it by up to 70% for low g_m values. Qualitative differences between the A/C_i and A/C_C parameterizations were observed under stressed conditions, when underestimated V_{cmax} and overestimated R_d of A/C_i parameterizations resulted in excessive mid-day depression of photosynthesis. Comparison with measured diurnal assimilation rates in the Mediterranean sclerophyll species *Quercus ilex* under drought further supported this bias of A/C_i parameterizations. While A/C_i parameterization predicted negative carbon balance at mid-day, actual measurements and simulations with the A/C_C approach yielded positive carbon gain under these conditions. In addition, overall variation captured by the best A/C_i parameterization was poor compared with the A/C_C approach. This analysis strongly suggests that for correct parameterization of daily time-courses of photosynthesis under realistic field conditions, g_m must be included in photosynthesis models.

Key words: Diffusion limits, drought, mid-day reduction of photosynthesis, model parameterization, photosynthesis model.

Introduction

The widely used steady-state model of C_3 photosynthesis of Farquhar and co-workers (Farquhar *et al.*, 1980) is based on the CO_2 concentration in chloroplasts (C_C) to predict the potential rates of carboxylation of ribulose-1,5-bisphosphate (RuBP) driven by Rubisco and the rate of photosynthetic electron transport. The model of Farquhar *et al.* (1980) has proved incredibly popular because it can be parameterized with gas-exchange data alone, and thus provides a simple and easy analysis of the *in vivo* biochemical limitations of photosynthesis (von Caemmerer, 2000). In addition to providing information on biochemical limitations of leaf-level photosynthesis, the equations, logic,

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* To whom correspondence should be addressed. E-mail: ylo.niinemets@emu.ee

and parameters of the model of Farquhar *et al.* (1980) are used to drive canopy-, landscape-, and biome-scale models of carbon exchange (Harley and Tenhunen, 1991; Harley and Baldocchi, 1995; Haxeltine and Prentice, 1996; Churkina and Running, 1998; Rambal *et al.*, 2003).

The beauty of the model of Farquhar *et al.* (1980) is a combination of its predictive power, in particular prediction of plant responses to CO_2 that is useful to simulate photosynthesis under globally changing environmental conditions, and ease of parameterization. However, both the model predictability and the simplicity of parameterization critically depend on identification and estimation of substrate concentration. In the case of photosynthesis, the correct substrate concentration is the CO_2 concentration at the site of carboxylation, in the chloroplasts (C_C). As the diffusion conductance between substomatal cavities (C_i) and chloroplasts (g_m) has traditionally been suggested to be large ($g_m \rightarrow \infty$) (Björkman, 1973; Laisk, 1977; but see Nobel, 1977), the model of Farquhar *et al.* is most often parameterized using the CO_2 concentrations in substomatal cavities (C_i), i.e. assuming that $C_i = C_C$. By now, we have known for several decades that the concentration of CO_2 in the chloroplasts is significantly lower than that in the substomatal cavities because of finite g_m (Bongi and Loreto, 1989; von Caemmerer and Evans, 1991). Although the use of C_i as a surrogate for the correct substrate concentration, C_C, is logically flawed, the majority of current model exercises are still based on C_i because it is quickly and easily estimated by conventional gas-exchange techniques.

The key question is whether the substitution of C_C by C_i affects the utility of the model of Farquhar *et al.* (1980). Although it is currently widely accepted that the CO_2 concentration in the chloroplasts is significantly lower than in the substomatal cavities, there is still no consensus in the way photosynthesis models should be parameterized; in particular, whether the derivation of key model parameters, V_{cmax} and J_{max}, from either A/C_i or A/C_C response curves has any influence over modelling photosynthesis under field conditions. Several studies have already highlighted that finite g_m affects the numerical values of V_{cmax} and J_{max} (Ethier and Livingston, 2004; Manter and Kerrigan, 2004; Flexas *et al.*, 2008; Warren, 2008*b*). These studies have also shown that while g_m may scale with the biochemical capacity for photosynthesis, there is a large variation among species in the CO_2 drawdown due to g_m, C_i–C_C, implying that the degree of mesophyll diffusion limitations does vary significantly (Ethier and Livingston, 2004; Niinemets and Sack, 2006; Warren and Adams, 2006; Niinemets *et al.*, 2009*a*, *b*). Recent studies have further shown that g_m and its relationship with stomatal conductance (g_s) and photosynthetic capacity are highly variable within species and are affected by a variety of environmental variables (Flexas *et al.*, 2007; Warren, 2008*b*). This responsiveness of g_m to environmental conditions precludes any simple or 'universal' correction factor from being applied for converting from C_i-based to C_C-based model parameters. Although modifications in g_m in response to environmental stresses, in particular to drought, greatly alter photosynthesis, such changes are not considered in current larger scale simulation analyses (Rambal *et al.*, 2003). In consequence, models based on infinite g_m may underestimate drought-induced reductions in photosynthesis.

In this analysis, earlier studies are built on by first highlighting the key effects of finite g_m on derivation of the model parameters of Farquhar *et al.* (1980), in particular asking whether the use of C_i versus C_C and whether fitting of V_{cmax} and J_{max} separately from different A/C_i curve parts versus simultaneously from the entire A/C_i curve affects the parameter estimates. Traditionally, V_{cmax} is estimated from the linear portion of an A/C_i response and J_{max} from the saturating part, but Ethier and Livingston (2004) have shown that this fitting can lead to severe underestimation of Rubisco activity such that the entire A/C_i response curve can be apparently limited by Rubisco activity. However, it is possible to estimate V_{cmax} and J_{max} simultaneously from the same A/C_i response, and it was hypothesized that this will reduce errors. Using these parameterizations, the sensitivity of daily photosynthesis to different parameterizations based on C_i and C_C is analysed. So far, the overall effect of g_m on parameterization of the model of Farquhar *et al.* (1980) has not been assessed under realistic field conditions, and there is still no consensus on the way photosynthesis models should be parameterized. The present analysis demonstrates that derivation of key model parameters, V_{cmax} and J_{max}, from A/C_i response curves, independently of fitting the A/C_i responses, can result both in biased estimates of carbon gain, and in fundamentally flawed conclusions with respect to photosynthetic limitations in the field.

Materials and methods

Simulation of net assimilation (A) versus CO_2 response curves

According to the steady-state photosynthesis model of Farquhar *et al.* (1980), the foliage net assimilation rate (A) at any given chloroplastic CO_2 concentration (C_C) is limited either by Rubisco or by photosynthetic electron transport (RuBP regeneration). For Rubisco-limited photosynthesis:

$$A = \frac{V_{cmax}(C_C - \Gamma^*)}{C_C + K_m} - R_d, \quad (1)$$

where V_{cmax} is the maximal carboxylase activity of Rubisco, R_d is the mitochondrial respiration rate, Γ^* is the hypothetical CO_2 compensation point of photosynthesis in the absence of R_d, and K_m is the effective Michaelis–Menten constant. K_m is expressed as $K_C(1+O/K_O)$, where K_C is the Michaelis–Menten constant for CO_2 and K_O that for oxygen, and O is the oxygen concentration. For electron transport-limited photosynthesis:

$$A = \frac{J(C_C - \Gamma^*)}{4(C_C + 2\Gamma^*)} - R_d \quad (2)$$

where J is the rate of photosynthetic electron transport. J depends on the capacity for photosynthetic electron transport (J_{max}) and photosynthetic quantum flux density (Q) according to a non-rectangular hyperbola:

$$J = \frac{\alpha Q + J_{max} - \sqrt{(\alpha Q + J_{max})^2 - 4\alpha\theta Q J_{max}}}{2\theta} \tag{3}$$

where α is the initial quantum yield and θ is the curvature of the light response. C_C and the CO_2 concentration in substomatal cavities (C_i) are related as:

$$C_C = C_i - A/g_m, \tag{4}$$

where g_m is the mesophyll diffusion conductance from substomatal cavities to chloroplasts. Combining Eqs 1, 2, and 4, A in dependence on C_i can be calculated as (Ethier and Livingston, 2004; Niinemets *et al.*, 2004):

$$A = \frac{-b - \sqrt{b^2 - 4ac}}{2a} \tag{5}$$

where

$$\begin{aligned} a &= 1/g_m \\ b &= (V_{max} - R_d)/g_m - C_i - K_m \\ c &= V_{max}(C_i - \Gamma^*) - R_d(C_i + K_m) \end{aligned} \tag{6}$$

for Rubisco-limited photosynthesis, and

$$\begin{aligned} a &= 4/g_m \\ b &= -(J - 4R_d)/g_m - 4C_i - 8\Gamma^* \\ c &= J(C_i - \Gamma^*) - 4R_d(C_i + 2\Gamma^*) \end{aligned} \tag{7}$$

for electron transport-limited photosynthesis.

Using Eqs 5–7, A versus C_i response curves were simulated for given values of V_{cmax}, J_{max}, R_d, and g_m at saturating light of 1500 μmol m^{-2} s^{-1}, leaf temperature of 25 °C, and 21% oxygen. K_C, K_O, and Γ^* values were those from Bernacchi *et al.* (2001). Although different values of biochemical constants are found in the literature (see Bernacchi *et al.*, 2001 for comparison of various Rubisco constants), the main results of the present analyses were independent of the specific Rubisco constants used (simulations with varying biochemical constants not shown). An initial quantum yield of 0.24 mol mol^{-1} [assuming four electrons per CO_2, leaf absorptance of 0.85, and inherent quantum yield of photosynthesis of 0.073 mol CO_2 mol^{-1} quanta (Ehleringer and Björkman, 1977)] and a curvature of 0.85 (Evans *et al.*, 1993) were used.

The A–C_i curves derived in this way, were further re-fitted by the model of Farquhar *et al.* (1980) (Equations 1 and 2), replacing C_C by C_i, i.e. using the standard approach of the modelling community that neglects g_m. Two fitting procedures were used. According to the first (conventional) technique, V_{cmax} and J_{max} were fitted separately using the initial, essentially linear part of the A/C_i response curve to derive the values of V_{cmax} and R_d, while the saturating part of the curve was used to determine J_{max}. According to the second fitting procedure, V_{cmax}, J_{max}, and R_d were fitted simultaneously using all data of an A/C_i response curve. Least square fitting procedures were used in all cases using the MS Excel 2002 Solver feature that employs a generalized reduced gradient algorithm for optimization of non-linear problems (Lasdon *et al.*, 1978).

These simulations were conducted with three representative values of g_m. A value of 0.15 mol m^{-2} s^{-1} corresponds to relatively high diffusion conductance observed in species with mesophytic leaves (see Niinemets *et al.*, 2009*a* for a review of species' g_m values). For the values of the model parameters of Farquhar *et al.* (1980) used (V_{cmax}=50 μmol m^{-2} s^{-1}, J_{max}=100 μmol m^{-2} s^{-1}, and R_d=0.75 μmol m^{-2} s^{-1} at 25 °C), this value of g_m results in CO_2 drawdown from substomatal cavities to chloroplasts (C_i–C_C=A/g_m) of 70 μmol mol^{-1} (at a C_i of 250 μmol mol^{-1} that is characteristic of non-stressed plants under current ambient CO_2 and at light saturation). A value of g_m=0.07 mol m^{-2} s^{-1} corresponds to moderately low diffusion conductance (C_i–C_C=111 μmol mol^{-1}), and a value of g_m=0.03 mol m^{-2} s^{-1} (C_i–C_C=151 μmol mol^{-1}) corresponds to the relatively low diffusion conductances observed in evergreen sclerophylls (see Niinemets *et al.*, 2009*a* for a review of species' g_m values).

Simulation of daily time courses of photosynthesis

Diurnal variations in net assimilation rates for different parameterizations of the model of Farquhar *et al.* (1980) were simulated using standard time-courses of light and temperature (Fig. 4c inset) for non-stressed (C_i was fixed at 270 μmol mol^{-1}) and for drought-stressed leaves. For the latter simulation, C_i was varied according to a bell-shaped sine function with minimum at mid-day (Fig. 4f inset). The Rubisco kinetic characteristics Γ^*, K_C, and K_O depend exponentially on temperature. In the current simulation analyses, the temperature dependencies of Bernacchi *et al.* (2001) were used. For R_d, V_{cmax}, and J_{max}, the shapes of the temperature response curves as parameterized in Niinemets and Tenhunen (1997) were used. g_m also depends on temperature (Bernacchi *et al.*, 2002; Warren and Dreyer, 2006). As g_m increases monotonically with temperature to ~35–40 °C (Bernacchi *et al.*, 2002; Warren, 2008*a*), the temperature-dependent increase in g_m was simulated by an exponential relationship with a Q_{10} value of 2.0. As various estimates of Q_{10} for g_m have been reported with values ranging from as low as 1.1–1.5 to as high as 3–4 (see Warren and Dreyer, 2006 for comparison of various estimates), and the temperature dependence of g_m is not routinely included in the models, a simulation with constant g_m throughout the day was also conducted to evaluate the importance of including the g_m temperature dependence in photosynthesis simulations.

To compare the simulations using the A/C_i and A/C_C approach in the field, the measurements in the Mediterranean evergreen sclerophyllous species *Quercus ilex* (Bertin *et al.*, 1997; Seufert *et al.*, 1997; Niinemets *et al.*, 2002*a*), conducted in August 1994 in Castelporziano, Rome, Italy (41°45′ N, 12°26′ E), were used. To parameterize these field data, the model of Farquhar *et al.* (1980) (Equations 1 and 2) was applied to the data either taking C_C equal to C_i (A/C_i approach) or using the model with g_m (A/C_C approach, Eqs 5–7). The shapes of temperature response

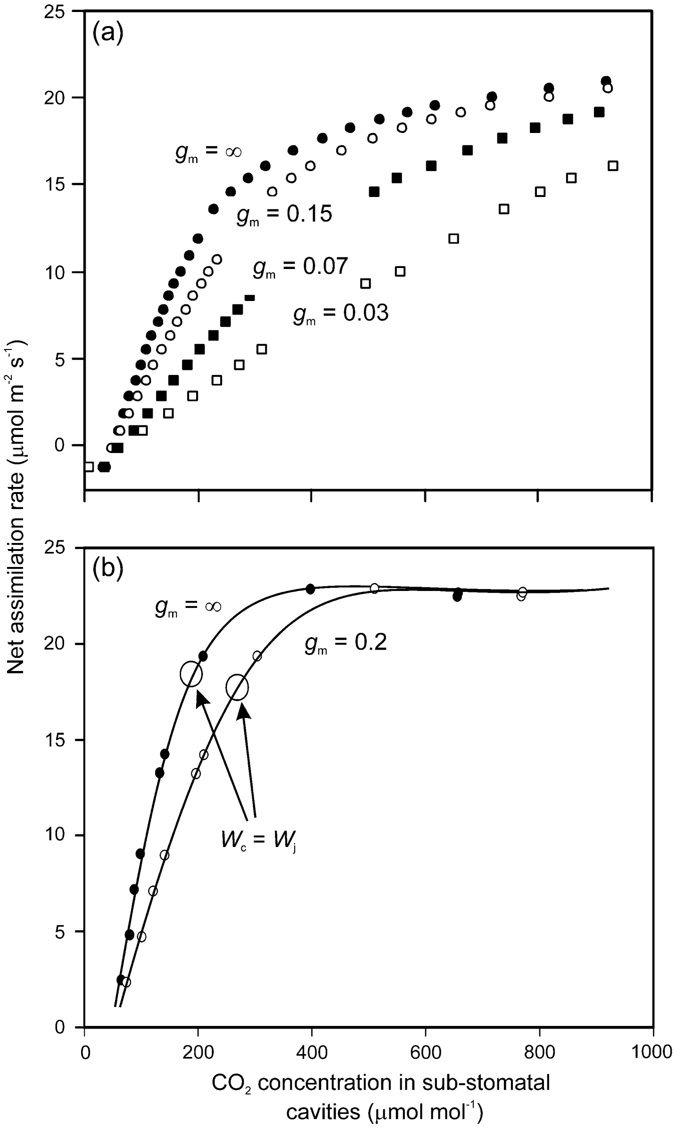

Fig. 1. Simulated (a) and measured (b) responses of the net assimilation rate (A) to CO$_2$ concentration in substomatal cavities (C_i) for differing values of mesophyll diffusion conductance (g_m, mol m^{-2} s^{-1}). The curves with $g_m = \infty$ correspond to A versus chloroplastic CO$_2$ concentration (C_C). The measurements were conducted in Mediterranean evergreen sclerophyll *Olea europaea* at a saturating quantum flux density of 1500 μmol m^{-2} s^{-1}, and leaf temperature of 25 °C (Diaz-Espejo *et al.*, 2007), while the simulation was conducted using the photosynthesis model of Farquhar *et al.* (1980) with the maximal carboxylase activity of Rubisco (V_{cmax}) of 100 μmol m^{-2} s^{-1}, capacity for photosynthetic electron transport (J_{max})of 200 μmol m^{-2} s^{-1}, and the mitochondrial respiration rate of 0.75 μmol m^{-2} s^{-1} for the same environmental conditions according to Eqs 5–7. The large open circles in (b) indicate the transition point between the Rubisco-limited rate of carboxylation (W_c) and the electron transport- (ribulose-1,5-bisphosphate regeneration) limited rate of carboxylation (W_j).

functions were those from Niinemets and Tenhunen (1997) for V_{cmax} and from Niinemets *et al.* (2002*b*) for J_{max} and R_d. In these simulations, C_i values were obtained from the measurements. For both simulation approaches, values of

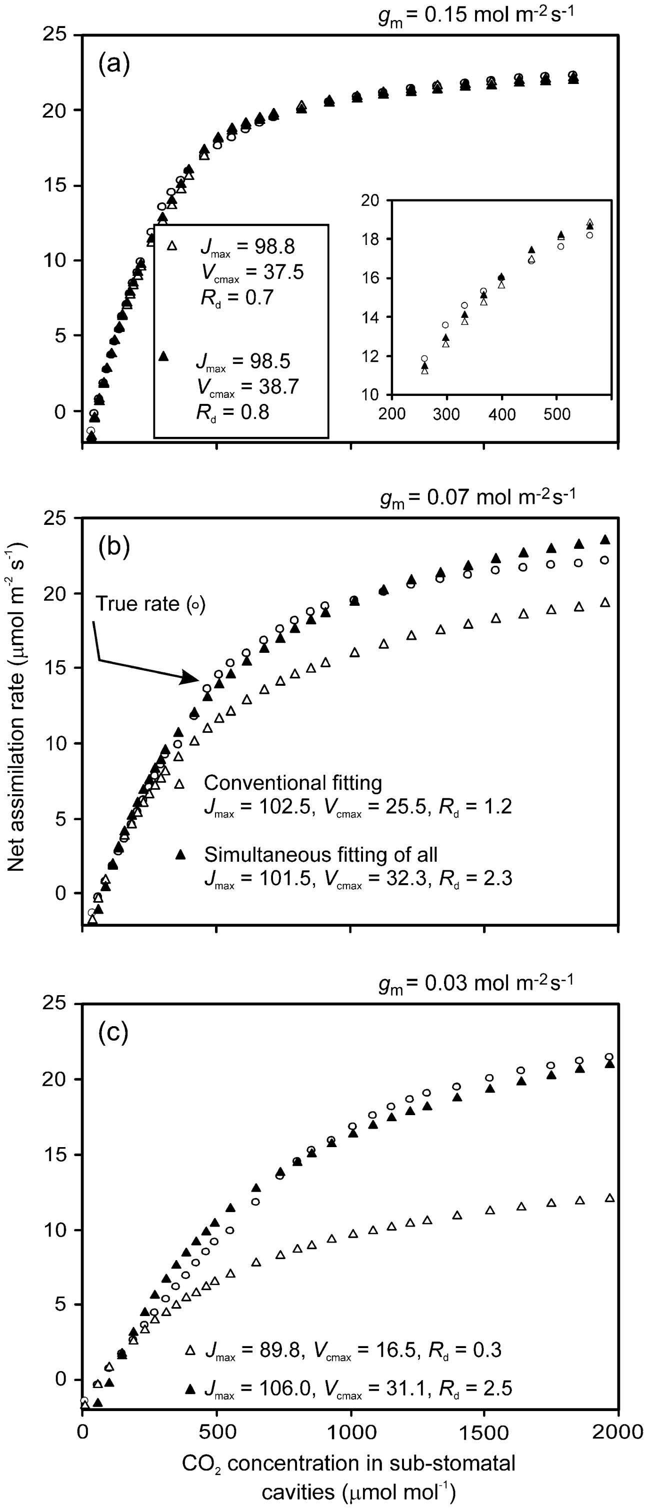

Fig. 2. Simulated 'true' A/C_i response curves (open circles) based on V_{cmax} (100 μmol m^{-2} s^{-1}), J_{max} (200 μmol m^{-2} s^{-1}), and R_d (0.75 μmol m^{-2} s^{-1}) (Eqs 5–7) and simulated response curves using these biochemical parameters estimated from A/C_i curves and assuming that $g_m = \infty$ (open circles) for three different leaves with varying values of mesophyll diffusion conductance. The A/C_i curve fitting was conducted by two contrasting methods. According to the conventional fitting technique, V_{cmax} and R_d were derived from the initial 'linear' part of the A/C_i response curve, and J_{max} from the saturating part (open triangles). According to the

V_{cmax}, J_{max}, and R_d that provided the best correspondence between measurements and predictions were derived. For the A/C_C approach, g_m was additionally fitted. For all these model parameters, estimates realistic to *Q. ilex* leaves exposed to high light were obtained (Niinemets *et al.*, 2006) (for *Q. ilex* model parameters and g_m values).

Results and Discussion

Influence of finite g_m *on* A/C_i *response curves*

Finite diffusion conductance, g_m, significantly alters the shape of an A/C_i response curve (Fig. 1a; Ethier and Livingston, 2004). This has major effects on the initial slope of an A/C_i response curve and R_d, with smaller effects on the saturating part of the curve that is limited by J_{max} (Fig. 1). Thus, the major consequence of having a finite g_m is that V_{cmax} calculated from an A/C_i response curve is always lower than that calculated from an A/C_C response curve (Fig. 1a). For instance, in young fully mature leaves of *Olea europaea* with relatively high g_m of 0.2 mol m^{-2} s^{-1}, V_{cmax} calculated on a C_C basis is 25% higher than V_{cmax} calculated on a C_i basis, while the differences in J_{max} are small (Fig. 1b).

For larger values of g_m, direct fitting of A/C_i response curves derived from A/C_C response curves according to Eq. 4 resulted in good fits between the predicted and 'true' (A/C_i response with g_m) values (Fig. 2a). The obtained estimates were similar using the conventional technique of separately determining V_{cmax} and R_d from the linear part of the curve, and J_{max} from the saturating part, and using a technique that simultaneously estimated all three parameters (Fig. 2a). Nevertheless, V_{cmax} was underestimated by ~30% for both fitting procedures, and this was reflected in 5–10% lower than predicted 'true' net assimilation at currently relevant C_i values between 200 μmol mol^{-1} and 300 μmol mol^{-1} (Fig. 2a inset). A/C_i estimation methods yielded larger discrepancies with decreasing g_m (Fig. 2b, c). In particular, the conventional fitting procedure underestimated V_{cmax} up to 3-fold in leaves with low g_m. As the result of this strong underestimation, entire A/C_i response curves simulated by such low V_{cmax} estimates were apparently limited by Rubisco activity. Estimation of all parameters by simultaneous fits underestimated V_{cmax} by as much as 1.6-fold (versus 3-fold for separate fits) (Fig. 2b, c). Therefore, V_{cmax} may be largely underestimated using the conventional fitting of the Farquhar *et al.* model whenever g_m is small (Flexas *et al.*, 2008).

Simultaneous fitting of all three model parameters overestimated R_d up to 3-fold. For conventional fitting, the R_d values obtained were variable and were overestimated for moderate g_m (Fig. 2b) and underestimated for low g_m (Fig. 2c). In the latter simulation, the derived characteristics depended somewhat on the set of data points included in the fitting. For instance, determining V_{cmax} and R_d from the part of the A/C_i curve between 10 μmol mol^{-1} and 250 μmol mol^{-1} (values that are common in fitting A/C_i response curves) yielded the parameters shown in Fig. 2c, while fitting over the range 10–500 μmol mol^{-1} yielded a larger V_{cmax} value of 19.5 μmol m^{-2} s^{-1} and a larger R_d value of 0.5 μmol m^{-2} s^{-1}.

In contrast to V_{cmax} and R_d, J_{max} values were relatively insensitive to fitting procedures, with moderate, generally <10%, under- or overestimation (Fig. 2). This small effect of fitting procedures on J_{max} is not surprising as this characteristic is determined by $A+R_d$ at high C_i (Eq 2).

These simulations collectively indicate that widely varying values of V_{cmax} and R_d, and less variable values of J_{max} can be obtained using A/C_i curve fitting for leaves with different g_m values. In particular, the conventional fitting procedure of deriving V_{cmax} and J_{max} separately from different parts of the same A/C_i curve results in large underestimations in V_{cmax} and in variable R_d values. Derivation of V_{cmax} and R_d values of more robust leaves with lower g_m values (Niinemets *et al.*, 2009*a*) is expected to be especially strongly affected.

These simulations were conducted using a constant g_m value for the entire A/C_C response curves, but recent data show that g_m may depend on the CO_2 concentration (Flexas *et al.*, 2007). The response of g_m to the CO_2 concentration reported in that study was a curve with a maximum at CO_2 substomatal concentrations between 100 μmol mol^{-1} and 300 μmol mol^{-1}, thus it is probable that the CO_2 effect on g_m will amplify the differences in A/C_i versus A/C_C curve parameters for the currently highly relevant CO_2 range.

Influence of g_m *on the* J_{max}/V_{cmax} *ratio*

The J_{max}/V_{cmax} ratio is a key characteristic indicating allocation of photosynthetic proteins between light and dark reactions of photosynthesis, and as such is widely reported and analysed in studies investigating the partitioning of photosynthetic resources (Dreyer *et al.*, 2001; Leuning, 2002; Misson *et al.*, 2006). J_{max}/V_{cmax} also determines the sensitivity of photosynthesis to light and temperature, i.e. the transition point at which one limitation goes over to the other (Hikosaka, 1997; Hikosaka *et al.*, 1999). Actual data and the simulation analysis (Figs 1b, 2) demonstrate that the apparent J_{max}/V_{cmax} ratio derived from A/C_i curves may be as large as 6.7. This is very high relative to the true A/C_C curve estimate of 2.0 (see Fig. 2c for conventional fitting). For this high estimate of the J_{max}/V_{cmax} ratio derived from A/C_i responses, photosynthesis is expected to become limited by Rubisco (light-saturated) at a quantum flux density of 160 μmol m^{-2} s^{-1}, whereas with the original estimate from A/C_C curves, photosynthesis becomes light-saturated at Q > 1000 μmol m^{-2} s^{-1}. Clearly, underestimation of Rubisco activity relative to electron transport results in biased estimates of photosynthetic sensitivity to light. This evidence emphasizes the fact that V_{cmax} and J_{max} are simplified mathematical descriptions of an A/C_i or A/C_C response. Although V_{cmax} and J_{max} are widely used as synonymous

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second method, J_{max}, V_{cmax}, and R_d were fitted simultaneously (filled triangles). The simulations were conducted for 25 °C and saturating light.

with Rubisco activity and RuBP regeneration, the meaning of these characteristics and usefulness in predicting the degree to which photosynthesis is limited either by light or by CO_2 strongly depends on the way these parameters are derived. In fact, most of the J_{max}/V_{cmax} ratios estimated from A/C_i response curves are overestimated, being commonly between 2.5 and 4, but awkwardly high values up to 10 have been reported (Wullschleger, 1993).

It is further important to note that interpretation of the J_{max}/V_{cmax} ratio and how it relates to photosynthesis strongly depends on g_m, irrespective of whether the parameters are C_i or C_C based. The sensitivity of photosynthesis to the J_{max}/V_{cmax} ratio is smaller in leaves with lower g_m values (Fig. 3). This is because with decreasing g_m, photosynthesis is generally more strongly limited by Rubisco than by electron transport due to lower CO_2 concentrations in the chloroplasts. Thus, in leaves with low g_m, the rate of photosynthesis can be increased more by preferential investments of N in Rubisco than in electron transport. In fact, in strongly sclerophyllous leaves with low g_m, large investments of nitrogen in Rubisco, even up to 50%, have been observed (Warren and Adams, 2004, 2005).

Effects of different parameterization methods for daily photosynthesis in the field

The implications of different parameterizations on simulations of daily photosynthesis were analysed (Fig. 4) using standard time-courses of light and temperature (inset in Fig. 4c) for a non-stressed (C_i fixed at a constant value of 270 μmol mol^{-1} throughout the day) and stressed scenario (C_i varied according to a sine function with minimum at mid-day, inset in Fig. 4f). For both non-stressed and stressed scenarios, a simulation for a hypothetical situation, with $g_m=\infty$, resulted in higher daily photosynthesis than any other parameterization. For a high g_m of 0.15 mol m^{-2} s^{-1}, reduction of daily photosynthesis due to g_m was ~10% for the non-stressed (Fig. 4a, comparison with A/C_C simulation with actual g_m) and ~25% for the stressed scenario (Fig. 4d, Table 1). Comparison of different parameterizations showed that for a high g_m of 0.15 mol m^{-2} s^{-1}, simulations based on A/C_C parameterizations yielded 6–8% lower daily photosynthesis than the two A/C_i parameterizations (Fig. 4a, d, Table 1). This reflected the drop of CO_2 concentration from C_i to C_C for A/C_C parameterizations, and a lower J_{max}/V_{cmax} ratio of A/C_C parameterizations, implying that photosynthesis saturated at higher light (Fig. 4a).

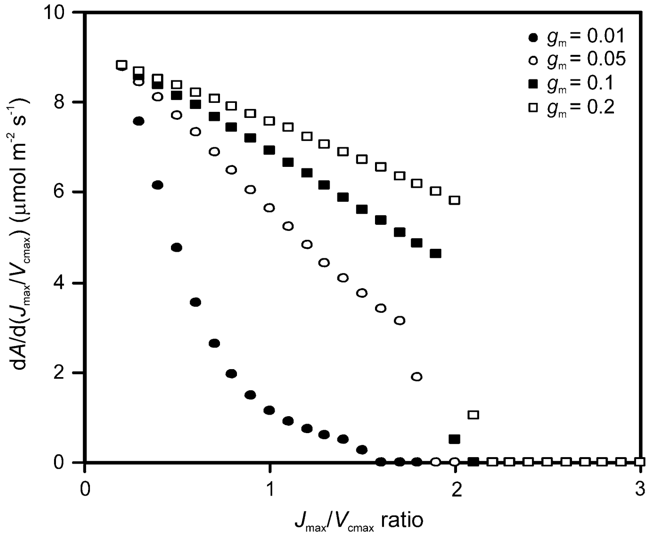

Fig. 3. Simulated sensitivity of the net assimilation rate to the J_{max}/V_{cmax} ratio in relation to J_{max}/V_{cmax} in leaves with varying values of g_m (mol m^{-2} s^{-1}). In this simulation, the ambient CO_2 concentration (C_a) was set to 385 μmol mol^{-1}, stomatal conductance to CO_2 (g_s) to 0.2 mol m^{-2} s^{-1}, leaf temperature to 25 °C, V_{cmax} to 50 μmol m^{-2} s^{-1}, and J_{max} was varied from 5 μmol mol^{-2} s^{-1} to 175 μmol m^{-2} s^{-1}. Net assimilation rate was calculated according to a completely iterative procedure: $A=f(C_C, V_{cmax}, J_{max}, R_d)$, $C_C=f(C_i, A)$, and $C_i=f(C_C, g_s, C_a)$. The simulations were conducted using the MS Excel 2002 successive substitution iterative procedure (calculations in iterative mode).

In contrast, for lower g_m, A/C_i parameterizations led to significantly lower estimates of daily assimilation than A/C_C parameterizations—due to underestimation of V_{cmax} in A/C_i parameterizations (Fig. 4b, c, e, f, Table 1). The differences were especially large for the stressed scenario and for the A/C_i parameterization in which V_{cmax}, J_{max}, and R_d were fitted simultaneously (Fig. 4e, f). In particular, at mid-day, A was seriously depressed for this parameterization, even becoming negative for the parameterization derived from the A/C_i curves with the lowest g_m (Fig. 4f). The very low mid-day assimilation for this parameterization reflected overestimated R_d (Fig. 2b, c) and, to a lesser extent, underestimated V_{cmax}. At higher temperatures at mid-day, the CO_2 compensation point, Γ^*, and the effective Michaelis–Menten constant for CO_2, K_m, also strongly increase, and this is expected to reduce the net assimilation rate further (Eq. 1). For instance, in the current simulations, leaf temperature was predicted to increase from 15 °C to 31 °C, and this results in changes of Γ^* from 30.2 μmol mol^{-1} to 57.0 μmol mol^{-1} and of K_m from 144.5 μmol mol^{-1} to 757.4 μmol mol^{-1}. Although V_{cmax} also increases with increasing temperature, V_{cmax} still remains too low in the A/C_i parameterizations to compensate for the reductions in Rubisco-limited photosynthesis due to temperature-dependent increases in K_m and Γ^*.

It has been suggested previously that simultaneous fitting of A/C_i response curve parameters is superior to the conventional fitting procedure which is based on division of the A/C_i curves into regions limited by V_{cmax} and J_{max} (Dubois *et al.*, 2007). While this simultaneous fitting approach underestimated less V_{cmax} values than the conventional fitting, it led to overestimation of R_d (Fig. 2). Daily simulations further suggested that simultaneous fitting yielded a parameterization that provided a better correspondence of daily photosynthesis with A/C_C parameterization under non-stressed conditions (Table 1). However, the correspondence was worse due to overestimated R_d under stressed conditions (Table 1). Thus, none of the two A/C_i

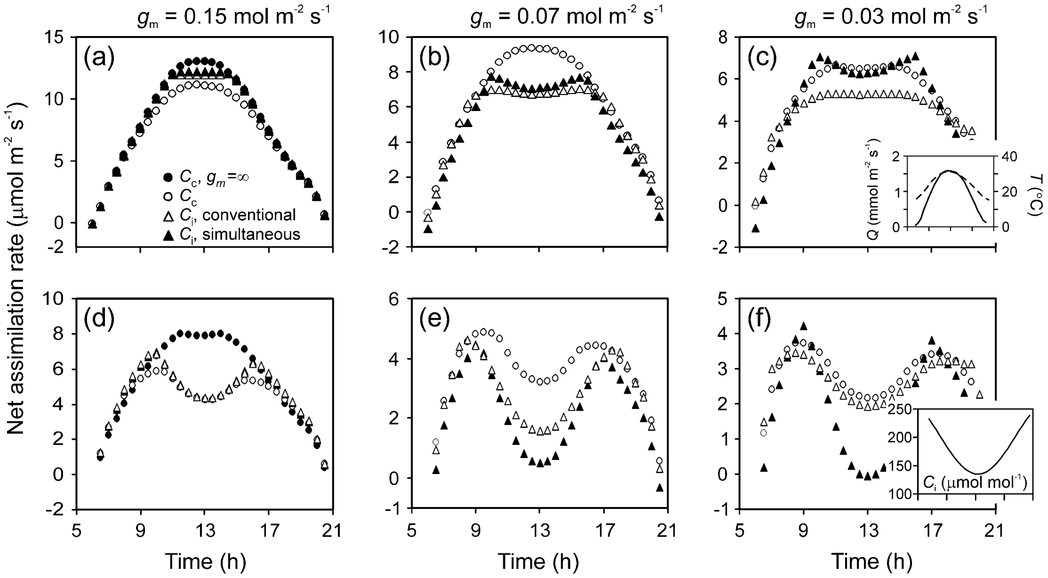

Fig. 4. Simulated diurnal variations in net assimilation for a non-stressed scenario (intercellular CO_2 concentration set to 270 μmol mol^{-1}, a–c) and for a stressed scenario [C_i varied as shown in the inset of (f) to describe mid-day stomatal closure in drought-stressed plant, d–f] for three representative values of g_m and for three sets of model parameters of Farquhar *et al.* (1980) and for given values of leaf temperature (dashed line in the inset of c) and quantum flux density (solid line in the inset of c). The three sets of model parameters are those derived in Fig. 2. The first set of parameters (open circles) corresponds to V_{cmax}, J_{max}, and R_d values of the A/C_C approach. The two other parameter sets are based on refitting of A/C_i curves taking C_i=C_C and without considering the modification of the shape of the response curve due to finite g_m according to the conventional technique, i.e. fitting the initial linear part and saturating part separately (open triangles), and fitting all three parameters simultaneously (filled triangles). In (a) and (d), the simulation for infinite g_m is also shown (filled circles). The values of daily integrated net assimilation rate (A_{int}) are provided in all cases in Table 1, except for the A/C_C simulation for the hypothetical situation of g_m=∞. For this scenario, A_{int}=0.421 mol m^{-2} d^{-1} for (a) and A_{int}=0.281 mol m^{-2} d^{-1} for (d).

Table 1. Simulated daily integrated net assimilation rates (A_{int}, mol m^{-2} d^{-1}) using parameterization of the photosynthesis model of Farquhar *et al.* (1980) on the basis of A/C_C response curves with either temperature-dependent mesophyll diffusion conductance (g_m) or assuming a constant g_m during the entire day, and on the basis of A/C_i responses for the model parameterization and either determining V_{cmax} and R_d from the initial part of the curve and J_{max} from the saturating part (conventional fitting) or fitting all three characteristics simultaneously

The simulationsly conducted scenarios for non-stressed and stressed situations are shown in Fig. 4.

Parameterization	**g_m=0.15 mol m^{-2} s^{-1}**		**g_m=0.07 mol m^{-2} s^{-1}**		**g_m=0.03 mol m^{-2} s^{-1}**	
	Non-stressed	**Stressed**	**Non-stressed**	**Stressed**	**Non-stressed**	**Stressed**
A/C_C, g_m=$f(T)$*	0.383	0.228	0.346	0.193	0.280	0.145
A/C_C, g_m=const.	0.377	0.224	0.334	0.189	0.263	0.144
A/C_i, convent.	0.413	0.242	0.287	0.148	0.232	0.138
A/C_i, simult.	0.412	0.241	0.277	0.115	0.254	0.096

* g_m value shown at 25 °C was scaled to any other temperature with a value of Q_{10}=2.0.

parameterization methods can be recommended as a universal solution to capture the modification in the A/C_i curve shape due to finite g_m.

Inclusion of temperature dependence of g_m affected daily integrated assimilation rates by 2–6% under non-stressed conditions, and only by 0.5–2% under stressed conditions (Table 1). This seems to suggest that the temperature dependence of g_m has minor effects on integrated assimilation rates, especially under stress. Nevertheless, a Q_{10} value for g_m of 2 was used, and clearly, for higher Q_{10} values (see Warren and Dreyer, 2006 for review of case studies), temperature effects on g_m would be quantitatively more important.

A/C_C and A/C_i simulations of daily assimilation were further compared with measured assimilation rates in the evergreen sclerophyll *Q. ilex* in Mediterranean drought conditions (Fig. 5). These simulations show that the best A/C_i parameterization underestimates photosynthesis at mid-day (Fig. 5c), while A/C_C parameterization correctly simulates the entire time course of photosynthesis, resulting in much better overall correspondence between simulations and measurements for the A/C_C approach (cf. Fig. 5e, f). In

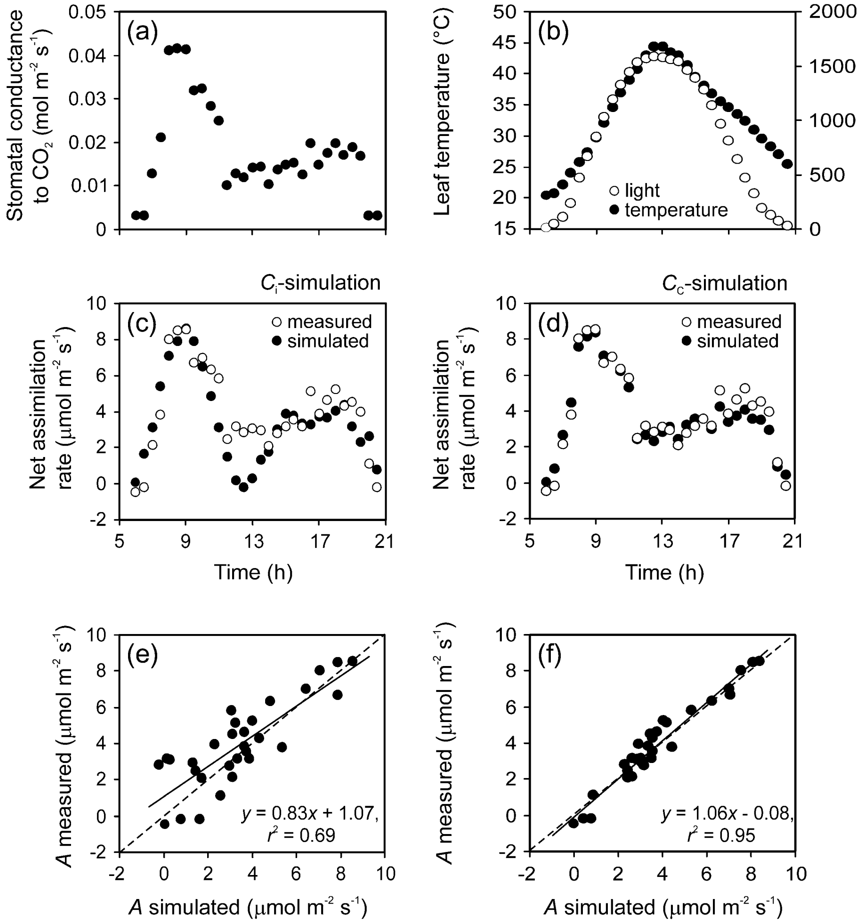

Fig. 5. Variation in stomatal conductance (a) and leaf irradiance and temperature (b) in drought-stressed Mediterranean evergreen sclerophyll species *Quercus ilex*, and comparison of fitting of diurnal variations in net assimilation rate using the parameterization based on C_i response curves (c, e) and on the basis of C_C (d, f). The measurements were conducted in Castelporziano, Italy in August 1994 when the plants suffered from severe drought (Bertin *et al.*, 1997; Seufert *et al.*, 1997; Niinemets *et al.*, 2002*a*). Best-fit values of biochemical model parameters used in these simulations (at 25 °C) were: R_d=0.8 µmol m^{-2} s^{-1}, V_{cmax}=41.1 µmol m^{-2} s^{-1}, and J_{max}=102.7 µmol m^{-2} s^{-1} for the A/C_i approach (c–e), and g_m=0.08 mol m^{-2} s^{-1}, R_d=0.8 µmol m^{-2} s^{-1}, V_{cmax}=113.8 µmol m^{-2} s^{-1}, and J_{max}=207.4 µmol m^{-2} s^{-1} for the A/C_C approach. These values were scaled to different temperatures using the shapes of V_{cmax} versus temperature from Niinemets and Tenhunen (1997) and R_d and J_{max} versus temperature from Niinemets *et al.* (2002*b*).

fact, difficulties in simulating mid-day depression in photosynthesis are well known (Tenhunen *et al.*, 1987*a*; Harley and Tenhunen, 1991; Misson *et al.*, 2004). The mid-day depression in Fig. 5c could be correctly parameterized by increasing the V_{cmax} value. However, because A/C_i parameterization underestimates V_{cmax} relative to J_{max}, correct parameterization of photosynthesis at mid-day would result in significant overestimation of photosynthesis during the rest of the day, as has been observed previously (Tenhunen *et al.*, 1987*a*; Harley and Tenhunen, 1991).

This simulation demonstrates qualitative differences in model outcomes between A/C_i and A/C_C parameterizations. Under Mediterranean drought conditions, leaf temperatures can reach 40–50 °C (Valladares and Niinemets, 2007), stomata are relatively closed, and mitochondrial respiration is high. The A/C_i parameterizations predict negative photosynthesis due to underestimated V_{cmax} and overestimated R_d. However, this is probably incorrect given that even under such highly stressful conditions, negative carbon balance has rarely (if ever) been observed (Beyschlag *et al.*, 1986, 1987; Harley *et al.*, 1987; Tenhunen *et al.*, 1987*b*). Although A/C_i parameterizations may give reasonable fits to experimental A/C_i data (e.g. good r^2), they may result in fundamentally flawed conclusions with respect to environmental effects in the field.

There are serious implications for the present finding that A/C_i parameterizations lead to comparatively poor simulations of photosynthesis in the field. It has been argued that even though A/C_i parameterizations are logically biased since they ignore g_m, they remain useful for simulating photosynthesis. The present modelling study clearly shows that this pragmatic view is flawed insofar as A/C_i parameterizations are never as good as A/C_C parameterizations in simulating daily photosynthesis. Currently, there is an increasing trend for measuring response curves of photosynthesis to environmental drivers, deriving V_{cmax} and J_{max} values from these curves, and considering these curves as a 'final truth' for uncritical simulation of photosynthesis under field conditions. As this study demonstrates, this is often an illusion. Photosynthesis model parameterizations must explicitly consider finite g_m and need verification with field measurements of diurnal time courses of photosynthesis.

More generally, the scientific community needs to rethink the logic of using A/C_i parameterizations of the model of Farquhar *et al.* (1980) for simulating and interpreting photosynthesis at leaf, canopy, landscape, and biome scales. The chief rationale for measuring A/C_i response curves and using these for modelling of photosynthesis in the field is that the 'biochemical' information (i.e. V_{cmax} and J_{max}) has serious explanatory power. However, if based on A/C_i parameterizations, this explanatory power and biochemical information is a mirage for several reasons.

(i) A/C_i parameterizations lead to large underestimates of the true V_{cmax} and smaller underestimates of J_{max} (Flexas *et al.*, 2008; Warren, 2008*b*; this study). This means that interpretation of C_i-derived V_{cmax} and J_{max} values is problematic since they contain information pertaining to g_m. In other words, they are no longer the pure biochemical parameters on which the model of Farquhar *et al.* is founded.

(ii) A/C_i parameterizations are frequently used to determine the limitations of photosynthesis, but their interpretation is affected by finite g_m. For example, g_m alters the sensitivity of photosynthesis to fluctuations in ambient CO_2 concentration (Fig. 1) and the share of photosynthetic limitations between dark and light reactions of photosynthesis.

(iii) V_{cmax} and J_{max} are affected not only by the choice of C_i versus C_C as substrate concentration, but also by the choice of curve-fitting method. Fitting V_{cmax} and J_{max} simultaneously or separately can lead to wildly different parameter estimates (Fig. 2), which can subsequently affect simulations of daily photosynthesis (Fig. 4). More importantly, this study supports the view that V_{cmax} and J_{max} are not biochemical constants that can be shared among studies, but are mathematical parameters that are highly sensitive to the method of curve fitting.

Inclusion of g_m in models of photosynthesis

The present model simulations clearly show that g_m ought to be included in models of photosynthesis, but how to do it? The key problem for modelling is that there seems to be no simple and accurate means of predicting g_m without measuring it. This is because g_m varies greatly among and within given species (Ethier and Livingston, 2004; Flexas *et al.*, 2008; Warren, 2008*b*; Niinemets *et al.*, 2009*a*). As maximal values of g_m are inherently constrained by leaf structure, e.g. mesophytic leaves with higher versus sclerophytic leaves with lower g_m (Terashima *et al.*, 2006; Flexas *et al.*, 2008; Warren, 2008*b*; Evans *et al.*, 2009; Niinemets *et al.*, 2009*a*), linking of g_m to leaf structure can provide a means to include g_m in photosynthesis models, in particular for large-scale models that only capture the key functional characteristics of chief plant functional types (Haxeltine *et al.*, 1996; Hickler *et al.*, 2008). However, g_m is affected by many of the environmental variables that drive models of canopy photosynthesis (e.g. temperature, drought, etc.; Warren, 2008*b*), and there is currently not enough physiological information for reliable parameterization of these dependencies. On the other hand, some of the environmental effects may be small or moderate relative to the overall effect of g_m on canopy photosynthesis. For instance, the effect of temperature on g_m appeared relatively small according to the present simulations (Table 1). Also, drought-dependent reductions commonly occur together with reductions in stomatal conductance (g_s) (Centritto *et al.*, 2003; Loreto *et al.*, 2003; Flexas *et al.*, 2008). As for the two conductances in series, the overall conductance will be dominated by the smaller component; the overall effect of drought-dependent reductions in g_m on daily photosynthesis is less pronounced.

There have not been many attempts to include g_m in photosynthesis models. Apart from complex numerical diffusion models (e.g. Cooke and Rand, 1980), Williams *et al.* (1996) were one of the first who embedded g_m into the photosynthesis model of Farquhar *et al.* (1980) to simulate photosynthesis of a mixed deciduous broad-leaved forest. In their simulations, a fixed value of g_m was used in all calculations (Williams *et al.*, 1996). More recently, Ohsumi *et al.* (2007) used two different approaches, one correlating g_m with leaf nitrogen content (basically scaling with leaf photosynthetic capacity), and the second correlating g_m with g_s. Cai *et al.* (2008) also used the correlation with g_s to include g_m in the model as well. Linking g_m to g_s can be promising, in particular as this approach significantly simplifies the calculation burden in simulation analyses; for entirely independent g_m and g_s values, several iterative loops are needed to simulate assimilation rates: $A=f(C_C, V_{cmax}, J_{max}, R_d)$, $C_C=f(C_i, A)$, and $C_i=f(C_C, g_s, C_a)$. However, as with g_m versus nitrogen and g_m versus photosynthetic capacity (Niinemets *et al.*, 2009*a*), the relationships between g_m and g_s are variable (Flexas *et al.*, 2008), in agreement with various CO_2 drawdowns due to g_m (C_i–C_C) across species and environmental conditions (Niinemets and Sack, 2006; Warren and Adams, 2006). While new information of the determinants of g_m is constantly accumulating, there is currently no justification for varying g_m in proportion to g_s. Nevertheless, for the time being, photosynthesis models should at least intend to describe correctly the mesophyll diffusion conductance relative to photosynthetic capacity (A/g_m), e.g. because of the limits set by leaf structure or aquaporin conductance (Evans *et al.*, 2009). Including such effects on the shape of A/C_i response curves will already result in significant improvements of canopy photosynthesis in the field (Fig. 5c versus d).

Conclusions

The present analysis adds to a growing body of literature highlighting the importance of g_m as a limitation of photosynthesis. The scientific community has assumed that V_{cmax} and J_{max} derived from A/C_{i} responses are synonymous with Rubisco activity and RuBP regeneration. While C_{i}-based V_{cmax} and J_{max} include some information on g_{m}, these estimates are strongly affected by the choice of curve-fitting method, and their usefulness in predicting photosynthesis under stressful conditions in the field decreases with increasing the degree to which g_{m} limits photosynthesis. Overall, the parameterizations of the model of Farquhar *et al* (1980) including g_{m} (A/C_{C} parameterizations) provide a more realistic description of daily photosynthesis relative to A/C_{i} parameterizations, in particular for stressed leaves sustaining mid-day stomatal closure.

As stated in the Introduction, the beauty of the model of Farquhar *et al.* is a combination of its predictive power and ease of parameterization; yet the results of this study suggest that there is instead a trade-off between predictive power and ease of parameterization. A/C_{i} parameterizations have minimal (biochemical) predictive power, but are very easy to parameterize. In contrast, A/C_{C} parameterizations have larger predictive power, but are more difficult to parameterize. Nevertheless, there may be no alternative to inclusion of g_{m} for correct simulation of photosynthesis in field stressful environments.

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References

Bernacchi CJ, Portis AR, Nakano H, von Caemmerer S, Long SP. 2002. Temperature response of mesophyll conductance. Implications for the determination of Rubisco enzyme kinetics and for limitations to photosynthesis *in vivo*. *Plant Physiology* **130,** 1992–1998.

Bernacchi CJ, Singsaas EL, Pimentel C, Portis Jr AR, Long SP. 2001. Improved temperature response functions for models of Rubisco-limited photosynthesis. *Plant, Cell and Environment* **24,** 253–259.

Bertin N, Staudt M, Hansen U, Seufert G, Ciccioli P, Foster P, Fugit JL, Torres L. 1997. Diurnal and seasonal course of monoterpene emissions from *Quercus ilex* (L.) under natural conditions—application of light and temperature algorithms. *Atmospheric Environment* **31,** 135–144.

Beyschlag W, Lange OL, Tenhunen JD. 1986. Photosynthese und Wasserhaushalt der immergrünen mediterranen Hartlaubpflanze *Arbutus unedo* L. im Jahreslauf am Freilandstandort in Portugal. I. Tagesläufe von CO_2-Gaswechsel und Transpiration unter natürlichen Bedingungen. *Flora* **178,** 409–444.

Beyschlag W, Lange OL, Tenhunen JD. 1987. Diurnal patterns of leaf internal CO_2 partial pressure of the sclerophyll shrub *Arbutus unedo* growing in Portugal. In: Tenhunen JD, Catarino FM, Lange OL, Oechel WC, eds. *Plant response to stress. Functional analysis in Mediterranean ecosystems.* NATO ASI series, series G: Ecological sciences, 15. Berlin: Springer-Verlag, 355–368.

Björkman O. 1973. Comparative studies on photosynthesis in higher plants. *Photophysiology* **8,** 1–63.

Bongi G, Loreto F. 1989. Gas-exchange properties of salt-stressed olive (*Olea europea* L.) leaves. *Plant Physiology* **90,** 1408–1416.

Cai T, Flanagan LB, Jassal RS, Black TA. 2008. Modelling environmental controls on ecosystem photosynthesis and the carbon isotope composition of ecosystem-respired CO_2 in a coastal Douglas-fir forest. *Plant, Cell and Environment* **31,** 435–453.

Centritto M, Loreto F, Chartzoulakis K. 2003. The use of low [CO_2] to estimate diffusional and non-diffusional limitations of photosynthetic capacity of salt-stressed olive saplings. *Plant, Cell and Environment* **26,** 585–594.

Churkina G, Running SW. 1998. Contrasting climatic controls on the estimated productivity of global terrestrial biomes. *Ecosystems* **1,** 206–215.

Cooke JR, Rand RH. 1980. Diffusion resistance models. In: Hesketh JB, Jones JW, eds. *Predicting photosynthesis for ecosystem models*, Vol. I. Boca Raton, FL: CRC Press, 93–121.

Diaz-Espejo A, Nicolás E, Fernández JE. 2007. Seasonal evolution of diffusional limitations and photosynthetic capacity in olive under drought. *Plant, Cell and Environment* **30,** 922–933.

Dreyer E, Le Roux X, Montpied P, Daudet FA, Masson F. 2001. Temperature response of leaf photosynthetic capacity in seedlings from seven temperate tree species. *Tree Physiology* **21,** 223–232.

Dubois J-JB, Fiscus EL, Booker FL, Flowers MD, Reid CD. 2007. Optimizing the statistical estimation of the parameters of the Farquhar–von Caemmerer–Berry model of photosynthesis. *New Phytologist* **176,** 402–414.

Ehleringer J, Björkman O. 1977. Quantum yields for CO_2 uptake in C_3 and C_4 plants. Dependence on temperature, CO_2 and O_2 concentration. *Plant Physiology* **59,** 86–90.

Ethier GJ, Livingston NJ. 2004. On the need to incorporate sensitivity to CO_2 transfer conductance into the Farquhar–von Caemmerer–Berry leaf photosynthesis model. *Plant, Cell and Environment* **27,** 137–153.

Evans JR, Jakobsen I, Ögren E. 1993. Photosynthetic light-response curves. 2. Gradients of light absorption and photosynthetic capacity. *Planta* **189,** 191–200.

Evans JR, Kaldenhoff R, Terashima I. 2009. Resistances along the CO_2 diffusion pathway inside leaves. *Journal of Experimental Botany* **60,** 2235–2248.

Farquhar GD, von Caemmerer S, Berry JA. 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. *Planta* **149,** 78–90.

Flexas J, Diaz-Espejo A, Galmés J, Kaldenhoff R, Medrano H, Ribas-Carbó M. 2007. Rapid variations of mesophyll conductance in response to changes in CO_2 concentration around leaves. *Plant, Cell and Environment* **30,** 1284–1298.

Flexas J, Ribas-Carbó M, Diaz-Espejo A, Galmés J, Medrano H. 2008. Mesophyll conductance to CO_2: current knowledge and future prospects. *Plant, Cell and Environment* **31,** 602–621.

Harley PC, Baldocchi DD. 1995. Scaling carbon dioxide and water vapour exchange from leaf to canopy in a deciduous forest. I. Leaf model parametrization. *Plant, Cell and Environment* **18,** 1146–1156.

Harley PC, Tenhunen JD. 1991. Modeling the photosynthetic response of C_3 leaves to environmental factors. In: Boote KJ, ed. *Modeling crop photosynthesis—from biochemistry to canopy.* CSSA Special Publication, No. 19. Madison: Agronomy and Crop Science Society of America, 17–39.

Harley PC, Tenhunen JD, Beyschlag W, Lange OL. 1987. Seasonal changes in net photosynthesis rates and photosynthetic capacity in leaves of *Cistus salvifolius*, a European mediterranean semi-deciduous shrub. *Oecologia* **74,** 380–388.

Haxeltine A, Prentice IC. 1996. BIOME3: an equilibrium terrestrial biosphere model based on ecophysiological constraints, resource availability, and competition among plant functional types. *Global Biogeochemical Cycles* **10,** 693–709.

Haxeltine A, Prentice IC, Creswell DI. 1996. A coupled carbon and water flux model to predict vegetation structure. *Journal of Vegetation Science* **7,** 651–666.

Hickler T, Smith B, Prentice IC, Mjöfors K, Miller P, Arneth A, Sykes MT. 2008. CO_2 fertilization in temperate FACE experiments not representative of boreal and tropical forests. *Global Change Biology* **14,** 1531–1542.

Hikosaka K. 1997. Modelling optimal temperature acclimation of the photosynthetic apparatus in C_3 plants with respect to nitrogen use. *Annals of Botany* **80,** 721–730.

Hikosaka K, Murakami A, Hirose T. 1999. Balancing carboxylation and regeneration of ribulose-1,5-bisphosphate in leaf photosynthesis: temperature acclimation of an evergreen tree, *Quercus myrsinaefolia*. *Plant, Cell and Environment* **22,** 841–849.

Laisk A. 1977. *Kinetika fotosinteza i fotodyhaniya C_3-rastenii.* (*Kinetics of photosynthesis and photorespiration in C_3-plants*). Moscow: Nauka.

Lasdon LS, Waren AD, Jain A, Ratner MI. 1978. Design and testing of a generalized reduced gradient code for nonlinear programming. *ACM Transactions on Mathematical Software (TOMS)* **4,** 34–50.

Leuning R. 2002. Temperature dependence of two parameters in a photosynthesis model. *Plant, Cell and Environment* **25,** 1205–1210.

Loreto F, Centritto M, Chartzoulakis K. 2003. Photosynthetic limitations in olive cultivars with different sensitivity to salt stress. *Plant, Cell and Environment* **26,** 595–601.

Manter DK, Kerrigan J. 2004. A/C_i curve analysis across a range of woody plant species: influence of regression analysis parameters and mesophyll conductance. *Journal of Experimental Botany* **55,** 2581–2588.

Misson L, Panek JA, Goldstein AH. 2004. A comparison of three approaches to modeling leaf gas exchange in annually drought-stressed ponderosa pine forests. *Tree Physiology* **24,** 529–541.

Misson L, Tu KP, Boniello RA, Goldstein AH. 2006. Seasonality of photosynthetic parameters in a multi-specific and vertically complex forest ecosystem in the Sierra Nevada of California. *Tree Physiology* **26,** 729–741.

Niinemets Ü, Cescatti A, Rodeghiero M, Tosens T. 2006. Complex adjustments of photosynthetic capacity and internal mesophyll conductance to current and previous light availabilities and leaf age in Mediterranean evergreen species *Quercus ilex*. *Plant, Cell and Environment* **29,** 1159–1178.

Niinemets Ü, Díaz-Espejo A, Flexas J, Galmés J, Warren CR. 2009*a*. Role of mesophyll diffusion conductance in constraining potential photosynthetic productivity in the field. *Journal of Experimental Botany,* **60,** 2249–2270.

Niinemets Ü, Hauff K, Bertin N, Tenhunen JD, Steinbrecher R, Seufert G. 2002*a*. Monoterpene emissions in relation to foliar photosynthetic and structural variables in Mediterranean evergreen *Quercus* species. *The New Phytologist* **153,** 243–256.

Niinemets Ü, Sack L. 2006. Structural determinants of leaf light-harvesting capacity and photosynthetic potentials. In: Esser K, Lüttge UE, Beyschlag W, Murata J, eds. *Progress in Botany*, Vol. 67. Berlin: Springer Verlag, 385–419.

Niinemets Ü, Seufert G, Steinbrecher R, Tenhunen JD. 2002*b*. A model coupling foliar monoterpene emissions to leaf photosynthetic characteristics in Mediterranean evergreen *Quercus* species. *New Phytologist* **153,** 257–276.

Niinemets Ü, Sonninen E, Tobias M. 2004. Canopy gradients in leaf intercellular CO_2 mole fractions revisited: interactions between leaf irradiance and water stress need consideration. *Plant, Cell and Environment* **27,** 569–583.

Niinemets Ü, Tenhunen JD. 1997. A model separating leaf structural and physiological effects on carbon gain along light gradients for the shade-tolerant species *Acer saccharum*. *Plant, Cell and Environment* **20,** 845–866.

Niinemets Ü, Wright I, Evans JR. 2009*b*. Leaf mesophyll diffusion conductance in 35 Australian sclerophylls covering a broad range of foliage structural and physiological variation. *Journal of Experimental Botany* **60,** 2433–2449.

Nobel PS. 1977. Internal leaf area and cellular CO_2 resistance: photosynthetic implications of variations with growth conditions and plant species. *Physiologia Plantarum* **40,** 137–144.

Ohsumi A, Hamasaki A, Nakagawa H, Yoshida H, Shiraiwa T, Horie T. 2007. A model explaining genotypic and ontogenetic variation of leaf photosynthetic rate in rice (*Oryza sativa*) based on leaf nitrogen content and stomatal conductance. *Annals of Botany* **99,** 265–273.

Rambal S, Ourcival JM, Joffre R, Mouillot F, Nouvellon Y, Reichstein M, Rocheteau A. 2003. Drought controls over conductance and assimilation of a Mediterranean evergreen ecosystem: scaling from leaf to a canopy. *Global Change Biology* **9,** 1813–1824.

Seufert G, Bartzis J, Bombol T, *et al*. 1997. An overview of the Castelporziano experiments. *Atmospheric Environment* **31,** 5–17.

Tenhunen JD, Beyschlag W, Lange OL, Harley PC. 1987*a*. Changes during summer drought in leaf CO_2 uptake rates of macchia shrubs growing in Portugal: limitations due to photosynthetic capacity, carboxylation efficiency, and stomatal conductance. In: Tenhunen JD, Catarino FM, Lange OL, Oechel WC, eds. *Plant response to stress.*

Functional analysis in Mediterranean ecosystems. NATO ASI series, series G: Ecological sciences, 15. Berlin: Springer-Verlag, 305–327.

Tenhunen JD, Harley PC, Beyschlag W, Lange OL. 1987*b*. A model of net photosynthesis for leaves of the sclerophyll *Quercus coccifera*. In: Tenhunen JD, Catarino FM, Lange OL, Oechel WC, eds. *Plant response to stress. Functional analysis in Mediterranean ecosystems.* NATO ASI series, series G: Ecological sciences, 15. Berlin: Springer-Verlag, 339–354.

Terashima I, Hanba YT, Tazoe Y, Vyas P, Yano S. 2006. Irradiance and phenotype: comparative eco-development of sun and shade leaves in relation to photosynthetic CO_2 diffusion. *Journal of Experimental Botany* **57,** 343–354.

Valladares F, Niinemets Ü. 2007. The architecture of plant crowns: from design rules to light capture and performance. In: Pugnaire FI, Valladares F, eds. *Handbook of functional plant ecology*. Boca Raton, FL: CRC Press, 101–149.

von Caemmerer S. 2000. *Biochemical models of leaf photosynthesis. Techniques in plant sciences, 2*. Collingwood: CSIRO Publishing.

von Caemmerer S, Evans JR. 1991. Determination of the average partial pressure of CO_2 in chloroplasts from leaves of several C_3 plants. *Australian Journal of Plant Physiology* **18,** 287–305.

Warren CR. 2008*a*. Does growth temperature affect the temperature response of photosynthesis and internal conductance to CO_2? A test with *Eucalyptus regnans*. *Tree Physiology* **28,** 11–19.

Warren CR. 2008*b*. Stand aside stomata, another actor deserves centre stage: the forgotten role of the internal conductance to CO_2 transfer. *Journal of Experimental Botany* **59,** 1475–1487.

Warren CR, Adams MA. 2004. What determines rates of photosynthesis per unit nitrogen in *Eucalyptus* seedlings? *Functional Plant Biology* **31,** 1169–1178.

Warren CR, Adams MA. 2005. What determines interspecific variation in relative growth rate of *Eucalyptus* seedlings? *Oecologia* **144,** 373–381.

Warren CR, Adams MA. 2006. Internal conductance does not scale with photosynthetic capacity: implications for carbon isotope discrimination and the economics of water and nitrogen use in photosynthesis. *Plant, Cell and Environment* **29,** 192–201.

Warren CR, Dreyer E. 2006. Temperature response of photosynthesis and internal conductance to CO_2: results from two independent approaches. *Journal of Experimental Botany* **12,** 3057–3067.

Williams M, Rastetter EB, Fernandes DN, Goulden ML, Wofsy SC, Shaver GR, Melillo JM, Munger JW, Fan SM, Nadelhoffer KJ. 1996. Modelling the soil–plant–atmosphere continuum in a *Quercus-Acer* stand at Harvard Forest: the regulation of stomatal conductance by light, nitrogen and soil/plant hydraulic properties. *Plant, Cell and Environment* **19,** 911–927.

Wullschleger SD. 1993. Biochemical limitations to carbon assimilation in C_3 plants—a retrospective analysis of the A/C_i curves from 109 species. *Journal of Experimental Botany* **44,** 907–920.