

1 **Photosynthesis: ancient, essential, complex, diverse .. and in need of**  
2 **improvement in a changing world**

3 **The 17th International Congress on Photosynthesis Research - Photosynthesis in a**  
4 **Changing World, 7-12 August, 2016, Maastricht, The Netherlands**

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22 The 17th Congress on Photosynthesis Research covered an extraordinarily broad range of  
23 topics from sub-molecular-scale biophysical details of energy transfer and basic chemistry of  
24 artificial photosynthesis to ecophysiology and crop physiology at whole leaf, plant and to  
25 global scales by remote sensing and modeling. Recognizing that photosynthesis is the key  
26 source of energy for life on Earth and the rapid pace of global environmental change and the  
27 pressure of an increasing human population (e.g., Tilman *et al.*, 2011; Alexandratos &  
28 Bruinsma, 2012), the photosynthesis research community faces two important challenges: 1)

29 understanding the mechanisms, vulnerabilities and potentials for improvement of the  
30 photosynthetic process, and 2) developing better techniques for monitoring, modeling and  
31 rapid screening of photosynthesis at scales ranging from the individual genotype (Fiorani &  
32 Schurr, 2013) to fields, bread-baskets (Guan *et al.*, 2016; Pinto *et al.*, 2016) and global  
33 vegetation units (Rogers *et al.*, 2016). Addressing these challenges is essential to identify and  
34 incorporate new genetic improvements in the basic mechanism and to understand and  
35 anticipate the role of photosynthesis in the responses of the global biosphere to climate and  
36 anthropogenic change. In this regard we take note of the decision made this year by the  
37 European Space Agency to build a satellite, FLuorescence EXplorer (FLEX) mission,  
38 intended specifically for studies of photosynthesis by monitoring a product of photosynthesis,  
39 chlorophyll fluorescence. While this will provide an unprecedented new measurement  
40 capability, there remain many questions about how to relate this measurement to  
41 photosynthesis (Schlau-Cohen & Berry, 2015) and this challenge will no doubt be a major  
42 issue in future congresses.

43 Another challenge to crop improvement is the fact that the photosynthetic process has  
44 been fine tuned by billions of years of natural selection, and is subject to deeply rooted  
45 genetic controls shaped in the native environments of the crop ancestors. These may be  
46 difficult to change and may not be optimal for current agro-ecosystems. This was nicely  
47 demonstrated at the meeting by Lisa Ainsworth (USDA ARS, USA) who reported on  
48 mechanisms underlying the historical 80 year improvement in soybean yield showing that  
49 soybean yield has been driven largely by a near doubling of harvest index. While carbon gain  
50 has increased somewhat in modern soybean cultivars, it has been due to increased stomatal  
51 conductance and lower water use efficiency (Koester *et al.*, 2016). Yet, photosynthesis is the  
52 only yield determinant that is not close to its biological limits (Zhu *et al.*, 2008; Ort *et al.*,  
53 2015). In the following, we focus on meeting highlights pertaining to rate-limiting processes  
54 for which improvement could increase crop yield, and on new advancements in monitoring  
55 and predictive modeling of plant photosynthesis.

56

### 57 **Limitation of photosynthesis by ribulose-1,5-bisphosphate carboxylase/oxygenase** 58 **(Rubisco) kinetics and photorespiration**

59 Plant photosynthesis is Rubisco-activity limited much of the time due to its slow catalytic  
60 turnover, and oxygenase activity that competes with carbon fixation, especially in conditions

61 of low chloroplastic CO<sub>2</sub> concentration and higher temperatures. That not all Rubiscos are  
62 identical has motivated work reported at this meeting on the extensive screening of natural  
63 diversity in Rubisco structure and kinetic properties (Galmés *et al.*, 2014b; Whitney *et al.*,  
64 2015; Hermida-Carrera *et al.*, 2016; Orr *et al.*, 2016). Such characterisation is essential to  
65 underpin attempts to tailor crops to future climates. In particular, model analyses suggest that  
66 for many crops replacement of the native Rubisco with the Rubisco of different kinetic  
67 properties would increase photosynthesis in future climates/atmospheres (Long *et al.*, 2006;  
68 Galmés *et al.*, 2014a). While low expression and poor assembly of transgenic Rubiscos has  
69 bedevilled these replacement efforts, Spencer Whitney (Australian National University)  
70 showed promising strategies to engineer foreign Rubisco into tobacco. The use of engineered  
71 tobacco plants to test the influence of altered Rubisco kinetics under future realistic  
72 environmental conditions was further reported by Danielle Way (University of Western  
73 Ontario, Canada). Whitney also emphasized the importance of Rubisco assembly within the  
74 chloroplasts and indicated that co-evolution of one of the chaperons Raf1 with Rubisco  
75 (Whitney *et al.*, 2015) might limit the assembly of foreign Rubisco when Rubisco without  
76 Raf1 is engineered into plants. Exciting progress in understanding the complex processes in  
77 assembly and activation of Rubisco was discussed in the plenary talk of Manajit Heyer-Hartl  
78 (Max Planck Institute of Biochemistry, Germany, Durao *et al.*, 2015; Hauser *et al.*, 2015),  
79 revealing promise that the mechanisms of Rubisco assembly may be soon resolved.

80 An alternative to modifying Rubisco is to directly improve photorespiration using  
81 novel biochemistries to recover carbon at less energetic cost (Kebeish *et al.*, 2007; Xin *et al.*,  
82 2015). This critically depends on the coordination of carbon metabolism and energy supply  
83 (Xin *et al.*, 2015). As Hermann Bauwe (University of Rostock, Germany) discussed, some  
84 photorespiratory bypasses engineered into plants by now disturb NADH supply to  
85 mitochondria and therefore, can be counterproductive due to suppression of mitochondrial  
86 ATP synthesis. To further complicate the matters, engineering photorespiration to future  
87 conditions also requires consideration of inorganic nitrogen assimilation. Florian Busch  
88 (Australian National University) suggested that linking nitrogen assimilation to  
89 photosynthesis by removal of a greater fraction of glycine from photorespiratory cycle  
90 enhances photosynthesis, which can be particularly relevant under conditions of high CO<sub>2</sub>  
91 when the use of nitrate assimilation is inhibited (Bloom *et al.*, 2010). The importance of the  
92 efforts to improve Rubisco and photorespiration was emphasized by Berkeley Walker  
93 (University of Düsseldorf, Germany) who showed that despite photorespiration of crops is  
94 predicted to decrease under future realistic climate scenarios due to the effect of increasing

95 CO<sub>2</sub> concentration being stronger than the higher temperature effect on Rubisco kinetics  
96 (Walker *et al.*, 2016), photorespiration will remain a very large drag on C<sub>3</sub> photosynthetic  
97 efficiency throughout this century.

98

## 99 **CO<sub>2</sub> diffusion limitations of photosynthesis**

100 For photosynthesis to occur, CO<sub>2</sub> has to diffuse from the surrounding leaves to the sites of  
101 CO<sub>2</sub> fixation, which are in the chloroplast of C<sub>3</sub> species and in the mesophyll cytosol in C<sub>4</sub>  
102 species. Compared with the control of photosynthesis by CO<sub>2</sub> diffusion from the air to  
103 intercellular airspaces due to stomatal conductance, the control by CO<sub>2</sub> diffusion from  
104 intercellular airspaces into chloroplasts due to limited mesophyll conductance is much less  
105 understood (Flexas *et al.*, 2012). John Evans (Australian National University) discussed the  
106 control of mesophyll conductance by leaf anatomical characteristics including the  
107 intercellular airspace exposed surface area of mesophyll cells, cell wall thickness and  
108 properties of the cell wall and membranes. These are traits that vary among plant functional  
109 types and underlay the functional type-specific differences of CO<sub>2</sub> sensitivity of  
110 photosynthesis (Niinemets *et al.*, 2011). Jennifer McElwain and Charilaos Yiotis (University  
111 College Dublin, Ireland) put such structural controls in an evolution context to explain the  
112 shifts in dominance of ferns, gymnosperms and angiosperms as plants encountered varying  
113 CO<sub>2</sub>:O<sub>2</sub> concentration ratio over geological time and are also predicted to alter the  
114 distribution of natural vegetation in future atmospheres (Niinemets *et al.*, 2011; Flexas *et al.*,  
115 2016).

116 It is currently less clear how we can explain rapid changes in mesophyll conductance,  
117 which have sometimes been observed. Several aquaporins have been shown to enhance CO<sub>2</sub>  
118 permeability of membranes and John Evans suggested that aquaporins may form part of the  
119 answer, but pointed out that our quantitative understanding is currently limited by the  
120 resolution of techniques that exist for measuring mesophyll conductance (Groszmann *et al.*,  
121 2016). Guillaume Théroux-Rancourt (University of California Davis, USA) further suggested  
122 that two-dimensional techniques used to quantify mesophyll conductance may lead to  
123 misleading inferences on light dependence of mesophyll conductance as different cell layers  
124 contribute to photosynthesis at low and high light, suggesting that 3D leaf anatomy needs to  
125 be considered in models calculating mesophyll conductance (see also Lloyd *et al.*, 1992).

126 Most of our information of mesophyll conductance has been obtained in C<sub>3</sub> species  
127 where several measuring techniques exist, including measurements of <sup>13</sup>C isotope  
128 discrimination or chlorophyll fluorescence combined with gas exchange. These techniques  
129 cannot be used to measure mesophyll conductance in C<sub>4</sub> species. An exciting advancement  
130 has been the use of stable isotopes of oxygen (<sup>18</sup>O) in CO<sub>2</sub> to estimate mesophyll conductance  
131 (Gillon & Yakir, 2000; Barbour *et al.*, 2016). Asaph Cousins and his team have used this  
132 technique to quantify mesophyll conductance in several C<sub>4</sub> species and demonstrate that  
133 mesophyll conductance in C<sub>4</sub> species is quantitatively similar to that of C<sub>3</sub> species at  
134 comparable photosynthetic rates. Furthermore, Cousins demonstrated that mesophyll  
135 conductance in C<sub>4</sub> species *Zea mays*, *Setaria viridis* and *Miscanthus x giganteus* strongly  
136 increased with temperature as had previously been observed for C<sub>3</sub> species (von Caemmerer  
137 & Evans, 2015).

138 Improving C<sub>3</sub> plant photosynthesis by introducing carbon-concentrating mechanisms  
139 (Atkinson *et al.*, 2016) or facilitating diffusion by increasing carbonic anhydrase activity  
140 (Terashima *et al.*, 2011) have also been suggested as possible targets for improving  
141 photosynthesis. However, the progress has been moderate (Atkinson *et al.*, 2016), and as  
142 Bernard Genty (Centre Energie Atomique et Energies Alternatives, France) demonstrated,  
143 CO<sub>2</sub> backdiffusion and lack of intrachloroplastic compartmentalization implies that faster  
144 inorganic carbon conversion to CO<sub>2</sub> has only limited effects on photosynthesis.

145

#### 146 **Improving inefficiencies due to limited response to dynamically changing environmental** 147 **drivers over short and long term**

148 Photosynthetic research in natural field environments and in crop plants has characteristically  
149 looked at photosynthesis in steady-state conditions. However, leaves in plant canopies have a  
150 light and temperature environment that is under constant fluctuation, and thus, photosynthesis  
151 is continually adjusting to such fluctuations. However, reaching full photosynthetic activation  
152 upon environmental changes is time-consuming, implying that the environment dynamicity  
153 has important consequences for daily carbon gain. Losses in productivity due to limited  
154 dynamic responses can result from slow activation of Calvin cycle enzymes, including  
155 Rubisco activation by Rubisco activase, time needed to fill up the metabolite pools of the

156 Calvin cycle, limited rate of relaxation of photoprotective non-photochemical quenching  
157 (NPQ), as well as sluggish stomatal movements.

158           Rebekka Wachter (Arizona State University, USA) described new mechanisms for the  
159 assembly and regulation of Rubisco activase from higher plants and explained the possible  
160 mechanisms underlying the Rubisco activation kinetics (Kuriata *et al.*, 2014; Wachter &  
161 Henderson, 2015), while Elizabete Carmo-Silva (Lancaster University, UK) reported on  
162 natural variation in the response of Rubisco and Rubisco activase that holds promise for  
163 improving daily carbon gain by maintaining a higher average Rubisco activation state in  
164 fluctuating light. Ana Lobo (Universidade Federal do Ceará, Brazil) tested the hypothesis that  
165 that overexpression of 2-carboxy-D-arabinitol-1-phosphate (CA1P) phosphatase (CA1Pase)  
166 to more rapidly dephosphorylate the Rubisco inhibitor CA1P to a non-inhibitory compound  
167 would stimulate Rubisco activity and photosynthesis by decreasing the amount of CA1P in  
168 wheat leaves. The surprising outcome was that CA1Pase overexpression resulted in a large  
169 drop in Rubisco abundance and photosynthesis, proving that not every good idea goes as  
170 planned.

171           Although there is considerable biological variability in the rate of NPQ relaxation  
172 (e.g. Adams *et al.*, 2001), there have been surprisingly few efforts to enhance it. Johannes  
173 Kromdijk (University of Illinois, USA) reported exciting results showing that the transgenic  
174 manipulation of three genes involved in regulating the formation and relaxation of non-  
175 photochemical quenching (NPQ) resulted in tobacco plants that had a significantly higher rate  
176 of NPQ relaxation, which in turn resulted in significantly higher carbon gain and biomass  
177 accumulation in fluctuating light.

178           Tracy Lawson (University of Essex, UK) showed that due to a mismatch between  
179 photosynthetic induction and stomatal response, species differences in stomatal kinetics  
180 strongly affect plant water use efficiency (McAusland *et al.*, 2016), and the implications of  
181 dynamic environmental conditions on simulated carbon gain at leaf and canopy scales were  
182 further considered by Silvere Vialet-Chabrand (University of Essex, UK) and Alejandro  
183 Morales Sierre (University of Wageningen, The Netherlands). Compared with past dynamic  
184 photosynthesis models (e.g., Pearcy *et al.*, 1997), these new models include mesophyll  
185 conductance, chloroplast movements and relaxation of fast and slow components of NPQ,  
186 providing tools for rigorous quantification of the dynamic adjustment of photosynthetic traits  
187 on plant productivity. Our general feeling from this meeting was that the photosynthesis

188 community is increasingly starting to appreciate the relevance of fast dynamic responses of  
189 photosynthesis and their implications for crop yield.

190         Regarding longer-term dynamics, it has long been thought that rising atmospheric  
191 CO<sub>2</sub> would improve crop yields particularly during drought events because it promotes  
192 stomatal closure and saves water. ISPR 2016 Calvin Benson Award winner Andrew Leakey  
193 (University of Illinois, USA) presented surprising results from 8 seasons of FACE  
194 experiments showing that increased sensitivity of stomata to physiological drought signals  
195 limited the usual CO<sub>2</sub> stimulation of photosynthesis and that an interaction of CO<sub>2</sub>  
196 enrichment, drought and the deployment of nitrogen-fixing root nodules in dry soil led to a  
197 nitrogen deficit in drought years. Thus, in wet years, elevated CO<sub>2</sub> improved soybean yield  
198 by more than 20%, but severe drought eliminated the stimulation altogether (Gray *et al.*,  
199 2016).

200

#### 201 **Sink-source activity and implications for whole plant photosynthesis**

202 Sink strength has long been recognized as an important regulator of photosynthetic carbon  
203 gain and yield. The ratio between the supply of carbohydrates by the leaves and the  
204 consumption by all plant organs has been found to be an important player and this  
205 source:sink ratio can up- or downregulate photosynthesis. Congming Lu (Chinese Academy  
206 of Sciences, China) reported highly convincing evidence that transgenic rice plants  
207 expressing the *Arabidopsis* phloem-specific sucrose transporter AtSUC2, which loads sucrose  
208 into the phloem, showed up to 16% increase in grain yield in field trials. Daisuke Sugiura  
209 (University of Tokyo, Japan) manipulated the source:sink balance of a range of species by  
210 continuously removing the new leaves, providing low levels of nutrients or high levels of  
211 light. Soluble sugars and starch accumulated relative to control plants, but a significant  
212 negative correlation with photosynthetic capacity was observed in only one of the species  
213 studied. He considered the effect of defoliation more closely and found that it strongly  
214 increased the total leaf mass and cell wall mass per area, but decreased stomatal and  
215 mesophyll conductance. The implication of these findings is that there could be various  
216 independent modes by which source and sink interact. Although sink-source effects were  
217 seemingly an underrepresented area at the Congress, resolving the controls of sink strength is  
218 of paramount significance for efforts targeted to improve crop photosynthesis under future  
219 climates.

## 220 **Improvements in predicting and monitoring photosynthesis**

221 The steady-state photosynthesis model of Farquhar et al. (1980) is now widely used in  
222 simulating photosynthesis at scales ranging from leaf to globe due to its simplicity, ease of  
223 parameterization and extensive validation. Since the model development, there have been  
224 uncertainties in description of the light reactions of photosynthesis, in particular, about how  
225 the balance of ATP and NADPH is achieved, and what limits photosynthesis in feedback-  
226 inhibited conditions (Sharkey, 1985). Xinyou Yin (University of Wageningen, The  
227 Netherlands) extended the Farquhar et al. (1980) steady-state model by including cyclic and  
228 pseudocyclic electron flows and distribution of light between photosystems I and II. David  
229 Kramer (Michigan State University, USA) described of an approach of using cost-effective  
230 distributed, cloud-linked instrumentation based on measurements of chlorophyll fluorescence  
231 for studies of photosynthesis in agriculture, ecology and climate research. Point  
232 measurements taken by a large number of operators all over the world are automatically  
233 reported to a central cloud based server where machine learning algorithms can be used to  
234 analyze simultaneous data from many locations, revealing hidden relationships and allowing  
235 for efficient gap-filling strategies and integrated estimates to be made. Measuring light  
236 reactions of photosynthesis is principally the only option for simple and fast screening of  
237 photosynthetic activity in multiple locations and for large-scale assessment of photosynthetic  
238 activity of plants (Guan *et al.*, 2016; Pinto *et al.*, 2016).

239 Several talks focused on remote sensing of fluorescence emitted from chlorophyll of  
240 photosynthetic organisms in response to passive solar illumination. Unlike other forms of  
241 remote sensing this measurement is tied specifically and mechanistically to the  
242 photosynthetic process (Schlau-Cohen & Berry, 2015). The fact that this signal can now be  
243 measured from space opens up new possibilities for investigating photosynthetic mechanisms  
244 at a planetary scale and new challenges to interpret this measurement. Presentations dealt  
245 with retrieval of fluorescence from the global oceans and from the land; with measurements  
246 at smaller scales designed to provide “ground-truth” for the global measurements, and with  
247 model development designed to bridge the gap between mechanistic studies of  
248 photosynthesis at the leaf or chloroplast scale with observations of fluorescence emitted from  
249 complex canopies. Uwe Rascher (Forschungszentrum Jülich, Germany) presented an  
250 overview of the (FLEX) mission recently selected by the European Space Agency (ESA).  
251 FLEX will be the first satellite designed specifically for retrieval of fluorescence from  
252 terrestrial plants, and is expected to launch in 2022. Rascher also described extensive ground



253 and aircraft based measurements that have paved the way for this mission. Most important is  
254 the visualization of large differences between vegetation types (e.g. crops and forests) in  
255 fluorescence that correspond to differences in their photosynthetic rates. Fluorescence was  
256 also shown to be a sensitive indicator of the presence of plant stress, supporting the prospect  
257 that this satellite will have a major impact on efforts to understand photosynthesis at a  
258 planetary scale. Christiaan van der Tol (University of Twente, The Netherlands) described a  
259 model developed to simulate and assimilate satellite observations to invert for important  
260 vegetation properties (like Rubisco levels) that are known to control productivity (Tol *et al.*,  
261 2014). While the recent progress in remote sensing by gauging solar-induced fluorescence  
262 has been truly amazing , there are still uncertainties in the mechanistic aspects of  
263 fluorescence especially the relative roles of PSI and PSII and non-photochemical quenching  
264 in the passive fluorescence observed by aircraft and satellites, and quantification of the  
265 relationship between fluorescence and productivity still remains challenging.

266

## 267 **Outlook and future perspectives**

268 The conference showed exciting examples where steps forward were made in understanding  
269 and manipulating components of the photosynthetic process, in part by clever  
270 experimentation, in part by changing genes or gene expression. What we hope to see for the  
271 next meeting in 2020 in Rotorua, New Zealand is that people are able to combine these  
272 insights to stack various changes onto each other, thereby reaching improvements in  
273 productivity that would go beyond the values that have been reported for those changes  
274 individually. Another great step would that satellite based remote sensing of traits of the  
275 photosynthetic process will indeed reveal new possibilities for investigating photosynthesis  
276 and photosynthetic mechanisms at a planetary scale.

277

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281

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