What is the most prominent factor limiting photosynthesis in different layers of a greenhouse cucumber canopy?

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INTRODUCTION

Improving productivity is a major goal in crop production. This can be achieved by genetic crop improvement or by the optimization of the cropping system. Important tasks to optimize the cropping system are to maximize crop photosynthesis at the canopy level (Murchie et al., 2009; Zhu et al., 2010; Reynolds et al., 2012) and to optimize the photosynthetic resource distribution within the canopy (Buckley et al., 2013). However, since it is difficult to measure canopy photosynthesis, modelling approaches are necessary for its study (Zhu et al., 2012). To date, several approaches for modelling canopy photosynthesis have been proposed: (1) big leaf models, where the whole canopy is assumed to consist of one leaf (Thornley et al., 1992); (2) sunlit–shaded models, where a plant canopy is represented by two leaves and where one of which is shaded by the other (de Pury and Farquhar, 1997; Peltoniemi et al., 2012); (3) multilayer models, where the plant canopy is divided into leaf clusters exposed to different light environments (Zhu et al., 2012); and (4) functional–structural plant models (FSPMs), where the plants and the canopy are constructed spatially explicitly at the organ level with geometry and topology, and the physiological functions of plants, e.g. photosynthesis, and interactions between canopy structure and environmental factors, such as light, are described (Vos et al., 2010; DeJong et al., 2011). A key feature of FSPMs is that the heterogeneities of microclimate, especially local light conditions, can be simulated and used to compute photosynthesis at the leaf level and upscale it to the canopy level.
For decades, plant physiologists have searched for methods to identify and to quantify the factors restricting photosynthesis (Jones, 1985). So far, it is not possible to ‘measure’ the photosynthetic limitation. The relative or quantitative magnitude of photosynthetic limitations can only be quantified by mathematical approaches (see Jones, 1985; Wilson et al., 2000; Grassi and Magnani, 2005; Grassi et al., 2009). By combining the Farquhar–von Caemmerer–Berry model (FvCB model; Farquhar 1980) with the state function method (Jones, 1985), Grassi and Magnani (2005) have dissected and quantified the contribution of different photosynthetic limitations. By their approach (in the following referred to as quantitative limitation analysis), the absolute total limitation of photosynthesis (percentage of a reference photosynthesis rate at ambient CO₂ concentration and saturating light, A_{\text{max,ref}}, μmol CO₂ m⁻² s⁻¹) can be quantitatively partitioned into stomatal, mesophyll and biochemical components. In their study, the reference photosynthesis rate is 16-8 μmol CO₂ m⁻² s⁻¹, and their finding is that the stomatal limitation of sun leaves in oak trees in summer is 9–14 % indicates that photosynthesis rates can be increased by 1-51–2-35 μmol CO₂ m⁻² s⁻¹ if the stomata were to open fully. Quantitative limitation analysis is a helpful methodology to quantify the photosynthetic limitations based on measured data and allows plant physiologist to disentangle the contributions of different physiological and environmental factors to photosynthetic limitations on the leaf level (Flexas et al., 2009; Egea et al., 2011). However, the results from the quantitative limitation analysis at leaf level would correspond to the photosynthetic limitations at the canopy level is questionable for two reasons. First, quantitative limitation analysis has only been applied under light-saturated (Rubisco-limited) conditions, but most leaves in the canopy (except for leaves grown in the upper part of the canopy) are normally exposed to non-saturating light conditions (RuBP-limited; Song et al., 2013). Secondly, a recent study has shown that the type and extent of photosynthetic limitations vary between different tree canopy layers (Cano et al., 2013). Therefore, the compositions of photosynthetic limitations at the canopy or whole-plant level may be quite different from those at the leaf level. To date, it is still unknown to what extent the different factors restrict photosynthesis in different canopy layers and at the whole-plant level.

Recently, a quantitative limitation analysis for the RuBP-limited phase of photosynthesis was proposed (Chen et al., 2013). In this approach, the influence of light on limiting photosynthesis is taken into account and the total limitation of leaf photosynthesis (A_L, % of A_{\text{max,ref}}) is partitioned into four components:

\[ A_L = S_L + M_L + B_L + L_L \]  

(1)

where S_L, M_L, B_L and L_L are stomatal, mesophyll, biochemical and light limitation, respectively. The fact that the contribution of different limitations calculated by this method can be treated additively (Grassi et al., 2009) allows straightforward interpretation and allows the computation of the photosynthetic limitation at canopy levels by summing up the limitations of all leaves of a plant. For example, the stomatal limitation of a plant with n leaves (S_{Lp}, μmol CO₂ per plant s⁻¹) can be calculated by:

\[ S_{Lp} = A_{\text{max,ref}} \sum_{k=1}^{n} (S_{L,k} \times L_A) \]  

(2)

where L_A is the area of leaf k and S_{L,k} is the stomatal limitation of leaf k. This approach allows the calculation of the total limitation of a plant (A_{Lp}, μmol CO₂ per plant s⁻¹):

\[ A_{Lp} = A_{\text{max,ref}} \sum_{k=1}^{n} [L_A \times (S_{L,k} + M_{L,k} + B_{L,k} + L_{L,k})] \]  

(3)

where M_{L,k}, B_{L,k} and L_{L,k} are the mesophyll, biochemical and light limitations of leaf k. This upscaling approach may provide insights into the sources of photosynthetic limitations in the cropping system. Since it is almost impossible to measure all of the parameters (light interception by the leaves, FvCB model parameters, stomatal and mesophyll conductance) required for the quantitative limitation analysis of all leaves of a plant, a modelling approach would be desirable for investigating the photosynthetic limitation of both different canopy layers and the whole plant. We suggest combining a structural model and the FvCB model, as has been done in several studies (Buck-Sorlin et al., 2011; Sarlikioti et al., 2011; Wiechers et al., 2011a; Song et al., 2013), to quantify different components of photosynthetic limitation at the canopy level.

Both experimental and model-based investigations have demonstrated that canopy photosynthesis and light use efficiency may be improved under diffuse light conditions (Alton et al., 2007; Wohlfart et al., 2008; Mercado et al., 2009). Greenhouse experiments have shown that transforming direct light entering the greenhouses to diffuse light by a plastic film results in a more even light distribution in the canopy and increases the yield of cucumber by 5 % (Hemming et al., 2008). However, the effects of diffuse radiation on canopy photosynthesis change with environmental and biological conditions. For example, they are less significant when the radiation above the canopy is low (Alton et al., 2007), and they depend on plant species and planting season (Jongschaap et al., 2006). Moreover, Wohlfart et al. (2008) have found that the effect of diffuse radiation on canopy photosynthesis is more significant in a canopy with a higher leaf area index, suggesting that canopy structure might influence the impact of diffuse radiation on canopy photosynthesis; however, this has not been examined.

In this work, we used data from plant digitization to construct a static FSPM, a representative cucumber canopy structure, using the interactive modelling platform GroIMP (Kniemeyer, 2008), and applied the quantitative limitation of photosynthesis (Chen et al., 2013), to (1) determine the most prominent factor limiting leaf and canopy photosynthesis; (2) quantify the variations in photosynthetic limitations at different canopy layers; (3) investigate the dependence of photosynthesis and light use efficiency on light interception at different canopy layers; and (4) examine the effect of diffuse light on leaf-level light interception and photosynthesis.

**MATERIALS AND METHODS**

*Constructing the virtual cucumber canopy*

The whole-plant architecture of cucumber plants (*Cucumis sativus* ‘Aramon’, Rijk Zwaan, De Lier, The Netherlands) with 21 mature leaves grown in a greenhouse experiment (treatment...
D1R2 in Kahlen and Stützel, 2007) was digitized. The reconstruction of the leaves using digitizing data is described by Wiechers et al. (2011b). In brief, the co-ordinates of 13 points per leaf lamina were digitized by a 3-D digitizer (Fastrak, Polhemus, Colchester, VT, USA). Each lamina was represented by a pre-defined set of triangles and was reconstructed using the commands FloatList and PolygonMesh in GroIMP (Kniemeyer, 2008). For construction of the virtual canopy structure, 18 cucumber plants placed at a density of one plant per square metre were distributed in three rows (Fig. 1). Distances between virtual plants in a row and between rows were 0.5 and 2 m, respectively. Furthermore, the whole canopy was divided into four canopy layers: (1) lower canopy (leaf rank 1–5); (2) middle–lower canopy (leaf rank 6–10); (3) middle–upper canopy (leaf rank 11–15); and (4) upper canopy (leaf rank 16–21). Leaf age (d) and leaf area (m²) are summarized in Table 1.

Simulating local light environment

The light environment was simulated according to Buck-Sorlin et al. (2011). In brief, the virtual canopy was surrounded by sun and sky, providing direct and diffuse light, respectively (Supplementary Data Fig. S1). The sun was a single object providing light in the direction of the corresponding location and time (in our simulation: Hannover, Germany, 52° 23′ N, 9° 37′ E, on 1 July at 1200 h). The sky was approximated by an array of 72 directional light sources arranged in a hemisphere. For computing the light distribution, a ray-tracer, integrated into GroIMP, was used with 10 million rays and a recursion depth of ten reflections (Buck-Sorlin et al., 2011). It was assumed that a leaf absorbs 87 %, transmits 7 % and reflects 6 % of the incident photosynthetically active radiation (PAR; Kahlen et al., 2008). Since the ground in the greenhouse of the experiment was covered by a white film, the ground in the model

Table 1. Characteristics of the leaves at different leaf ranks

<table>
<thead>
<tr>
<th>Leaf rank</th>
<th>Leaf age (d)</th>
<th>Leaf area (m²)</th>
<th>Canopy layer</th>
<th>Canopy layer leaf area (m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40</td>
<td>0.0462</td>
<td>Lower</td>
<td>0.3630</td>
</tr>
<tr>
<td>2</td>
<td>38.4</td>
<td>0.0725</td>
<td>Lower</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>36.8</td>
<td>0.0747</td>
<td>Lower</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>35.2</td>
<td>0.0774</td>
<td>Lower</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>33.6</td>
<td>0.0922</td>
<td>Lower</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>32</td>
<td>0.1016</td>
<td>Middle–lower</td>
<td>0.4788</td>
</tr>
<tr>
<td>7</td>
<td>30.4</td>
<td>0.0953</td>
<td>Middle–lower</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>28.8</td>
<td>0.1063</td>
<td>Middle–lower</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>27.2</td>
<td>0.0900</td>
<td>Middle–lower</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>25.6</td>
<td>0.0855</td>
<td>Middle–lower</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>24</td>
<td>0.0799</td>
<td>Middle–upper</td>
<td>0.3349</td>
</tr>
<tr>
<td>12</td>
<td>22.4</td>
<td>0.0719</td>
<td>Middle–upper</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>20.8</td>
<td>0.0593</td>
<td>Middle–upper</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>19.2</td>
<td>0.0589</td>
<td>Middle–upper</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>17.6</td>
<td>0.0650</td>
<td>Middle–upper</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>16</td>
<td>0.0550</td>
<td>Upper</td>
<td>0.2734</td>
</tr>
<tr>
<td>17</td>
<td>14.4</td>
<td>0.0560</td>
<td>Upper</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>12.8</td>
<td>0.0417</td>
<td>Upper</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>11.2</td>
<td>0.0454</td>
<td>Upper</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>9.6</td>
<td>0.0382</td>
<td>Upper</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>8</td>
<td>0.0371</td>
<td>Upper</td>
<td></td>
</tr>
<tr>
<td>1–21</td>
<td>–</td>
<td>–</td>
<td>Whole plant</td>
<td>1.4501</td>
</tr>
</tbody>
</table>

Leaf age (day after leaf appearance) is calculated by assuming a constant leaf appearance rate (0.625 d leaf⁻¹). Canopy layer leaf area is the sum of the leaf area in the part of the canopy.
Modelling leaf photosynthesis

Two assumptions were made for all simulations: (1) leaf temperature = 25°C and (2) constant ambient CO₂ concentration (Cᵅ = 380 μmol mol⁻¹). To simulate the stomatal conductance to CO₂ (gₛₑ, mol m⁻² s⁻¹), the model proposed by Medlyn et al. (2011) was used:

\[ gₛₑ = g₀ + (1 + g₁/D) \times (A/Cᵅ) \]  (4)

where D is leaf to air vapour pressure deficit (0.87 kPa assuming the relative humidity in the greenhouse is around 70 %), parameters g₀ and g₁ are 0.009 mol m⁻² s⁻¹ and 3.51 (unitless), respectively (T.-W. Chen et al., Leibniz Universität Hannover, unpubl. res.), and A (μmol CO₂ m⁻² s⁻¹) is the minimum of the Rubisco-limited (Aᵅ, μmol CO₂ m⁻² s⁻¹) or RuBP regeneration-limited (Aᵢ, μmol CO₂ m⁻² s⁻¹) photosynthesis rate (Farquhar et al., 1980):

\[ Aᵅ = Vᶜₘₐₓ × (Cᵅ − Γ*)/[Cᵅ + Kᵅ(1 + O/Kₐ)] − R_d \]  (5a)

\[ Aᵢ = J × (Cᵅ − Γ*)/(4Cᵅ + 8J)* − R_d \]  (5b)

where \( Vᶜₘₐₓ \) is the maximum rate of Rubisco carboxylation (μmol CO₂ m⁻² s⁻¹), \( Γ^* \) is the CO₂ compensation point in the absence of dark respiration (for cucumber: 43.02 μmol mol⁻¹; Singsaas et al., 2003), \( Kᵅ \) (404 μmol mol⁻¹) and \( Kᵦ \) (278 μmol mol⁻¹) are Michaelis– Menten constants of Rubisco for CO₂ and O₂, O (210 μmol mol⁻¹) is the mole fraction of O₂ at the site of carboxylation, \( R_d \) is the respiration rate (1.08 μmol CO₂ m⁻² s⁻¹; T.-W. Chen et al., unpubl. res.), \( C_c \) (chloroplastic CO₂ concentration, μmol mol⁻¹) and J (rate of electron transport, μmol m⁻² s⁻¹) were calculated by (Archontoulis et al., 2012):

\[ C_c = C_a − A(1/gₛₑ + 1/g_m) \]  (6)

\[ J = (Kₑₛₑ × Iₐₓₑ + Jₘₐₓ) − \sqrt{(Kₑₛₑ × Iₐₓₑ + Jₘₐₓ)^2 − 4θIₐₓₑ × Kₑₛₑ × Iₐₓₑ}]/(2θ) \]  (7)

where \( g_m \) is mesophyll conductance (mol m⁻² s⁻¹), \( Iₐₓₑ \) is intercepted PAR (μmol photons m⁻² s⁻¹), \( Jₘₐₓ \) is the maximum electron transport rate (μmol e⁻ m⁻² s⁻¹), \( Kₑₛₑ \) is a constant describing the conversion efficiency of \( Iₐₓₑ \) to J (0.425 mol e⁻ mol⁻¹ photon; Wieschers et al., 2011a) and \( θ \) is a constant convexity factor describing the response of J to \( Iₐₓₑ \) (0.7; Wieschers et al., 2011a). The dependency of \( Vᶜₘₐₓ \), \( Jₘₐₓ \) and \( g_m \) on leaf age is fitted to a log-normal curve (Irving and Robinson, 2006):

\[ X(t) = Xₘₐₓ × \exp[-0.5[log(t/b)/c]^2] \]  (8)

where \( Xₘₐₓ \) is the maximum of the variables (Table 2), t is leaf age (d), b is the time (8.56 d) when the \( Xₘₐₓ \) occurs, and c is the curve standard deviation (0.952). These parameters for cucumber were taken from the work of Wieschers (2011). From our previous study (T.-W. Chen et al., unpubl. res.; see also Egea et al., 2011; Buckley et al., 2013), parameters b and c for \( Vᶜₘₐₓ \), \( Jₘₐₓ \)

<table>
<thead>
<tr>
<th>( Aᵅₑₑ ) (μmol m⁻² s⁻¹)</th>
<th>( Jₘₐₓₑ )</th>
<th>( Vᶜₘₐₓₑ )</th>
<th>( g_mₑₑ ) (mol m⁻² s⁻¹)</th>
<th>( g_mₑₑ ) (mol m⁻² s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>26.7*</td>
<td>191.1</td>
<td>168.8</td>
<td>140.7</td>
<td>0.37</td>
</tr>
</tbody>
</table>

*\( Aᵅₑₑ \) is calculated by Eqn (13).

and \( g_m \) were not significantly different and were well correlated. Therefore, the same parameter set for these three variables was used. Finally, \( Aᵅ \), \( Aᵢ \), \( gₛₑ \) and \( C_c \) were obtained by solving Eqns (4)–(6) analytically.

Evaluation of the photosynthesis model

To evaluate the photosynthesis model, leaf gas exchange measurements were conducted in a greenhouse experiment in 2013. Cucumber seedlings at the three-leaf stage were transplanted into the greenhouses of the Institute of Horticultural Production Systems, Leibniz Universität Hannover, Germany (52°23′N, 9°37′E) on 20 March 2013. In our model, leaf 21 was 8 d past leaf appearance (approx. 5 cm in leaf length). In the experiment, leaf 21 appeared on 17 April, and photosynthesis was measured on 24 April 2013. The experimental set-up was similar to the experiment described by Kahlen and Stützel (2007). Root mean squared deviation of the photosynthesis rate (μmol m⁻² s⁻¹) and accuracy (%) were calculated according to Kahlen and Stützel (2011).

Leaves on ten ranks (1, 3, 5, 7, 9, 11, 14, 17, 19 and 21) were measured using a portable gas exchange system (Li-6400; Licor, Lincoln, NE, USA) at \( C_a = 380 \) μmol mol⁻¹, leaf temperature = 25°C and 70 % relative humidity, corresponding to the model conditions. Four leaves per rank were measured at the corresponding light conditions simulated in the model. For example, when the PAR above the canopy in the model was 100, 500, 900 and 1300 μmol photon m⁻² s⁻¹, leaf 5 absorbed on average 27, 133, 247 and 346 μmol photons m⁻² s⁻¹, respectively. Therefore, leaf 5 in the experiment was measured at 27, 133, 247 and 346 μmol photon m⁻² s⁻¹ (the input PAR in the Li-6400 chamber was 1.15 times these values, corresponding to 87 % leaf absorbance). All measurements were done between 0900 and 1400 h.

Quantitative limitation analyses

To test if the rate of photosynthesis is limited by the Rubisco carboxylation rate or by the RuBP regeneration rate, \( C_c \) and the intersection point of the FvCB model (Cᵥₑₑ: Dubois et al., 2007) were compared:

\[ Cᵥₑₑ = [Kᵢ(J[Kᵦ + O] − 8KᵦΓ*Vᶜₘₐₓ)/[Kᵦ(4Vᶜₘₐₓ − J)] \]  (9)

When \( C_c < Cᵥₑₑ \), photosynthesis is Rubisco limited. In this case, quantitative limitation analysis for saturating light conditions, proposed by Grassi and Magnani (2005), was used. When
where \( S_l, M_l, B_l \) and \( L_a \) are the contributions of stomatal conductance, mesophyll conductance, biochemical capacity and light to photosynthetic limitation, \( A_l \) is the total limitation, \( I_s, I_m \) and \( I_j \) are the relative limitations of stomatal and mesophyll conductance and of the electron transport rate, \( J_{af} \) and \( J_{al} \) are the changes of electron transport rate due to biochemical capacity and irradiance, respectively, and \( \Delta A_j/A_j, \Delta g_{sc}/g_{sc}, \Delta g_m/g_m, J_{df}/J \) and \( J_{af}/J \) are approximated by:

\[
d\Delta A_j/A_j \approx (A_j,\text{ref} - A_j)/A_j,\text{ref} \tag{12a}
\]

\[
d\Delta g_{sc}/g_{sc} \approx (g_{sc,\text{ref}} - g_{sc})/g_{sc,\text{ref}} \tag{12b}
\]

\[
d\Delta g_m/g_m \approx (g_{m,\text{ref}} - g_m)/g_{m,\text{ref}} \tag{12c}
\]

\[
n\Delta j_{df}/J \approx (J_{s,\text{ref}} - J_j)/J_s,\text{ref} \tag{12d}
\]

\[
n\Delta j_{af}/J \approx (J_s - J_j)/J_s,\text{ref} \tag{12e}
\]

where \( g_{sc,\text{ref}} \) and \( g_{m,\text{ref}} \) are the reference values of stomatal and mesophyll conductance, \( J_s \) is the electron transport rate at saturating light conditions (PAR = 1300 \( \mu \)mol m\(^{-2}\) s\(^{-1}\), achieving > 95 % of the photosynthesis rate for cucumber), \( J_{s,\text{ref}} \) is the electron transport rate with maximum \( J_{\text{max}} \) [with the highest biochemical capacity, Eqn (7)] at saturating light conditions and \( A_j,\text{ref} \) is calculated by solving the following function:

\[
A_j,\text{ref} = J_{s,\text{ref}}[C - A_j,\text{ref}(1/g_{sc,\text{ref}} + 1/g_{m,\text{ref}}) - \Gamma^*]/
\]

\[
\times (4[C - A_j,\text{ref}(1/g_{sc,\text{ref}} + 1/g_{m,\text{ref}}) + 8\Gamma^*] - R_d
\tag{13}
\]

Reference values are listed in Table 2. Furthermore, the sensitivity of stomatal limitation to model parameters \( g_1 \) and \( D \) [in Eqn (4)] was tested. These parameters were chosen because their changes have no influence on the reference photosynthesis rate \( [A_j,\text{ref} \text{ in Eqn (13)]}. Simulations for sensitivity analyses were run for leaves intercepting 700 \( \mu \)mol m\(^{-2}\) s\(^{-1}\) PAR, on days 15, 25 and 35 after leaf appearance.

Simulation and statistical analysis

To investigate the compositions of photosynthetic limitations at the leaf level, PAR above the virtual canopy was assumed to be 100, 500, 900 and 1300 \( \mu \)mol photon m\(^{-2}\) s\(^{-1}\), consisting of 79 % direct and 21 % diffuse light. These four scenarios were also used to upscale the photosynthetic limitations from leaf to the canopy layers and to the whole plant by using Eqns (2) and (3). Canopy light interception (\( I_{c}, \mu \)mol photon per canopy layer s\(^{-1}\)), canopy photosynthesis (\( A_{c}, \mu \)mol CO\(_2\) per canopy layer s\(^{-1}\)) and canopy light use efficiency (LUE\(_{c}, \mu \)mol CO\(_2\) \( \mu \)mol\(^{-1}\) photon) were calculated by:

\[
I_{c} = \sum_{k=1}^{n}(I_{c} \times L_{A_k}) \tag{14a}
\]

\[
A_{c} = \sum_{k=1}^{n}(A \times L_{A_k}) \tag{14b}
\]

\[
\text{LUE}_{c} = A_{c}/I_{c} \tag{14c}
\]

To investigate the relationship between \( I_{c}, A_{c} \) and LUE\(_{c}\), a wide range of PAR above the virtual canopy was simulated (between 100 and 1500 \( \mu \)mol photon m\(^{-2}\) s\(^{-1}\)). For the analysis of the influence of diffuse light on canopy photosynthesis, PAR above the virtual canopy was assumed to be 1000 \( \mu \)mol photon m\(^{-2}\) s\(^{-1}\) and the diffuse light consisted of 0 and 100 % of the total light only.

The simulated results from the two plants in the centre of the middle row (Fig. 1A) were taken for statistical analysis. To avoid model artefacts, simulations for each scenario were repeated ten times, each run with a slight difference in orientation (±30°) of the tested plants in the virtual canopy. Averages, standard errors and regression analyses were calculated in R (v.2.12.0; R Foundation for Statistical Computing).

RESULTS

Influence of light regimes on light interception

Simulated PAR interception at the leaf level with different PAR above the canopy is shown in Fig. 2A. For the leaves on the top and at the bottom of the canopy, values of PAR interception (\( \mu \)mol m\(^{-2}\) leaf area s\(^{-1}\)) were about 97 and 21 % of the PAR values above the canopy (\( \mu \)mol m\(^{-2}\) ground area s\(^{-1}\)), respectively. The lower, middle–lower, middle–upper and upper canopy received 14, 30, 25 and 31 %, respectively, of the light intercepted by the whole canopy.

Under 100 % diffuse light, most of the leaves in the canopy intercepted more PAR than under 100 % direct light, especially the leaves in the lower canopy (Fig. 2B). The increase of light interception was most prominent at leaf ranks 10, 13 and 16 (86, 114 and 118 %, respectively).

Evaluation of the photosynthesis model

A clear linear relationship was found between measured and simulated photosynthesis rates (Fig. 3A; \( R^2 = 0.98, P < 0.001 \)). The 95 % confidence intervals of the slope and the intercept were 0.94–1.04 and 0.37–1.42, respectively. Root mean squared deviation was 1.30 \( \mu \)mol m\(^{-2}\) s\(^{-1}\) and accuracy was 86 %. The model slightly underestimated the photosynthesis rate.
No relationships between model errors and PAR (Fig. 3B) or leaf rank (Fig. 3C) were found. Furthermore, in all simulations, photosynthesis was limited by the RuBP regeneration rate ($\frac{\text{g}}{\text{mol}}$) of ten simulations with slightly rotated (less than or equal to $\pm 30^\circ$) plants; bars represent standard deviation.

Photosynthetic limitations at the single-leaf level

The compositions of photosynthetic limitations changed strongly with the light condition above the canopy and with canopy depth (Fig. 4). In general, stomatal limitations ($S_L$) decreased photosynthesis of the leaves in the upper canopy by 2–4 %, and this reduction increased with leaf age to 8 % (Fig. 4A). Furthermore, $S_L$ decreased when light interception increased. The maximal mesophyll limitation ($M_L$) was about 3 % (Fig. 4B). In contrast to $S_L$, $M_L$ increased with the light above the canopy. Diffusional limitations of photosynthesis ($D_L = S_L + M_L$) were stronger in the lower than in the upper canopy (Fig. 4C). Biochemical limitations ($B_L$) increased with canopy depth (Fig. 4D) and restricted photosynthesis of the lowest leaves by $>60 \%$. Interestingly, although the leaves in the lower canopy received less light than those located in the upper canopy (Fig. 2A), photosynthesis of lower leaves was less restricted by light (Fig. 4E). Reduction of the light above the canopy increased the light limitation ($L_L$) of all leaves, especially of the upper ones. Total limitation ($A_L$) reduced with leaf rank and light (Fig. 4F). In all cases, $B_L$ and $L_L$ were the most prominent components (80–93 %) in $A_L$.

An increase in water vapour deficit enhanced $S_L$ (Fig. 5A), whereas an increase of $g_1$ reduced $S_L$ (Fig. 5B). Changes in
FIG. 4. Changes of (A) stomatal, (B) mesophyll, (C) diffusional (stomatal + mesophyll), (D) biochemical, (E) light and (F) total (diffusional + biochemical + light) limitation with leaf rank and light conditions above the canopy (100, 500, 900 and 1300 μmol photon m^{-2} s^{-1}, 79 % direct light and 21 % diffuse light, as indicated in the key). n = 10.

FIG. 5. Sensitivity of stomatal limitation to (A) water vapour deficit and (B) parameter \( g_1 \) [in Eqn (4)]. Simulations were run for leaves on days 15, 25 and 35 after leaf appearance, as indicated in the key. The vertical solid lines indicate the default parameter values used for analysing the canopy photosynthetic limitation.
water vapour deficit and $g_1$ influenced $S_L$ by up to 12 and 8 % of the reference photosynthesis rate, respectively. $S_L$ in upper leaves was as sensitive as it was in lower leaves to $g_1$ and to water vapour deficit. Intercepted light had negligible effects on the sensitivity of $S_L$ to water vapour deficit (data not shown). Furthermore, these two parameters had very small effects on $M_L$, $B_L$, and $L_L$ (<1 %).

Photosynthetic limitations on different canopy layers and the whole plant

Table 3 shows photosynthesis and the compositions of photosynthetic limitations on different canopy layers and the whole plant. Stomatal limitation contributed about 10 % of the total limitation. This contribution was stronger in young canopies and at high light conditions than in old canopies and at low light conditions. The middle–lower canopy contributed more than one-third to the whole-plant stomatal limitation. Mesophyll limitations contributed <4 % of the total limitation on the different canopy layers and the whole plant. Light conditions above the canopy had little effect on biochemical limitations. Both the middle–lower and lower canopy contributed about 40 % of the biochemical limitation to the whole plant. Light limitations in all parts of the canopy were decreased when PAR above the canopy increased, especially in the upper canopy. At the whole-plant level, about 30 % of the light limitation occurred in the upper and middle–upper part of the canopy (Table 3). Independently of the PAR above the canopy, the total limitation in the different parts of the canopy ranged as follows: middle–lower > lower > middle–upper > upper. The upper canopy made the highest contribution to the whole-plant photosynthesis (>30 % of whole-plant photosynthesis), but both the middle–upper and middle–lower canopy also assimilated >25 % of the whole-plant photosynthetic products.

Canopy light interception, photosynthesis rate and light use efficiency

Responses of canopy photosynthesis ($A_C$, $\mu$mol CO$_2$ per plant s$^{-1}$) and light use efficiency ($\text{LUE}_C$, $\mu$mol CO$_2$ $\mu$mol$^{-1}$ photon) to incident light ($I_C$, $\mu$mol photon per plant s$^{-1}$) on different canopy layers were essentially different from those at the whole-plant level (Fig. 6). The maximum $A_C$ and $\text{LUE}_C$ occurred at the upper canopy and decreased with canopy depth (Table 4).

Influence of diffuse light on canopy photosynthesis

Under 100 % diffuse light, leaves in the lower canopy intercepted more PAR than under 100 % direct light. This increase in light interception only resulted in an approx. 20 % increase of leaf photosynthesis (Fig. 7). Interestingly, diffuse light had the most significant effects on the leaves at ranks 10, 13 and 16. Under 100 % diffuse light, these leaves intercepted 86, 113 and 117 %, respectively, more light than under 100 % direct light (Fig. 2B), and their photosynthesis increased 28, 54 and 55 %, respectively (Fig. 7). These leaves made the biggest contribution to the increase of canopy photosynthesis under 100 % diffuse light.

DISCUSSION

The quantitative limitation analysis is a useful tool to disentangle the contributions of different physiological and environmental factors to photosynthetic limitations. However, it requires complicated calculations. To aid other researchers in conducting this analysis, we provide a Microsoft Excel file for this calculation in the Supplementary Data (Excel S1).

This is the first approach to quantify the components of photosynthetic limitations of different canopy layers and the whole plant. Methodological considerations of quantitative limitation analysis have been sufficiently discussed in previous papers (Grassi and Magnani, 2005; Grassi et al., 2009). To our knowledge, it is not possible to validate our approach experimentally because photosynthetic limitations cannot be measured directly. Therefore, we took a step back and evaluated our model for photosynthesis. The slight underestimation of photosynthesis rates in our model (about 0.5–1.0 $\mu$mol m$^{-2}$ s$^{-1}$, i.e. 2–4 % of the reference photosynthesis rate, Fig. 3, Table 2) may result in small overestimations of photosynthetic limitations. One reason for this slight inaccuracy could be that $R_d$ in our photosynthetic model was constant. In many modelling works, $R_d$ is scaled with $V_{\text{max}}$ (e.g. Buckley et al., 2013; Cano et al., 2013), which is a function of leaf age [Eqn (8)]. Implementing the dependency of $R_d$ on leaf age in the photosynthetic model may reduce the underestimation of the simulated photosynthesis in the middle and lower canopy (Fig. 3C). However, quantitative limitation analysis for the RuBP regeneration-limited phase of photosynthesis may underestimate the total limitation by 0.5–3 % (T.-W. Chen et al., unpubl. res.). This indicates that the errors from the photosynthesis model could be counterbalanced, but not amplified, by the quantitative limitation analysis. Furthermore, fruit and stem structures, which may contribute to the whole-plant carbon

<table>
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<th>$M_L$</th>
<th>$B_L$</th>
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$S_L$, $M_L$, $B_L$, and $L_L$ are stomatal, mesophyll, biochemical and light limitation, respectively. The leaf area of different canopy layers is shown in Table 1.
assimilation (Aschan and Pfanz, 2003), were not included in the architectural model. Due to this simplification, the whole-plant photosynthesis might be slightly overestimated because more light may reach the leaves. However, the absence of non-foliar carbon assimilation in the model would counterbalance this effect.

In this work, we combined a light model, a static structural model of a cucumber canopy, an FvCB photosynthesis model and the quantitative limitation analysis of photosynthesis to examine the following questions.

**What is the most prominent factor limiting greenhouse cucumber leaf and canopy photosynthesis?**

The most prominent factors limiting cucumber photosynthesis were \( B_L \) and \( L_L \), and they changed strongly with leaf rank. It seems to be contradictory that the leaves in the upper canopy received the highest light intensities (Fig. 2A) but their photosynthesis could be more restricted by light (Fig. 4E) than those in the lower canopy. This can be explained by the fact that the electron transport rate \( J \) in Eqn (7), which is determined by the biochemical capacity \( J_{\text{max}} \) and light interception \( I_{\text{Int}} \), of the older leaves is mainly reduced by low \( J_{\text{max}} \). Therefore, an increase in \( I_{\text{Int}} \) of the leaves below rank 10 may only increase their photosynthesis rate by up to 20% of the reference.

Here we found that diffusional resistances had less importance in limiting photosynthesis (8–14% of total limitation; Table 3) than biochemical capacity and light interception under non-stressed conditions. In the model used here, parameter \( g_1 \) was
assumed to be constant, but in reality it could decrease with the leaf age. According to Eqn (4), a reduction in \( g_l \) results in a lower \( g_{sc} \) and an increased \( S_L \) (Fig. 5B). This indicates that \( S_L \) could be higher than our estimates in the lower canopy and lower in the upper canopy. In all our simulations, \( M_L \) comprised <4% of the total limitation. This results conflicts with the recently prevailing opinion that \( g_m \) would be the target for increasing photosynthesis and water use efficiency (Flexas et al., 2013). This contradiction may be the consequence of the following: (1) in our study, plants were assumed to grow under non-stressed conditions, comparable, for example, with \( M_L \) of 5% estimated in non-stressed grape (Flexas et al., 2009); (2) cucumber has a relatively high \( g_m \) in comparison with other plant species (Loreto et al., 1992); and (3) all leaves in our simulations were in the RuBP regeneration-limited phase of photosynthesis (Supplementary Data Fig. S2). In this phase, increasing \( C_C \) is less effective in enhancing the net photosynthesis rate than in the Rubisco carboxylation-limited phase because the slope of \( A_C - C_C \) function at \( C_C > C_{ctr} \) is, in general, lower than the slope of \( A_C - C_C \) function at \( C_C < C_{ctr} \).

Sensitivity analysis showed that young and old leaves had a similar sensitivity of \( S_L \) to water vapour deficit (Fig. 5A). This indicates that changes in water vapour deficit may affect whole-plant photosynthesis by up to 10%.

**Do the compositions of photosynthetic limitations vary between different canopy layers?**

Our results showed strong variations in the compositions of photosynthetic limitations between different canopy layers and between different light regimes. The upper canopy, where the young leaves were located (Table 1), had the smallest \( B_L \) and \( S_L \) (Fig. 4A, D). This is the reason why the upper canopy had the highest maximum LUE\(_C\) (Table 4). Our simulations, showing that \( M_L \) increased with canopy depth, are in accordance with the results of a recent publication by Cano et al. (2013), who suggested that in beech and sessile oak \( M_L \) in the lower canopy is twice as high as in the upper canopy.

**How can cucumber canopy photosynthesis be improved?**

Based on analysing the virtual canopy, we suggest three possibilities to improve canopy photosynthesis in a high-wire cucumber cropping system. First, an increase in leaf size of the upper canopy could improve whole-plant photosynthesis. The upper canopy had lower \( B_L \) and higher LUE\(_C\) (Fig. 6) than other canopy layers, but a small photosynthetic apparatus (leaf area, Table 1). Thus, it would be very interesting to investigate the factors limiting the final leaf size of the upper canopy. Possible causes might be the competition between vegetative and generative growth (Wiechers et al., 2011a). However, an increase in leaf size of the upper canopy could shade the lower canopy layers and increase their light limitation. Thus, it is worth using dynamic structural models to find out the optimal leaf area profile (see also Kahlen and Stützel, 2011). Using a Y-shape training system, instead of a single-stem system, might be a possibility to increase the leaf area of the upper canopy. Secondly, improving light interception of the middle–lower and middle–upper canopy layers would also be of importance for increasing whole-plant photosynthesis, because \( L_L \) may contribute up to 55% of the total limitation of these canopy layers (Table 3). This could explain why inter-lighting seems to be more efficient than top-lighting in such a production system (Hovi et al., 2004; Hovi-Pekkanen and Tahvonen, 2008; de Visser et al., 2014). Finally, maintaining the biochemical capacity of the middle–lower canopy layer would be of special importance for increasing whole-plant photosynthesis. It is often observed that the final size of an individual leaf in the single-stem high-wire cropping system reaches its maximum at rank 6–10 and then decreases with leaf rank.
(see also Table 1). Therefore, the middle–lower canopy in this system has the largest photosynthetic apparatus. Using genotypes with a higher value of parameter $c$ [in Eqn (8)] may reduce $B_L$ in the middle–lower and lower canopy because this parameter has a strong influence on the shape of the $B_L$ profile (Fig. 4D). A recent study has revealed that this parameter varies 2-fold between different genotypes (Khaembah et al., 2013). Using inter-lighting would be another method to maintain the photosynthetic capacity of the middle canopy as leaves acclimatizing to a better light environment may maintain their photosynthetic capacity (Trouwborst et al., 2011).

### Perspectives and limitations of combining FSPM and quantitative limitation analysis

We would like to stress that our results may not be generalized to all plant species, although we suppose that similar results may be obtained by analysing other greenhouse crops (e.g. melon, tomato, pepper and aubergine). However, our approach, combining FSPM and quantitative limitation analyses (for both saturating and non-saturating light conditions), can be applied to all plant species and we merely use cucumber as a model plant to demonstrate this approach. It will be fruitful to apply this analysis to investigate other plant species and the influence of horticultural practices on canopy photosynthesis or to search the optimal cropping systems for yield maximization, e.g. row distance, plant density and training system. Another question might be the necessity for supplemental lighting and the efficiency of its energy use. Furthermore, implementing the physiological responses to temperature would aid in revealing the importance of temperature to canopy photosynthesis. It is very likely that temperature is the key factor determining whether photosynthesis is at the Rubisco-limited or RuBP-limited phase in the FvCB model (von Caemmerer, 2013). Carmo-Silva and Salvucci (2012) also showed that temperature strongly affects the position of the transition point ($C_{\text{crit}}$) in the FvCB model. However, temperature changes the reference photosynthesis rate and this makes the comparison difficult. It would be interesting to implement stress responses of $g_{\text{sc}}$ and $g_{\text{m}}$ into the model to investigate the changes in $D_L$ on the canopy level under stress. In general, $g_{\text{sc}}$ and $g_{\text{m}}$ decrease under stress conditions. These decreases may result in (1) an increase in $D_L$, (2) a decrease in $C_a$ and (3) a higher leaf temperature due to a lower $g_{\text{sc}}$ and transpiration. Since photosynthesis tends to be Rubisco limited at low $C_a$ and high temperature and $D_L$ is more prominent at the Rubisco-limited phase (see above), $D_L$ would be significantly higher under stress conditions.

Moreover, implementing this analysis in a dynamic structural model (Kahlen and Stützel, 2011; Wiechers et al., 2011a) would enable us to explore the effect of developmental stage on photosynthetic limitations at the canopy level. How does diffuse light improve the leaf and canopy photosynthesis?

Our findings suggest that the increase in whole-plant photosynthesis under diffuse light is not the result of a higher LUE (with respect to leaf area) but a higher light interception per plant or unit ground area. Indeed, leaves use direct light more efficiently than diffuse light (Brodersen et al., 2008; Brodersen and Vogelmann, 2010), and this was not taken into account in our model. Diffuse light might increase canopy photosynthesis by improving light interception of leaves directly shaded by leaves above them in the canopy. This might explain why the effects of diffuse light were most significant on leaves (Fig. 2B, Fig. 7) which were directly shaded by the leaves above them in the virtual canopy (Supplementary Data Fig. S3). Furthermore, leaves in the lower canopy may acclimatize to the light environment under diffuse light and maintain their photosynthetic capacity (Trouwborst et al., 2011). Therefore, we speculate that the long-term effect of diffuse light could reduce $B_L$ in the lower and middle–lower canopy.

### Conclusions

Our novel model approach, combining an FSPM with quantitative limitation analysis of photosynthesis, allows us to quantify the different photosynthetic limitations at the leaf level and to upscale them to the canopy level. Under non-stressed conditions, the biochemical capacity is the most prominent limitation in the lower canopy, whereas light interception is the most important factor limiting photosynthesis in the upper canopy and diffusional limitations contribute less to total limitation. Methods for maintaining the biochemical capacity of the middle–lower canopy and optimizing the vertical leaf area profile would be promising strategies to improve canopy photosynthesis. Further analyses using our model approach would provide insights into the influence of horticultural practices on canopy photosynthesis and the design of optimal cropping systems.

### SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Text S1: a full mathematical derivation of the limitation analysis under saturating and non-saturating light conditions. Excel S1: two Excel spreadsheets to calculate the different components of photosynthetic limitation. Figure S1: graphical description of the light model from a side view and a top view. Figure S2: relationship between $C_a$ and $C_{\text{crit}}$ in all simulations. Figure S3: top view of the virtual canopy.

### ACKNOWLEDGEMENTS

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### LITERATURE CITED


