

Using a reaction-diffusion model to estimate day respiration and reassimilation of (photo)respired CO₂ in leaves

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Summary

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Received: 15 October 2018

Accepted: 5 April 2019

New Phytologist (2019) 223: 619–631

doi: 10.1111/nph.15857

Key words: C₃ photosynthesis, mesophyll conductance, photorespiration, reaction-diffusion model, reassimilation, respiration.

- Methods using gas exchange measurements to estimate respiration in the light (day respiration R_d) make implicit assumptions about reassimilation of (photo)respired CO₂; however, this reassimilation depends on the positions of mitochondria.
- We used a reaction-diffusion model without making these assumptions to analyse datasets on gas exchange, chlorophyll fluorescence and anatomy for tomato leaves. We investigated how R_d values obtained by the Kok and the Yin methods are affected by these assumptions and how those by the Laisk method are affected by the positions of mitochondria.
- The Kok method always underestimated R_d . Estimates of R_d by the Yin method and by the reaction-diffusion model agreed only for nonphotorespiratory conditions. Both the Yin and Kok methods ignore reassimilation of (photo)respired CO₂, and thus underestimated R_d for photorespiratory conditions, but this was less so in the Yin than in the Kok method. Estimates by the Laisk method were affected by assumed positions of mitochondria. It did not work if mitochondria were in the cytosol between the plasmamembrane and the chloroplast envelope. However, mitochondria were found to be most likely between the tonoplast and chloroplasts.
- Our reaction-diffusion model effectively estimates R_d , enlightens the dependence of R_d estimates on reassimilation and clarifies (dis)advantages of existing methods.

Introduction

Quantifying respiration is important for accurately predicting net ecosystem productivity, as respiratory losses can account for ≤ 40% of gross primary production (Gifford, 2003). At the leaf level, respiration in the light, also called ‘day respiration’ (R_d), is an important term in the model of Farquhar, von Caemmerer & Berry (‘FvCB model’; Farquhar *et al.*, 1980), which is used widely as the basic model component for predicting ecosystem productivity. Unlike leaf respiration in the dark (R_{dk}), day respiration occurs simultaneously with photosynthetic CO₂-assimilation and is difficult to determine by gas-exchange measuring systems. Uncertainties have arisen over years with regard to, for example, whether R_d differs from R_{dk} and whether R_d varies with different conditions (Griffin & Turnbull, 2013). Efforts continued recently in updating the biochemical processes underlying R_d (Tcherkez *et al.*, 2017a,b) and in how to better measure or quantify this parameter (Buckley *et al.*, 2017; Farquhar & Busch, 2017; Tcherkez *et al.*, 2017a,b; Gong *et al.*, 2018; Way *et al.*, 2019).

Techniques have been developed to measure R_d directly (Loreto *et al.*, 1999, 2001; Pärnik & Keerberg, 2007; Gong *et al.*, 2015; Tcherkez *et al.*, 2017a; Gauthier *et al.*, 2018), but these techniques usually require access to sophisticated and expensive isotope discrimination measuring devices and are often unavailable. Methods exist to indirectly estimate R_d in C₃ leaves from conventional gas-exchange measurements (Kok, 1948; Laisk, 1977; Brooks & Farquhar, 1985), sometimes combined with chlorophyll fluorescence measurements (Yin *et al.*, 2009). The Laisk method (Laisk, 1977; Brooks & Farquhar, 1985) has become the most common one. It explores the linear part of several $A_N - C_i$ curves at low C_i concentrations (C_i is the intercellular CO₂ partial pressure), measured at difference irradiances. The negative net CO₂ assimilation rate A_N at the point at which the linear $A_N - C_i$ curves intersect is the estimated R_d . C_i at the intersection point (C_i^*) is often used as the CO₂ compensation point Γ^* , at which the amount of CO₂ produced by photorespiration equals the amount of CO₂ consumed by ribulose biphosphate (RuBP) carboxylation. The theoretical basis of the Laisk method is the FvCB model:

$$A = \frac{(C_c - \Gamma^*)X_1}{C_c + X_2} - R_d, \quad \text{Eqn 1}$$

where C_c is the CO₂ partial pressure at the carboxylating sites of Rubisco. The terms X_1 and X_2 depend on whether carboxylation is limited by Rubisco activity, electron transport (Farquhar *et al.*, 1980), or triose phosphate utilization (Sharkey, 1985). If it is limited by electron transport, X_1 is a function of incident irradiance (I_{inc}) and Eqn 1 can generate the Laisk plot. However, theoretically, the Laisk method works only if $C_c = C_i$. This was a common assumption at the time when the method was proposed as mesophyll resistance (r_m) was then believed to be negligible compared with stomatal resistance to CO₂ transfer (r_s). Nowadays, r_m (and its inverse, mesophyll conductance g_m) is proven to be relevant under a wide range of conditions and across different species (Evans *et al.*, 1986; Flexas *et al.*, 2008; Niinemets *et al.*, 2009). Consequently, there is a large CO₂ gradient between the intercellular spaces and the chloroplasts (Von Caemmerer & Evans, 1991; Von Caemmerer *et al.*, 1994):

$$C_c = C_i - \frac{A_N}{g_m}. \quad \text{Eqn 2}$$

Combining the nonlinear Eqn 1 and the linear Eqn 2 results in a solution in which $A_N - C_i$ curves at different irradiances will not necessarily intersect at the same value of the A_N axis. Tholen *et al.* (2012) pointed out that the method of calculating g_m according to Eqn 2 implicitly assumes that CO₂ produced by respiration and photorespiration (which will be called '(photo)respired CO₂' hereafter), and the CO₂ molecules from intercellular air-spaces experience the same mesophyll resistance. However, (photo)respired CO₂, if being reassimilated, probably experiences the chloroplast resistance component (r_{ch}) only. By contrast, the CO₂ molecules from intercellular air-spaces experience cell wall and plasma-membrane resistance (r_{wp}) as well as r_{ch} (the sum of r_{wp} and r_{ch} makes the total r_m). Therefore, Tholen *et al.* (2012) concluded that g_m as defined by Eqn 2, is an apparent parameter. They demonstrated that if this scheme for r_m resistance components is considered, there is no guarantee that $A_N - C_i$ curves at different I_{inc} will intersect at the same C_i and at the same A_N . This implies that R_d estimated by the Laisk method may depend on the resistance scheme, which, in turn, depends on the cellular position of (photo)respired CO₂ release (see later in this Introduction). Moreover, as pointed out by Yin *et al.* (2011), the Laisk method has the practical problem that all measurements are at lower than ambient-air CO₂ concentrations (C_a), requiring the correction of gas exchange data for CO₂ leakage (Flexas *et al.*, 2007).

An alternative method to estimate R_d is the Kok method (Kok, 1948). This method exploits the fact that the response of A_N to irradiance is approximately linear at low irradiances. R_d is calculated as the intercept of this linear relationship. However, at irradiances close to the light compensation point or lower, this slope may become steeper (Kok, 1948; Farquhar & Busch, 2017; Tcherkez *et al.*, 2017a). In order to avoid this so-called Kok

effect, irradiances under which this method is applied should be above this breakpoint (Brooks & Farquhar, 1985). If evaluated from the electron-transport limited form of Eqn 1, the Kok method actually assumes that the quantum yield of Photosystem II electron transport (Φ_2) is constant over the same range of irradiances. However, Φ_2 has been observed to decline with increasing irradiances (Genty & Harbinson, 1996) even under low-irradiance conditions (Yin *et al.*, 2011, 2014). To account for this decline, Yin *et al.* (2009) proposed a method which also exploits the $A_N - I_{\text{inc}}$ curve at low irradiance, but combines it with simultaneously measured chlorophyll fluorescence to assess Φ_2 , and R_d is estimated as the intercept of the linear regression of A_N vs $\Phi_2 I_{\text{inc}}/4$. To distinguish it from the Kok method, it has been called the Yin method (Tcherkez *et al.*, 2017b). Theoretically, both Kok and Yin methods work only for nonphotorespiratory conditions (Yin *et al.*, 2011), or for photorespiratory conditions if C_c is made to be constant across irradiance intensities. They are sometimes also used for photorespiratory conditions where C_c varies, because usually the plot of measured A_N vs I_{inc} or vs $\Phi_2 I_{\text{inc}}/4$ seems linear.

However, when the Yin or Kok methods are applied directly to photorespiratory conditions where only C_a is controlled, the problem associated with the variation of C_i or C_c with I_{inc} may become relevant. This is because linear regression of A_N against I_{inc} (Kok method) or against $\Phi_2 I_{\text{inc}}/4$ (Yin method) implicitly assumes that C_c does not vary with I_{inc} within the data range used. The present understanding of stomatal conductance (g_s) and g_m shows that g_s and g_m can have very low values at low I_{inc} ; when combined with the FvCB model, the low g_s and g_m values predict that C_c decreases sharply with increasing I_{inc} within the low I_{inc} range (Farquhar & Busch, 2017). It is known also that a combined FvCB and conductance model can implicitly account for reassimilation of (photo)respired CO₂ (Von Caemmerer, 2013). Therefore, both the Kok and Yin methods, when applied to photorespiratory conditions, may implicitly assume that there is no reassimilation of (photo)respired CO₂ as they assume that C_c remains constant under a range of low light intensities. In fact, there is both experimental (Loreto *et al.*, 1999; Haupt-Herting *et al.*, 2001; Pärnik & Keerberg, 2007; Busch *et al.*, 2013) and theoretical (Tholen *et al.*, 2012; Ho *et al.*, 2016; Berghuijs *et al.*, 2017; Yin & Struik, 2017) evidence that a substantial fraction of the (photo)respired CO₂ is used for RuBP carboxylation in the chloroplasts before it can escape to the atmosphere. If recycling of CO₂ is not accounted for to determine R_d , the true R_d is possibly underestimated (Loreto *et al.*, 1999; Gong *et al.*, 2018). Instead of using simple linear regressions, using the combined FvCB and g_m model to fit types of experimental data (that each method relies on) under photorespiratory conditions would give an estimation of R_d while simultaneously considering reassimilation. However, r_m , let alone its components, r_{wp} and r_{ch} , is not known beforehand. In fact, an estimation of r_m or g_m would require an estimate of R_d beforehand (Harley *et al.*, 1992; Yin & Struik, 2009).

According to the resistance model of Tholen *et al.* (2012), the fraction of reassimilation of (photo)respired CO₂ depends on the

relative magnitude of individual resistance components along the path from leaf surface to Rubisco carboxylation sites. These resistances include r_s , r_{wp} , r_{ch} and r_{cx} , where r_{cx} is carboxylation resistance that can be expressed from Eqn 1 as $(C_c + X_2)/X_1$ (Tholen *et al.*, 2012). Because at least r_s and r_{cx} are known to depend on CO₂ concentration and irradiance, reassimilation also may be affected by environmental variables. A similar statement can be made for any impact of physiological parameters on reassimilation. Yin & Struik (2017) extended the model of Tholen *et al.* (2012) and pointed out that the fraction of reassimilation of (photo)respired CO₂ not only depends on the relative magnitude of the resistance components but also on intracellular arrangements of chloroplasts and mitochondria. They highlighted that the impact of such intracellular arrangements of organelles is hard to be dealt with by resistance models when the chloroplast coverage of mesophyll areas is low. The resistance model of Tholen *et al.* (2012) assumes either that there is no CO₂ gradient in the cytosol (Tholen *et al.*, 2014) or that the mitochondria are located in a cytosol layer between the cell wall and the chloroplasts (Berghuijs *et al.*, 2015, 2016). In reality, the mitochondria are mostly located between the chloroplasts and the tonoplasts (Hatakeyama & Ueno, 2016), intimately associated with chloroplasts (Sage & Sage, 2009). Previous studies (Berghuijs *et al.*, 2017; Xiao & Zhu, 2017; Yin & Struik, 2017) showed that the modelled position of mitochondria relative to the chloroplasts can substantially affect A_N , reassimilation of photorespired CO₂ and g_m .

The CO₂ diffusion pathway between the intercellular airspaces and the chloroplasts is rather complex. Various methods to estimate R_d and other physiological parameters of the FvCB model make simplifying assumptions about this pathway. Such simplification results either in not considering the reassimilation of (photo)respired CO₂ at all or in implicit assumptions about the location of (photo)respired CO₂ release. This problem can be avoided by using reaction-diffusion models that describe the CO₂ diffusion pathway within mesophyll cells in sufficient detail that they do not have to make these implicit assumptions.

Most reaction-diffusion models for photosynthesis (Tholen & Zhu, 2011; Ho *et al.*, 2016; Retta *et al.*, 2017; Xiao & Zhu, 2017) are complex and have lengthy computational times. We previously developed a simple reaction-diffusion model that can be used as an alternative to mesophyll resistance-based models to estimate photosynthetic parameters (Berghuijs *et al.*, 2017). Here we will use this model as a tool to assess whether the Kok method, the Yin method and the Laisk method underestimate R_d due to their assumptions with regard to reassimilation. Given that reassimilation and mesophyll resistance are affected by the assumed position of the mitochondria relative to the chloroplasts (Berghuijs *et al.*, 2017; Xiao & Zhu, 2017), any assumption about the location of mitochondria in mesophyll cells may affect the estimates of the photosynthetic parameters. Therefore, we will also identify the most likely position of the release of (photo)respired CO₂, relative to the position of the chloroplasts.

Materials and Methods

Experimental data

We used published datasets from two experiments (Berghuijs *et al.*, 2015; Ho *et al.*, 2016), both consisting of simultaneous measurements of gas exchange and chlorophyll fluorescence (Table 1). The Berghuijs *et al.* (2015) dataset contains measurements taken from the distal leaflet from 15- and 25-d-old leaves from the tomato (*Solanum lycopersicum*) cultivars Admiro, Doloress and Growdena. The Ho *et al.* (2016) dataset contains measurements taken from leaves of the same cultivars as in the experiment of Berghuijs *et al.* (2015). For each cultivar, two types of leaflets were used for measurements. The first was the distal leaflet of the uppermost fully expanded leaf (the ‘upper leaf’). The second was the most distal leaflet from a leaf four layers below the upper leaf (the ‘lower leaf’). In both experiments, gas exchange measurements were taken under photorespiratory and nonphotorespiratory conditions; but leaf anatomical measurements were taken in the experiment of Berghuijs *et al.* (2015) only.

Mesophyll microstructural model and CO₂ reaction-diffusion model

We used measured anatomical properties to parameterize the model for the leaf microstructure of each leaf type (three cultivars × two leaf ages/positions × two datasets = 12 leaf types in total). We used measurements of t_{wall} (cell wall thickness), t_{cyt} (cytosol thickness), t_{str} (stroma thickness), S_c/S_m (surface area ratio of exposed chloroplasts to exposed mesophyll) and S_m/S (surface area of exposed mesophyll to leaf) to parameterize the model for leaf types from the Berghuijs *et al.* (2015) dataset. As the Ho *et al.* (2016) dataset lacks measurements of these parameters, we assumed for each leaf type in this dataset that $t_{wall} = 120$ nm, $t_{cyt} = 250$ nm, $t_{str} = 2.5$ μm, $S_c/S_m = 0.90$ and $S_m/S = 16$,

Table 1 Overview of the two experimental datasets used in this study.

Source:	Berghuijs <i>et al.</i> (2015)	Ho <i>et al.</i> (2016)
Cultivars:	Admiro, Doloress, Growdena	Admiro, Doloress, Growdena
Leaf types:	15-d-old leaves, 25-d-old leaves	Upper leaves, lower leaves
Anatomical measurements:	Yes	No
A – C _a curves		
PR	$I_{inc} = 1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ O = 21 kPa	$I_{inc} = 1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ O = 21 kPa
NPR	$I_{inc} = 1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ O = 2 kPa	$I_{inc} = 1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ O = 2 kPa
A – I _{inc} curves		
PR	C _a = 40 Pa O = 21 kPa	C _a = 38 Pa O = 21 kPa
NPR	C _a = 100 Pa O = 2 kPa	C _a = 100 Pa O = 2 kPa

PR, photorespiratory conditions; NPR, nonphotorespiratory conditions.

which are within the same range of the values of Berghuijs *et al.* (2015). For all types, we assumed that the Michaelis–Menten coefficient for carboxylation by Rubisco, K_{mC} equals 26.7 Pa (Ho *et al.*, 2016), the Michaelis–Menten coefficient for oxygenation by Rubisco, K_{mO} equals 16.4 kPa (Ho *et al.*, 2016), and Rubisco specificity $S_c/o = 2.6 \text{ kPa Pa}^{-1}$ (Tholen *et al.*, 2012). We ran simulations for three different scenarios. (Photo)respired CO_2 is released either in the inner cytosol (layer between chloroplasts and tonoplast), in cytosol gaps (spaces between two neighbouring chloroplasts) or in the outer cytosol (layer between the chloroplasts and the plasma membrane). Further details on the reconstruction of the leaf geometry, modelling of the scenarios for (photo)respired CO_2 release, the calculation of the fraction of (photo)respired CO_2 that is reassimilated, and the reaction-diffusion model are provided by Berghuijs *et al.* (2017).

Parameterization and validation of the reaction-diffusion model

The linear electron transport rate was calculated as $J = sI_{\text{inc}}\Phi_2$, where I_{inc} is the irradiance for each measurement, Φ_2 is the quantum yield of Photosystem II, and s is a proportionality coefficient, which is calculated as the slope of the linear regression between A_N and $I_{\text{inc}}\Phi_2/4$ under nonphotorespiratory conditions (Yin *et al.*, 2009).

For both datasets, we used only irradiance data $\leq 150 \mu\text{mol m}^{-2} \text{ s}^{-1}$, the range usually used for linear regression by the Kok and Yin. We estimated day respiration rate R_d with the reaction-diffusion model by minimizing the sum of the squared residuals of the measured and the simulated CO_2 assimilation rates. R_d was estimated for each scenario for the location of the release of (photo)respired CO_2 . For this optimization, we used the MATLAB (The Mathworks, Natick, USA) function `lsqnonlin()`. Supporting Information Notes S1 contains documentation of the source code (Notes S2) for this procedure, with a user guide. The values estimated by the reaction-diffusion model were compared with the values of R_d estimated by the Yin and Kok methods using linear regression on the same experimental data.

The rate of triose phosphate utilization T_p was determined as $(A_p + R_d)/3$, where A_p is the mean observed value at the highest C_a of the CO_2 response curve measured under photorespiratory conditions. The reaction-diffusion model also was used to estimate V_{cmax} (maximum rate of Rubisco carboxylation) for each scenario, by minimizing the squared difference between the predicted and the measured A_N , using only data from the CO_2 response curve measured for $O = 21 \text{ kPa}$ and $C_a < 30 \text{ Pa}$. The remaining gas exchange data for each leaf type were used to validate the model.

Simulations of CO_2 assimilation under conditions of the Laisk method

For using the reaction-diffusion model to simulate CO_2 assimilation under the conditions only at which the Laisk method is applied, we adjusted the boundary condition at the interface of

the intercellular airspace, such that C_i was used as input for the model, rather than g_s and C_a . In line with the assumptions of the Laisk method, we assumed that photosynthesis is limited by electron transport. We simulated CO_2 response curves at four intensities of I_{inc} : 150, 100, 50 and $25 \mu\text{mol m}^{-2} \text{ s}^{-1}$, using a prefixed value for Γ^* and the R_d estimates obtained by the reaction-diffusion model as input. From measurements of Φ_2 in the light response curve and the estimate of s , we calculated the rate of linear electron transport for these irradiances, according to $J = s\Phi_2 I_{\text{inc}}$, as 52.0, 36.3, 19.3 and $9.1 \mu\text{mol m}^{-2} \text{ s}^{-1}$, and used these as input for simulation. We ran these simulations for each of the three scenarios with regard to the location of (photo)respired CO_2 release. The results were used to investigate how these scenarios would affect the estimates of R_d if the Laisk method is applied to similar experimental conditions.

Response of g_m and reassimilation to C_a and I_{inc}

We used the reaction-diffusion model to calculate the apparent g_m for each leaf type and each scenario of (photo)respired CO_2 release. We first used the model to calculate A_N , C_i and C_c as described by Berghuijs *et al.* (2017). Next, we re-arranged Eqn 2 to $g_m = A_N/(C_i - C_c)$ to calculate g_m . We calculated both g_m and the fraction of (photo)respired CO_2 that is reassimilated, f_{reass} , as described by Berghuijs *et al.* (2017), for various levels of C_a , O and I_{inc} .

Method to identify most likely locations of (photo)respired CO_2 release

We calculated the Akaike's Information Criterion (AIC) (Akaike, 1974) for each combination of measured and simulated response curves, for each leaf type and for each scenario. For details, see Methods S1.

Results

Estimation of R_d

We used the reaction-diffusion model to estimate R_d for the leaf types in the datasets of Berghuijs *et al.* (2015) (Fig. 1a,b; Table S1) and Ho *et al.* (2016) (Fig. 1c,d; Table S2). Additionally, we estimated R_d by linear regression for the Yin and Kok methods. In 11 of 12 cases, the R_d values estimated by the reaction-diffusion model under photorespiratory conditions were higher than the R_d values under nonphotorespiratory conditions.

The values of R_d estimated by the reaction-diffusion model did not differ much for the different assumed positions of (photo)respired CO_2 release. In all instances, the values of R_d estimated by the Yin method were higher than the R_d values estimated by the Kok method. In all cases, the values of R_d estimated by the Yin method under nonphotorespiratory conditions were close to the values estimated by the reaction-diffusion model (Fig. 1b,d). Under photorespiratory conditions, this was not

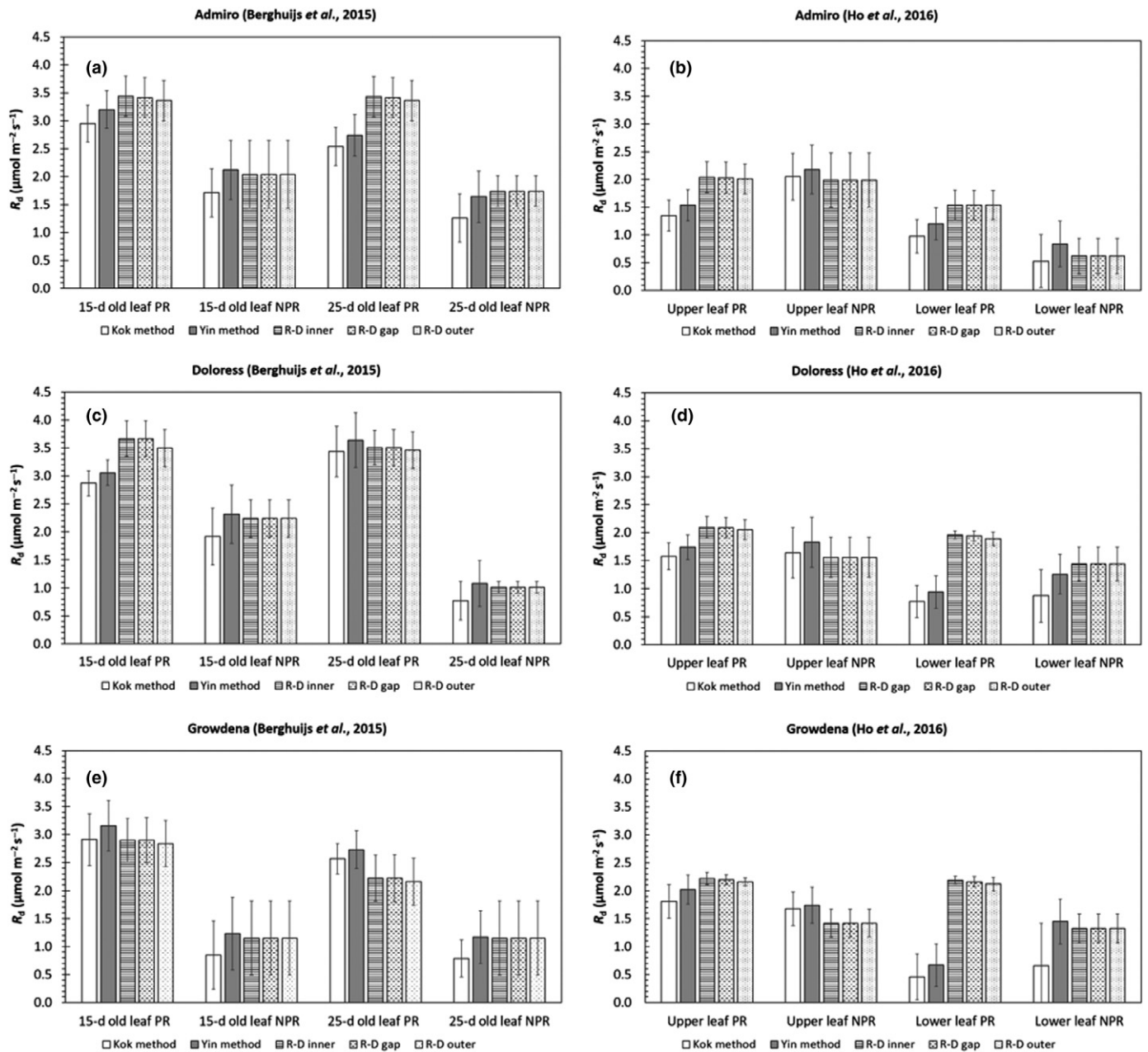


Fig. 1 Estimates of day respiration, R_d , either estimated by the Kok (1948) method, the Yin *et al.* (2009) method or by the reaction-diffusion model (R-D). Data collected by Berghuijs *et al.* (2015) (a, c, e) and Ho *et al.* (2016) (b, d, f) for three cultivars (a,b: Admiro, c,d: Doloress, e,f: Growdena) and two leaf ages (either 15- and 25-d-old leaves or upper leaves and lower leaves) were used for estimation. Estimates were made both for photorespiratory (PR) and nonphotorespiratory (NPR) conditions. R_d was estimated by the reaction-diffusion for three different scenarios: (photo)respired CO_2 was released either in the inner cytosol (RD-inner), the cytosol gaps (RD-gaps) or the outer cytosol (RD-outer). In each panel, the length of the error represents one standard deviation.

always the case (Fig. 1a,c). The values of R_d estimated by all of the methods did not differ consistently between leaf ages or leaf types.

Determination of T_p and V_{cmax}

Because of the similar estimates for R_d , there were also almost no differences for the estimates of T_p for the same leaf types

among different assumed locations of (photo)respired CO_2 release (Table S3). The estimate of V_{cmax} for each leaf type was lower if the (photo)respired CO_2 release was assumed to take place in the inner cytosol than if it was to take place in the cytosol gaps (Table S3). When (photo)respired CO_2 release took place in the outer cytosol, the estimate of V_{cmax} was always of the same order of magnitude as its standard error.

Model validation

Figs S1 and S2 show a comparison between measured and simulated CO₂ and light response curves, respectively, for each scenario of the location (photo)respired CO₂ release. They display only the part of the curves for which the measured data were not used for parameterization. Under most conditions, there was a good agreement between the measured and simulated net CO₂ assimilation rate for any scenario. However, under photorespiratory conditions the model that assumes (photo)respired CO₂ release in the outer cytosol tended to underestimate the net CO₂ assimilation rate more than the other two scenarios, under low CO₂ concentrations in the CO₂ response curves and high irradiances in the light response curves.

Response of g_m , and reassimilation to C_a and I_{inc}

Figure 2 shows how g_m responded to C_a and to I_{inc} for the case of 15-d-old leaves of cv Admiro. The relationship for other leaf types showed a similar trend, and is therefore not shown here. If (photo)respired CO₂ release took place in the outer cytosol or in the cytosol gap, g_m increased with increasing C_a . If (photo)respired CO₂ release took place in the inner cytosol, g_m decreased with C_a . g_m was always larger if (photo)respiratory CO₂ release took place in the inner cytosol than in the cytosol gaps, and in the cytosol gaps than in the outer cytosol. For each scenario, g_m tended to approach an equilibrium value at a high C_a , and this equilibrium value was the same for 21 and 2 kPa O₂ conditions for the same leaf type. See Methods S2 for further comments.

Figure 3 shows the response curves of f_{reass} to C_a and to I_{inc} , for 15-d-old Admiro leaves from Berghuijs *et al.* (2015), and this relationship are similar for the other leaf types. The relationship with C_a was sigmoidal, and that with I_{inc} was in a saturation shape, under both oxygen concentrations.

Simulations under conditions of the Laisk method

We simulated $A_N - C_i$ curves under different irradiances, for 15-d-old Admiro leaves (Fig. 4) using the R_d values that were previously estimated for each scenario of (photo)respired CO₂ release (Figs S1, S2). If (photo)respired CO₂ release took place in the inner cytosol, the curves had about the same intersection point at $C_i = 2.1$, Pa. If (photo)respired CO₂ release took place in the cytosol gaps, the curves had about the same intersection point in $C_i = 3.0$, Pa. For both scenarios, the common intersection points of the curves also were intersected with the line $A_N = -R_d$. If (photo)respired CO₂ release took place in the outer cytosol, the curves did not intersect at the same value of C_i . Instead, individual curves intersected in intercellular CO₂ partial pressures of 4.3, 4.7, 5.1, 5.4 and 5.8 Pa, respectively. The values of A_N in these intersection points were higher than $-R_d$. The intersection points were not obtained by the line $C_i = \Gamma^*$ in any of the three scenarios.

Likely locations of (photo)respired CO₂

We calculated ΔAIC for each combination of leaf type and scenario for each measured response curve type (Tables S4, S5). The ΔAIC values are bold if $\Delta AIC \leq 2$, indicating the corresponding scenario has substantial support (Burnham & Anderson, 2004). There was only one case (Admiro lower leaf CO₂ response curves at ambient O₂; Table S5) in which the scenario that assumed (photo)respired CO₂ release in the outer cytosol had substantially greater support than the scenario that assumed the release in the inner cytosol. There also was only one case in which the scenario that assumed (photo)respired CO₂ release in the cytosol gaps had more support than the other two scenarios. In the other 46 out of 48 cases, the model in which (photo)respired CO₂ release took place in the inner cytosol had either the most support or substantial support relative to the best model. In all cases, all three scenarios had substantial support for the light response curves under nonphotorespiratory conditions.

Discussion

Use of a simple reaction-diffusion model as a tool to assess R_d

Reaction-diffusion models have been used as powerful tools to investigate mesophyll conductance (g_m) and its response to various environmental and physiological factors (Tholen & Zhu, 2011; Ho *et al.*, 2016). Here, we explored using the simple reaction-diffusion model of Berghuijs *et al.* (2017) to investigate the day respiration rate (R_d). Reaction-diffusion models certainly have limitations. The most relevant one in the context of this study is that these models require prefixed diffusion coefficients as input, whose values are hard to measure. Therefore, we had to adopt these from previous studies (Gutknecht *et al.*, 1977; Evans *et al.*, 2009; Fanta *et al.*, 2012; Ho *et al.*, 2016). Additionally, we simplified the leaf structure to a single rectangular cuboid chloroplast, surrounded by a cytosol layer (Berghuijs *et al.*, 2017). This simplification simulates the leaf tissue as a 2D computational domain, while assuming that the third dimension is homogeneous. These simplifications can potentially affect the simulated results. However, Berghuijs *et al.* (2017) validated the simple model by comparing the results with those generated by a complex 3D model (Ho *et al.*, 2016). Here, we further validated the model by comparing measured (Berghuijs *et al.*, 2015; Ho *et al.*, 2016) and simulated net CO₂ assimilation rates (Figs S1, S2). Our model had various advantages. First, computational time was greatly reduced, which made it feasible to use the model directly to estimate R_d . Second it can be parameterized using a limited number of leaf anatomical parameters. We showed that the model, when combined with anatomical parameters, gas exchange and chlorophyll fluorescence data, adds to the literature by providing an additional method to indirectly estimate R_d under either photorespiratory or nonphotorespiratory conditions. We chose leaf anatomical properties that have been measured in combination with gas exchange in various previous studies

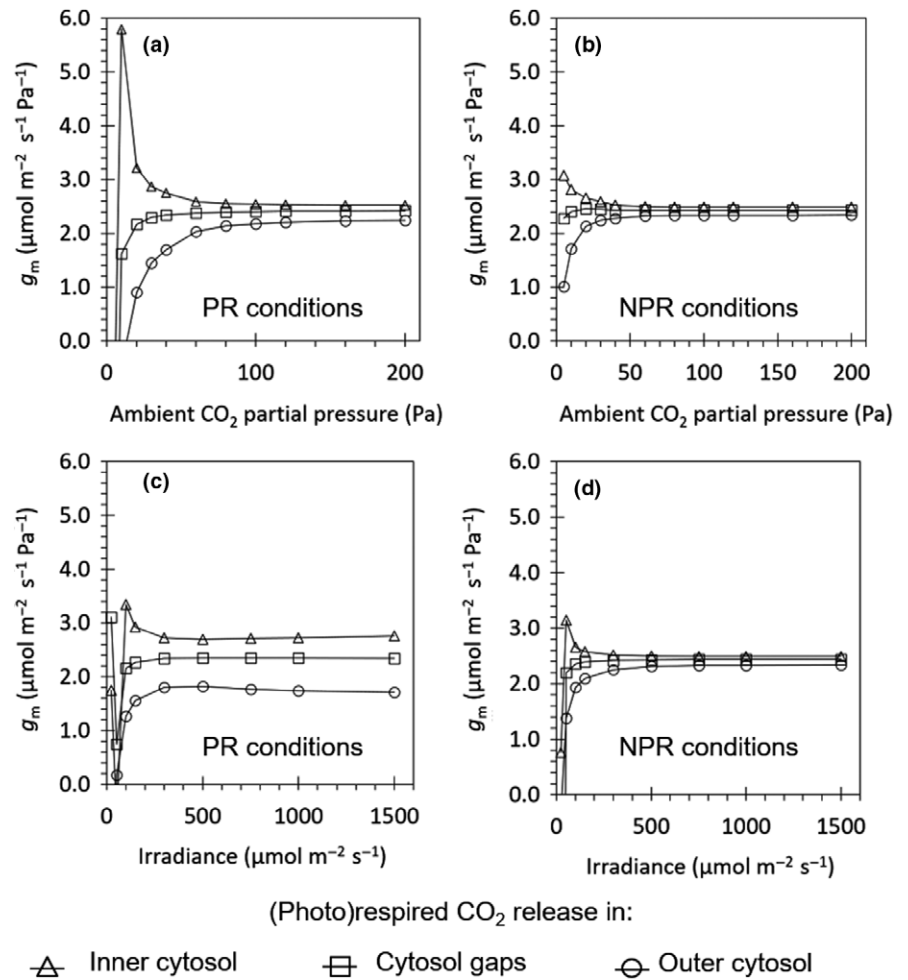


Fig. 2 Response of the simulated apparent mesophyll conductance (g_m) to increased ambient CO₂ concentrations (a,b) or light intensities (c,d) under ambient oxygen ($O = 21$ kPa) concentrations (a, c) and low oxygen ($O = 2$ kPa) concentrations (b, d). The CO₂ response curves were measured under saturating light ($I_{inc} = 1500 \mu\text{mol m}^{-2} \text{s}^{-1}$) in 15-d-old Admiro leaves from the Berghuijs *et al.* (2015) dataset. The light response curves at low oxygen concentrations were simulated under high CO₂ concentrations ($C_a = 40$ Pa). Light response curves at high oxygen concentrations were measured at ambient CO₂ concentrations ($C_a = 40$ Pa). The release of (photo)respiratory CO₂ is assumed to take place either in the inner cytosol (triangles), the cytosol gaps (squares) or the outer cytosol (circles). The lines connect each of the triangles, squares or circles.

(Syvertsen *et al.*, 1995; Tosens *et al.*, 2012; Galmes *et al.*, 2013; Retta *et al.*, 2016b; Ouyang *et al.*, 2017).

Estimation of R_d by the Kok method and the Yin method

Reassimilation and other processes can compromise the indirect estimation of R_d based on gas exchange data. We first examined estimates for nonphotorespiratory conditions where reassimilation is not relevant because then CO₂ released by (photo)respiration does not contribute much to increasing ribulose biphosphate (RuBP) carboxylation (Busch *et al.*, 2013). In most cases, R_d values estimated by the Kok method were smaller than estimates by the reaction-diffusion model, although these differences are sometimes small as the standard deviations overlap. The estimates of R_d obtained from the reaction diffusion model and the ones from the Yin method under nonphotorespiratory conditions are very similar (Figs 1,2). R_d estimates by the Kok method under photorespiratory conditions also were smaller than the estimates by the Yin method (Fig. 1), in line with Yin *et al.* (2011). Our reaction-diffusion model, like the Yin method, considers the decrease of Φ_2 with increasing irradiance (Genty & Harbinson, 1996), which occurs even within the low-irradiance range (Yin *et al.*, 2009, 2011). The Kok method underestimates R_d because

it neglects this dependence of Φ_2 on irradiance. Recognizing the decrease of Φ_2 with increasing irradiance also avoids the underestimation of the quantum yield of CO₂-assimilation (Yin *et al.*, 2014).

For photorespiratory conditions, estimates of R_d by the reaction-diffusion model were higher than those by the Yin method, let alone by the Kok method, for most leaf types (Fig. 1). The underestimation of R_d by the Yin method relative to the R_d estimated by the reaction-diffusion was 10.5–13.0%, depending on the scenario that the reaction-diffusion model assumed with regard to the location of (photo)respired CO₂ release (Fig. S3). Under photorespiratory conditions, chloroplast CO₂ concentration (C_c) decreases significantly with increased light under low light intensities (Farquhar & Busch, 2017). Such variation was generated using the FvCB model coupled with the g_m model of Eqn 2, which has a similar form as a stomatal conductance (g_s) model. Therefore, the generated variation of C_c with increasing irradiance is similar to the measured pattern for the decrease of intercellular CO₂ concentration (C_i) with light intensity (Berghuijs *et al.*, 2015). The decrease of C_c with irradiance is a common result when g_m is finite and is greatest when g_m is smallest (Farquhar & Busch, 2017). Similarly, our reaction-diffusion framework, explicitly modelling CO₂ sources, diffusion and

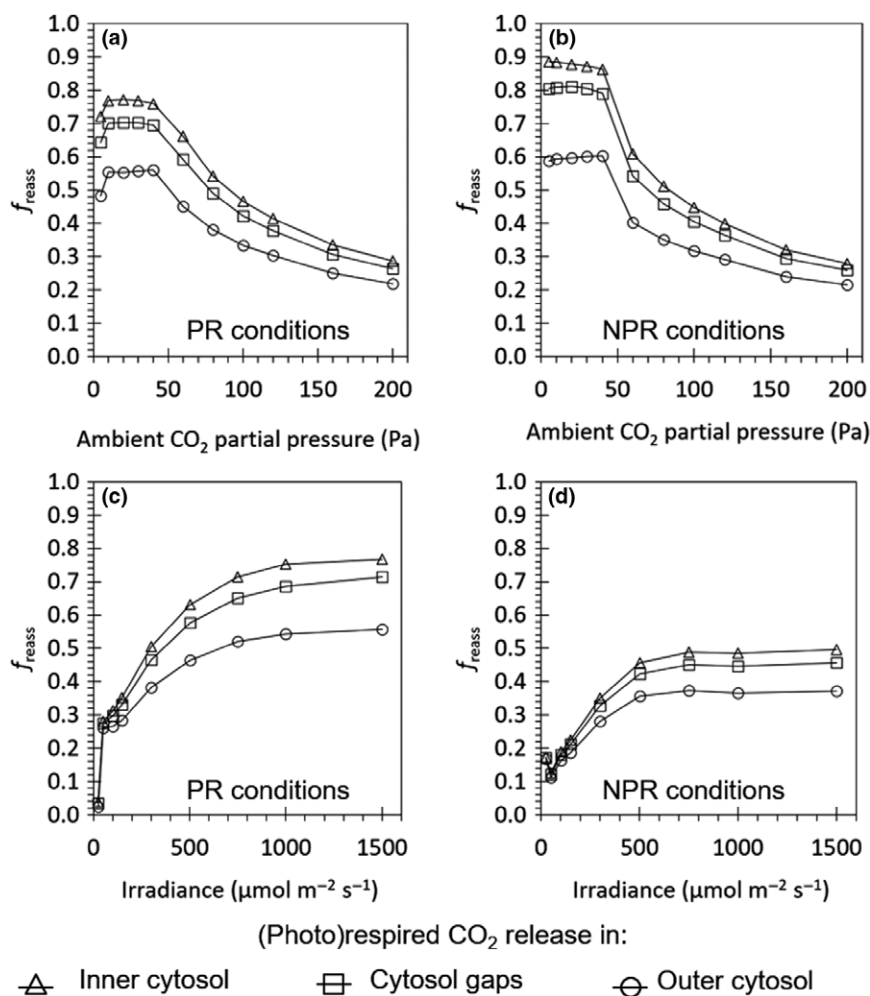


Fig. 3 Response of the simulated fraction of reassimilation (photo)respired CO₂ (f_{reass}) to increased ambient CO₂ concentrations (a,b) or light intensities (c,d) under ambient oxygen ($O = 21\text{ kPa}$) concentrations (a, c) and low oxygen ($O = 2\text{ kPa}$) concentrations (b, d). The CO₂ response curves were measured under saturating light ($I_{\text{inc}} = 1500 \mu\text{mol m}^{-2} \text{s}^{-1}$) in 15-d-old Admiro leaves from the Berghuijs *et al.* (2015) dataset. The light response curves at low oxygen concentrations were simulated under high CO₂ concentrations ($C_a = 40\text{ Pa}$). Light response curves at high oxygen concentrations were measured at ambient CO₂ concentrations ($C_a = 40\text{ Pa}$). The release of (photo)respiratory CO₂ is assumed to either take place in the inner cytosol (triangles), the cytosol gaps (squares) or the outer cytosol (circles). The lines connect each of the triangles, squares or circles.

sinks, accounts for the variation of C_c with increasing irradiance. By contrast, the linear regression procedure of the Kok method or the Yin method implicitly assumes that C_c does not vary with irradiance, when applied under photorespiratory conditions. Ignoring this variation of C_i or C_c has been shown by Kirschbaum & Farquhar (1987) and Farquhar & Busch (2017) to lead to an underestimation of R_d (see also Buckley *et al.*, 2017). Therefore, we conclude that the Yin method underestimates R_d for photorespiratory conditions by neglecting the variation of C_c with increasing irradiance.

To what extent is this variation of C_c with increasing irradiance associated with the reassimilation by (photo)respired CO₂? For nonphotorespiratory conditions that are achieved with a very low O₂ concentration, both a g_m model and the reaction-diffusion model can predict a decline of C_c with increasing irradiance (results not shown), but with a negligible effect on leaf photosynthesis. By contrast, for photorespiratory conditions, this decline is highly relevant as it affects the rate of RuBP carboxylation. The FvCB model, when combined with g_s and g_m , accounts for re-assimilation of (photo)respired CO₂ (Tholen *et al.*, 2012; Von Caemmerer, 2013; Yin & Struik, 2017). A high g_m value could predict little drawdown of C_c from C_i with increasing irradiance and a low intracellular re-assimilation. Therefore, for

photorespiratory conditions, the modelled variation of C_c with irradiance indirectly reflects the contribution of (photo)respired CO₂ release to C_c , therefore, to re-assimilation. This assertion is supported by the similarity between the above-stated percentages of R_d underestimation by the Yin method (10.5–13.0%) and the values of f_{reass} we estimated for the low-irradiance range (Fig. 3). The small difference in f_{reass} at low light among the three possible positions of mitochondria (Fig. 3) is also in line with the small difference among R_d estimates in different scenarios (Fig. 1). Although the placement of mitochondria is known to affect f_{reass} (Yin & Struik, 2017), our reaction-diffusion model predicts that such an effect of the scenario is most expressed under high-light conditions (Fig. 3).

Because of the above differences in handling the irradiance-dependence of C_c and re-assimilation, the relative value of R_d estimated for the photorespiratory vs nonphotorespiratory conditions by the Yin method and the reaction-diffusion model differed. The estimates of R_d by the Yin method were either higher or lower in one than in the other conditions, whereas those by the reaction-diffusion model were always lower for non-photorespiratory than for photorespiratory conditions (Fig. 1). This is in agreement with results from Buckley *et al.* (2017), who showed that R_{dk} was higher at 21% than at 2% O₂ in developing

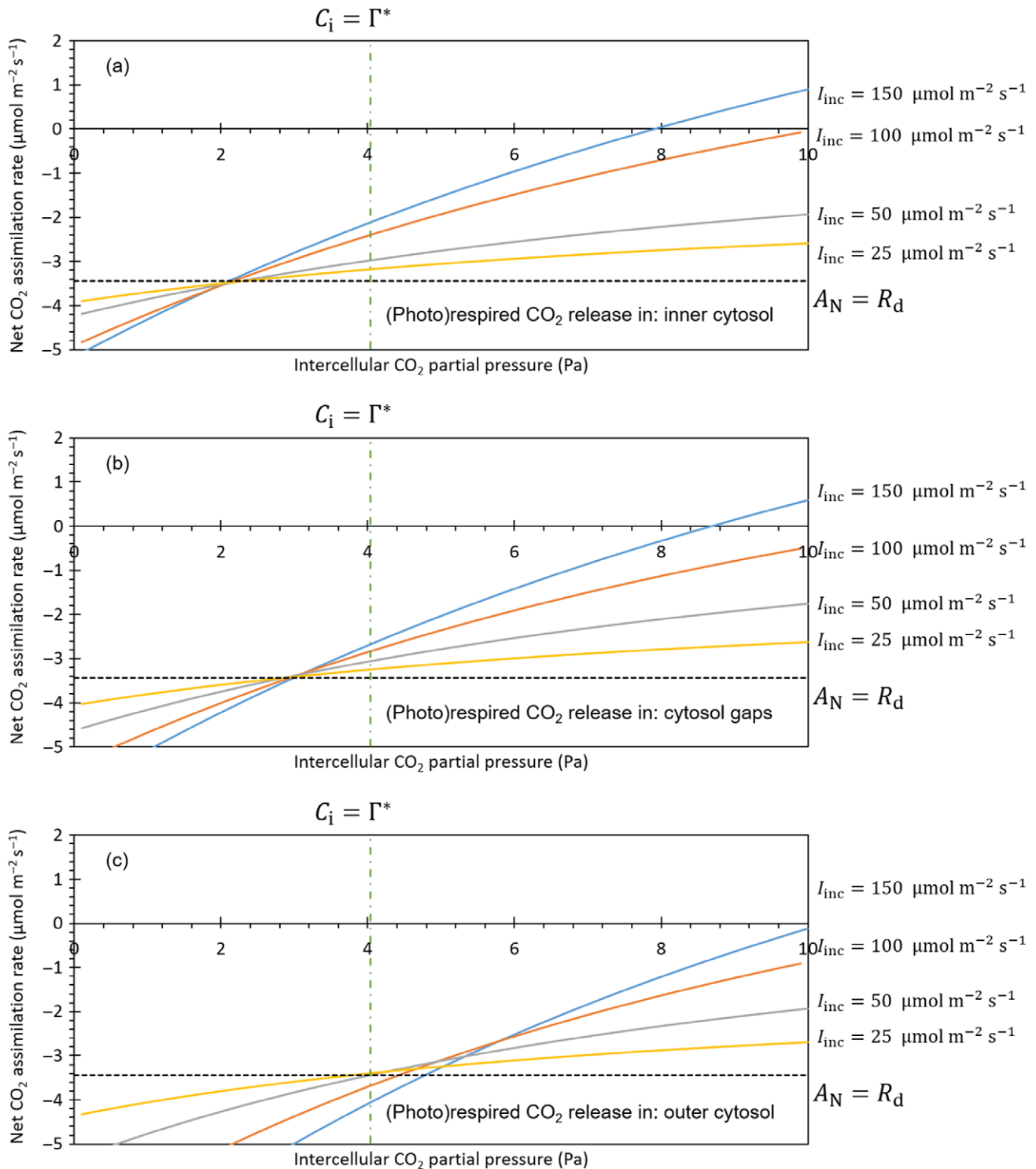


Fig. 4 Simulated response curve of the net CO₂ assimilation rate (A_N) to intercellular CO₂ partial pressures (C_i) under the conditions of the Laisk method for different scenarios of (photo)respired CO₂ release. The solid lines represent CO₂ response curves simulated at different irradiances I_{inc} (150, 100, 50 and 25 μmol m⁻² s⁻¹). The dashed line represents the net CO₂ assimilation rate which equals the negative input value of the day respiration rate (R_d). The dashed-dotted line represents the intercellular partial pressure which equals the CO₂ compensation point (Γ^*). (Photo)respired CO₂ is released either in the inner cytosol (a), the cytosol gaps (b) or the outer cytosol (c).

leaves of *Vicia faba*. Respiration is a process where O₂ is the substrate (Tcherkez *et al.*, 2017b) and respiratory rates measured in terms of O₂ and CO₂ exchange may not be equal (Gauthier

et al., 2018). However, the amount of respiratory CO₂ release at the low O₂ concentration, as applied for measurements under nonphotorespiratory conditions, will likely decrease relative to

that under ambient O_2 conditions. The Yin method is theoretically valid for nonphotorespiratory conditions only (Yin *et al.*, 2011). However, it is previously unknown to what extent R_d estimated from nonphotorespiratory conditions can be used for photorespiratory conditions. Based on our results and those found in literature (Buckley *et al.*, 2017), we conclude that the R_d estimate obtained under nonphotorespiratory conditions by the Yin method cannot be used as a replacement for R_d under photorespiratory conditions.

This conclusion also applies to the Kok method. One assumption when using the Kok method or the Yin method under photorespiratory conditions is that C_c is constant for different light intensities. However, practically, it is impossible to design an experiment where C_c is maintained constant across various irradiances because g_s , g_m and A_N are not known beforehand (Buckley *et al.*, 2017). Our analysis shows the power of using reaction-diffusion models parameterized with standard diffusion coefficients (Berghuijs *et al.*, 2017) and leaf anatomical measurements (Berghuijs *et al.*, 2015) to estimate R_d , which can account for the decrease of both Φ_2 and C_c with increasing irradiance.

Estimation of R_d by the Laisk method

The Laisk method relies on measurements at low C_i , the conditions having high photorespiration, but it theoretically has problems if r_m is significant, especially under the framework of multiple components of r_m (Tholen *et al.*, 2012). Yin *et al.* (2011) stated that R_d estimated by the Laisk method was comparable with the estimates by the Yin method for photorespiratory conditions. Gong *et al.* (2018) showed that the Laisk method underestimates R_d when compared with their isotopic disequilibrium method that directly estimates R_d .

The results of our simulations of CO_2 response curves under the conditions of the application of the Laisk method (Fig. 4) actually show that assumptions regarding the location of (photo) respired CO_2 release affect the estimates it obtains. If (photo) respired CO_2 is assumed to be released in the outer cytosol, the curves do not intersect in a single point. Moreover, each of the

intersection points between two curves has a higher net CO_2 assimilation rate than the prefixed $-R_d$. Based on their model, which implicitly assumes (photo) respired CO_2 release in the outer cytosol, Tholen *et al.* (2012) also indicates that the Laisk method will underestimate R_d . Our simulations show that in the other two scenarios, CO_2 response curves actually do intersect in $A_N = -R_d$. This shows that if one of these two scenarios is true, Laisk's method yields a good estimate of R_d .

In an application of the Laisk plot, it is still required that all CO_2 response curves share a single intersection point. This issue can be solved by the fitting procedure as described by Yin *et al.* (2011) for the Laisk method or the slope-intercept regression analysis as applied by Walker & Ort (2015). However, the Laisk linear plot to estimate R_d should be made as a function of C_c ; for that g_m needs to be known. A dilemma is that g_m can be estimated only after R_d is known (Harley *et al.*, 1992). Again, the reaction-diffusion model does not have this problem as it does not require g_m as an input beforehand. Nevertheless, relying on g_m values indirectly derived from an established relationship between g_m and g_s , Gong *et al.* (2018) showed that R_d estimated by the Laisk method does not depend on whether it is based on C_i or C_c .

Estimates of photosynthetic parameters and mesophyll conductance in relation to the position of mitochondria

The estimate of V_{cmax} was always higher if (photo)respiratory CO_2 release took place in the cytosol gap than in the inner cytosol (Table S3). Because the reassimilation of (photo)respiratory CO_2 was higher if (photo)respiratory CO_2 was released in the inner cytosol than in the cytosol gaps (Fig. 3), the model compensated for the lower reassimilation by a higher RuBP carboxylation under Rubisco limited conditions, thereby resulting in a higher estimated V_{cmax} . If (photo)respiratory CO_2 was released in the outer cytosol, the standard error was very high (Table S3), possibly because the model cannot fully compensate for the discrepancy between its prediction of A_N and the measured A_N for this scenario by estimating a high value for V_{cmax} .

Table 2 Overview of advantages and disadvantages of estimation methods for R_d .

Method	Advantages	Disadvantages
Kok method	Does not require chlorophyll fluorescence measurements Does not require leaf anatomical measurements	Does not consider the increase of C_c with decreased irradiance Is theoretically only valid under nonphotorespiratory conditions Does not account for the decrease of Φ_2 with increased irradiance
Yin method	Does not require leaf anatomical measurements Accounts for the decrease of Φ_2 with increased irradiance	Requires chlorophyll fluorescence measurements Does not consider the increase of C_c with decreased irradiance Is theoretically only valid under nonphotorespiratory conditions
Laisk method	Does not require chlorophyll fluorescence measurements Does not require leaf anatomical measurements Partly considers the reassimilation of photorespired CO_2 Applies to photorespiratory conditions	Requires an assumption of no mesophyll resistance Estimate is affected by the position of mitochondria relative to the chloroplasts Is applied at very low CO_2 concentrations and requires gas leakage corrections
Reaction diffusion model	Does not require an estimate of mesophyll conductance Accounts for the decrease of Φ_2 with increased radiation The placement of mitochondria relative to the chloroplasts can be defined explicitly Is theoretically valid under photorespiratory conditions	Requires chlorophyll fluorescence measurements Requires leaf anatomical parameters to parameterize the geometry Requires CO_2 diffusion coefficients for different mesophyll compartments Has to be solved numerically

For all leaf types, our reaction-diffusion model generated the same trend in the response of g_m to different values of C_a and I_{inc} (Fig. 2). If (photo)respired CO_2 release was assumed to take place in the inner cytosol, g_m decreased with an increase in C_a . The shape of this response was similar to the response of g_m to C_i (when C_i was above certain values) reported in various studies (Flexas *et al.*, 2007; Yin *et al.*, 2009; Tholen & Zhu, 2011). The apparent g_m model as used in these studies was Eqn 2. This model assumes that the mitochondria are located closely behind the chloroplasts as if that (photo)respired CO_2 were released in the same compartment as RuBP carboxylation does (Tholen & Zhu, 2011; Yin & Struik, 2017). If (photo)respired CO_2 was to release in the outer cytosol or in the cytosol gaps, the shape of the response was more similar to the one calculated by Tholen *et al.* (2012) using a resistance model based on the same assumption. Xiao & Zhu (2017) also found similar differences in the shape of the response curve of g_m to C_i depending on the position of the mitochondria relative to the chloroplasts.

The most likely position of mitochondria relative to the chloroplasts

In a vast majority of cases, the scenario for (photo)respired CO_2 release in the outer cytosol had less support than the scenario that assumed (photo)respired CO_2 release in the inner cytosol (Tables S4 and S5). The consequences of this finding is that, at least in tomato, two-resistance models (Tholen *et al.*, 2012; Berghuijs *et al.*, 2015) that implicitly assume (photo)respired CO_2 release in the outer cytosol are less likely than the classical single mesophyll resistance models.

This assertion agrees generally with experimental observations like (Hatakeyama & Ueno, 2016), who reported that for 10 C_3 grasses, on average 80% of the mitochondria are located closely on the vacuole side of chloroplasts in mesophyll cells. Sage & Sage (2009) and Busch *et al.* (2013) had a similar observation for rice and wheat, who even indicated that chloroplast covers > 95% of the mesophyll periphery with a high $S_c : S_m$ ratio that provides an effective mechanism to trap and re-assimilate (photo)respired CO_2 . The classical resistance model, Eqn 2, works best if mitochondria are exclusively located closely behind chloroplasts and the $S_c : S_m$ ratio is very close to 1.0, whereas the two-resistance model of Tholen *et al.* (2012) works if mitochondria are located predominantly in the outer cytosol combined with a low $S_c : S_m$ and little cytosol resistance (Yin & Struik, 2017). Although the reality may be somewhere between these two extremes, our analysis in Tables S4 and S5 suggests that the classical g_m model, Eqn 2, is closer to reality in the two experiments for tomato. It should be noted that the $S_c : S_m$ measurements that were used in this study (between 0.84 and 0.96) (Berghuijs *et al.*, 2015) were all at the higher end of the values mentioned in literature for various species. For instance, considerably lower ranges have been reported for *Arabidopsis thaliana* (0.43–0.75) (Tholen *et al.*, 2008). As $S_c : S_m$ decreases with leaf aging and varies with species (Busch *et al.*, 2013) and with environment (Ouyang *et al.*, 2017), it may be hard to ascertain which model of the two is closer to reality.

Concluding remarks

Our reaction-diffusion model can estimate R_d without making implicit assumptions regarding re-assimilation, position of mitochondria, mesophyll conductance and the variability of C_c at low light conditions. In these aspects, our model provides a better tool to estimate R_d than the Laisk, Kok and Yin methods. However, if there are no leaf anatomical data available, it depends on the available data which of the existing models has to be applied. Table 2 shows an overview of the advantages and disadvantages of each method. With leaf anatomical data available, reaction-diffusion models have previously shown to be useful to study the mechanisms of mesophyll conductance and re-assimilation (Tholen & Zhu, 2011; Ho *et al.*, 2016; Retta *et al.*, 2016a, 2017; Berghuijs *et al.*, 2017). We demonstrate here that they also can estimate R_d and photosynthetic parameters. We recommend further research to collect datasets containing leaf anatomical parameters in combination with gas exchange and chlorophyll fluorescence measurements to make the best possible use of our method.

Acknowledgements

Wageningen-based authors thank the BioSolar Cells programme for financial support. Leuven-based authors thank the Research Council of the KU Leuven for financial support (project C16/16/002). We thank Bob Douma, Pepijn van Oort, Wopke van der Werf and Willemien Lommen for a useful discussion on the interpretation of Akaike's Information Criterion.

Author contributions

HNCB planned the research and wrote the first draft of the manuscript; HNCB and XY ran the simulations; HNCB, XY, QTH, MAR, BMN and PCS interpreted the simulations; and HNCB, XY, QTH, MAR, BMN and PCS wrote the final manuscript.

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References

- Akaike H. 1974. New look at statistical-model identification. *IEEE Transactions on Automatic Control* **Ac19**: 716–723.
- Berghuijs HNC, Yin X, Ho QT, Retta MA, Verboven P, Nicolai BM, Struik PC. 2017. Localization of (photo) respiration and CO_2 re-assimilation in tomato leaves investigated with a reaction-diffusion model. *PLoS ONE* **12**: e0183746.

- Berghuijs HNC, Yin X, Ho QT, van der Putten PEL, Verboven P, Retta MA, Nicolai BM, Struik PC. 2015. Modelling the relationship between CO₂ assimilation and leaf anatomical properties in tomato leaves. *Plant Science* 238: 297–311.
- Berghuijs HNC, Yin XY, Ho QT, Driever SM, Retta MA, Nicolai BM, Struik PC. 2016. Mesophyll conductance and reaction-diffusion models for CO₂ transport in C₃ leaves; needs, opportunities and challenges. *Plant Science* 252: 62–75.
- Brooks A, Farquhar GD. 1985. Effect of temperature on the CO₂/O₂ specificity of ribulose-1,5-bisphosphate carboxylase oxygenase and the rate of respiration in the light – estimates from gas-exchange measurements on spinach. *Planta* 165: 397–406.
- Buckley TN, Vice H, Adams MA. 2017. The Kok effect in *Vicia faba* cannot be explained solely by changes in chloroplastic CO₂ concentration. *New Phytologist* 216: 1064–1071.
- Burnham KP, Anderson DR. 2004. Multimodel interference – understanding AIC and BIC in model selection. *Sociological Methods & Research* 33: 261–304.
- Busch FA, Sage TL, Cousins AB, Sage RF. 2013. C₃ plants enhance rates of photosynthesis by reassimilating photorespired and respired CO₂. *Plant, Cell & Environment* 36: 200–212.
- Evans JR, Kaldenhoff R, Genty B, Terashima I. 2009. Resistances along the CO₂ diffusion pathway inside leaves. *Journal of Experimental Botany* 60: 2235–2248.
- Evans JR, Sharkey TD, Berry JA, Farquhar GD. 1986. Carbon isotope discrimination measured concurrently with gas-exchange to investigate CO₂ diffusion in leaves of higher plants. *Australian Journal of Plant Physiology* 13: 281–292.
- Fanta SW, Vanderlinden W, Abera MK, Verboven P, Karki R, Ho QT, De Feyter S, Carmeliet J, Nicolai BM. 2012. Water transport properties of artificial cell walls. *Journal of Food Engineering* 108: 393–402.
- Farquhar GD, Busch FA. 2017. Changes in the chloroplastic CO₂ concentration explain much of the observed Kok effect: a model. *New Phytologist* 214: 570–584.
- Farquhar GD, Caemmerer SV, Berry JA. 1980. A biochemical model of photosynthetic CO₂ assimilation in leaves of C₃ species. *Planta* 149: 78–90.
- Flexas J, Díaz-Espejo A, Galmés J, Kaldenhoff R, Medrano H, Ribas-Carbó M. 2007. Rapid variations of mesophyll conductance in response to changes in CO₂ concentration around leaves. *Plant, Cell & Environment* 30: 1284–1298.
- Flexas J, Ribas-Carbó M, Díaz-Espejo A, Galmés J, Medrano H. 2008. Mesophyll conductance to CO₂: current knowledge and future prospects. *Plant, Cell & Environment* 31: 602–621.
- Galmes J, Ochogavia JM, Gago J, Roldan EJ, Cifre J, Conesa MA. 2013. Leaf responses to drought stress in Mediterranean accessions of *Solanum lycopersicum*: anatomical adaptations in relation to gas exchange parameters. *Plant, Cell & Environment* 36: 920–935.
- Gauthier PPG, Battle MO, Griffin KL, Bender ML. 2018. Measurement of gross photosynthesis, respiration in the light, and mesophyll conductance using (H₂O)-O-18 labeling. *Plant Physiology* 177: 62–74.
- Genty B, Harbinson J. 1996. Regulation of light utilization for photosynthetic electron transport. In: Baker NR, ed. *Photosynthesis and the environment*. Dordrecht, the Netherlands: Kluwer Academic, 67–99.
- Gifford RM. 2003. Plant respiration in productivity models: conceptualisation, representation and issues for global terrestrial carbon-cycle research. *Functional Plant Biology* 30: 171–186.
- Gong XY, Schaufele R, Feneis W, Schnyder H. 2015. ¹³CO₂/¹²CO₂ exchange fluxes in a clamp-on leaf cuvette: disentangling artefacts and flux components. *Plant, Cell & Environment* 38: 2417–2432.
- Gong XY, Tcherkez G, Wenig J, Schaufele R, Schnyder H. 2018. Determination of leaf respiration in the light: comparison between an isotopic disequilibrium method and the Laisk method. *New Phytologist* 218: 1371–1382.
- Griffin KL, Turnbull MH. 2013. Light saturated RuBP oxygenation by Rubisco is a robust predictor of light inhibition of respiration in *Triticum aestivum* L. *Plant Biology* 15: 769–775.
- Gutknecht J, Bisson MA, Tosteson FC. 1977. Diffusion of carbon-dioxide through lipid bilayer membranes - Effects of carbonic-anhydrase, bicarbonate, and unstirred layers. *Journal of General Physiology* 69: 779–794.
- Harley PC, Loreto F, Di Marco G, Sharkey TD. 1992. Theoretical considerations when estimating the mesophyll conductance to CO₂ flux by analysis of the response of photosynthesis to CO₂. *Plant Physiology* 98: 1429–1436.
- Hatakeyama Y, Ueno O. 2016. Intracellular position of mitochondria and chloroplasts in bundle sheath and mesophyll cells of C₃ grasses in relation to photorespiratory CO₂ loss. *Plant Production Science* 19: 540–551.
- Haupt-Herting S, Klug K, Fock HP. 2001. A new approach to measure gross CO₂ fluxes in leaves. Gross CO₂ assimilation, photorespiration, and mitochondrial respiration in the light in tomato under drought stress. *Plant Physiology* 126: 388–396.
- Ho QT, Berghuijs HNC, Watte R, Verboven P, Herremans E, Yin XY, Retta MA, Aernouts B, Saeyns W, Helfen L *et al.* 2016. Three-dimensional microscale modelling of CO₂ transport and light propagation in tomato leaves enlightens photosynthesis. *Plant, Cell & Environment* 39: 50–61.
- Kirschbaum MUF, Farquhar GD. 1987. Investigation of the CO₂ dependence of quantum yield and respiration in *Eucalyptus pauciflora*. *Plant Physiology* 83: 1032–1036.
- Kok B. 1948. A critical consideration of the quantum yield of *Chlorella* photosynthesis. *Enzymologia* 13: 1–56.
- Laisk A. 1977. *Kinetics of photosynthesis and photorespiration in C₃ plants (in Russian)*. Moscow, Russia: Nauka.
- Loreto F, Delfine S, Di Marco G. 1999. Estimation of photorespiratory carbon dioxide recycling during photosynthesis. *Australian Journal of Plant Physiology* 26: 733–736.
- Loreto F, Velikova V, Di Marco G. 2001. Respiration in the light measured by ¹²CO₂ emission in ¹³CO₂ atmosphere in maize leaves. *Australian Journal of Plant Physiology* 28: 1103–1108.
- Niinemetts U, Diaz-Espejo A, Flexas J, Galmes J, Warren CR. 2009. Role of mesophyll diffusion conductance in constraining potential photosynthetic productivity in the field. *Journal of Experimental Botany* 60: 2249–2270.
- Ouyang W, Struik PC, Yin X, Yang J. 2017. Stomatal conductance, mesophyll conductance, and transpiration efficiency in relation to leaf anatomy in rice and wheat genotypes under drought. *Journal of Experimental Botany* 68: 5191–5205.
- Pärnik T, Keerberg O. 2007. Advanced radiogasometric method for the determination of the rates of photorespiratory and respiratory decarboxylations of primary and stored photosynthates under steady-state photosynthesis. *Physiologia Plantarum* 129: 34–44.
- Retta MA, Ho QT, Yin X, Verboven P, Berghuijs HNC, Struik PC, Nicolai BM. 2017. Exploring anatomical controls of C₄ leaf photosynthesis using a 3D reaction-diffusion model. *Acta Horticulture* 1154: 171–178.
- Retta M, Ho QT, Yin XY, Verboven P, Berghuijs HNC, Struik PC, Nicolai BM. 2016a. A two-dimensional microscale model of gas exchange during photosynthesis in maize (*Zea mays* L.) leaves. *Plant Science* 246: 37–51.
- Retta M, Yin XY, van der Putten PEL, Cantre D, Berghuijs HNC, Ho QT, Verboven P, Struik PC, Nicolai BM. 2016b. Impact of anatomical traits of maize (*Zea mays* L.) leaf as affected by nitrogen supply and leaf age on bundle sheath conductance. *Plant Science* 252: 205–214.
- Sage TH, Sage RF. 2009. The functional anatomy of rice leaves: implications for refixation of photorespiratory CO₂ and efforts to engineer C₄ photosynthesis into rice. *Plant and Cell Physiology* 50: 756–772.
- Sharkey TD. 1985. Photosynthesis in intact leaves of C₃ plants: physics, physiology and rate limitations. *Botanical Review* 51: 53–105.
- Syvertsen JP, Lloyd J, Mcconchie C, Kriedemann PE, Farquhar GD. 1995. On the relationship between leaf anatomy and CO₂ diffusion through the mesophyll of hypostomatous leaves. *Plant, Cell & Environment* 18: 149–157.
- Tcherkez G, Gauthier P, Buckley TN, Busch FA, Barbour MM, Bruhn D, Heskell MA, Gong XY, Crous K, Griffin KL *et al.* 2017a. Tracking the origins of the Kok effect, 70 years after its discovery. *New Phytologist* 214: 506–510.
- Tcherkez G, Gauthier P, Buckley TN, Busch FA, Barbour MM, Bruhn D, Heskell MA, Gong XY, Crous KY, Griffin K *et al.* 2017b. Leaf day respiration: low CO₂ flux but high significance for metabolism and carbon balance. *New Phytologist* 216: 986–1001.

- Tholen D, Boom C, Noguchi K, Ueda S, Katase T, Terashima I. 2008. The chloroplast avoidance response decreases internal conductance to CO₂ diffusion in *Arabidopsis thaliana* leaves. *Plant, Cell & Environment* 31: 1688–1700.
- Tholen D, Éthier G, Genty B. 2014. Mesophyll conductance with a twist. *Plant, Cell & Environment* 37: 2456–2458.
- Tholen D, Éthier G, Genty B, Pepin S, Zhu XG. 2012. Variable mesophyll conductance revisited: theoretical background and experimental implications. *Plant, Cell & Environment* 35: 2087–2103.
- Tholen D, Zhu XG. 2011. The mechanistic basis of internal conductance: a theoretical analysis of mesophyll cell photosynthesis and CO₂ diffusion. *Plant Physiology* 156: 90–105.
- Tosens T, Niinemets U, Westoby M, Wright IJ. 2012. Anatomical basis of variation in mesophyll resistance in eastern Australian sclerophylls: news of a long and winding path. *Journal of Experimental Botany* 63: 5105–5119.
- Von Caemmerer S. 2013. Steady-state models of photosynthesis. *Plant, Cell & Environment* 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. *Australian Journal of Plant Physiology* 18: 287–305.
- 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.
- Walker BJ, Ort DR. 2015. Improved method for measuring the apparent CO₂ photocompensation point resolves the impact of multiple internal conductances to CO₂ to net gas exchange. *Plant, Cell & Environment* 38: 2462–2474.
- Way DA, Aspinwall MJ, Drake JE, Crous KY, Company CE, Ghannoum O, Tissue DT, Tjoelker MG. 2019. Responses of respiration in the light to warming in field-grown trees: a comparison of the thermal sensitivity of the Kok and Laik methods. *New Phytologist* 222: 132–143.
- Xiao Y, Zhu XG. 2017. Components of mesophyll resistance and their environmental responses: a theoretical modelling analysis. *Plant, Cell & Environment* 40: 2729–2742.
- Yin X, Belay DW, van der Putten P, Struik PC. 2014. Accounting for the decrease of photosystem photochemical efficiency with increasing irradiance to estimate quantum yield of leaf photosynthesis. *Photosynthesis Research* 122: 323–335.
- 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 & Environment* 32: 1513–1524.
- Yin X, Struik PC. 2017. Simple generalisation of a mesophyll resistance model for various intracellular arrangements of chloroplasts and mitochondria in C₃ leaves. *Photosynthesis Research* 132: 211–220.
- 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 & Environment* 32: 448–464.
- Yin X, Sun ZP, Struik PC, Gu JF. 2011. Evaluating a new method to estimate the rate of leaf respiration in the light by analysis of combined gas exchange and chlorophyll fluorescence measurements. *Journal of Experimental Botany* 62: 3489–3499.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Measured vs simulated CO₂ response curves under photorespiratory and nonphotorespiratory conditions.

Fig. S2 Measured vs simulated light response curves under photorespiratory and nonphotorespiratory conditions.

Fig. S3 Day respiration rate estimates obtained by the reaction diffusion model vs estimates obtained by the Yin method.

Fig. S4 Schematic overview of the flow of the program that was used to estimate parameter values with the reaction diffusion model.

Methods S1 Determination of Akaike's Information Criterion.

Methods S2 Comments on g_m .

Notes S1 Source code to estimate R_d and V_{cmax} .

Notes S2 Code of M files.

Table S1 Estimates of the lumped calibration factors and the day respiration rates obtained by various methods using data from Berghuijs *et al.* (2015).

Table S2 Estimates of the lumped calibration factors and the day respiration rates obtained by various methods using data from Ho *et al.* (2016).

Table S3 Estimates of the maximum RuBP carboxylation rate by Rubisco and the triose phosphate utilization rates obtained by the reaction-diffusion model for different scenarios of (photo) respired CO₂ release.

Table S4 Akaike's information criteria for different combinations of leaf age, cultivar, photorespiratory conditions and scenarios for the release of (photo) respired CO₂ using the reaction-diffusion model and data from Berghuijs *et al.* (2015).

Table S5 Akaike's information criteria for different combinations of leaf age, cultivar, photorespiratory conditions, and scenarios for the release of (photo) respired CO₂ using the reaction-diffusion model and data from Ho *et al.* (2016).

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