






# Using a reaction-diffusion model to estimate day respiration and reassimilation of (photo)respired CO<sub>2</sub> in leaves

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## Summary

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**Key words:** C<sub>3</sub> 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 CO<sub>2</sub>; 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 CO<sub>2</sub>, 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  $\leq 40\%$  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 CO<sub>2</sub>-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 C<sub>3</sub> 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 CO<sub>2</sub> partial pressure), measured at difference irradiances. The negative net CO<sub>2</sub> 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 CO<sub>2</sub> compensation point  $\Gamma^*$ , at which the amount of CO<sub>2</sub> produced by photorespiration equals the amount of CO<sub>2</sub> 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  $\text{CO}_2$  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 ( $I_{\text{inc}}$ ) 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  $\text{CO}_2$  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  $\text{CO}_2$  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  $\text{CO}_2$  produced by respiration and photorespiration (which will be called '(photo)respired  $\text{CO}_2$ ' hereafter), and the  $\text{CO}_2$  molecules from intercellular air-spaces experience the same mesophyll resistance. However, (photo)respired  $\text{CO}_2$ , if being reassimilated, probably experiences the chloroplast resistance component ( $r_{\text{ch}}$ ) only. By contrast, the  $\text{CO}_2$  molecules from intercellular air-spaces experience cell wall and plasma-membrane resistance ( $r_{\text{wp}}$ ) as well as  $r_{\text{ch}}$  (the sum of  $r_{\text{wp}}$  and  $r_{\text{ch}}$  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  $I_{\text{inc}}$  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  $\text{CO}_2$  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  $\text{CO}_2$  concentrations ( $C_a$ ), requiring the correction of gas exchange data for  $\text{CO}_2$  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 ( $\Phi_2$ ) is constant over the same range of irradiances. However,  $\Phi_2$  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  $\Phi_2$ , 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  $I_{\text{inc}}$  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  $I_{\text{inc}}$  may become relevant. This is because linear regression of  $A_N$  against  $I_{\text{inc}}$  (Kok method) or against  $\Phi_2 I_{\text{inc}}/4$  (Yin method) implicitly assumes that  $C_c$  does not vary with  $I_{\text{inc}}$  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  $I_{\text{inc}}$ ; when combined with the FvCB model, the low  $g_s$  and  $g_m$  values predict that  $C_c$  decreases sharply with increasing  $I_{\text{inc}}$  within the low  $I_{\text{inc}}$  range (Farquhar & Busch, 2017). It is known also that a combined FvCB and conductance model can implicitly account for reassimilation of (photo)respired  $\text{CO}_2$  (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  $\text{CO}_2$  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  $\text{CO}_2$  is used for RuBP carboxylation in the chloroplasts before it can escape to the atmosphere. If recycling of  $\text{CO}_2$  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,  $r_{\text{wp}}$  and  $r_{\text{ch}}$ , 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  $\text{CO}_2$  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 CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> and  $g_m$ .

The CO<sub>2</sub> 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 CO<sub>2</sub> at all or in implicit assumptions about the location of (photo)respired CO<sub>2</sub> release. This problem can be avoided by using reaction-diffusion models that describe the CO<sub>2</sub> 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 CO<sub>2</sub>, 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 CO<sub>2</sub> 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 <sub>a</sub> 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 <sub>inc</sub> curves		
PR	C <sub>a</sub> = 40 Pa O = 21 kPa	C <sub>a</sub> = 38 Pa O = 21 kPa
NPR	C <sub>a</sub> = 100 Pa O = 2 kPa	C <sub>a</sub> = 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  $\text{CO}_2$  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  $\text{CO}_2$  release, the calculation of the fraction of (photo)respired  $\text{CO}_2$  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  $I_{\text{inc}}$  is the irradiance for each measurement,  $\Phi_2$  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  $\text{CO}_2$  assimilation rates.  $R_d$  was estimated for each scenario for the location of the release of (photo)respired  $\text{CO}_2$ . 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  $\text{CO}_2$  response curve measured under photorespiratory conditions. The reaction-diffusion model also was used to estimate  $V_{\text{cmax}}$  (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  $\text{CO}_2$  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 $\text{CO}_2$ assimilation under conditions of the Laisk method

For using the reaction-diffusion model to simulate  $\text{CO}_2$  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  $\text{CO}_2$  response curves at four intensities of  $I_{\text{inc}}$ : 150, 100, 50 and  $25 \mu\text{mol m}^{-2} \text{ s}^{-1}$ , using a prefixed value for  $\Gamma^*$  and the  $R_d$  estimates obtained by the reaction-diffusion model as input. From measurements of  $\Phi_2$  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  $\text{CO}_2$  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 $I_{\text{inc}}$

We used the reaction-diffusion model to calculate the apparent  $g_m$  for each leaf type and each scenario of (photo)respired  $\text{CO}_2$  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  $\text{CO}_2$  that is reassimilated,  $f_{\text{reass}}$ , as described by Berghuijs *et al.* (2017), for various levels of  $C_a$ ,  $O$  and  $I_{\text{inc}}$ .

### Method to identify most likely locations of (photo)respired $\text{CO}_2$ release

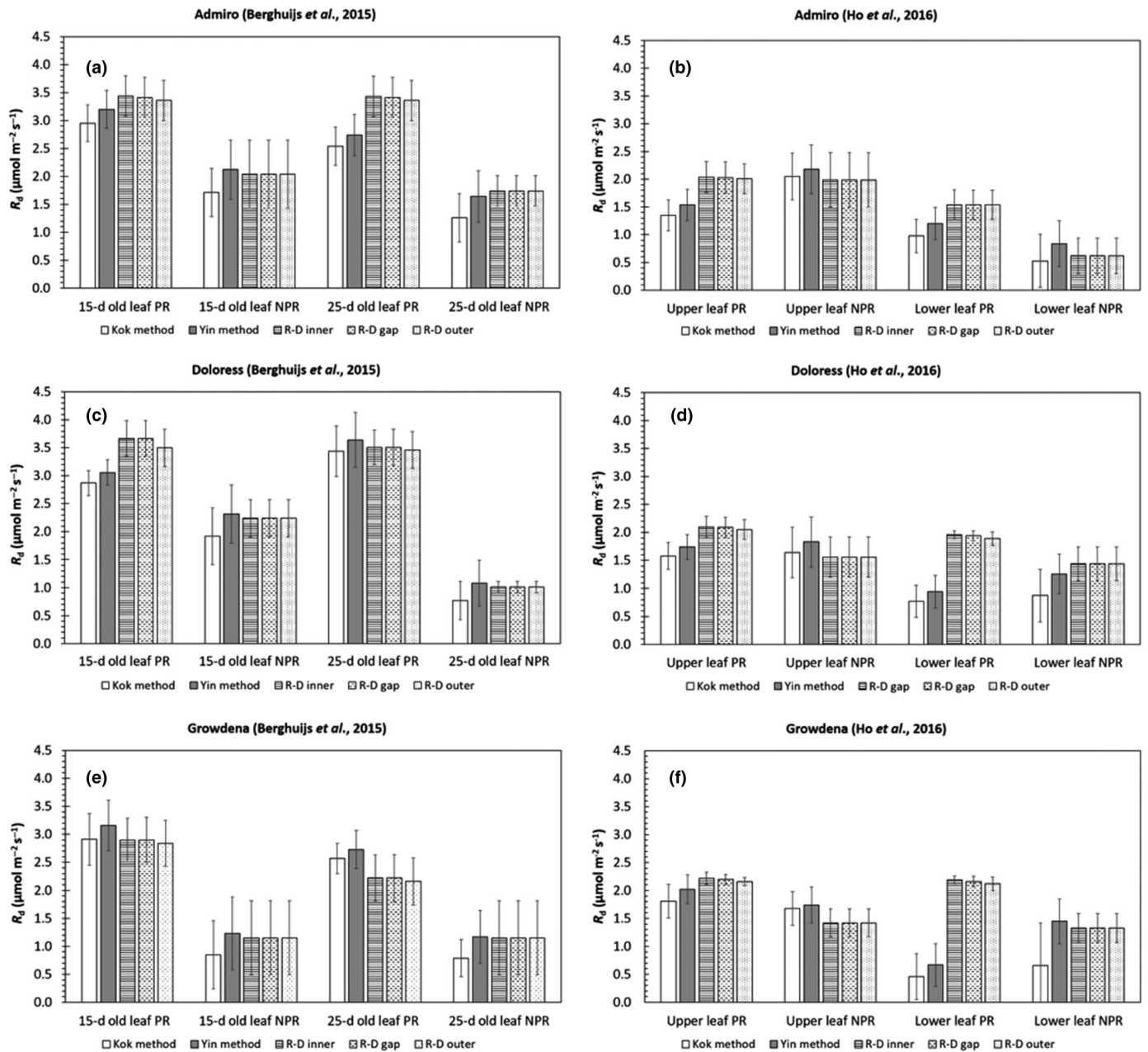
We calculated the Akaike's Information Criterion (AIC) (Akaike, 1974) for each combination of measured and simulated response curves, for each leaf type and for each scenario. For details, see Methods S1.

## 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  $\text{CO}_2$  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  $\text{CO}_2$  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 $V_{\text{cmax}}$

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  $\text{CO}_2$  release (Table S3). The estimate of  $V_{\text{cmax}}$  for each leaf type was lower if the (photo)respired  $\text{CO}_2$  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  $\text{CO}_2$  release took place in the outer cytosol, the estimate of  $V_{\text{cmax}}$  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 CO<sub>2</sub> and light response curves, respectively, for each scenario of the location (photo)respired CO<sub>2</sub> 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 CO<sub>2</sub> assimilation rate for any scenario. However, under photorespiratory conditions the model that assumes (photo)respired CO<sub>2</sub> release in the outer cytosol tended to underestimate the net CO<sub>2</sub> assimilation rate more than the other two scenarios, under low CO<sub>2</sub> concentrations in the CO<sub>2</sub> 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 CO<sub>2</sub> release took place in the outer cytosol or in the cytosol gap,  $g_m$  increased with increasing  $C_a$ . If (photo)respired CO<sub>2</sub> release took place in the inner cytosol,  $g_m$  decreased with  $C_a$ .  $g_m$  was always larger if (photo)respiratory CO<sub>2</sub> 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 O<sub>2</sub> 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 CO<sub>2</sub> release (Figs S1, S2). If (photo)respired CO<sub>2</sub> release took place in the inner cytosol, the curves had about the same intersection point at  $C_i = 2.1$ , Pa. If (photo)respired CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> 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.

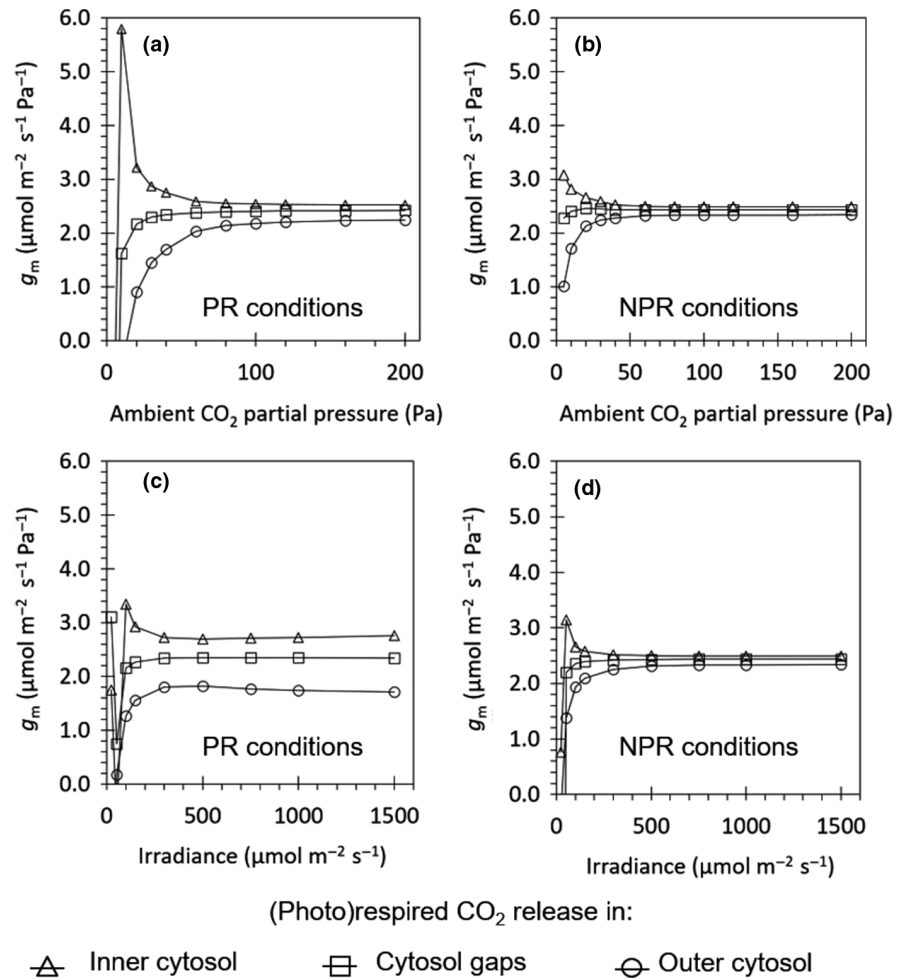
## Likely locations of (photo)respired CO<sub>2</sub>

We calculated  $\Delta AIC$  for each combination of leaf type and scenario for each measured response curve type (Tables S4, S5). The  $\Delta AIC$  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 CO<sub>2</sub> response curves at ambient O<sub>2</sub>; Table S5) in which the scenario that assumed (photo)respired CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> 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  $\text{CO}_2$  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  $\text{CO}_2$  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  $\text{CO}_2$  concentrations ( $C_a = 40$  Pa). Light response curves at high oxygen concentrations were measured at ambient  $\text{CO}_2$  concentrations ( $C_a = 40$  Pa). The release of (photo)respiratory  $\text{CO}_2$  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.

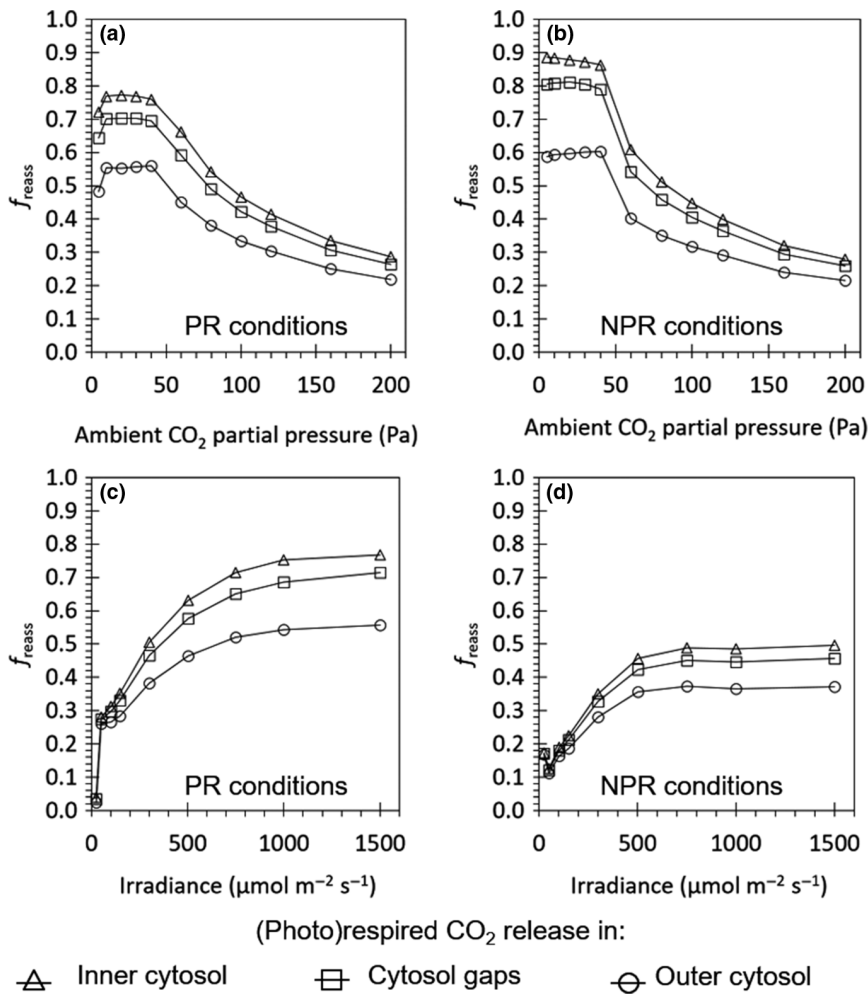
(Syvertsen *et al.*, 1995; Tosens *et al.*, 2012; Galmes *et al.*, 2013; Retta *et al.*, 2016b; Ouyang *et al.*, 2017).

#### 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  $\text{CO}_2$  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  $\Phi_2$  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  $\Phi_2$  on irradiance. Recognizing the decrease of  $\Phi_2$  with increasing irradiance also avoids the underestimation of the quantum yield of  $\text{CO}_2$ -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  $\text{CO}_2$  release (Fig. S3). Under photorespiratory conditions, chloroplast  $\text{CO}_2$  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  $\text{CO}_2$  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  $\text{CO}_2$  sources, diffusion and



**Fig. 3** Response of the simulated fraction of reassimilation (photo)respired  $\text{CO}_2$  ( $f_{\text{reass}}$ ) to increased ambient  $\text{CO}_2$  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  $\text{CO}_2$  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  $\text{CO}_2$  concentrations ( $C_a = 40 \text{ Pa}$ ). Light response curves at high oxygen concentrations were measured at ambient  $\text{CO}_2$  concentrations ( $C_a = 40 \text{ Pa}$ ). The release of (photo)respiratory  $\text{CO}_2$  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.

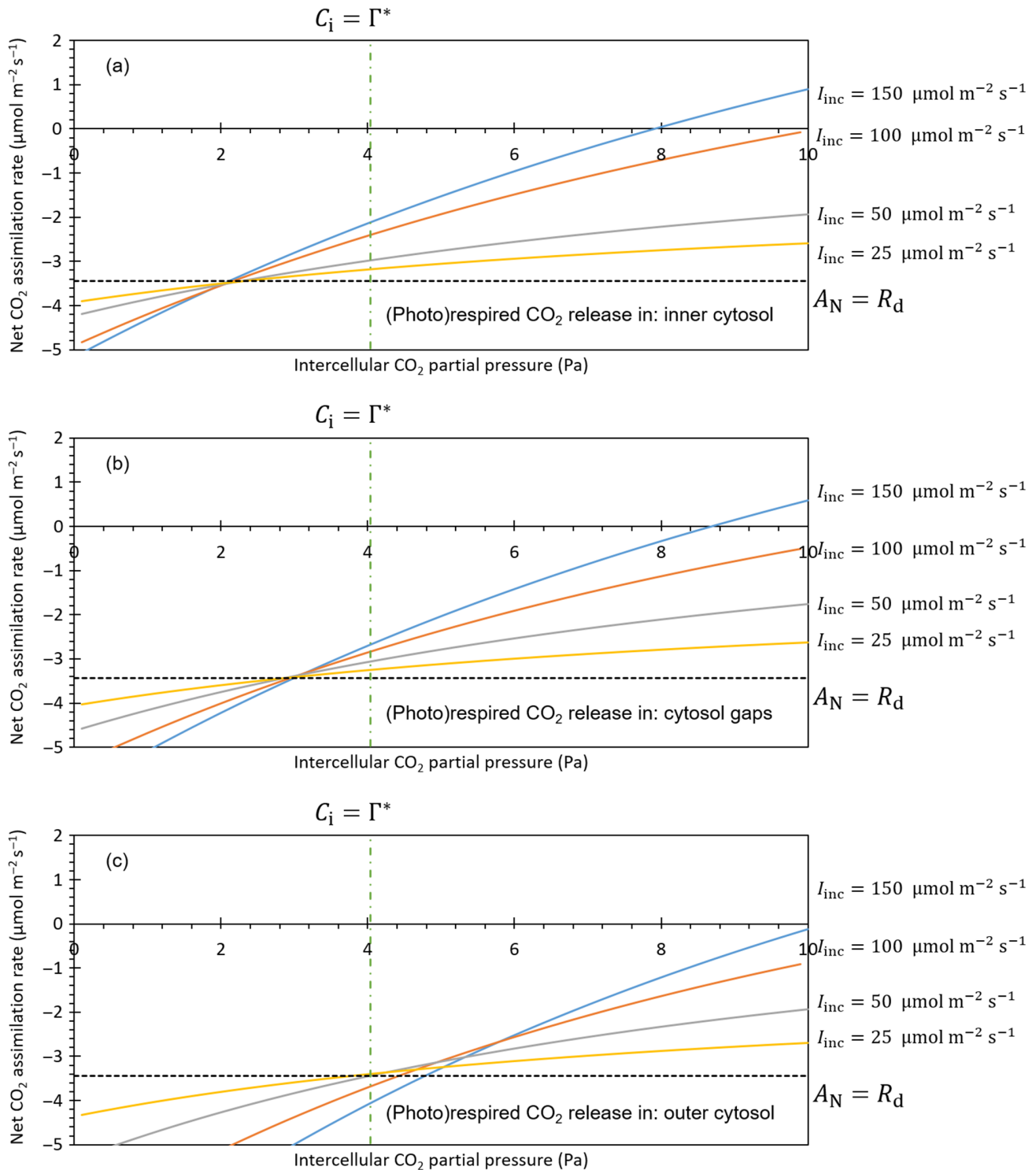
sinks, accounts for the variation of  $C_c$  with increasing irradiance. By contrast, the linear regression procedure of the Kok method or the Yin method implicitly assumes that  $C_c$  does not vary with irradiance, when applied under photorespiratory conditions. Ignoring this variation of  $C_i$  or  $C_c$  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  $\text{CO}_2$ ? For nonphotorespiratory conditions that are achieved with a very low  $\text{O}_2$  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  $\text{CO}_2$  (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  $\text{CO}_2$  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  $f_{\text{reass}}$  we estimated for the low-irradiance range (Fig. 3). The small difference in  $f_{\text{reass}}$  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  $f_{\text{reass}}$  (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  $R_{\text{dk}}$  was higher at 21% than at 2%  $\text{O}_2$  in developing





**Fig. 4** Simulated response curve of the net CO<sub>2</sub> assimilation rate ( $A_N$ ) to intercellular CO<sub>2</sub> partial pressures ( $C_i$ ) under the conditions of the Laisk method for different scenarios of (photo)respired CO<sub>2</sub> release. The solid lines represent CO<sub>2</sub> response curves simulated at different irradiances  $I_{inc}$  (150, 100, 50 and 25 μmol m<sup>-2</sup> s<sup>-1</sup>). The dashed line represents the net CO<sub>2</sub> 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 CO<sub>2</sub> compensation point ( $\Gamma^*$ ). (Photo)respired CO<sub>2</sub> 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 O<sub>2</sub> is the substrate (Tcherkez *et al.*, 2017b) and respiratory rates measured in terms of O<sub>2</sub> and CO<sub>2</sub> exchange may not be equal (Gauthier

*et al.*, 2018). However, the amount of respiratory CO<sub>2</sub> release at the low O<sub>2</sub> 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  $\Phi_2$  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 $\Phi_2$ with increased irradiance
Yin method	Does not require leaf anatomical measurements Accounts for the decrease of $\Phi_2$ 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 $\Phi_2$ 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.

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### 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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## 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 CO<sub>2</sub> 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 CO<sub>2</sub> release.

**Table S4** Akaike's information criteria for different combinations of leaf age, cultivar, photorespiratory conditions and scenarios for the release of (photo) respired CO<sub>2</sub> 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 CO<sub>2</sub> using the reaction-diffusion model and data from Ho *et al.* (2016).

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