# S5 Text. The impact of simplifications in the leaf geometry and transport processes on $A\_{N}$ and $f\_{rec}$

The model described in the main text of this manuscript makes various simplifications about both the leaf structure and the processes that take place in the leaf. These simplifications are:

1). It is assumed that (photo)respiration takes place in a cytosol compartment, rather than a loose mitochondrion in this compartment.

2). It is assumed that the light absorption does not vary with the leaf depth.

3). It is assumed that there is full CO2 transport facilitation by carbon anhydrase.

4). It is assumed that $q$, $S\_{m}/S$, $S\_{c}/S\_{m}$, $t\_{wall}$, $t\_{cyt}$ and $t\_{str}$ do not vary in the $z$-dimension.

In Supplementary text 4, it is already shown that modelling loose mitochondria in either the inner cytosol or outer cytosol hardly affects the values of $A\_{N}$ and $f\_{rec}$ predicted by the default model. This demonstrates that, in the case of tomato, simplification 1 is reasonable. The aim of the current supplementary text is to show that the remaining three limitations also will not affect $A\_{N}$. This will be done by comparing the values of $A\_{N}$ predicted by the model in this study with the values predicted by the model in [1] that does not have simplifications 2, 3, and 4.

## S5.1 Summary description 3-D model in [1]

The model described in [1] describes CO2 transport, production, and consumption in tomato leaves. The leaf geometry is a discretized 3-D tomography [1], which was obtained by X-ray synchrotron microscopy [2]. Next, the mesophyll cells from the obtained 3-D leaf geometry were compartmented into a chloroplast layer that is exposed to the intercellular air space,

cytosol layer, and a vacuole. Finally, the chloroplast layer was subdivided into spherical chloroplasts and cytosol compartments in between. Additionally, the remaining compartments were subdivided into intercellular air space and the epidermis. Monte-Carlo ray tracing was applied to calculate the light absorption gradient within this geometry [3]. Stomatal opening was modelled by making a cylindrical air hole in the epidermis that connects the intercellular air space with the ambient air. This air hole is the stomatal aperture. Over this discretized geometry, a system of partial differential equations for CO2 transport and HCO3- transport were solved. The equations that were used are listed below; the notation of symbols is adjusted in such a way that the notation is the same as the symbols used in the 2-D model from the current study:

$$\begin{array}{c}∇∙D\_{CO\_{2},gas}∇\left[CO\_{2}\right]=0\#\left(S5.1\right)\end{array}$$

$$\begin{array}{c}∇∙p\_{eff,i}ζ\_{i}D\_{CO\_{2},water}∇\left[CO\_{2}\right]-w\_{i}+r\_{p,i}+r\_{d,i}-B=0\#\left(S5.2\right)\end{array}$$

$$\begin{array}{c}∇∙p\_{eff,i}ζ\_{i}D\_{HCO\_{3},water}∇\left[HCO\_{3}\right]+B=0\#\left(S5.3\right)\end{array}$$

where $B$ is the conversion rate of CO2 into HCO3-. The subscript $i$ indicates that the value depends on the compartment. $D\_{CO\_{2},gas}$ is the diffusion coefficient of CO2 in the gas phase.

For the simulations with the 3-D model that are considered in this supplementary text, it is assumed that CO2 transport is facilitated by carbon anhydrases in the cytosol and the stroma. In the presence of carbon anhydrases, $B$ was represented as [1,4]:

$$\begin{array}{c}B=\frac{k\_{CA}\left[CA\right]\left(\left[CO\_{2}\right]-\frac{\left[H^{+}\right]\left[HCO\_{3}^{-}\right]}{K\_{eq}}\right)}{K\_{CA,CO\_{2}}+\frac{K\_{CA,CO\_{2}}\left[HCO\_{3}^{-}\right]}{K\_{CA,HCO\_{3}^{-}}}+\left[CO\_{2}\right]}\#\left(S5.4\right)\end{array}$$

where $k\_{CA}$, $K\_{eq}$, and $\left[CA\right]$ are the turnover rate, the equilibrium constant and the concentration of carbon anhydrases, respectively. $K\_{CA,CO\_{2}}$ and $K\_{CA,HCO\_{3}^{-}}$ are the Michaelis-Menten constants of hydration and dehydration, respectively. Equation (S5.4) implicitly assumes that the further dehydration of HCO3- into CO32- is negligible under the pH levels in leaves.

## S5.2 Quantification parameter values in the 2-D model and in the 3-D model

The parameter values in equation (S5.1 - S5.4) can be found in the supplementary material of the study in [1]. For the simulations in the current study, the same parameter values were used for $s$, $S\_{c\O} ^{\*}$, $R\_{d}$, $V\_{cmax}$, and $T\_{p}$ as by [1]. For the anatomical parameters, it was assumed that $S\_{m}/S=16$, $t\_{str}=2.5 μm$, and $t\_{cyt}=250 nm$. The values $t\_{wall}=200 nm$ and $S\_{c}/S\_{m}=0.90$ were adopted from [1]. In the 3-D model in [1], it is assumed that the radius of the stomatal pore does not change with increased $C\_{a}$. Unlike the 2-D model in the current study, the 3-D model does not use stomatal conductance as an implicit input value in the 3-D model. In order to use the same stomatal conductance as input for the 2-D model as for the model in [1], first $C\_{i}$ and $A\_{N}$ were calculated for each value of $C\_{a}$ from the solution of the 3-D model:

$$\begin{array}{c}W=\left(∭\_{Stroma}^{}w dx dy dz\right)S^{-1}\#\left(S5.5\right)\end{array}$$

$$\begin{array}{c}R\_{p}=\left(∭\_{Stroma}^{}\frac{wγ^{\*}}{\left[CO\_{2}\right]} dx dy dz\right)S^{-1}\#\left(S5.6\right)\end{array}$$

$$\begin{array}{c}R\_{d}=\left(∭\_{Cytosol}^{}r\_{d} dx dy dz\right)S^{-1}\#\left(S5.7\right)\end{array}$$

$$\begin{array}{c}C\_{i}=RT\left(∭\_{Intercellular air space}^{}\left[CO\_{2}\right] dx dy dz\right)\left(∭\_{Intercellular air space}^{}dx dy dz \right)^{-1}\#\left(S5.8\right)\end{array}$$

$$\begin{array}{c}A\_{N}=W-R\_{p}-R\_{d}\#\left(S5.9\right)\end{array}$$

$$\begin{array}{c}g\_{s}=\frac{A\_{N}}{C\_{a}-C\_{i}}\#\left(S5.10\right)\end{array}$$

## S5.3 Comparison of simple 2-D model to complex 3-D model

For each combination of $C\_{a}$ calculated values of $g\_{s}$ (equations (S5.5-S5.10)) were used as input values for the 2-D model. Furthermore, the values of $J$, calculated by the 3-D model, were used as input for the 2-D model. The calculations are done for three of the six tomato leaf types examined in [1]. These leaf types are “Admiro lower leaf”, “Doloress lower leaf”, and “Growdena lower leaf”. Fig A shows diagrams, in which the values of $A\_{N}$ for each value of $C\_{a}$ predicted by the 2-D model are plotted against $A\_{N}$ values for the same $C\_{a}$ predicted by the 3-D model. This shows that all values of $A\_{N}$, with a possible exception of the highest values of $C\_{a}$ ($C\_{a}=100 Pa$ and $C\_{a}=150 Pa$) for Doloress lower leaf and Growdena lower leaf, are about the same for both the 2-D and the 3-D model.

|  |  |
| --- | --- |
| A | B |
| DC |  |
| FE |  |
| **Fig A:** Net CO2 assimilation rate predicted by the 2-D model is plotted against the net CO2 assimilation rate predicted by the 3-D model in [1] for three leaf types. These are “Admiro lower leaf” (A, D), “Doloress lower leaf” (B, E), “Growdena lower leaf” (C, F). Simulation with the 2-D model were run for two scenarios; (photo)respiratory CO2 release takes place in the inner cytosol (A-C) or in the cytosol gaps (D-F). The solid line is the 1 to 1 line. |

## References

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4. Tholen D, Zhu XG (2011) The mechanistic basis of internal conductance: a theoretical analysis of mesophyll cell photosynthesis and CO2 diffusion. Plant Physiology 156: 90-105.