3-D microscale modeling of CO₂ transport and light propagation in tomato leaves

enlightens photosynthesis

QUANG TRI HO¹, HERMAN N.C. BERGHUIJS²,³, RODRIGO WATTÉ¹,
PIETER VERBOVEN¹, ELS HERREMANS¹, XINYOU YIN²,³, MOGES A. RETTA¹,
BEN AERNOUTS¹, WOUTER SAEYS¹, LUKAS HELFEN⁴,⁵, GRAHAM D. FARQUHAR⁶, PAUL
C. STRUIK²,³ AND BART M. NICOLAÏ¹

² Centre for Crop Systems Analysis, Wageningen University, P.O. Box 430, 6700 AK Wageningen, The Netherlands.
³ BioSolar Cells, P.O. Box 98, 6700 AB Wageningen, The Netherlands.
⁴ IPS / ANKA, Karlsruhe Institute of Technology, PO Box 3640, 76021 Karlsruhe, Germany.
⁵ European Synchrotron Radiation Facility, 38043 Grenoble Cedex, France.
⁶ Research School of Biology, The Australian National University, Canberra, ACT 2601, Australia.

Corresponding author:
Email: bart.nicolai@biw.kuleuven.be
Tel: +32 16 322375
Fax: +32 16 322955

ABSTRACT

We present a combined 3-D model of light propagation, CO₂ diffusion and photosynthesis in tomato (Solanum lycopersicum L.) leaves. The model incorporates a geometrical representation of the actual leaf microstructure that we obtained with synchrotron radiation X-ray laminography, and was
evaluated using measurements of gas exchange and leaf optical properties. The combination of the 3-D microstructure of leaf tissue and chloroplast movement induced by changes in light intensity affects the simulated CO$_2$ transport within the leaf. The model predicts extensive reassimilation of CO$_2$ produced by respiration and photorespiration. Simulations also suggest that carbonic anhydrase could enhance photosynthesis at low CO$_2$ levels but had little impact on photosynthesis at high CO$_2$ levels. The model confirms that scaling of photosynthetic capacity with absorbed light would improve efficiency of CO$_2$ fixation in the leaf, especially at low light intensity.

**Key-words:** 3D model, light propagation, gas diffusion, photosynthesis, synchrotron radiation X-ray computed laminography, photosynthetic capacity, tomato (*Solanum lycopersicum* L.)

**INTRODUCTION**

Photosynthesis in plants involves transport of CO$_2$ from the atmosphere through the leaf to the chloroplast stroma. Understanding the mechanisms of CO$_2$ transport and leaf photosynthesis is increasingly important given efforts to increase crop yield (Long et al., 2006; Griffiths & Helliker, 2013), predict effects of climate change on photosynthetic carbon uptake (Wright et al., 2004; Bloom et al., 2010; Pons, 2012; Wheeler & von Braun, 2013), and contextualize the evolutionary drivers of photosynthesis (West-Eberhard et al., 2011; Busch et al., 2013; Griffiths & Helliker, 2013).

In photosynthesis studies, the biochemical model at the chloroplast level has been commonly scaled up to the whole leaf level by incorporating a resistance model of gas exchange. Although Farquhar *et al.* (1980) initially assumed a uniform light intensity and a constant photosynthesis capacity throughout the leaf, it was realized that this was unnecessary (Farquhar, 1989) since photosynthesis capacity could vary in proportion to light intensity inside the leaf (Terashima & Inoue, 1985; Farquhar, 1989). The profile of CO$_2$ fixation through the leaf might be affected by light penetration, CO$_2$ diffusion and leaf microstructure (Vogelmann & Evans, 2002; Evans & Vogelmann, 2003). Because light penetration in leaves is usually limited and the absorption profile follows an exponential decay, photosynthetic capacity could adapt to vary with leaf depth (Terashima & Inoue, 1985; Farquhar, 1989; Vogelmann & Evans, 2002) and, hence, affect the CO$_2$ fixation profile. However, an impact of partitioning of photosynthetic capacity within the leaf on the efficiency of CO$_2$ fixation is still far from clear.
At the cellular level, CO₂ fixation by ribulose-1,5-bisphosphate carboxylase (RuBisCo) is competitively inhibited by O₂. Under normal ambient conditions, the partial catalytic activity of RuBisCo fixing O₂ ultimately results in photorespiratory release of CO₂ in the mitochondria (Tholen et al., 2012), in addition to the CO₂ release from respiration in these organelles. Recent research has shown that only part of the (photo)respired CO₂ may diffuse into the intercellular airspace and subsequently disappear into the atmosphere, while the remainder is re-used for carboxylation (Busch et al., 2013). Consequently, the release of (photo)respired CO₂ by the mitochondria into the mesophyll cytosol and its partial reassimilation affect the estimation of mesophyll conductance \( g_m \) (Tholen et al., 2012). In addition, the spatial distribution of chloroplasts along the part of the mesophyll cell wall that is exposed to intercellular air spaces also affects light absorption, CO₂ diffusion and photosynthesis in leaves (Oguchi et al., 2005). Although chloroplasts are mainly distributed along the cell wall, they can reorient in the mesophyll cell in response to changes in light intensity within minutes (Wada et al., 2003; Tsuboi et al., 2009; Kong et al., 2013). Chloroplast movement is a common and dynamic phenomenon found in all land plants tested (Wada et al., 2003; Tsuboi et al., 2009; Kong et al., 2013). The effect of this chloroplast movement in response to light on CO₂ diffusion in the mesophyll cells and photosynthesis in leaves is, however, not well understood.

As it is difficult to experimentally investigate gas transport processes in leaves noninvasively at a high spatial resolution, computer models have been used instead. In other plant organs such as fruits, roots, and seeds, in silico experiments have shown that gas exchange critically depends on the 3-D structural arrangement of cells and tissues within the organ (Armstrong & Beckett, 2011; Ho et al., 2011; Verboven et al., 2013). The most advanced models for photosynthesis in leaves, however, have been limited to 2-D only (Ho et al., 2012), oversimplify the real microstructure (Aalto & Juurola, 2002), or consider only a single cell instead of the whole tissue (Tholen & Zhu, 2011). The importance of the 3-D architecture of the leaf and the distribution of the capacity for gas exchange, light harvesting and photosynthesis has been shown (Evans & Vogelmann, 2003; Zhou et al., 2013). We, therefore, developed and experimentally validated in this study a model that incorporates the actual 3-D leaf microstructure, and accounts for light penetration, CO₂ transport and photosynthesis. We used this model to investigate photosynthesis taking into account partitioning of the photosynthetic capacity in tomato (Solanum lycopersicum L.) leaves at a yet unseen spatial resolution.
MATERIALS AND METHODS

Leaf microstructure

Synchrotron X-ray computed microlaminography using propagation-based phase contrast (Helfen et al., 2009) was employed to obtain 3-D images of the leaf at a resolution of 0.75 µm (Verboven et al., 2015). This allowed cells to be clearly distinguished, and to visualize their size, shape and distribution through local variations in electron density (Cloetens et al., 2006; Verboven et al., 2008; Verboven et al., 2013). The experiment was performed at the parallel-beam imaging beamline ID19 (Weitkamp et al., 2010) of the European Synchrotron Radiation Facility in Grenoble, France. The 3-D data sets revealing the microstructure of tomato leaf tissues was reconstructed from a series of radiographs using a filtered back-projection algorithm (Myagotin et al., 2013). The resulting 3-D laminography images were visualised and analysed using the Avizo image-processing software (Visualization Group Sciences, France). The anatomical features of synchrotron-derived geometries were reported by Verboven et al. (2015). Since there was insufficient contrast to discriminate organelles in a cell, vacuoles and disk shaped chloroplasts were artificially created in the mesophyll cells. Mitochondria were assumed to be uniformly distributed in the cytoplasm and were not modeled explicitly. More details are provided in Supporting information, Text S1.

Gas exchange and chlorophyll fluorescence measurements

Three commercial tomato cultivars – Admiro, Doloress (both De Ruiter Seeds, Bergschenhoek, The Netherlands), and Growdena (Syngenta, Bergen op Zoom, The Netherlands) – were used for photosynthesis measurements. Plants were grown in the greenhouse research facility UNIFARM of Wageningen University under natural plus supplemental light and the photoperiod was 16 hours per day. The relative humidity was maintained at about 70% and the day/night temperature inside the greenhouse was 21/16°C during the whole growing period. Details of gas exchange and chlorophyll fluorescence measurements are given in Supporting information, Text S2. Variables and parameters are listed in Supporting information Tables S1-S2. The estimated photosynthetic parameters are given in Supporting information Tables S3.

Microscale model
The absorption of light within tomato plant leaves was computed using a meshed Monte Carlo photon transport method solved over the 3-D microstructure of a leaf. Details of the light propagation model, the model parameters and the validation are given in Supporting information Text S3, Text S4 and Table S4. The computed light absorption was used as an input to the FvCB model for describing the CO₂ fixation rate in the chloroplasts of C₃ plants as the minimum of the RuBisCo-limited carboxylation rate, the RuBP-regeneration or electron transport limited rate, and the triose phosphate utilization limited rate (Farquhar et al., 1980; Bernacchi et al., 2013) (more details in Supporting information Text S4).

Microscale diffusion of CO₂ and HCO₃⁻ in the intercellular spaces and cells was described by a set of reaction-diffusion equations:

\[ \nabla \cdot \left( D_{\text{CO}_2} \nabla [\text{CO}_2] \right) - w^* + R_{\text{f}}^* - B = 0 \]  

(1)

\[ \nabla \cdot \left( D_{\text{HCO}_3^-} \nabla [\text{HCO}_3^-] \right) + B = 0 \]  

(2)

Here, \([\text{CO}_2]\) and \([\text{HCO}_3^-]\) are the CO₂ and HCO₃⁻ concentrations, \(\nabla\) is the gradient operator, \(D_{\text{CO}_2}\) is the CO₂ diffusivity which is different in the intracellular gas phase and in the various compartments of the mesophyll cells, and \(D_{\text{HCO}_3^-}\) is the HCO₃⁻ diffusivity in the various compartments of the mesophyll cells. The second term \(w^*\) in Eq. (1) is the volumetric CO₂ consumption by RuBP carboxylation (described by the FvCB model) in the chloroplasts while the third term \(R_{\text{f}}^*\) and fourth term \(R_{\text{f}}^*\) are the volumetric CO₂ production by photorespiration and respiration in mitochondria, respectively. The photorespiration defined by half of the rate of RuBP oxygenation by RuBisCo was also accounted for by the FvCB model (Farquhar et al., 1980; Bernacchi et al., 2013). The last term \(B\) in Eqs. (1) and (2) is the net hydration rate of CO₂ to HCO₃⁻. Note that Eq. (2) applies only to cells and not to the intercellular space. The volumetric CO₂ production by respiration \(R_{\text{f}}^*\) is assumed to be constant and, hence, is independent to photorespiration.

The hydration of CO₂ to HCO₃⁻ takes place in the cytoplasm. In the absence of carbonic anhydrase (CA), the hydration rate of CO₂ is defined by:
\[ B = \dot{k}_2 [CO_2] - k_2 \left( \frac{[H^+] [HCO_3^-]}{K} \right) = \dot{k}_0 \left( [CO_2] - \frac{[H^+] [HCO_3^-]}{K_{eq}} \right) \]  

(3)

where \( k_1, k_2 \) and \( K \) are the rate constants of the CO\(_2\) to HCO\(_3^-\) conversion (for further details see Ho et al. (2011)).

The hydration of CO\(_2\) to HCO\(_3^-\) can be catalyzed by CA. Although CA may potentially affect mesophyll conductance (Evans et al., 2009), and thus photosynthesis, the supporting evidence is ambiguous (Flexas et al., 2012; Tosens et al., 2012; Tomás et al., 2013). We therefore wanted to test the hypothesis that CA activity affects photosynthesis. The net hydration rate of CO\(_2\) to HCO\(_3^-\) by CA is described as follows (Tholen & Zhu, 2011):

\[ B = \frac{k_{CA} [CA] [CO_2] - [HCO_3^-] [H^+]}{K_{eq}} \]

(4)

where \( k_{CA}, K_{eq}, [CA] \) are the turnover rate, equilibrium constant and CA concentration, respectively; \( K_{CA,CO_2} \) and \( K_{CA,HCO_3} \) are Michaelis-Menten constants of CA, for dehydration and hydration, respectively (Tholen & Zhu, 2011).

The relationship between the equilibrium CO\(_2\) concentration in the gas and liquid phase followed Henry’s law (Lide, 1999). We took into account the resistance to gas transport of the cell wall and cell membrane at the boundary of a mesophyll cell. For instance, flux \( \phi^* \) at the boundary of a mesophyll cell is described as follows:

\[ \phi^* = -P_{rw} \left( [CO_2]^*_{m} - [CO_2] \right) \]

(5)

where \([CO_2]^*_{m}\) is the equilibrium liquid CO\(_2\) concentration of the outer cell wall of the mesophyll cell. \( P_{rw} \) is the effective permeability of the membrane and the cell wall. Values of the physical properties that appear in the microscale model are given in Supporting information Table S5.

To calculate relative CO\(_2\) fixation along the leaf depth, a volumetric slice \( \Delta V(l) \) of the leaf with a thickness \( \Delta l \) and cross sectional area \( S_{leaf}(l) \) at the depth \( l \) is defined as follows:

\[ \Delta V(l) = S_{eq}(l) \cdot \Delta l \]

(6)
The thickness $\Delta l$ was set to 0.75 $\mu$m (voxel thickness of the laminography images).

The relative CO$_2$ fixation along the depth $l$ of the leaf is computed from the microscale model as:

$$R_\nu(l) = \frac{\int \nu^+ \, dV}{\int \nu^- \, dV}$$

(7)

where $V_{\text{leaf}}$ is the volume of the leaf. We used the shorthand notation $\int \nu \, dV = \int \int \int \nu(x, y, z) \, dx \, dy \, dz$. The numerator is the total CO$_2$ assimilation rate for a volumetric slice $\Delta V(l)$; the denominator is the total CO$_2$ assimilation rate of the leaf. More details are provided in Supporting Information Text S4, as well as details of the numerical solution.

RESULTS

Profiles of light and photosynthetic capacity within the leaf

To explore the distribution of the light profile, electron transport rate and CO$_2$ concentration in a photosynthesizing leaf, we first created high resolution (0.75 $\mu$m) 3-D geometrical models of tomato leaves using synchrotron radiation X-ray computed laminography (Fig. 1a). We then computed the absorption of light within these leaves using an advanced Monte Carlo photon transport model (Fig. 1b) and used the computed light absorption as input into the FvCB model for describing the CO$_2$ fixation rate in the chloroplasts.

We compared the predicted total reflectance and total transmittance of leaves by the light penetration model with measured values (Supporting information Fig. S1). The simulations clearly show that the light absorption declines significantly in the spongy mesophyll cells near the abaxial surface (Fig. 2a), and, thus, affects the electron transport rate and CO$_2$ fixation across the leaf (Fig. 3). The oscillations are due to the spatial periodicity of the position of the chloroplasts and the fact that the few palisade cells within the computational domain were lined up.

As stated before, the photosynthesis capacity may adapt to light absorption by varying with depth in order to maximise the whole-leaf CO$_2$ fixation (Farquhar, 1989). To test this hypothesis, we solved the model for three scenarios: (I) chloroplasts have the same photosynthetic capacity regardless of their position inside the leaf (‘uniform distribution’), (II) the photosynthetic capacity scales to the
absorbed irradiance (‘optimal distribution’), and (III) a distinction of the photosynthetic capacity between palisade and spongy mesophyll cells. We implemented this scaling process by assuming that the maximum potential electron transport rate, the maximum RuBisCo activity limited carboxylation and the maximum rate of triose phosphate utilisation follow the same pattern as the light absorption (a precise definition of photosynthetic capacity is included in the Supporting Information Text S5)).

The local photosynthesis capacity $P_c$ – defined as the fraction of photosynthetic capacity within a thin slice of leaf tissue at a certain depth $l$ – varies considerably with depth (Fig. 2b). $P_c$ profile is rather uniform along leaf depth for scenario (I) while $P_c$ is large in palisade mesophyll cells near the adaxial surface and declines significantly in the spongy mesophyll cells for scenarios (II) and (III). The $P_c$ profiles of the two scenarios (II) and (III) look similar to the measured photosynthetic capacity profile of spinach leaves (Sun & Nishio, 2001; Evans & Vogelmann, 2003).

**Distribution of CO$_2$ fixation along the leaf depth**

The relative CO$_2$ fixation profiles computed from the microscale model taking into account CA facilitation are shown in Fig. 3a–3c. In scenario (II), photosynthetic capacity scales with the light absorption; hence, CO$_2$ fixation is high adjacent to the adaxial surface receiving light. In scenario (I), CO$_2$ fixation occurring in half of the leaf depth near the adaxial surface is rather uniform while CO$_2$ fixation in scenario (III) is high in the palisade parenchyma and rapidly decreases at the transition between the palisade and spongy parenchyma. CO$_2$ fixation of scenario (II) is similar to the pattern of CO$_2$ fixation in spinach leaves under blue light described by Evans and Vogelmann (Evans and Vogelmann, 2003). In scenario (II), the profile obtained under 1000 $\mu$mol m$^{-2}$ s$^{-1}$ slightly differed from that under 200 $\mu$mol m$^{-2}$ s$^{-1}$ by having its maximum displaced to greater depth. This is due to CO$_2$ diffusion limitation occurring in the palisade mesophyll cells at high light intensity. Additional simulations were carried out with two different geometries of Admiro leaves (Supporting information Fig. S2a). We found that the CO$_2$ fixation rate consistently decreased from the palisade parenchyma to the spongy parenchyma.

At low light intensity of 200 $\mu$mol m$^{-2}$ s$^{-1}$, photosynthesis was mainly limited by electron
transport rate in all scenarios. The net photosynthesis rate $A$ of scenarios (II) and (III) was 7% and 3.7% higher than that of scenario (I). At a saturating light intensity of 1000 µmol m$^{-2}$ s$^{-1}$, photosynthesis in the palisade parenchyma region was mainly limited by the Rubisco rate. $A$ of scenario (II) was 0.8% higher than that of scenario (I) while $A$ of scenario (III) remained 0.5% lower than that of scenario (I). Our results indicate that scaling of photosynthesis capacity with light absorption would indeed result in improved whole-leaf photosynthesis compared with a uniform distribution, particularly under low light intensity. Plots of $A$ at different CO$_2$ levels at 21% O$_2$, $I_{inc}$ =1000 µmol m$^{-2}$ s$^{-1}$ and $T$=25°C are shown in Fig. 3d. Scenario (I) predicted a lower rate of photosynthesis at high CO$_2$ level (C$_i$>500 µmol mol$^{-1}$) of $A$-C$_i$ curve than scenarios (II) and (III).

We then compared predicted values of the net photosynthesis rate $A$ to experimentally derived ones using gas exchange measurements. For the simulations, the photosynthesis capacity scaling with light absorption (optimal distribution) was chosen (Supporting information Fig. S3). We found a good agreement between predicted and measured values of $A$ for a wide range of intercellular CO$_2$ concentrations (0-1500 µmol mol$^{-1}$) at 21% O$_2$ and an irradiance $I_{inc}$ of 1000 µmol m$^{-2}$ s$^{-1}$ in different leaves (Fig. 4). $A$ rapidly increases with an increase in CO$_2$ concentration at low CO$_2$ levels, but saturates at high CO$_2$ levels for the three different cultivars.

**CO$_2$ exchange within the leaf**

Fig. 1c shows the simulation results of 3-D microscale CO$_2$ gas transport in a tissue sample. Since the epidermis has a low permeability due to the absence of intercellular spaces, import of CO$_2$ occurs mostly through stomata. An almost uniform CO$_2$ concentration was found within the intercellular space except in unconnected pores. Gradients in the intercellular spaces mainly existed at the boundaries between mesophyll cells and pores (Supporting information Fig. S4). Inside the leaf, only one palisade layer was observed with long cylindrical cells embedded beneath the adaxial epidermis occupying about 45% of the thickness of the mesophyll cell layer (Fig. 1a). The oblong palisade cells with thin lateral cell walls, while lined up in parallel, only touched neighboring cells slightly so that most of their surface area was exposed to the intercellular air space. The spongy mesophyll was loosely packed and had large, highly interconnected intercellular spaces that facilitate gas diffusion.
The low CO₂ concentration in the mesophyll cells, and in the chloroplast clusters in particular, was due to CO₂ assimilation. Low CO₂ concentrations can also be observed where cells touch each other (Figs. 1c, d).

In the palisade mesophyll cells touched each other (Fig. 1c), decreasing both the exchange surface as well increasing the diffusion path length. In addition, CO₂ fixation was high in this region (Fig. 5a). As a consequence, the CO₂ concentration decreased (Fig. 5b). The spongy mesophyll was loosely packed and the intercellular space was thus large and highly interconnected so that the CO₂ fixation rate was limited by the electron transport rate, rather than by diffusion. The distribution of the photosynthetic activity along the leaf depth had a significant effect on the CO₂ profile and fixation across leaves. In scenario (I), the CO₂ fixation of the palisade mesophyll cell was about 50-53% of the total leaf CO₂ fixation. However, the value was about 65-69% when photosynthetic capacity scales with light absorption (scenario (II)). In the other extreme case (scenario (III)), the CO₂ fixation of the palisade mesophyll cell was about 69-72% of the total leaf CO₂ fixation.

Effect of CA on CO₂ transport and photosynthesis

To evaluate the effect of CA activity we carried out simulations with and without CA facilitation (Fig. 5c & 5d). The chloroplast concentration \( C_c \) was up to 9% larger when CA was included (Fig. 5d). This implies that CA significantly facilitates cellular CO₂ diffusion. At CO₂ levels below 500 µmol mol⁻¹ the net photosynthesis rate computed from the model with CA was up to 9.6% larger than that computed from the model without CA. However, the difference in net photosynthesis between the model with and without CA was less than 4% at high CO₂ levels \((C_c > 600 \text{ µmol mol}^{-1})\) when the response of photosynthesis to \( C_c \) leveled off (Fig. 5c, Fig. S5, Supporting information). These results suggest that CA helps to maintain a high rate of photosynthesis only when the internal CO₂ level is reduced.

Effect of chloroplast position as affected by light intensity on CO₂ profile and photosynthesis within the leaf
Although chloroplasts are mainly distributed along the cell wall, they may move away from the light along the anticlinal cell walls of the mesophyll cell to avoid photodamage of the photosynthetic machinery when the light intensity is high. At low light intensities, they move towards the light by gathering along the periclinal cell wall in palisade cells, hence reducing the $S_c/S_m$ ratio and maximizing photon harvest (Kasahara et al., 2002; Kong et al., 2013). Here, $S_c$ and $S_m$ are the total chloroplast and mesophyll surface area exposed to the intercellular space, respectively (Supporting information Table S2). We wanted to test whether the chloroplast position in the mesophyll cell would affect the net photosynthesis rate in tomato leaves using the microscale model. We thus solved the model equations in the same lower leaf geometry of cv. Admiro for three assumed scenarios: (a) $I_{inc} = 65 \, \mu\text{mol m}^{-2}\, \text{s}^{-1}$ in combination with a low $S_c/S_m$ value (0.41), (b) $I_{inc} = 1000 \, \mu\text{mol m}^{-2}\, \text{s}^{-1}$ in combination with an intermediate $S_c/S_m$ value (0.76), and (c) $I_{inc} = 2000 \, \mu\text{mol m}^{-2}\, \text{s}^{-1}$ in combination with a high $S_c/S_m$ value (0.90) (Supporting information Fig. S6). In all scenarios, the volume fraction of chloroplasts to the mesophyll cells was constant at 0.24 (See Supporting information Text S1); so, the different $S_c/S_m$ scenarios were due to a different chloroplast position in response to a difference in light intensity. In addition, simulations with a constant $S_c/S_m$ value (0.76) at different light intensities from 65 \, \mu\text{mol m}^{-2}\, \text{s}^{-1} to 2000 \, \mu\text{mol m}^{-2}\, \text{s}^{-1} were carried out to separate the effect of light intensity and chloroplast position. At a low $S_c/S_m$ ratio (0.41), a large CO$_2$ gradient in the palisade mesophylls was found (Supporting information Fig. S7). When the chloroplasts were mainly exposed to intercellular air spaces ($S_c/S_m$ ratio of 0.76 and 0.90), the CO$_2$ gradient inside the leaf reduced. The model predicted that under high light conditions ($I_{inc} = 2000 \, \mu\text{mol m}^{-2}\, \text{s}^{-1}$), the net photosynthesis with $S_c/S_m$ of 0.90 was only 0.7% larger than the one with $S_c/S_m$ of 0.76. The results indicated not much difference in net photosynthesis in the range of $S_c/S_m$ from 0.76 to 0.90. Under low light intensity ($I_{inc} = 65 \, \mu\text{mol m}^{-2}\, \text{s}^{-1}$) with the 0.41 $S_c/S_m$ scenario the model underestimated the net photosynthesis, while the model overestimated the net photosynthesis rate with constant $S_c/S_m$ (0.76) (Fig. 6a). The experimental and simulation results suggest that the photosynthesis is highly affected by chloroplast arrangement inside the mesophyll cell. At low light intensity of 65 \, \mu\text{mol m}^{-2}\, \text{s}^{-1}, the chloroplast distribution might have $S_c/S_m$ between 0.41 and 0.76.
The CO₂ fixation in the palisade mesophylls is in all cases much larger than that of spongy mesophylls (Figs. 6c & d). The model with a higher $S_c/S_m$ would increase photosynthesis even at low light intensity.

**Mitochondrial CO₂ is recycled**

The release of (photo)respired CO₂ by the mitochondria into the mesophyll cytosol and its partial reassimilation affect the CO₂ profile within the leaf. As respiratory CO₂ release is usually assumed to be constant across irradiance and CO₂ levels, we carried out simulations in two contrasting conditions of photorespiratory CO₂ release (21% O₂ versus 2% O₂). At 21% O₂, the partial catalytic activity of RuBisCo fixing O₂ is accompanied by a significant CO₂ production in the cytosol, the latter incorporating the mitochondria in the simulation. The simulation results indicate that at 21% O₂ the CO₂ concentration gradients inside the mesophyll cells were smaller than at 2% O₂ (Figs. 1c, d). At an atmospheric CO₂ concentration of 350 µmol mol⁻¹ and $I_{inc}$=1000 µmol m⁻² s⁻¹, the average CO₂ concentration gradient from intercellular space to chloroplasts was reduced by 23% at 21% O₂ ($\Delta C = 48.8$ µmol mol⁻¹) compared with values at 2% O₂ ($\Delta C = 63.7$ µmol mol⁻¹). We additionally simulated an *in silico* experiment with ambient air containing $^{13}$CO₂ to quantify recycling of (photo)respired CO₂ (Supporting information Fig. S8). Surprisingly, we found that as much as 61.7% of (photo)respired CO₂ was reassimilated by RuBisCo at $S_c/S_m$ of 0.76. Photorespiration mainly occurred in the palisade mesophyll where RuBisCo capacity was large and $^{13}$CO₂ concentration was low. *In silico* simulations with air containing $^{13}$CO₂ were also carried out for lower and higher $S_c/S_m$ values (0.41 and 0.90). For an ambient concentration of 350 µmol mol⁻¹ CO₂ and 21% O₂, reassimilation of (photo)respired CO₂ decreased to 36.5% when $S_c/S_m$ was 0.41. At the high $S_c/S_m$ value (0.90), the recycling of (photo)respired CO₂ increased to 62.1%.

**DISCUSSION**

Photosynthesis inside a leaf may be affected by light penetration, CO₂ diffusion and leaf
microstructure. Most previous photosynthetic studies so far were based on a simple internal conductance/resistance model (Evans et al., 2009; Tholen et al., 2012). These models usually do not consider the spatial nature of diffusion explicitly but incorporate such features in apparent parameters such as conductances; even if they do (Tholen & Zhu, 2011) they assume an idealised geometrical representation for a single mesophyll cell rather than the actual microscale geometry of the tissue as in our model. Therefore they provide less detailed information about the actual CO₂ transport process and its consequences for photosynthesis.

Using synchrotron radiation X-ray computed laminography we obtained high resolution (0.75 μm) 3-D images of tomato leaves which we then used for discretizing and solving the light propagation and CO₂ gas exchange models. The simulations show that the 3-D microscale topology of leaf tissue including the variable distribution of the photosynthetic capacity along the leaf depth affects gas exchange and photosynthesis considerably. Note that a previously published 2-D model of CO₂ diffusion showed a lack of interconnectivity among the pores and could not fully capture gas transport through and from discrete stomata (Ho et al., 2012). The CO₂ concentration inside mesophyll cell obtained from the 2-D model was lower than that of the 3-D model and, therefore, the 2D model underestimated photosynthesis (Fig. S9). The 3-D model in this study that incorporates the actual 3-D leaf microstructure provides more mechanistic insight into gas exchange and photosynthesis.

Leaves have been proposed and shown to adjust to changes in the external environment through changes in their biochemistry (Terashima & Inoue, 1985; Chow & Anderson, 1987; Farquhar, 1989; Ogren & Evans, 1993). The photosynthetic capacity has been shown to be high at the mesophyll parenchyma adjacent to the adaxial surface receiving light, then decreases strongly and finally levels off (Sun & Nishio, 2001; Evans & Vogelmann, 2003). Palisade mesophyll tends to have a higher photosynthetic capacity than spongy mesophyll (Terashima & Inoue, 1985). Note that photosynthetic properties of chloroplasts should be similar within a cell but might be different among cell layers (Terashima et al., 2006). The present analysis assumed constant resistance to diffusion of CO₂ across chloroplasts. At a smaller scale, ultrastructural differences between sun and shade chloroplasts in Alocasia adapted to high and low light intensities were reported (Chow et al., 1988). At high light intensity, a large proportion of the chloroplast area is stroma, consistent with a high content of
Rubisco. At the other extreme, at low light intensity, chloroplasts possess large granal stacks (Chow et al., 1988). The clear gradient of the photosynthetic capacity of chloroplasts in the leaf is similar to sun and shade plant chloroplasts adapting to the light environment within a canopy (Terashima & Inoue, 1985). Our simulation results confirm that scaling of the photosynthetic capacity with the light absorption efficiently improves photosynthetic productivity.

Variation of the photosynthetic capacity within leaves shows a significant effect on the pattern of CO₂ and carbon fixation across leaves. When the photosynthetic activity scaled with the light absorption, simulation results showed that CO₂ fixation rate in the palisade parenchyma region accounted for 68% and 65% of total CO₂ fixation rate at $I_{inc}$ of 200 μmol m⁻² s⁻¹ and 1000 μmol m⁻² s⁻¹, respectively. The CO₂ fixation profile does not fully match the photosynthetic activity profile due to CO₂ diffusion limitation. The total CO₂ fixation rate at $I_{inc}$ equal to 200 μmol m⁻² s⁻¹ and 1000 μmol m⁻² s⁻¹ were 10.3 μmol m⁻² s⁻¹ and 12.7 μmol m⁻² s⁻¹, respectively.

The benefits of CA activity with respect to photosynthesis have been suggested before (Cowan, 1986; Evans et al., 2009). Earlier studies (Price et al., 1994; Williams et al., 1996) found only a minor effect of chloroplastic CA levels on photosynthetic performance. Tosens et al. (2012) and Tomas et al. (2013) concluded that CA facilitation did not play an important role in the mesophyll diffusion resistance. However, CA-mediated diffusion was illustrated in some leaves of tobacco, soybean and oak (Gillon & Yakir, 2000). In our simulations, we found that CA significantly facilitated CO₂ diffusion in chloroplasts and, hence, affected the CO₂ level in the stroma at low CO₂ levels but not at high levels as hypothesized by Studer et al. (2014). Gillon & Yakir (2000) proposed that the relative contribution of CA to photosynthetic efficiency may be species dependent and not always clear. Indeed, CA was shown not to limit photosynthesis in leaves of the C₄ plant Zea mays at ambient CO₂ levels (Studer et al., 2014).

The chloroplast distribution along the mesophyll cell wall represented by the $S_c/S_m$ ratio may not only depend on the light intensity, but also on the shape of the mesophyll cells (for example, the shape of palisade mesophyll cells obtained from shade-grown or sun-grown leaves). Oblong palisade cells of sun-grown leaves having a large surface of lateral cell walls adjacent to intercellular air spaces have been shown to increase their $S_c/S_m$ ratio under high light intensity (Oguchi et al., 2005), thereby
improving \( \text{CO}_2 \) diffusion and photosynthesis within the leaf. Oguchi et al. (2005) observed that the fraction of mesophyll cell surface covered by chloroplasts \((S_c/S_m)\) increased following transfer to high irradiance levels, which diminished the path length for \( \text{CO}_2 \) diffusion. They concluded that an increase in \( S_c \) was a primary factor leading to an increase in photosynthesis capacity after transfer to high irradiance levels, and, hence, increased photosynthesis. However, Tholen et al. (2008) found that a sudden increase in light intensity initially leads to a smaller \( S_c \) to protect chloroplasts from photodamage, and, hence, decreases the internal conductance for \( \text{CO}_2 \) in \textit{Arabidopsis thaliana} leaves. The results reported by Oguchi et al. (2005) contradict those of Tholen et al. (2008). To explain this contradiction, Tholen et al. (2008) suggested that a possible decrease in \( S_c \) due to a chloroplast avoidance response is specific to shade-grown leaves. The \textit{A. thaliana} leaves used by Tholen et al. (2008) had only one layer of fairly round palisade cells. The lateral cell walls of round-shaped palisade cells in \textit{A. thaliana} leaves described by Tholen et al. (2008) were observed to be in contact with neighboring cells, whereas the basal cell walls of mesophyll cells were mainly adjacent to intercellular air spaces. In such a leaf, the face position of the chloroplast distribution would result in a larger \( S_c \) compared with the profile position. On the other hand, sun-grown leaves could have large surfaces of lateral cell walls adjacent to intercellular airspaces characterized by one or multiple layers of oblong palisade cells (Oguchi et al., 2005). Therefore, the profile position of chloroplast in such leaves is more likely to result in a larger \( S_c \) compared with the face position. In our tomato leaves, the typical length of oblong palisade cells was twice that of the spongy cells. Only a few internal air spaces neighboring the boundary periclinal cell walls of the palisade cells touched the adaxial epidermis while intercellular airspaces were mainly adjacent to the lateral cell walls in the palisade parenchyma region (See Supporting information Fig. S4). A larger \( S_c/S_m \) ratio (0.76 and 0.90) implies that chloroplasts gather along the lateral sides of the palisade cells that are exposed to the intercellular air spaces, corresponding to a profile rather than face distribution.

Our simulation results suggest that \( \text{CO}_2 \) drawdown from the intercellular spaces to the chloroplasts \((C_i - C_c)\) might partially change due to chloroplast repositioning in response to light. At low light intensities, they might move towards the light by gathering along the periclinal cell wall in palisade cells (Chow et al., 1988; Kasahara et al., 2002; Kong et al., 2013). When the light intensity is
High, chloroplasts could move away from the light along the anticlinal cell walls of the mesophyll cell to avoid photodamage of the photosynthetic machinery. Since the lateral walls of typical oblong palisade cells in tomato leaf are largely exposed to intercellular air spaces, such movements at high light level would increase the $S_c/S_m$ ratio, and hence improve photosynthesis. For a high $S_c/S_m$ ratio, the model predicted a very low concentration gradient in the chloroplasts. However, chloroplast positioning in response to changes of the light intensity might be species specific. It needs to be quantified by advanced measurement techniques.

Our model predicts that 36.5-62.1% of (photo)respired CO$_2$ is reassimilated by RuBisCo at $S_c/S_m$ values from 0.41 to 0.9. Note that mitochondria were not explicitly modelled as a separate compartment because this would require a very fine computational mesh and thus an excessive amount of computer time to solve the diffusion model. The mitochondria were, instead, considered as a continuum and merged with the cytoplasm. Since mitochondria are often partially or fully enveloped by chloroplasts, our model might thus even underestimate recycling of photorespired CO$_2$. In contrast, Haupt-Herting et al. (2001) found that 23-29% of (photo)respired CO$_2$ was recycled by RuBisCo in tomato leaves when assuming that the $^{12}$CO$_2$ assimilation rate was equal to the product of $^{13}$CO$_2$ assimilation rate and the ratio of the intercellular concentration of $^{12}$CO$_2$ and $^{13}$CO$_2$. However, they also assumed implicitly that the latter is equal to the ratio of the chloroplast concentration of $^{12}$CO$_2$ and $^{13}$CO$_2$ which is highly unlikely as predicted by our model. Our simulations predicted that the ratio of $^{12}$CO$_2$ and $^{13}$CO$_2$ concentration in the chloroplasts was 1.7 fold higher than that in the intercellular space. This might explain the lower percentage of CO$_2$ recycling calculated by Haupt-Herting et al. (2001). Busch et al. (2013) found that the overall CO$_2$ reassimilation at a CO$_2$ concentration of 350 μmol mol$^{-1}$ was 46-51%. They did not measure the $^{12}$CO$_2$ photorespiration rate and $^{12}$CO$_2$ release rate from the leaf directly, but calculated it by extrapolation of the CO$_2$ fluxes derived from measured $^{12}$CO$_2$ concentrations which may explain the discrepancy with our calculations. Note that a high $S_c/S_m$ (0.9) slightly increased the $^{13}$CO$_2$ assimilation rate in the chloroplasts and, hence, slightly decreased photorespiration. This could be explained by a competition
between CO₂ and O₂ for RuBisCo. A slight reduction of the photorespiration rate and a leakage of ¹²CO₂ from the chloroplasts to the intercellular air spaces by CA caused a slight decrease of the (photo)respired CO₂ recycling, thus apparently cancelling the increase of photo(respired) CO₂ recycling at high $S_c/S_m$. A similar trend of decreasing photo(respired) CO₂ recycling with increasing CO₂ level was found by Busch *et al.* (2013). The simulations support the hypothesis that exposing chloroplasts to the intercellular space improves the trapping CO₂ mechanism (Sage & Sage, 2009; Busch *et al.*, 2013). The profile distribution of chloroplasts with a high $S_c/S_m$ thus results in an efficient trapping ability of (photo)respired CO₂ released from mitochondria under conditions favorable to photorespiration.

Our simulations were all done on the same section of a single leaf. Extending the simulation to a whole leaf was not possible because the field of view of the synchrotron laminography images was not sufficiently large to image the whole leaf while maintaining the required spatial resolution; also the amount of computer time necessary to solve the model over such a large computational domain would be prohibitively expensive. We carried out additional simulations with sections of three different leaves to investigate how representative these simulations were and obtained quite similar results indeed (Supporting information Fig. S2b). The model did not account for the presence of vascular bundles. Verboven *et al.* (2015) found that they occupied as much as 10.5% of the total cross-section area of 3-D images generated by synchrotron laminography. The vascular bundles were clearly surrounded by spongy mesophyll cells and connected to palisade mesophyll cells located at the upper side next to the adaxial epidermis (Verboven *et al.*, 2015). The presence of vascular bundles would likely affect the local gas exchange, and future models should incorporate vascular bundles to explain their effect on leaf photosynthesis.

In conclusion, our results demonstrate that the complex system of leaf photosynthetic machinery can be unraveled by micro-mechanistic modeling of the leaf apparatus. The results open up new possibilities for *in silico* approaches to improving insights into leaf carbon uptake and to predicting climatic impacts on crop yield and vegetation.

**REFERENCES**

cells of a silver birch leaf. *Plant, Cell and Environment* 1399–1409


Acknowledgements

The authors thank the Research Council of the K.U. Leuven (OT 12/055) and the Research Fund Flanders (project G.0645.13) and the ARC Centre of Excellence for Translational Photosynthesis for financial support. The authors thank R.K. Thapa for his contribution to data collection. Rodrigo Watté is funded by a PhD grant from the Agency for Innovation by Science and Technology (IWT Flanders – project SB 101552). Synchrotron X-ray laminography was performed at the ESRF (Grenoble, France) by means of a beam time grant (experiment EC687). We would like to thank Prof. John Evans and Prof. Wah Soon Chow for helpful discussions on profiles of light absorption and photosynthetic capacity within the leaf, and Dr. Florian A. Busch for helpful discussions on photorespiration and reassimilation of photorespired and respired CO$_2$.

Figure legends

Figure 1. Microscale geometry and simulated CO$_2$ distribution in a lower tomato leaf (cv. Admrio).

(a) Reconstructed 3-D microscale geometry based on synchrotron radiation X-ray computed laminography. Chl, chloroplasts; Cyt, cytosol; E, epidermis; Vac, vacuole. (b) Light absorbance of leaf tissue with color bar on the right indicating the logarithm of the fraction of the irradiance absorbed by the leaf. The fraction of absorbed photons was calculated on cubical elements with edges of 0.75 µm. (c) shows the CO$_2$ distribution in the mesophyll cells at 21% O$_2$. (d) visualizes the CO$_2$ distribution in the mesophyll cells at 2% O$_2$. The color bar below (c) and (d) indicates the CO$_2$ concentration (µmol mol$^{-1}$). In all simulations, $C_a = 350$ µmol mol$^{-1}$, $I_{inc} = 1000$ µmol m$^{-2}$ s$^{-1}$ and $T = 25^\circ$C, and the $S_e/S_m$ ratio was 0.76. Large gradients of the CO$_2$ concentration are visible, especially at 2% O$_2$. For the simulations the optimal distribution of the photosynthetic capacity (scenario II) inside
the leaf was assumed (See Supporting information Text S5).

**Figure 2.** Simulated fraction of absorbed photons (a) and distribution of photosynthetic capacity (b) (see Supporting information Text S5 for its definition) along the depth of an Admiro tomato lower leaf. For the simulation the same light spectrum was used as for the measurements (10% at 470 nm, 90% at 665 nm), and the $S_c/S_m$ ratio was 0.76. In panel (b), three scenarios were assumed for distribution of photosynthetic capacity: scenario (I) uniform distribution, scenario (II) optimal distribution, and scenario (III) distinct photosynthetic capacity between palisade and spongy mesophyll cells (See Supporting information Text S5). The arrows (1), (2) and (3) in panels (a) and (b) indicate the transition from adaxial epidermis to palisade parenchyma, from palisade parenchyma to spongy parenchyma, and from spongy parenchyma to abaxial epidermis, respectively.

**Figure 3.** Distribution of relative CO$_2$ fixation rate along the depth and simulated $A$-$C_i$ curves of an Admiro tomato lower leaf for scenario (I) (uniform distribution, panel (a)), scenario (II) (optimal distribution, panel (b)), and scenario (III) (distinct photosynthetic capacity of palisade and spongy mesophyll cells, panel (c)) (Supporting information Text S5). Simulations were carried out at 350 µmol mol$^{-1}$ CO$_2$, 21% O$_2$, $I_{inc}$ of 200 and 1000 µmol m$^{-2}$ s$^{-1}$ and $T=25^\circ$C, and the $S_c/S_m$ ratio was 0.76. Panels (d) Simulated $A$-$C_i$ curves with different scenarios at 21% O$_2$, $I_{inc}$=1000 µmol m$^{-2}$ s$^{-1}$ and $T=25^\circ$C. The symbols (o) and the lines represent the measured data and model predictions, respectively. The error bars represent the standard error ($n = 4$).

**Figure 4.** Simulations versus measurements of the net rate of whole-leaf photosynthesis $A$ (a, b, c) of different leaves at different CO$_2$ levels at 21% O$_2$, $I_{inc}$=1000 µmol m$^{-2}$ s$^{-1}$ and $T=25^\circ$C. The symbols (o) represent the measured data. The error bars represent the standard error ($n = 4$). The solid (—) lines represent model predictions. Panels (a), (b) and (c) represent the results of Admiro, Doloress and Growdena lower leaves, respectively. The corresponding geometrical models had an $S_c/S_m$ ratio of 0.76, 0.71 and 0.71, respectively. Optimal distribution of the photosynthetic capacity inside the leaf was assumed in the simulations.

**Figure 5.** (a) and (b) CO$_2$ fixation rate $W$ and CO$_2$ drawdown from intercellular spaces to the chloroplasts ($C_i$-$C_c$) in palisade, spongy mesophyll and whole leaf, respectively. (c) and (d) Comparison of net rate of photosynthesis $A$, and CO$_2$ drawdown from intercellular spaces to the
chloroplasts with and without CA facilitation. Simulations were carried out for Admiro lower leaves at different CO$_2$ levels at 21% O$_2$, $I_{zz} = 1000$ μmol m$^{-2}$ s$^{-1}$ and $T=25^\circ$C, and the $S_c/S_m$ ratio was 0.76.

In panels (c) and (d), the symbols (o) represent the measured data while the solid (——) lines and dashed (---) lines represent model predictions with and without CA facilitation. Optimal distribution of photosynthetic capacity inside the leaf was assumed in the simulations. The error bars represent the standard error ($n=4$).

**Figure 6.** Photosynthesis in response to incident light irradiation $I_{inc}$ in tomato leaves (cv. Admiro). (a) and (b) net photosynthesis $A$ and CO$_2$ drawdown from intercellular spaces to the chloroplasts ($C_i$-$C_c$) as a function of $I_{inc}$. Symbol (o) in panel (a) shows the measurement while symbols (+) and (×) represent the model with variable $S_c/S_m$ ($S_c/S_m = 0.41, 0.76$ and $0.90$) and constant $S_c/S_m$ ($0.76$). $I_{inc}$ was 65, 500, 1000 and 2000 μmol m$^{-2}$ s$^{-1}$, respectively. Panel (c) and (d) show the CO$_2$ fixation rate $W$ of the whole leaf, palisade and spongy mesophyll for both variable and constant $S_c/S_m$. Optimal distribution of photosynthetic capacity inside the leaf was assumed in the simulations. The error bars represent the standard error ($n=4$).

**Supporting information**

**Text S1** Geometrical model of tomato leaf

**Text S2** Gas exchange and chlorophyll fluorescence measurements

**Text S3** Light penetration measurement

**Text S4** Models for light penetration, photosynthesis kinetics and CO$_2$ diffusion

**Text S5** Partitioning of photosynthesis capacity along the leaf depth

**Text S6** Recycling of CO$_2$

**Figure S1.** Comparison of measured (solid lines) and simulated (stars) transmittance ($M_t$) and reflectance ($M_r$) spectra for five tomato leaves (cv. Growdena).

**Figure S2.** Simulated distribution of relative CO$_2$ fixation rate along the depth (a) and $A$-$C_i$ curves (b) of three different geometries of Admiro leaves.

**Figure S3.** Relative photosynthetic capacity along the depth and intracellular CO$_2$ distribution of
different tomato leaves.

**Figure S4.** CO$_2$ distributions in the air phase (a) and mesophyll cells (b) of Admiro lower leaf.

**Figure S5** Simulations of the net rate of whole-leaf photosynthesis $A$ with and without CA facilitation of different leaves at different CO$_2$ levels at 21% O$_2$, $I_{\text{PAR}}=1000$ μmol m$^{-2}$ s$^{-1}$ and $T=25^\circ$C.

**Figure S6.** Microscale geometry of Admiro lower leaf generated with different chloroplast distributions.

**Figure S7.** Simulated CO$_2$ distribution in response to different levels of incident light in lower leaves (cv. Admiro).

**Figure S8.** Simulated $^{12}$CO$_2$(a) and $^{13}$CO$_2$ (b) distribution in a tomato leaf (cv. Admiro).

**Figure S9.** 2D model versus 3D model.

**Table S1.** List of model variables, their symbols, definitions and units

**Table S2.** List of model parameters, their symbols, definitions and units

**Table S3.** Estimated photosynthetic parameters (± standard error if applicable) of leaves of different tomato cultivars and leaf ages.

**Table S4.** Computed optical properties of the different compartments of the leaf model.

**Table S5.** Physical parameters of the microscale gas exchange model.
Microscale geometry and simulated CO$_2$ distribution in a lower tomato leaf (cv. Admiro). (a) Reconstructed 3-D microscale geometry based on synchrotron radiation X-ray computed laminography. Chl, chloroplasts; Cyt, cytosol; E, epidermis; Vac, vacuole. (b) Light absorbance of leaf tissue with color bar on the right indicating the logarithm of the fraction of the irradiance absorbed by the leaf. The fraction of absorbed photons was calculated on cubical elements with edges of 0.75 µm. (c) shows the CO$_2$ distribution in the mesophyll cells at 21% O$_2$. (d) visualizes the CO$_2$ distribution in the mesophyll cells at 2% O$_2$. The color bar below (c) and (d) indicates the CO$_2$ concentration (µmol mol$^{-1}$). In all simulations, $C_a = 350$ µmol mol$^{-1}$, $I_{inc} = 1000$ µmol m$^{-2}$ s$^{-1}$ and $T = 25°C$, and the $Sc/Sm$ ratio was 0.76. Large gradients of the CO$_2$ concentration are visible, especially at 2% O$_2$. For the simulations the optimal distribution of the photosynthetic capacity (scenario II) inside the leaf was assumed (See Supporting information Text S5).

160x161mm (72 x 72 DPI)
Simulated fraction of absorbed photons (a) and distribution of photosynthetic capacity (b) (see Supporting information Text S5 for its definition) along the depth of an Admiro tomato lower leaf. For the simulation the same light spectrum was used as for the measurements (10% at 470 nm, 90% at 665 nm), and the $Sc/Sm$ ratio was 0.76. In panel (b), three scenarios were assumed for distribution of photosynthetic capacity: scenario (I) uniform distribution, scenario (II) optimal distribution, and scenario (III) distinct photosynthetic capacity between palisade and spongy mesophyll cells (See Supporting information Text S5). The arrows (1), (2) and (3) in panels (a) and (b) indicate the transition from adaxial epidermis to palisade parenchyma, from palisade parenchyma to spongy parenchyma, and from spongy parenchyma to abaxial epidermis, respectively.
Distribution of relative CO\textsubscript{2} fixation rate along the depth and simulated A-C\textsubscript{i} curves of an Admiro tomato lower leaf for scenario (I) (uniform distribution, panel (a)), scenario (II) (optimal distribution, panel (b)), and scenario (III) (distinct photosynthetic capacity of palisade and spongy mesophyll cells, panel (c)) (Supporting information Text S5). Simulations were carried out at 350 µmol mol\textsuperscript{-1} CO\textsubscript{2}, 21% O\textsubscript{2}, I\textsubscript{inc} of 200 and 1000 µmol m\textsuperscript{-2} s\textsuperscript{-1} and T=25°C, and the Sc/Sm ratio was 0.76. Panels (d) Simulated A-C\textsubscript{i} curves with different scenarios at 21% O\textsubscript{2}, 1000 µmol m\textsuperscript{-2} s\textsuperscript{-1} and T=25°C. The symbols (o) and the lines represent the measured data and model predictions, respectively. The error bars represent the standard error (n = 4).
Simulations versus measurements of the net rate of whole-leaf photosynthesis $A$ (a, b, c) of different leaves at different CO$_2$ levels at 21% O$_2$, $I_{inc}=1000$ μmol m$^{-2}$ s$^{-1}$ and T=25°C. The symbols (o) represent the measured data. The error bars represent the standard error ($n=4$). The solid (―) lines represent model predictions. Panels (a), (b) and (c) represent the results of Admiro, Doloress and Growdena lower leaves, respectively. The corresponding geometrical models had an Sc/Sm ratio of 0.76, 0.71 and 0.71, respectively. Optimal distribution of the photosynthetic capacity inside the leaf was assumed in the simulations.
(a) and (b) CO2 fixation rate $W$ and CO2 drawdown from intercellular spaces to the chloroplasts ($C_i-C_c$) in palisade, spongy mesophyll and whole leaf, respectively. (c) and (d) Comparison of net rate of photosynthesis $A$, and CO2 drawdown from intercellular spaces to the chloroplasts with and without CA facilitation. Simulations were carried out for Admiro lower leaves at different CO2 levels at 21% O2, $I_{inc}=1000 \, \mu$mol m$^{-2}$ s$^{-1}$ and $T=25^\circ$C, and the $Sc/Sm$ ratio was 0.76. In panels (c) and (d), the symbols (o) represent the measured data while the solid (―) lines and dashed (—) lines represent model predictions with and without CA facilitation. Optimal distribution of photosynthetic capacity inside the leaf was assumed in the simulations. The error bars represent the standard error ($n=4$).

ScholarOne Image Spy version 007
Photosynthesis in response to incident light irradiation $I_{\text{inc}}$ in tomato leaves (cv. Admiro). (a) and (b) net photosynthesis $A$ and CO$_2$ drawdown from intercellular spaces to the chloroplasts ($C_i-C_e$) as a function of $I_{\text{inc}}$. Symbol (o) in panel (a) shows the measurement while symbols (+) and (×) represent the model with variable $Sc/Sm$ ($Sc/Sm = 0.41, 0.76$ and $0.90$) and constant $Sc/Sm$ ($0.76$). $I_{\text{inc}}$ was $65, 500, 1000$ and $2000$ μmol m$^{-2}$ s$^{-1}$, respectively. Panel (c) and (d) show the CO$_2$ fixation rate $W$ of the whole leaf, palisade and spongy mesophyll for both variable and constant $Sc/Sm$. Optimal distribution of photosynthetic capacity inside the leaf was assumed in the simulations. The error bars represent the standard error ($n = 4$).