Simple generalisation of a mesophyll resistance model for various intracellular arrangements of chloroplasts and mitochondria in C₃ leaves

Xinyou Yin¹ · Paul C. Struik¹

Received: 8 July 2016 / Accepted: 17 January 2017 / Published online: 14 February 2017
© The Author(s) 2017. This article is published with open access at Springerlink.com

Keywords  CO₂ transfer · Internal conductance · Mesophyll resistance

Abstract  The classical definition of mesophyll conductance (gₘ) represents an apparent parameter (gₘ,app) as it places (photo)respired CO₂ at the same compartment where the carboxylation by Rubisco takes place. Recently, Tholen and co-workers developed a framework, in which gₘ better describes a physical diffusional parameter (gₘ,dif). They partitioned mesophyll resistance (rₘ,dif = 1/gₘ,dif) into two components, cell wall and plasmalemma resistance (rₜₚ) and chloroplast resistance (rₜₜ), and showed that gₘ,app is sensitive to the ratio of photorespiratory (F) and respiratory (Rₜ) CO₂ release to net CO₂ uptake (A): gₘ,app = gₘ,dif/[1 + ω(F + Rₜ)/A], where ω is the fraction of rₜₜ in rₘ,dif. We herein extend the framework further by considering various scenarios for the intracellular arrangement of chloroplasts and mitochondria. We show that the formula of Tholen et al. implies either that mitochondria, where (photo)respired CO₂ is released, locate between the plasmalemma and the chloroplast continuum or that CO₂ in the cytosol is completely mixed. However, the model of Tholen et al. is still valid if ω is replaced by ω(1−σ), where σ is the fraction of (photo)respired CO₂ that experiences rₜₜ in addition to rₜₚ and stomatal resistance if this CO₂ is to escape from being refixed. Therefore, responses of gₘ,app to (F + Rₜ)/A lie somewhere between no sensitivity in the classical method (σ=1) and high sensitivity in the model of Tholen et al. (σ=0).

Introduction

The biochemical C₃ photosynthesis model of Farquhar, von Caemmerer and Berry (1980), the FvCB model hereafter, has been widely used to interpret leaf physiology from gas exchange measurements. The model calculates the net rate of leaf photosynthesis (A) as the minimum of the Rubisco carboxylation activity-limited rate (Aₜ) and the electron (e⁻) transport-limited rate (Aₑ) of photosynthesis (see Appendix A). The partial pressure of CO₂ at the intercellular air spaces (Cᵢ) is a required input variable to calculate both Aₜ and Aₑ in the model. The drawdown of Cᵢ, relative to the CO₂ level in the ambient air (Cᵢ₀), depends not only on stomatal conductance for CO₂ transfer (gₛ), but also on the mesophyll conductance for CO₂ transfer between substomatal cavities and the site of CO₂ carboxylation (gₘ). According to Fick’s diffusion law, gₘ can be expressed as follows (von Caemmerer and Evans 1991; von Caemmerer et al. 1994):

\[
gₘ = A/(Cᵢ − Cᵢ₀)
\]  

where Cᵢ is the partial pressure of CO₂ at the intercellular air spaces.

This simple gas diffusion equation has been combined with the FvCB model to estimate gₘ (Pons et al. 2009), based on combined data of A−Cᵢ curves and chlorophyll fluorescence measurements on photosystem II e⁻ transport efficiency Φ₂ (Harley et al. 1992; Yin and Struik 2009) or on combined gas exchange and carbon isotope discrimination measurements (Evans et al. 1986). When the gₘ estimation is based on combined gas exchange and chlorophyll
fluorescence measurements (e.g. the ‘variable J method’, Harley et al. 1992), the $A_j$ part of the FvCB model is used, in which the linear $e^−$ transport rate ($J$) is estimated from chlorophyll fluorescence signals. Using this method, it has been reported that $g_m$ can decrease with increasing $C_i$ or with decreasing incoming irradiance $I_{inc}$ (Flexas et al. 2007; Vrábl et al. 2009; Yin et al. 2009). Similar patterns of variable $g_m$ have been reported with the isotope discrimination method (Vrábl et al. 2009), although with less consistency (Tazoe et al. 2009).

Equation (1) is based on net photosynthesis and assumes that respiratory and photorespiratory CO$_2$ release occurs in the same compartment as CO$_2$ fixation by Rubisco. However, CO$_2$ fixation occurs in the chloroplast stroma, whereas (photo)respiratory CO$_2$ is released in the mitochondria. The first step of photorespiration, the O$_2$ fixation, takes place in the chloroplast to form phosphoglycolate. Phosphoglycolate is converted to glycolate and glyoxylate, and then to glycine in the peroxisome; glycine moves to the mitochondria to form phosphoglycine. Phosphoglycine is decarboxylated there into CO$_2$, NH$_3$ and serine (Kebeish et al. 2007). The CO$_2$ released in mitochondria, either respiration or photorespiration, can be partially refixed by Rubisco in the chloroplast stroma, whereas the remaining portion escapes to the atmosphere (Busch et al. 2013).

To quantify mesophyll resistance $r_m$ (the reciprocal of $g_m$), there is a need to specify resistance components within the cell imposed by walls, plasmalemma, cytosol, chloroplast envelope and stroma (Evans et al. 2009; Terashima et al. 2011). Unlike the CO$_2$ that comes from the substomatal cavities, the CO$_2$ from the mitochondria does not need to cross the cell wall and plasmalemma, and thus experiences a different resistance. Considering this difference, Tholen et al. (2012) developed a theoretical framework to analyse $g_m$ as described below.

The total mesophyll diffusional resistance ($r_{m,dif}$) can be described as the sum of a series of physical resistances comprising of intercellular air space, cell wall, plasmalemma, cytosol, chloroplast envelope and chloroplast stroma components (Evans et al. 2009): $r_{m,dif} = r_{i,as} + r_{wall} + r_{plasmalemma} + r_{cytosol} + r_{envelope} + r_{stroma}$. The resistance imposed by the gas phase component and the cytosol is generally small (Tholen et al. 2012), and may therefore be ignored.

Tholen et al. (2012) combined $r_{wall}$ and $r_{plasmalemma}$ into the resistance at the cell wall–plasma membrane interface ($r_{wp}$), and $r_{envelope}$ and $r_{stroma}$ into the total chloroplast resistance ($r_{ch}$), so that $r_{m,dif} = r_{wp} + r_{ch}$. Based on Fick’s diffusion law and considering two different resistance components encountered by CO$_2$ from substomatal cavities and CO$_2$ from the mitochondria, Tholen et al. (2012) derived the following relationship (their Eq. 6):

$$C_c = C_i - A(r_{wp} + r_{ch}) - (F + R_d)r_{ch}$$

where $F$ is the photorespiratory CO$_2$ release and $R_d$ is the CO$_2$ release in the light other than by photorespiration, both in the mitochondria. The model Eq. (2) is still a simplification of true resistance pathways, because (i) diffusion is a continuous process and there are many parallel pathways (Tholen et al. 2012) and (ii) the model ignores that some respiratory flux originates in the chloroplast (Tcherkez et al. 2012) and that there may be small activity of phosphoenolpyruvate carboxylase in cytosol (Douthe et al. 2012; Tholen et al. 2012).

Here we let $r_{ch} = \omega r_{m,dif}$; then $r_{wp} = (1-\omega)r_{m,dif}$, where $\omega$ is the relative contribution of $r_{wp}$ to the total mesophyll resistance $r_{m,dif}$ ($=r_{wp}+r_{ch}$). Equation (2) then becomes

$$C_c = C_i - Ar_{m,dif} - \omega(F + R_d)r_{m,dif}$$

(3)

Solving ($C_c - C_i$) from Eq. (3) and substituting it into Eq. (1) give

$$g_m = \frac{1}{r_{m,dif} \left(1 + \frac{F + R_d}{A}\right)}$$

(4)

Equation (4) is equivalent to Eq. (9) of Tholen et al. (2012), in which $g_{wp}$ and $g_{ch}$ (i.e. the inverse of $r_{wp}$ and $r_{ch}$, respectively) are used. We prefer Eq. (4) because it allows (i) to analyse how $g_m$ varies for a given total mesophyll resistance and (ii) to provide an analogue to an extended model that will be developed later.

Both Eq. (4) and Tholen et al.’s Eq. (9) tell that $g_m$, as defined by Eq. (1), is influenced by the ratio of (photo) respiratory CO$_2$ from the mitochondria to net CO$_2$ uptake ($F + R_d$)/$A$, thereby resulting in an apparent sensitivity of $g_m$ to CO$_2$ and O$_2$ levels (Tholen et al. 2012). This sensitivity does not imply a change in the intrinsic diffusion properties of the mesophyll; so, $g_m$ as defined by Eqs. (1) and (4) is apparent, and we denote it as $g_{m,app}$ hereafter. The sensitivity depends on $\omega$: the higher is $\omega$ the more sensitive is $g_{m,app}$ to ($F + R_d$)/$A$. If $\omega=0$, then $g_{m,app}$ is no longer sensitive to ($F + R_d$)/$A$, Eq. (3) becomes Eq. (1) and $g_{m,app}$ becomes $g_{m,dif}$—the intrinsic mesophyll diffusion conductance ($=1/r_{m,dif}$). In such a case, carboxylation and (photo) respiratory CO$_2$ release occur in the same organelle compartment or if occurring in separate compartments, the chloroplast exerts a negligible resistance to CO$_2$ transfer.

Equations (1) and (2) have been considered as two basic scenarios for CO$_2$ diffusion path in C$_3$ leaves (von Caemmerer 2013), both representing a simplified view on CO$_2$ diffusion in the framework of whole leaf resistance models. Detailed views on the mechanistic basis of CO$_2$ diffusion in relation to intracellular organelle positions could best be investigated using reaction–diffusion models (e.g. Tholen and Zhu 2011). However, uncertainties in the value of many required input diffusion coefficients and the complexity in nature are the major limitations of using these.
reaction–diffusion models (see Berghuijs et al. 2016 for discussions on simple resistance vs. reaction–diffusion models). We herein discuss an extended, yet simple, resistance model by considering various scenarios with regard to intracellular arrangement of organelles: (1) the relative positions of mitochondria and chloroplasts and (2) gaps between individual chloroplasts. We also discuss implications of these scenarios in estimating the fraction of (photo) respired CO₂ being refixed.

A generalised model

To develop a generalised model, we consider two possibilities of chloroplast distribution (either continuous or discontinuous) and three possibilities of mitochondria location (outer, inner or both outer and inner layers of cytosol). This gives six cases with regard to the arrangement of organelles within mesophyll cells (Fig. 1). In each scenario, mitochondria are intimately associated with chloroplasts, as commonly observed for real leaves (Sage and Sage 2009; Hatakeyama and Ueno 2016). Within our simple generalised model, we stay with the same notation of \( r_{wp} \) and \( r_{ch} \), the two-resistance components as the essence of the model of Tholen et al. (2012). However, as we discuss later on, instead of assuming that \( r_{cytosol} \) is negligible, we followed the approach of Berghuijs et al. (2015) that lumps part of \( r_{cytosol} \) into \( r_{wp} \) and the remaining part of \( r_{cytosol} \) into \( r_{ch} \). Given the position of mitochondria shown in Fig. 1, nearly all cytosolic resistance, i.e. along the diffusion path length from plasmalemma to chloroplast outer membrane, can be lumped into \( r_{wp} \), whereas only a small remaining portion of \( r_{cytosol} \) is lumped into \( r_{ch} \).

Case I

In this case, the coverage of chloroplasts is continuous and all mitochondria locate in the outer layer of cytosol (Fig. 1a). For this case, the net CO₂ influx \( (A) \) from the intercellular air spaces is driven by the gradient between \( C_i \) and \( C_{m(outer)} \) (where \( C_{m(outer)} \) is the CO₂ partial pressure at the outer layer of the mesophyll cytosol facing chloroplast envelope), whereas the gradient between \( C_{m(outer)} \) and \( C_c \) drives the carboxylation flux \( (V_c) \). Therefore, equations for the CO₂ gradient between the compartments and involved resistance components are as follows: \( C_c = C_{m(outer)} - V_r ch \) and \( C_{m(outer)} = C_i - Ar wp \). In the FvCB model, \( A \) is formulated as \( A = V_c - F - R_d \). Combining these three equations actually gives rise to Eq. (2), from which Eq. (4) for the sensitivity of \( g_m app \) to \( (F + R_d)/A \) was derived. Therefore, formulae for this Case I are in line with the framework as described by Tholen et al. (2012).

Tholen et al. (2012) also showed, based on their model framework, that the fraction of (photo)respired CO₂ that is refixed by Rubisco can be quantified using the resistance components. We use \( x(F + R_d) \) to denote the partial pressure of (photo)respired CO₂ in mesophyll cytosol, where \( x \) is a conversion factor from flux to partial pressure for (photo)respired CO₂ and has a unit of bar (mol m⁻² s⁻¹). CO₂ molecules from (photo)respiration can diffuse towards Rubisco but will experience \( r_{ch} \) and a resistance derived from the carboxylation itself \( (r_{cx}) \); so the refixation rate \( (R_{refix}) \) is \( x(F + R_d)/(r_{ch} + r_{cx}) \). A portion of the (photo)respired CO₂ molecules can also escape from refixation and move out of the stomata to the atmosphere, experiencing \( r_{wp} \) and the stomatal resistance for CO₂ transfer \( r_{sc} \) (including a small boundary layer resistance); so the rate of this leak or escape \( (R_{escape}) \) is \( x(F + R_d)/(r_{wp} + r_{sc}) \). The fraction of (photo)respired CO₂ that is refixed by Rubisco \( (f_{refix}) \) can be calculated by

\[
\begin{align*}
\frac{R_{refix}}{R_{refix} + R_{escape}} &= \frac{\frac{1}{r_{ch} + r_{cx}}}{\frac{1}{r_{ch} + r_{cx}} + \frac{1}{r_{wp} + r_{sc}}} = \frac{r_{sc} + r_{wp}}{r_{sc} + r_{wp} + r_{ch} + r_{cx}} \\
\end{align*}
\]

(5)

This compares with Eq. (14) of Tholen et al. (2012) and shows that the refixation fraction can be calculated simply as the ratio of the resistance components that the escaped (photo)respired CO₂ molecules have experienced to the total resistance along the full diffusion pathway.
Case II

The coverage of chloroplasts is continuous and all mitochondria locate in the inner layer of cytosol, closely behind chloroplasts (Fig. 1b). In this case, since there are no mitochondria between the plasma membrane and chloroplasts, in essence, \( r_{\text{ch}} \) and \( r_{\text{wp}} \) can be combined and the flux involved is the same for the CO₂ gradient between \( C_i \) and \( C_{\text{m(outter)}} \) and between \( C_{\text{m(outter)}} \) and \( C_{\text{c}} \), i.e. \( A = V_c - F - R_d \). This corresponds to the classical model, Eq. (1), that has commonly been used for estimating \( g_m \) (von Caemmerer and Evans 1991; von Caemmerer et al. 1994).

In this case, all (photo)respired CO₂ molecules have to experience \( r_{\text{ch}} \), in addition to \( r_{\text{wp}} \) and \( r_{\text{sc}} \), if they are to escape from being refixed. As mitochondria locate closely behind chloroplasts and mitochondria and chloroplasts are treated essentially as one compartment in the classical model, (photo)respired CO₂ molecules that diffuse towards Rubisco can be considered to experience \( r_{\text{cx}} \) only; so \( R_{\text{refix}} \) is \( x(F + R_d)/r_{\text{cx}} \). The remaining (photo)respired CO₂ that escape from refixation experience \( r_{\text{ch}} \), \( r_{\text{wp}} \) and \( r_{\text{sc}} \); so, \( R_{\text{escape}} \) is \( x(F + R_d)/(r_{\text{ch}} + r_{\text{wp}} + r_{\text{sc}}) \). Then, \( f_{\text{refix}} \) can be calculated by

\[
f_{\text{refix}} = \frac{R_{\text{refix}}}{R_{\text{refix}} + R_{\text{escape}}} = \frac{1}{r_{\text{cx}}} + \frac{1}{r_{\text{ch}} + r_{\text{wp}} + r_{\text{sc}}} = \frac{r_{\text{sc}} + r_{\text{wp}} + r_{\text{ch}}}{r_{\text{sc}} + r_{\text{wp}} + r_{\text{ch}} + r_{\text{cx}}}
\]

(6)

Obviously, this predicts a higher refixation fraction than Eq. (5) does.

Case III

The coverage of chloroplasts is continuous and mitochondria locate in both inner and outer layers of cytosol (Fig. 1c). Let \( \lambda \) be the fraction of mitochondria that locate closely behind chloroplasts in the inner cytosol. Then \( (1-\lambda) \) is the fraction of mitochondria that locate in the outer cytosol. The flux associated with the gradient between \( C_{\text{m(outter)}} \) and \( C_c \) is the carboxylation flux \( (V_c) \) minus the efflux of (photo)respired CO₂ from the inner layer \( \lambda(F + R_d) \), while the flux associated with the gradient between \( C_i \) and \( C_{\text{m(outter)}} \) is still \( A \). Therefore, equations for the CO₂ gradients between the compartments and involved resistance components are as follows:

\[
C_c = C_{\text{m(outter)}} - [V_c - \lambda(F + R_d)]r_{\text{ch}}
\]

(7)

\[
C_{\text{m(outter)}} = C_i - A r_{\text{wp}}
\]

(8)

Equation (7) without \( R_d \) would be comparable to the third equation in Fig. 4 of von Caemmerer (2013) for modelling the photosynthetic bypass engineered by Kebeish et al. (2007). Combining Eqs. (7) and (8) with \( V_c = A + F + R_d \) gives rise to an equation in analogy to Eq. (2):

\[
C_c = C_i - A(r_{\text{wp}} + r_{\text{ch}}) - (1 - \lambda)(F + R_d)r_{\text{ch}}
\]

(9)

The same logic as for Eqs. (3) and (4) gives

\[
g_{m,\text{app}} = \frac{1}{r_{\text{m,\text{diff}}} \left[ 1 + \omega(1 - \lambda) \frac{F + R_d}{A} \right]}
\]

(10)

Equation (10) suggests that the apparent \( g_m \) as defined by Eq. (1) is still sensitive to \((F+R_d)/\lambda\), although the sensitivity factor changes from \( \omega \) for Case I to \( \omega(1-\lambda) \) now for Case III.

For this case, either refixed or escaped (photo)respired CO₂ molecules have two parts, one part from the inner and the other from outer cytosol, and they experience different resistant components. Assuming for the purpose of simplicity that mitochondria are distributed in such a way that any variation in \( x \) between inner and outer cytosol is negligible, the refixed (photo)respired CO₂ molecules \( R_{\text{refix}} \) can easily be expressed as \( \lambda x(F + R_d)r_{\text{cx}} \), while the escaped (photo)respired CO₂ \( R_{\text{escape}} \) can be expressed as \( \lambda x(F + R_d)/(r_{\text{ch}} + r_{\text{wp}} + r_{\text{sc}}) \). Then, \( f_{\text{refix}} \) can be calculated by

\[
f_{\text{refix}} = \frac{R_{\text{refix}}}{R_{\text{refix}} + R_{\text{escape}}} = \frac{\lambda}{r_{\text{cx}}} + \frac{1-\lambda}{r_{\text{ch}} + \frac{r_{\text{ch}} + r_{\text{cx}}}{r_{\text{ch}} + r_{\text{wp}} + r_{\text{sc}}}} + \frac{1-\lambda}{r_{\text{wp}} + r_{\text{sc}}}
\]

(11)

This expression for \( f_{\text{refix}} \) looks rather unwieldy but it covers Eqs. (5) and (6) for the previous two cases when \( \lambda = 0 \) and 1, respectively.

Case IV

This is the most general case, in which the coverage of chloroplasts is discontinuous and mitochondria locate in both inner and outer layers of cytosol (Fig. 1d). If chloroplast coverage is discontinuous, it is possible that some mitochondria lie exactly in the chloroplast gaps. This situation can be simplified by assigning part of (photo)respired CO₂ in the gaps to be introduced to account for the direct effect of the chloroplast gaps. This expression for \( f_{\text{refix}} \) looks rather unwieldy but it covers Eqs. (5) and (6) for the previous two cases when \( \lambda = 0 \) and 1, respectively.

\[
C_c = C_{\text{m(outter)}} - [V_c - k\lambda(F + R_d)]r_{\text{ch}}
\]

(12)

While Eq. (8) remains unchanged. Then, equations for case IV, equivalent to Eqs. (9–11) for case III, can be easily defined by replacing the places of \( \lambda \) with \( k\lambda \). This also
means that the fraction of outer (photo)respired CO$_2$ now becomes $(1-k\lambda)$.

In fact, the lumped $k\lambda$ can be re-defined as a single factor $\sigma$, which refers to the fraction of (photo)respired CO$_2$ molecules that have to experience reaction–diffusion model. Again if $\sigma$ also refers to the fraction of outer (photo)respired CO$_2$ molecules that have to experience reaction–diffusion model. Then, a more general form of Eq. (3) or Eq. (9) becomes

$$C_c = C_i - Ar_{m,dif} - \omega(1-\sigma)(F + R_d)r_{m,dif}$$

and a more general form of Eqs. (10) and (11) becomes

$$g_{m,app} = \frac{1}{r_{m,dif} \left[ 1 + \omega(1-\sigma)\frac{F + R_d}{A} \right]}$$

$$f_{\text{refix}} = \frac{R_{\text{refix}}}{R_{\text{refix}} + R_{\text{escape}}} = \frac{\frac{\sigma}{r_{cx}} + \frac{1-\sigma}{r_{cx} + r_{sc}}}{\frac{\sigma}{r_{cx} + r_{sc} + r_{cx} + r_{sc}} + \frac{1-\sigma}{r_{cx} + r_{sc}}}$$

As $\sigma$ has a value between 0 and 1, it follows that the factor $k$ varies between 0 and 1/\lambda. This suggests that the lower the $\lambda$ is, the more likely it is that $k>1$. However, the exact value of $k$ and how $k$ modifies $\lambda$ (e.g. via the path between the chloroplasts vs through the chloroplast) are hard to quantify from the simple resistance model. As large gaps between chloroplasts decrease $S_c/S_m$, the ratio of chloroplast surface area to mesophyll surface area exposed to the intercellular air spaces (Sage and Sage 2009; Tholen et al. 2012; Tomas et al. 2013), the value of $k$ must be associated with $S_c/S_m$. However, $k$ may also depend on factors such as the CO$_2$ influx from the intercellular air spaces. These dependences of $k$ on $\lambda$, $S_c/S_m$, and other factors could best be analysed using reaction–diffusion models like the one by Tholen and Zhu (2011).

Two more special cases

Now we consider two more special cases. The first instance is the case in which the coverage of chloroplasts is discontinuous and all mitochondria locate in the inner layer of cytosol (Fig. 1e), and the second is that the coverage of chloroplasts is discontinuous and all mitochondria locate in the outer layer of cytosol (Fig. 1f). The diffused amount of (photo)respired CO$_2$ from the inner to the outer cytosol (for the first instance) or from the outer to the inner cytosol (for the second instance) could be analysed by the use of a reaction–diffusion model. Again if $\sigma$ also refers to the fraction of (photo)respired CO$_2$ molecules that have to experience reaction–diffusion model. Then, a more general form of Eqs. (13–15) also apply to these two special cases.

Results and discussion

Dependence of $A$ and $g_{m,app}$ on $\omega$ and $\sigma$ values

Equations for all illustrations in this section are all given in Appendix A. Figure 2 shows the initial section of simulated $A-C_i$ curves for various combinations of $\omega$ and $\sigma$ values, indicating that a change in $\sigma$ (i.e. the arrangement of chloroplasts and mitochondria in mesophyll cells) had a same magnitude of the effect as a change in $\omega$ (i.e. the physical resistance of chloroplast components relative to the total mesophyll resistance). Increasing $\sigma$ (Fig. 2a) or decreasing $\omega$ (Fig. 2b) increased $A$ for a given $g_{m,dif}$. This is largely caused by varying amounts of refixation of (photo)respired CO$_2$, which become increasingly important with decreasing $C_i$. For example, the estimated $f_{\text{refix}}$ (Eq. 15) was 0.385, 0.333 and 0.285 for the three cases corresponding to solid, long-dashed and short-dashed lines of Fig. 2a, respectively (where $r_{sc}$ was set to have the same value as 1/g$_{m,dif}$ and $r_{cx}$ was calculated as $(C_i + x_2)/x_1$, also see Eq. B2 in Tholen et al. 2012). $f_{\text{refix}}$ can also be calculated for the three cases of Fig. 2b. Such
differences in \( f_{\text{fix}} \) can produce a significant difference in \( A \) (when \( C_i \) is low) and in CO2 compensation point \( \Gamma \) (Fig. 2). Differences in \( \Gamma \) was already shown by von Caemmerer (2013) between two special cases, i.e. Case I (Fig. 1a) versus Case II (Fig. 1b). With increasing \( C_i \), refixation becomes less important, and differences in \( A \) are increasingly negligible (results not shown).

\( g_{\text{m,app}} \), calculated from Eq. (14), decreased with decreasing \( C_i \), although \( g_{\text{m,dir}} \) was fixed as constant (Fig. 3). This variation did not occur only if \( \sigma = 1 \) (the horizontal line in Fig. 3a) or \( \omega = 0 \) (the horizontal line in Fig. 3b), suggesting the classical \( g_{\text{m}} \) model can arise either from \( \sigma = 1 \) (all mitochondria stay closely behind chloroplasts as if carboxylation and (photo)respiratory CO2 release occur in one compartment) or from \( \omega = 0 \) (the chloroplast component in total mesophyll resistance is negligible). The short-dashed line in Fig. 3a represents the case when \( \sigma = 0 \), corresponding to the original model of Tholen et al. (2012) that applies to the case where all mitochondria locate in the outer cytosol. A change in organelle arrangement within a mesophyll cell resulted in a change in sensitivity of \( g_{\text{m,app}} \) to \( C_i \) as shown by the long-dashed line in Fig. 3a, which lies between the horizontal line and the short-dashed line.

The model of Tholen et al. (2012) as special case of the generalised model

It is evident from our analysis above that the original model of Tholen et al. (2012) applies to a special case of our generalised model, where (photo)respired CO2 is entirely released in the outer cytosol between the plasmalemma and the chloroplast layer. However, this case can hardly be observed in real leaves, where mitochondria occur mostly in the cell interior, closely behind chloroplasts (Sage and Sage 2009; Hatakeyama and Ueno 2016).

In our model, as stated earlier for the purpose of retaining model simplicity, a large part of \( r_{\text{cytosol}} \) is lumped into \( r_{\text{wp}} \), and the remaining part is lumped into \( r_{\text{ch}} \). For their model, Tholen et al. (2012) assumed that cytosolic resistance is negligible. Although this assumption was made, as described by Tholen et al. (2012), only for the purpose of simplicity, it has implications. If \( r_{\text{cytosol}} \) is so small that it can be neglected, then CO2 diffusion is so fast that the CO2 concentration anywhere in the cytosol should be the same independent of where the mitochondria are located, provided the cytosol is continuous (for example, allowed by an \( S/S_m \) lower than 1). Then the position of the mitochondria does not have any effect on \( f_{\text{fix}} \). Practically, the four cases for scenarios (a), (d), (e) and (f) in Fig. 1 would all be equivalent to the original Tholen et al. model (\( \sigma = 0 \)). This is because \( \lambda = 0 \) in the case of Fig. 1a, or \( k = 0 \) in cases of Fig. 1d,e, or both \( \lambda \) and \( k = 0 \) in the case of Fig. 1f. In this context, the original model of Tholen et al. (2012) would become an alternative special case of our model, that is, assuming that CO2 in the cytosol is completely mixed. If \( r_{\text{cytosol}} \) is indeed negligible, then cases in Fig. 1d,e,f are no longer needed for developing the generalised model.

Can parameters \( \omega \) and \( \sigma \) in the generalised model be measured?

In real cells, \( r_{\text{cytosol}} \) may be very high (Peguero-Pina et al. 2012; Berghuijs et al. 2015) and therefore cannot be neglected. Then, \( r_{\text{cytosol}} \) should appear in the model, making it dependent on the detailed morphology of the cell and location of mitochondria and chloroplasts, and this would require the use of a reaction–diffusion model. Within the resistance model framework, Tholen et al. (2012, in their Appendix C) and Tomas et al. (2013) analysed the possible effects of \( r_{\text{cytosol}} \) in relation to \( S/S_m \) on \( g_{\text{m}} \). In our generalised model, any significant \( r_{\text{cytosol}} \) value would mainly be lumped into parameter \( \omega \), while parameter \( \sigma \) encompasses any combination of chloroplast–mitochondria arrangement and \( S/S_m \). This means that parameters \( \omega \) and \( \sigma \) in our model can be experimentally measured, at least approximately.

Individual physical resistance components \( r_{\text{wall}}, r_{\text{plasmalemma}}, r_{\text{cytosol}}, r_{\text{envelope}} \) and \( r_{\text{stroma}} \) have been calculated.
from microscopic measurements on leaf anatomy (Peguero-Pina et al. 2012; Tosens et al. 2012a, b; Tomas et al. 2013; Berghuijs et al. 2015), despite the uncertainties in the value of gas diffusion coefficients used for the calculation. These measurements can provide basic data to derive $\omega$. For example, Berghuijs et al. (2015) showed that for tomato leaves, $\omega$ was about 0.65. Parameter $\sigma$ depends on both $S_j/S_m$ and the relative position of mitochondria to chloroplasts. In most annuals especially when leaves are young, $S_j/S_m$ is high (close to 1; Sage and Sage 2009; Terashima et al. 2011; Berghuijs et al. 2015), $\sigma$ should be predominantly determined by the relative position of mitochondria (i.e. $\sigma \approx \lambda$, the proportion of mitochondria lying in the inner cytosol). Hatakeyama and Ueno (2016) showed that for 10 C3 grasses most mitochondria are located on the vacuole side of chloroplast in mesophyll cells and their data suggested that $\lambda$ varies from 0.61 to 0.92 among these species, with an average of 0.8. Assuming these values are representative for young leaves of annual C3 species, then the collective value of $\omega(1-\sigma)$ in our model is about 0.13, a value closer to what the classical model represents (0) than the model of Tholen et al. (2012) does.

However, in woody species (e.g. Tosens et al. 2012a) or in old leaves of annual species (Busch et al. 2013), $S_j/S_m$ can be as low as 0.4. The chloroplast coverage is low, especially when combined with a low $r_{cytosol}$ (Tosens et al. 2012b), $\omega(1-\sigma)$ must be close to what the model of Tholen et al. (2012) represents. However, parameter $\sigma$ is hard to determine directly for this case as its component $k$ may be interdependent on its other component $\lambda$. In such a case, $\sigma$ may only be a “fudge factor” that lumps $\lambda$ and $S_j/S_m$ in a complicated manner, which may be elucidated by using reaction–diffusion models. Alternatively, the collective value of $\omega(1-\sigma)$ could be estimated (together with $g_{mdiff}$) by fitting Eq. (18) in AppendixA to gas exchange data at various $O_2$ levels, and then $\sigma$ could be calculated if anatomical measurements reliably estimate $\omega$, but this approach needs to be tested.

Can two-resistance models exclusively explain observed variable $g_{m,app}$?

Compared with the classical model that uses a single resistance parameter, both Tholen et al. (2012) model and our generalised model partition mesophyll resistance into two components. In Fig. 3, we have shown the dependence in the sensitivity of $g_{m,app}$ on both $\omega$ and $\sigma$ values. Our illustration for the general case (Fig. 3) still agrees qualitatively with Tholen et al. (2012), who, based on their two-resistance model, clearly showed the sensitivity of $g_{m,app}$ to the ratio of $(F+R_d)$ to $A$. They suggested that this sensitivity could explain the commonly observed decrease of $g_{m,app}$ with decreasing $C_i$ with a low $C_i$ range (e.g. Flexas et al. 2007; Yin et al. 2009). Since the $(F+R_d)/A$ ratio also varies with irradiance and temperature, one might wonder if their model explains any variation of $g_{m,app}$ with these factors. However, their framework, as stated by Tholen et al. (2012), cannot explain the commonly observed responses of $g_{m,app}$ to a change in $C_i$ within the higher $C_i$ range (e.g. Flexas et al. 2007) or in $I_{nc}$ (e.g. Yin et al. 2009; Douthe et al. 2012) or in temperature (e.g. Bernacchi et al. 2002; Yamori et al. 2006; Evans and von Caemmerer 2013; von Caemmerer and Evans 2015). In fact, Gu and Sun (2014) showed that even the response of $g_{m,app}$ to a change in $C_i$ (including the low $C_i$ range) could be simply due to possible errors in measuring $A$, $J$ and $C_i$, or to possible errors in estimating $R_d$ and $S_{clp}$, or could be due to the use of the NADPH-limited form of the FvCB model by the variable $J$ method when the true form is the ATP-limited equation.

In the absence of any measurement errors, can the sensitivity of $g_{m,app}$ to the $(F+R_d)/A$ ratio be considered as the only explanation of $g_{m,app}$ sensitivity to $C_i$ within the low $C_i$ range? Here we want to (re-)state that the decline of $g_{m,app}$ with decreasing $C_i$ below a certain level, as assessed by the variable $J$ method of Harley et al. (1992), can also be accounted for by the fact that the method is based only on the $A_j$ equation of the FvCB model (Yin et al. 2009). When $C_i$ is decreasing towards the $CO_2$ compensation point, $A$ is increasingly limited by $A_c$ rather than by $A_j$. Under such conditions, part of the $e^-$ fluxes may become alternative $e^-$ transport not used in support of $CO_2$ fixation and photorespiration. So, use of the variable $J$ method, which is based on Eq. (1) and the $A_j$ equation of the FvCB model, may lead to underestimation of $g_{m,app}$. This is shown in Fig. 4a, in which for a given fixed $g_{mdiff}$ (0.4 mol m$^{-2}$ s$^{-1}$ bar$^{-1}$), $g_{m,app}$ decreased with decreasing $C_i$ as expected from Eq. (14); but $g_{m,app}$ decreased more sharply if $A_j$ part of the model was applied to the low $C_i$ range which was actually $A_c$-limited. One would expect that $g_{mdiff}$ calculated back from using the simulated $A$ should be equal to the pre-fixed $g_{mdiff}$ (0.4 mol m$^{-2}$ s$^{-1}$ bar$^{-1}$). However, the calculated $g_{mdiff}$ if using only the $A_j$ part of the model as in the variable $J$ method gave artifically lower $g_{mdiff}$ values for the $A_c$-limited part (Fig. 4b). In this calculation shown in Fig. 4, $J$ was assumed to be a constant across $C_i$ levels, whereas actual fluorescence measured $J$ may decline slightly with lowering $C_i$ in the low $C_i$ range (e.g. Cheng et al. 2001), probably reflecting a feedback effect of Rubisco limitation on electron transport. However, the feedback is not so complete that the variable $J$ method, if applied to the low $C_i$ range, always tends to underestimate the actual mesophyll conductance. For these reasons, Yin and Struik (2009) stated that the proposal of the variable $J$ method to be applied to the lower range of $A-C_i$ curve where $J$ is variable (Harley et al. 1992) is inappropriate. A good correlation between values of $g_m$ estimated from the
The model of Tholen et al. (2012) considers the partitioning of intrinsic diffusion resistance but with little explicit consideration of intracellular organelle arrangements, especially not intracellular position of mitochondria and chloroplasts. We introduced the parameter $\sigma$ for defining the fraction of (photo) respiring $\text{CO}_2$ molecules that have to experience all $r_{\text{cp}}$ in addition to $r_{\text{wp}}$ and $r_{sc}$, if these $\text{CO}_2$ molecules are to escape from being refixed. $\sigma$ has a value between 0 and 1, depending on the arrangement of organelles within mesophyll cells, i.e., (1) the relative position of chloroplasts and mitochondria and (2) the size of the gaps between chloroplasts. This provides a simple generalised form of the Tholen et al. model in a way that the latter model, Eq. (4), is still valid for all organelle arrangement scenarios if $\omega$ is replaced by $\omega(1-\sigma)$. The two parameters of our generalised model can be amenable to experimental estimation for young leaves of annual species where chloroplast coverage continues along the mesophyll cell periphery ($S_c/S_m = 1$). The model of Tholen et al. (2012) is the special case of our model when $\sigma = 0$, which arises either from $\lambda=0$ (no mitochondria in the inner cytosol) combined with $S_c/S_m = 1$ or from a negligible $r_{\text{cytosol}}$ combined with $S_c/S_m < 1$. Our model shows that the sensitivity of $g_{\text{m,app}}$ to $(F+R_d)/A$ lies somewhere in between the classical method ($\omega = 0$ or $\sigma = 1$, non-sensitive) and the Tholen et al. model ($\sigma = 0$, highly sensitive). Therefore, Tholen et al. (2012) may have overstated that the sensitivity of $g_{\text{m,app}}$ on $(F+R_d)/A$ in their model explains the commonly reported decline of $g_{\text{m,app}}$ with decreasing $C_i$ in the low $C_i$ range. In fact, the decline, if not due to measurement or parameter–estimation errors, could also be attributed, at least partly, to the variable J method that is wrongly applied to low $C_i$ range where $\text{CO}_2$ assimilation is actually limited by Rubisco activity.

**Conclusions**

The model of Tholen et al. (2012) considers the partitioning of intrinsic diffusion resistance but with little explicit consideration of intracellular organelle arrangements, especially not intracellular position of mitochondria and chloroplasts. We introduced the parameter $\sigma$ for defining the fraction of (photo) respiring $\text{CO}_2$ molecules that have to experience all $r_{\text{cp}}$ in addition to $r_{\text{wp}}$ and $r_{sc}$, if these $\text{CO}_2$ molecules are to escape from being refixed. $\sigma$ has a value between 0 and 1, depending on the arrangement of organelles within mesophyll cells, i.e., (1) the relative position of chloroplasts and mitochondria and (2) the size of the gaps between chloroplasts. This provides a simple generalised form of the Tholen et al. model in a way that the latter model, Eq. (4), is still valid for all organelle arrangement scenarios if $\omega$ is replaced by $\omega(1-\sigma)$. The two parameters of our generalised model can be amenable to experimental estimation for young leaves of annual species where chloroplast coverage continues along the mesophyll cell periphery ($S_c/S_m = 1$). The model of Tholen et al. (2012) is the special case of our model when $\sigma = 0$, which arises either from $\lambda=0$ (no mitochondria in the inner cytosol) combined with $S_c/S_m = 1$ or from a negligible $r_{\text{cytosol}}$ combined with $S_c/S_m < 1$. Our model shows that the sensitivity of $g_{\text{m,app}}$ to $(F+R_d)/A$ lies somewhere in between the classical method ($\omega = 0$ or $\sigma = 1$, non-sensitive) and the Tholen et al. model ($\sigma = 0$, highly sensitive). Therefore, Tholen et al. (2012) may have overstated that the sensitivity of $g_{\text{m,app}}$ on $(F+R_d)/A$ in their model explains the commonly reported decline of $g_{\text{m,app}}$ with decreasing $C_i$ in the low $C_i$ range. In fact, the decline, if not due to measurement or parameter–estimation errors, could also be attributed, at least partly, to the variable J method that is wrongly applied to low $C_i$ range where $\text{CO}_2$ assimilation is actually limited by Rubisco activity.

**Acknowledgements** This research is financed in part by the Bio-Solar Cells open innovation consortium, supported by the Dutch Ministry of Economic Affairs, Agriculture and Innovation. We thank the reviewers and the coordinating editor (Dr. A.B. Cousins) for their very useful comments on the previous versions of the manuscript.

**Open Access** This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

**Appendix A**

**Equations used for simulation in this paper**

The FvCB model calculates carboxylation rate ($V_c$) as $C_i x_i/(C_c + x_i)$, and photorespiratory $\text{CO}_2$ release $F$ as $(F/C_c)V_c$; so, $F$ can be expressed as follows:

$$F = \frac{\Gamma_x x_1}{C_c + x_2}$$

where $\Gamma_x$ is the $\text{CO}_2$ compensation point in the absence of $R_d$ ($\Gamma_x$ depends on $O_2$ partial pressure $O$ and Rubisco specificity $S_{\text{cpo}}$ as $0.5O/S_{\text{cpo}}$), $x_1 = J/4$ and $x_2 = 2\Gamma_x$ for the $A_c$-limited conditions and $x_1 = V_{\text{max}}$ (maximum carboxylation activity of Rubisco) and $x_2 = K_{mC}(1+O/K_{mO})$ for the $A_c$-limited conditions ($K_{mC}$ and $K_{mO}$ are Michaelis–Menten constants of Rubisco for $\text{CO}_2$ and $O_2$, respectively).

Also $C_c$ can be solved from the FvCB model as

$$C_c = \frac{\Gamma_x x_1 + x_2(A + R_d)}{x_1 - (A + R_d)}$$

Combining Eqs. (16–17) with Eq. (13) and solving the combined equations for $A$, step by step, result in a quadratic solution:
\[ A = \frac{(-b - \sqrt{b^2 - 4ac})}{2a} \]  
(18)

where \( a = x_2 + \Gamma_s[1 - \omega(1 - \sigma)] \)

\[
b = \omega(1 - \sigma)\left( R_d x_2 + \Gamma_s x_1 \right) - \left\{ x_2 + \Gamma_s[1 - \omega(1 - \sigma)] \right\} \\
\left( x_1 - R_d \right) - g_{m,\text{diff}} \left( C_1 + x_2 \right) \left( x_2 + \Gamma_s \right)
\]

\[
c = -\omega(1 - \sigma) \left( R_d x_2 + \Gamma_s x_1 \right) \left( x_1 - R_d \right) + g_{m,\text{diff}} \left( x_2 + \Gamma_s \right) \left[ x_1 \left( C_1 - \Gamma_s \right) - R_d \left( C_1 + x_2 \right) \right]
\]

Equation (18) was used to generate \( A \)–\( C \) response curves as shown in Fig. 2 for a given set of input parameter values. When \( A \) is solved, \( C \) can be solved from Eq. (18), and the solved \( C \) was used as input to Eq. (1) to calculate \( g_{m,\text{app.}} \). Alternatively, \( g_{m,\text{app.}} \) can be calculated directly from Eq. (14) with \( F \) calculated from eqn (16). This gave \( g_{m,\text{app.}} \) as shown in Figs. 3 and 4a.

When \( A \), \( C \), and \( F \) are all known, one can calculate back for \( g_{m,\text{diff.}} \):

\[
g_{m,\text{diff.}} = \frac{A + \omega(1 - \sigma)(F + R_d)}{C_1 - C_c} \]  
(19)

When \( C \), and \( F \) are both based on the \( A \)-limited part of the FvCB model, then eqn (19) gives an equation that calculates \( g_{m,\text{diff.}} \) like the variable \( J \) method of Harley et al. (1992) for calculating \( g_{m,\text{app.}} \). This was used to generate Fig. 4b.

References

Berghuijs HNC, Yin X, Ho QT, van der Putten PEL, Verboven P, Retta MA, Nicolai BM, Struik PC (2015) Modeling the relationship between \( CO_2 \) assimilation and leaf anatomical properties in tomato leaves. Plant Sci 238:297–311

Berghuijs HNC, Yin X, Ho QT, Driever SM, Retta MA, Nicolai BM, Struik PC (2016) Mesophyll conductance and reaction-diffusion models for \( CO_2 \) transport in \( C_3 \) leaves; needs, opportunities and challenges. Plant Sci 252:62–75

Benzacchi CJ, Portis AR, Nakano H, von Caemmerer S, Long SP (2002) Temperature response of mesophyll conductance. Implication for the determination of Rubisco enzyme kinetics and for limitations to photosynthesis in vivo. Plant Physiol 130:1992–1998

Busch FA, Sage TL, Cousins AB, Sage RF (2013) \( C_3 \) plants enhance rates of photosynthesis by reassimilating photospired and respirated \( CO_2 \). Plant Cell Environ 36:200–212

Cheng L, Fuchigami LH, Breen PJ (2001) The relationship between photosystem II efficiency and quantum yield for \( CO_2 \) assimilation is not affected by nitrogen content in apple leaves. J Exp Bot 52:1865–1872

Douthe C, Dreyer E, Brendel O, Warren CR (2012) Is mesophyll conductance to \( CO_2 \) in leaves of three \( Eucalyptus \) species sensitive to short-term changes of irradiance under ambient as well as low \( O_2 \)? Funct Plant Biol 39:435–448

Evans JR, von Caemmerer S (2013) Temperature response of carbon isotope discrimination and mesophyll conductance in tobacco. Plant Cell Environ 36:745–756

Evans JR, Sharkey TD, Berry JA, Farquhar GD (1986) Carbon isotope discrimination measured concurrently with gas exchange to investigate \( CO_2 \) diffusion in leaves of higher plants. Aust J Plant Physiol 13:281–292

Evans JR, Kaldenhoff R, Genty B, Terashima I (2009) Resistances along the \( CO_2 \) diffusion pathway inside leaves. J Exp Bot 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-Elsepe A, Galmes J, Kaldenhoff R, Medrano H, Ribas-Carbó M (2007) Rapid variation of mesophyll conductance in response to changes in \( CO_2 \) concentration around leaves. Plant Cell Environ 30:1284–1298

Guz L, Sun Y (2014) Artefactual responses of mesophyll conductance to \( CO_2 \) and irradiance estimated with the variable \( J \) and online isotope discrimination methods. Plant Cell Environ 37:1231–1249

Hatakeyama Y, Ueno O (2016) Intracellular position of mitochondria and chloroplasts in bundle sheath and mesophyll cells of \( C_3 \) grasses in relation to photosynthetic \( CO_2 \) flux by analysis of the response of photosynthesis to \( CO_2 \). Plant Physiol 98:1429–1436

Kebeish R, Niessen M, Thirshavkeni K, Bari R, Hirsch H-J, Rosenkranz R, Stäbler N, Schönfeld B, Kreuzaler F, Peterhansel C (2007) Chloroplastic photosynthetic bypass increases photosynthesis and biomass production in \( Arabidopsis \) thaliana. Nature Biotechnol 25:593–599

Peguero-Pina JJ, Flexas J, Galme J, Niinemets Ü, Sancho-Knapik D, Barredo G, Villarroya D, Gil-Pelegrin E (2012) Leaf anatomical properties in relation to differences in mesophyll conductance to \( CO_2 \) and photosynthesis in two related Mediterranean \( Abies \) species. Plant Cell Environ 35:2121–2129

Pons TL, Flexas J von Caemmerer S, Evans JR, Genty B, Ribas-Carbó M, Brugnoli E (2009) Estimating mesophyll conductance to \( CO_2 \): methodology, potential errors, and recommendations. J Exp Bot 60:2217–2234

Sage TL, Sage RF (2009) The functional anatomy of rice leaves: implications for refixation of photosynthetic \( CO_2 \) and effects to engineer \( C_4 \) photosynthesis into rice. Plant Cell Physiol 50:756–772

Takao Y von Caemmerer S, Badger MR, Evans JR (2009) Light and \( CO_2 \) do not affect the mesophyll conductance to \( CO_2 \) diffusion in wheat leaves. J Exp Bot 60:2291–2301

Tcherkez G, Boex-Fontvieille E, Mahe A, Hodges M (2012) Respiratory carbon fluxes in leaves. Curr Opin Plant Biol 15:308–314

Terashima I, Hanba YT, Tholen D, Niinemets Ü (2011) Leaf functional anatomy in relation to photosynthesis. Plant Physiol 155:108–116

Tholen D, Zhu X-G (2011) The mechanistic basis of internal conductance: a theoretical analysis of mesophyll cell photosynthesis and \( CO_2 \) diffusion. Plant Physiol 156:90–105

Tholen D, Ethier G, Genty B, Pepin S, Zhu X-G (2012) Variable mesophyll conductance revisited: theoretical background and experimental implications. Plant Cell Environ 35:2087–2103

Tomás M, Flexas J, Copolovici L, Galmes J, Hallik L, Medrano H, Ribas-Carbó M, Tosens T, Vislapi V, Niinemets Ü (2013) Importance of leaf anatomy in determining mesophyll diffusion conductance to \( CO_2 \) across species: quantitative limitations and scaling up by models. J Exp Bot 64:2269–2281
Tosens T, Niinemets Ü, Vislap V, Eichelmann H, Castro Diez P (2012a) Developmental changes in mesophyll diffusion conductance and photosynthetic capacity under different light and water availabilities in *Populus tremula*: how structure constrains function. Plant Cell Environ 35:839–856

Tosens T, Niinemets Ü, Westoby M, Wright IJ (2012b) Anatomical basis of variation in mesophyll resistance in eastern Australian sclerophylls: news of a long and winding path. J Exp Bot 63:5105–5119

von Caemmerer S (2013) Steady-state models of photosynthesis. Plant Cell Environ 36:1617–1630

von Caemmerer S, Evans JR (1991) Determination of the average partial pressure of CO₂ in chloroplasts from leaves of several C₃ plants. Aust J Plant Physiol 18:287–305

von Caemmerer S, Evans JR (2015) Temperature responses of mesophyll conductance differ greatly between species. Plant Cell Environ 38:629–637

von Caemmerer S, Evans JR, Hudson GS, Andrews TJ (1994) The kinetics of ribulose-1,5-bisphosphate carboxylase/oxygenase in vivo inferred from measurements of photosynthesis in leaves of transgenic tobacco. Planta 195:88–97

Vrábl D, Vašková M, Hronková M, Flexas J, Šantrůček J (2009) Mesophyll conductance to CO₂ transport estimated by two independent methods: effect of variable CO₂ concentration and abscisic acid. J Exp Bot 60:2315–2323

Yamori W, Noguchi K, Hanba YT, Terashima I (2006) Effects of internal conductance on the temperature dependence of the photosynthetic rate in spinach leaves from contrasting growth temperatures. Plant Cell Physiol 47:1069–1080

Yin X, Struik PC (2009) Theoretical reconsiderations when estimating the mesophyll conductance to CO₂ diffusion in leaves of C₃ plants by analysis of combined gas exchange and chlorophyll fluorescence measurements. Plant Cell Environ 32:1513–1524 with corrigendum in 33:1595

Yin X, Struik PC, Romero P, Harbinson J, Evers JB, van der Putten PEL, Vos J (2009) Using combined measurements of gas exchange and chlorophyll fluorescence to estimate parameters of a biochemical C₃ photosynthesis model: a critical appraisal and a new integrated approach applied to leaves in a wheat (*Triticum aestivum*) canopy. Plant Cell Environ 32:448–464