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Letters

New calculations for photosynthesis measurement systems: what's the impact for physiologists and modelers?

Measurement of carbon dioxide (CO_2) and water (H_2O) vapor exchanged by leaves has been tremendously important for advancing understanding of plant physiology. Typically, photosynthesis measurement systems estimate the concentration of CO_2 and H_2O in air that has passed over a leaf inside a leaf chamber. Together with additional information such as flow rate, leaf area, and measurement of environmental variables, the calculations of von Caemmerer & Farquhar (1981), hereafter vCF1981, are used to estimate CO_2 and H_2O fluxes between the leaf and atmosphere. The combination of the vCF1981 calculations and photosynthesis measurement systems has enabled significant advances in the understanding and model representation of leaf-level photosynthesis and respiration.

Recently, the theory underlying the vCF1981 calculations has been updated (Márquez *et al.*, 2021), hereafter M2021. Changing the core calculations in photosynthesis measurement systems could have a marked impact on the measurement of leaf-level CO_2 and H_2O fluxes, and subsequent effects on derived parameters such as the maximum carboxylation capacity of Rubisco (V_{cmax}). Here, we highlight the implications of the M2021 theory for the plant physiology, crop breeding, and terrestrial biosphere modeling communities. We assess the effect of using the M2021 theory in photosynthesis measurement systems and identify which of the many measurements and derived parameters are affected.

What changes in the M2021 theory?

The foundation of the vCF1981 and M2021 theories rests on the shared transport pathways of H_2O and CO_2 through the leaf epidermis. The H_2O vapor concentration inside the leaf is typically assumed to be at saturation, that is, the relative humidity is 100%. This assumption enables the gradients of H_2O vapor to be calculated from measurements made at the leaf surface and enables estimation of the leaf water conductance (g_{lw}). Because H_2O and CO_2 share the same transport pathway, leaf CO_2 conductance (g_{lc}) can be inferred by accounting for the difference in diffusivity between CO_2 and H_2O. In the vCF1981 theory, g_{lc} is assumed to equal to the stomatal conductance to CO_2 (g_{sc}) and is calculated as:

$$g_{lc} = \frac{g_{lw}}{1.6} \qquad \text{Eqn 1}$$

where 1.6 is the ratio of the diffusivities of H_2O to CO_2 in air (Jarvis, 1971).

In the M2021 theory, g_{lc} considers the cuticular transport pathway and is calculated as:

$$g_{lc} = \frac{g_{sw}}{1.6} + \frac{g_{cw}}{20} \qquad \text{Eqn 2}$$

where g_{sw} is the stomatal conductance to H_2O, g_{cw} is the cuticular conductance to H_2O, and 20 is the ratio of the conductance through the cuticle of H_2O to CO_2, which is markedly higher than the ratio in air (Márquez *et al.*, 2021). In other words, the diffusivity of H_2O and CO_2 through the stomata is of the same order of magnitude (a ratio of 1.6), but the cuticle is a stronger barrier to CO_2 than H_2O where the diffusivity of H_2O is much higher than that of CO_2 (a ratio of 20). For simplicity, we have used the conservative estimate (20) of the ratio of H_2O and CO_2 conductance through the cuticle provided by Márquez *et al.* (2021) throughout this letter; however, this value has been shown to vary between 20 and 40 depending on species and growth conditions (Boyer *et al.*, 1997; Boyer, 2015a,b; Márquez *et al.*, 2021). Using values higher than 20 would increase the magnitude of the effects of applying the M2021 theory that we discuss later.

For the same g_{lw}, considering solely the stomatal pathway or including the cuticular pathway can make a large difference to g_{lc}, particularly when the $g_{cw} : g_{sw}$ ratio is markedly above zero. This is the most important difference between the vCF1981 and M2021 theories. Other differences between M2021 and vCF1981 have a smaller effect on calculated fluxes but are still notable: (1) The concentration of H_2O and CO_2 at the leaf surface is now explicitly calculated in M2021, which was not the case with vCF1981; (2) The M2021 theory better represents the molecular collisions between gases – known as the ternary effect – which can limit gas transport.

Theoretical changes: The M2021 theory now includes the cuticular conductance pathway which was not considered by the vCF1981 theory. It also improves representation of the conditions at the leaf surface and better represents the collision between gas molecules.

Which measured gas exchange variables are impacted by M2021?

Table 1 gives the list of variables of both theories as well as the expected impact due to application of the M2021 theory. The H_2O and CO_2 concentration at the leaf surface (w_s and C_s, respectively) are an output of M2021 calculations which were not directly given by vCF1981 calculations. The vCF1981 calculations only gave the H_2O and CO_2 concentrations in the atmosphere (w_a and C_a,

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Table 1 Variables calculated by the vCF1981 and M2021 theories and the expected difference in calculated values resulting from the application of the M2021 theory with $g_{cw} > 0$.

vCF1981	M2021	Definition	Effect
A	A	Net CO_2 assimilation rate	No difference
E_T	E_T	Total leaf transpiration rate	No difference
—	E_s	Leaf transpiration rate through the stomata	New variable
—	E_c	Leaf transpiration rate through the cuticle	New variable
C_a	C_a	CO_2 concentration in the atmosphere	No difference
—	C_s	CO_2 concentration at the leaf surface	New variable
C_i	C_i	CO_2 concentration inside the leaf	Difference (decrease)
g_{bc}	g_{bc}	Boundary layer conductance to CO_2	No difference
g_{bw}	g_{bw}	Boundary layer conductance to water	No difference
g_{lw}	g_{lw}	Total leaf water vapor conductance	Difference (slight increase)
g_{sw}	g_{sw}	Stomatal water vapor conductance	Difference (decrease)
—	g_{cw}	Cuticular water vapor conductance	New variable
g_{lc}	g_{lc}	Total leaf conductance to CO_2	Difference (decrease)
g_{sc}	g_{sc}	Stomatal conductance to CO_2	Difference (decrease)
—	g_{cc}	Cuticular conductance to CO_2	New variable
w_a	w_a	Water vapor concentration in the atmosphere	No difference
—	w_s	Water vapor concentration at the leaf surface	New variable
w_i	w_i	Water vapor concentration inside the leaf	No difference

respectively). The difference between w_a, w_s and C_a, C_s is expected to be low under typical measurement conditions (high flow rates and high boundary layer conductance). In the M2021 theory the total leaf water transpiration (E_T) is separated into transpiration through the stomata (E_s) and through the cuticle (E_c) but E_T remains the same for both theories. Measurements of g_{lw} only change slightly with the M2021 theory, and thus g_{sw}, which was previously assumed to equal g_{lw}, will change with a difference of approximately the value of g_{cw}. Importantly, the measured CO_2 assimilation rate (A) does not change. Relative to application of the vCF1981 theory, there is potential for a marked effect on g_{lc} when the M2021 theory is applied. As a consequence of changes in g_{lc},

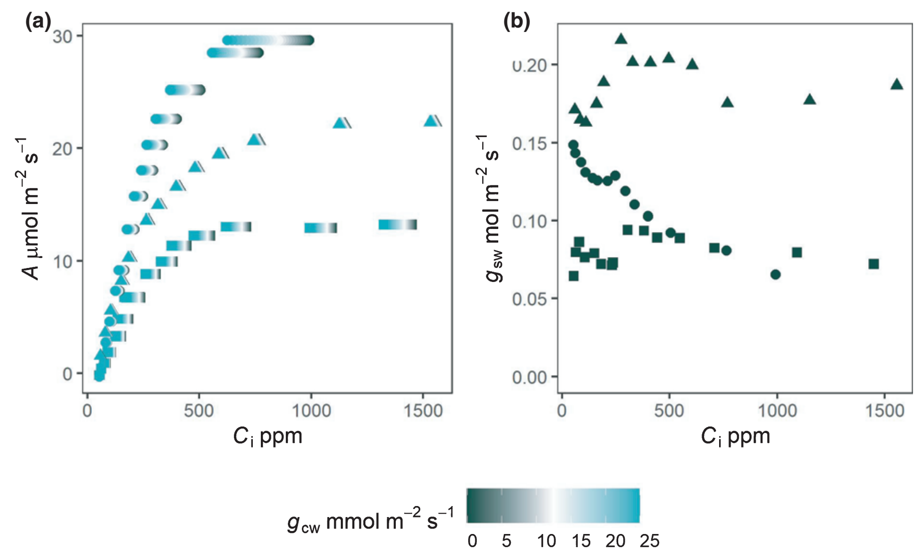

Fig. 1 Example of recalculation of A–C_i curves (a) measured on plants with different stomatal conductance (b). (a) Recalculation of A–C_i curves using the M2021 theory for different values of cuticular conductance (g_{cw}, color ramp) for three different species from an arctic (*Petasites frigidus*, triangles), temperate (*Quercus cocinear*, circles) and tropical (*Guatteria dumetorum*, squares) environment. The A–C_i curve for *G. dumetorum* was measured in the San Lorenzo Protected Area in the Republic of Panama at 28°C with a LICOR 6800 (Lamour *et al*., 2021). The A–C_i curves for *P. frigidus* and *Q. cocinear* were measured at a leaf temperature of 12°C in Alaska, USA and 22°C in New York, USA, using a LICOR 6400XT (Rogers *et al*., 2017b; Burnett *et al*., 2021). The A–C_i curves recalculated with a g_{cw} of 0 mol m^{-2} s^{-1} are equivalent to the value resulting from application of the vCF1981 theory and correspond to the original output given by the photosynthesis measurement instruments which use the vCF1981 theory. The range of g_{cw} used here is similar to the range presented by Duursma *et al*. (2019) and measured by Márquez *et al*. (2021; pers. comm.). The effect of g_{cw} on C_i in the three A–C_i curves depends on several factors. The most important of these is stomatal conductance (g_{sw}, panel b), the lower the stomatal conductance, the greater the effect of g_{cw} on C_i. It also depends on E_T, A, and the boundary layer inside the instrument during the measurements (see Box 1 and M2021 theory, Márquez *et al*., 2021). Note that A is not modified by g_{cw} on the A–C_i curves. (b) The stomatal conductance (g_{sw}) estimated using the vCF1981 theory ($g_{cw} = 0$) for the A–C_i curves is presented in (a).

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notable reductions in C_i can be realized (see fig. 5 of Márquez *et al.*, 2021). Note that an increase in C_i is also possible when A is negative.

For physiological studies, this means that the protocols that will be impacted by the M2021 theory are mainly those dependent upon C_i. This includes, as mentioned by Márquez *et al.* (2021), the estimation of the mesophyll conductance and the CO_2 concentration inside chloroplasts (C_c) which is based upon measurement of C_i (Pons *et al.*, 2009). Importantly, it also impacts parameters estimated from photosynthetic CO_2 response curves (A–C_i or A–C_c curves) which are frequently used to parameterize crop and terrestrial biosphere models. Measurement of variables that are not dependent on C_i or C_c such as the CO_2 assimilation rate at saturating irradiance (A_{sat}), the directly measured dark-adapted respiration rate (R_{dark}) or parameters derived from fitting models that do not rely on C_i or C_c will not be impacted (e.g. parameters derived from light response curves, or leaf H_2O conductance measurements).

Measurements: The main impact of applying the M2021 theory is a reduction in the estimation of intercellular CO_2 concentration during photosynthesis (i.e. when $A > 0$). Measurements of the photosynthetic rate and transpiration are not affected.

Box 1 Equations of gas transport between the leaf and the leaf surface

Fick's law of diffusion

$$C_i = C_s - \frac{A}{g_{lc}} \quad \text{Eqn 3}$$

vCF1981 theory

$$C_i = \frac{C_s\left(g_{lc} - \frac{E_T}{2}\right) - A}{g_{lc} + \frac{E_T}{2}} \quad \text{Eqn 4}$$

Compared to Fick's law of diffusion, the vCF1981 theory considers a ternary effect which hinders the diffusion of CO_2 from the leaf surface toward the inside of the leaf through the stomata aperture due to collision between CO_2 molecules and the much higher number of H_2O molecules flowing in the opposite direction. This effect is represented in the equations by the terms $-E_T/2$ and $+E_T/2$. Note that if those terms are considered negligible, then we retrieve the Fick's law of diffusion (Eqn 4 corresponds to Eqn 3 when $E_T/2 = 0$). In this theory and in Fick's representation, $g_{lc} = g_{sc}$ and g_{cc} is assumed to be zero (Márquez *et al.*, 2021). Note that in photosynthesis measurement devices, C_s is not calculated by the vCF1981 theory (Table 1) and we assumed it was equal to C_a here.

M2021 theory

$$C_i = \frac{C_s\left(g_{sc} + g_{cc} - \frac{E_s}{2}\right) - A}{g_{sc} + g_{cc} + \frac{E_s}{2}} \quad \text{Eqn 5}$$

Compared to the vCF1981 theory, the cuticular conductance pathway is considered. The ternary effect which modifies the flux of CO_2 and water into and out of the leaf is taken into account by the $E_s/2$ terms. Compared to the vCF1981 model, the transpiration through the stomata (E_s) and the transpiration through the cuticle are also distinguished. Note that if $g_{cw} = 0$, then $g_{cc} = 0$, $g_{sc} = g_{lc}$ and $E_T = E_s$ and therefore, Eqn 5 would correspond to Eqn 4.

Importantly, the three equations (Eqns 3–5) depend upon the conductance variables for CO_2 (g_{sc} and g_{cc}). Note that the conductance for CO_2 is often inferred from the conductance to H_2O and that g_{sc} corresponds to $g_{sw}/1.6$ but that g_{cc} ranges from $g_{cw}/40$ to $g_{cw}/20$ (Boyer *et al.*, 1997; Boyer, 2015a,b; Márquez *et al.*, 2021) such as $g_{sc} + g_{cc} \neq (g_{sw} + g_{cw})/1.6$ if g_{cw} is above zero.

Impact of g_{cw} on A–C_i measurements

To illustrate the effect of the g_{cw} on C_i and on parameters dependent on estimation of C_i, we recomputed previously published A–C_i curves (Rogers *et al.*, 2017b; Lamour *et al.*, 2021; Burnett *et al.*, 2021) using the M2021 theory considering a range of hypothetical g_{cw} (0–25 mmol m^{-2} s^{-1}, Fig. 1). We then estimated the following parameters from the recalculated A–C_i data: the maximum rate of carboxylation (V_{cmax}), the maximum rate of electron transport (J_{max}), the triose phosphate utilization (TPU) rate, and the CO_2 release from the leaf in the light (R_{day}). Fig. 1 shows that indeed the new theory modifies C_i. The effect differed among the curves. This is expected by the theory, and a stronger effect is expected when the $g_{cw} : g_{sw}$ ratio is high. Here, the arctic species (*Petasites frigidus*) was the least affected by consideration of g_{cw} and had the highest stomatal conductance during the measurement (around 0.20 mol m^{-2} s^{-1}, Fig. 1(a,b), triangles). In oak (*Quercus coccinear*) the highest C_i on the A–C_i curve (Fig. 1a, high × ordinate, circle) showed the greatest response to g_{cw}, as it had the lowest conductance during the measurement (Fig. 1b). Note that the measured rates of E_T and A – while not directly affected by application of the M2021 theory – also influence the size of the g_{cw} effect on C_i (see Box 1).

Fig. 2 shows the effect on parameters derived from A–C_i curves and highlights the potential for a marked increase in V_{cmax} when g_{cw} is high. This result is critical for estimation of photosynthetic traits because the assumption that g_{cw} is zero (in the vCF1981 theory) can bias the estimation of V_{cmax} in leaves for which g_{cw} is markedly higher than zero. Note that the parameter R_{day} is also impacted as it corresponds to the value of A when C_i equals the CO_2 compensation point in the absence of nonphotorespiratory CO_2 release (Γ^*). However, note that R_{day} fitted during estimation of the other A–C_i parameters is usually not used in physiological studies and other methods of estimating R_{day} are preferred. Interestingly, this also means that estimation of R_{day} using the approach of Laisk (1977), that is, the intersection of several A–C_i curves, could be impacted by the M2021 theory. However, measurements using the Kok method (Kok, 1948) which are based on the response of A to irradiance, and which therefore do not rely on C_i, will not be impacted. Obviously, direct measurement of dark-adapted R_{dark} is also unaffected by the new theory.

Derived parameters: Only parameter estimates that are dependent upon measurement of C_i will be impacted by the M2021 theory. Notably, this includes V_{cmax} and the estimation of the mesophyll conductance. The magnitude of the effect increases with g_{cw}.

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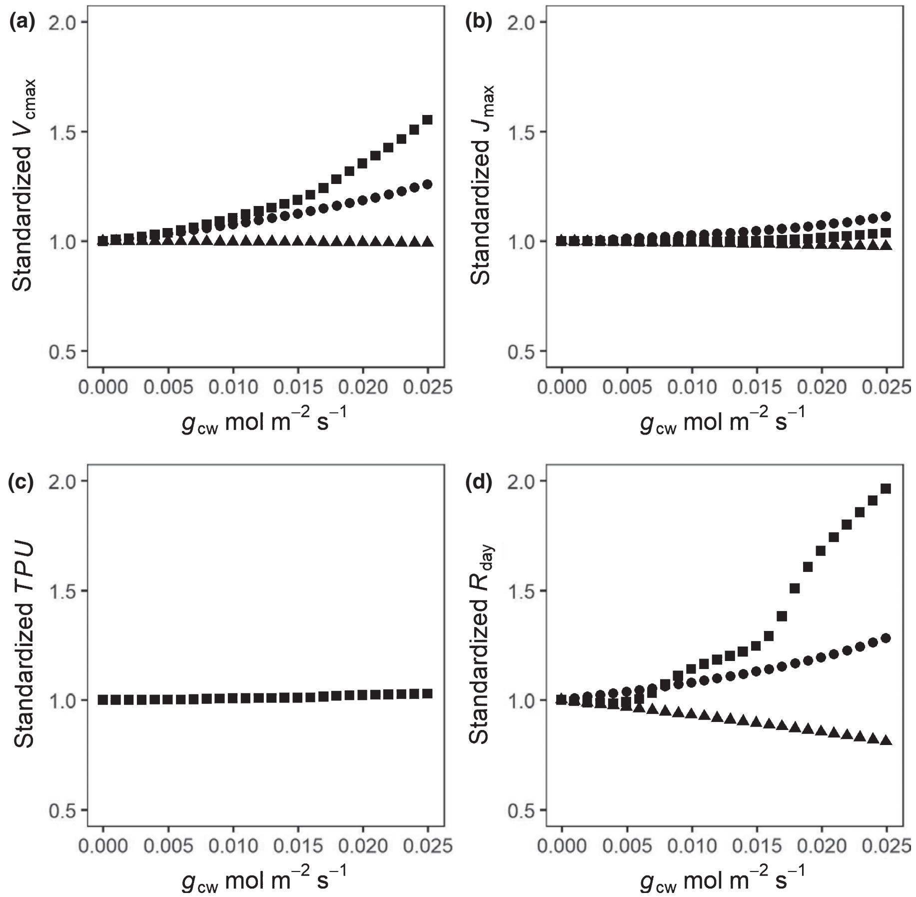

Fig. 2 Impact of g_{cw} on the parameters of the FvCB model (Farquhar *et al.*, 1980), V_{cmax}, J_{max}, TPU and R_{day} (a–d, respectively) estimated from the A–C_i curves described in Fig. 1. The A–C_i curves were measured on three different species (*Petasites frigidus*, triangles; *Quercus cocinear*, circles; *Guatteria dumetorum*, squares). The parameters estimated from the A–C_i curves measured with the M2021 theory were standardized by dividing their values with the ones estimated using the vCF1981 theory and are unitless. Only *G. dumetorum* showed a TPU limitation so only squares are displayed in (c).

Impact of the M2021 theory on the simulation of photosynthesis

To simulate photosynthesis, leaf gas exchange models typically link a leaf gas transport model (e.g. Fick, 1855), a leaf conductance model (e.g. Medlyn *et al.*, 2011) and a photosynthesis model such as the FvCB model (Farquhar *et al.*, 1980). The leaf gas exchange models are end-users of the parameters estimated from data collected by photosynthesis measurement systems and will be affected by changes in measurements related to the use of the M2021 theory. In particular, changing the parameterization of the FvCB model to account for the effect of the M2021 theory on estimates of A–C_i parameters (Fig. 2) would alter the simulation of gas exchange, as V_{cmax} is one of the most influential parameters in terrestrial biosphere models (Rogers, 2014; Walker *et al.*, 2021).

However, this is not the only impact of the M2021 theory. Márquez *et al.* (2021) focused on the theory of gas transport for improving calculations in photosynthesis measurement systems. Importantly, the representation of gas transport is also used in leaf gas exchange models. Today, in most models, it is represented by Fick's law of diffusion (Collatz *et al.*, 1991; Yin & Struik, 2009; Prieto *et al.*, 2012; Oleson *et al.*, 2013) which is a simpler representation than that used in both the vCF1981 and M2021 models (Box 1). Leaf gas exchange models typically do not represent gas transport through the cuticle, and share the flaws that motivated the publication of the M2021 theory. For example, at present, the effect of the cuticular conductance is imperfectly taken into account by the parameter g_0 in leaf stomatal conductance models (Lombardozzi *et al.*, 2017) and the transport of H_2O and CO_2 through the cuticle is represented through the stomatal pathway. Fig. 3 shows an example of differentiating the pathways in simulations of gas exchange by using the M2021 transport model with a hypothetical range of g_{cw} values (0–25 mmol m^{-2} s^{-1}). Changing the transport model substantially modified simulations of A and E_T (Fig. 3) by modifying g_{lc} and C_i. Generally, simulated A decreased, and E_T increased as a consequence of using the M2021 model in place of the Fick's law of diffusion. This resulted in greater simulated transpiration for a given rate of photosynthesis, that is, a reduced water use efficiency. The effect was particularly evident at high temperature and low humidity (Fig. 3c,d,g,h). Therefore, consideration of the M2021 theory could be particularly important when modeling the response of CO_2 and H_2O cycles to drought. A key advantage of the M2021 theory is an improved representation of g_{cw} effects in photosynthesis models, which will open the door to improved representation of the impact of drought (Márquez *et al.*, 2021), temperature (Schreiber, 2001; Schuster *et al.*, 2016) or leaf aging (Duursma *et al.*, 2019) on g_{cw}. We also want to emphasize that if the M2021 theory is used in photosynthesis measurement systems, photosynthesis models will have to be updated so the calculations used for parametrization and simulation are the same (Rogers *et al.*, 2017a).

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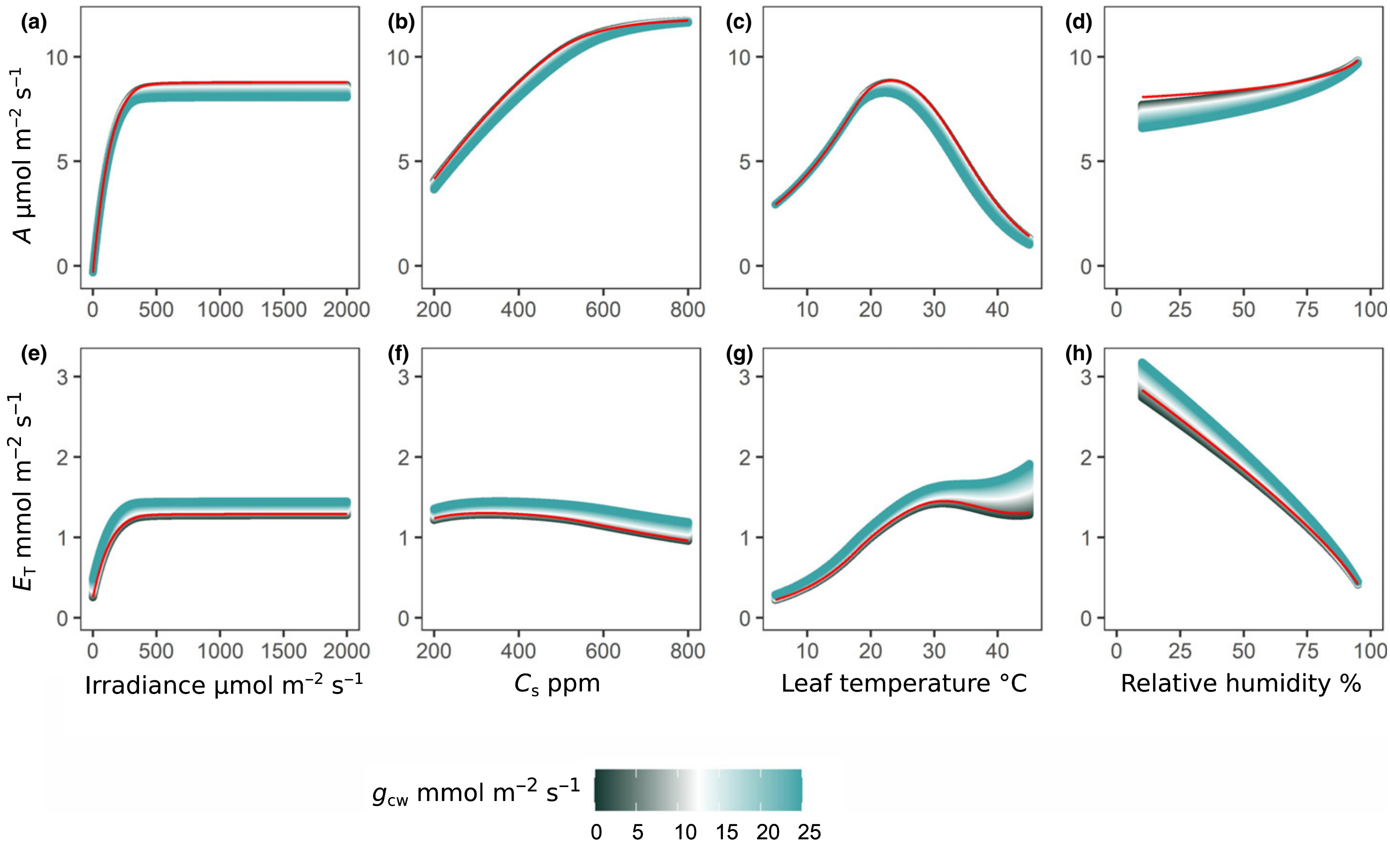

Fig. 3 Impact of the choice of the representation of water and CO_2 transport between the leaf and atmosphere on modeled net assimilation rate (*A*, panels a–d) and transpiration (E_T, panels e–h). Fick's law is represented by a red line. The M2021 model is represented by a color ramp for different g_{cw} values. Note that using a g_{cw} of 0 mol m^{-2} s^{-1} corresponded here to the vCF1981 theory. The standard conditions for the simulations were irradiance = 2000 µmol m^{-2} s^{-1}, relative humidity = 70%, leaf and air temperature = 25°C, C_s = 400 ppm. The following FvCB parameters measured at 25°C for *Guatteria dumetorum* were used: V_{cmax} = 36.7 µmol m^{-2} s^{-1}, J_{max} = 66.9 µmol m^{-2} s^{-1}, TPU = 4.08 µmol m^{-2} s^{-1} and R_{day} = 0.33 µmol m^{-2} s^{-1} (Fig. 2, vCF1981). We used the USO model of leaf conductance (Medlyn *et al*., 2011), with a g_1 of 1.89 kPa$^{0.5}$ which corresponded to the value measured in *G. dumetorum* (Fig. 2). The leaf conductance parameter g_0 was set to 0.03 mol m^{-2} s^{-1} for Fick's law of diffusion and for the vCF1981 theory. This parameter is thought to represent the cuticular conductance and the conductance due to unclosed stomata at low irradiance. Therefore, for the M2021 theory, g_0 was set to 0.03 – g_{cw} so the parametrization is comparable between simulations and gas transport models.

Modeled fluxes: The transport of gases in leaves is commonly represented by Fick's law of diffusion in crop and terrestrial biosphere models. The M2021 theory improves representation of gas transport and explicitly accounts for fluxes through the leaf cuticle which are currently accounted for using stomatal models.

Conclusion

The new M2021 theory could replace the way gas exchange is calculated in photosynthesis measurement systems. This would enable a better estimation of mesophyll conductance and of parameters derived from A–C_i or A–C_c response curves. The work of Márquez *et al.* (2021) therefore highlights that improved measurement and understanding of g_{cw} is not only important for drought mortality studies (e.g. Martin-StPaul *et al*., 2017; Choat *et al*., 2018) but also for accurate estimation of important photosynthetic parameters that determine leaf-level and global CO_2 and H_2O fluxes. In addition, the M2021 theory corrects the representation of gas fluxes through the cuticle. This advance can be implemented in crop and terrestrial biosphere models that currently represent cuticular fluxes through the stomatal pathway. A significant barrier for the broad scale adoption of the M2021 theory in gas exchange systems is the necessity to measure g_{cw} for which the best methods are still debated (Boyer, 2015a; Duursma *et al*., 2019; Márquez *et al*., 2022). Recent advances, emerging datasets and new methods for measuring g_{cw} should accelerate the adoption of this new theory (Duursma *et al*., 2019; Machado *et al*., 2021; Márquez *et al*., 2022; Slot *et al*., 2021). Importantly, some aspects of gas exchange calculations are still uncertain and could change in the future. For example, plant physiologists and modelers typically assume that the H_2O content inside the leaf (w_i) is at saturation, which in some circumstances is an oversimplification that can result in biased estimation of C_i (Canny & Huang, 2006; Cernusak *et al*., 2018; Buckley & Sack, 2019). The work of Márquez *et al.* (2021) and potential continued development of the calculations used to describe the exchange of CO_2 and H_2O underscores the need to preserve the raw gas exchange data produced by photosynthesis measurement systems (Ely *et al*., 2021).

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Data availability

The gas exchange measurements are available online, linked to the published datasets associated with the original studies (Rogers *et al.*, 2017b; Burnett *et al.*, 2021). The A–C_i data for *Guatteria dumetorum* is publicly available (Lamour *et al.*, 2021). The code used to process the data is available online (Lamour, 2021).

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