

High-throughput estimation of incident light, light interception and radiation-use efficiency of thousands of plants in a phenotyping platform

Llorenç Cabrera-Bosquet, Christian Fournier, Nicolas Brichet, Claude Welcker, Benoît Suard, François Tardieu

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1 **METHODS PAPER**

2

3 **High throughput estimation of incident light, light interception and radiation-**
4 **use efficiency of thousands of plants in a phenotyping platform**

5

6 Llorenç Cabrera-Bosquet, Christian Fournier, Nicolas Brichet, Claude Welcker,
7 Benoît Suard & François Tardieu

8

9 *UMR LEPSE, INRA, Montpellier SupAgro, 34000, Montpellier, France*

10

11 Author for correspondence:

12 Llorenç Cabrera-Bosquet, Tel. : +33 499 612 956, Fax: +33 467 612 116,

13 Email: llorenc.cabrera@supagro.inra.fr

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20 **Summary**

- 21 • Light interception and radiation use efficiency (RUE) are essential components of
22 plant performance. Their genetic dissections require novel high-throughput
23 phenotyping methods.
- 24 • We have developed a suite of methods to evaluate (i) the spatial distribution of
25 incident light as experienced by hundreds of plants in a greenhouse, by simulating
26 sun beam trajectories through greenhouse structures every day of the year (ii) the
27 amount of light intercepted by maize (*Zea mays*) plants, via a functional-structural
28 model using 3D reconstructions of each plant placed in a virtual scene reproducing
29 the canopy in the greenhouse and (iii) RUE, as the ratio of plant biomass to
30 intercepted light.
- 31 • The spatial variation of direct and diffuse incident light in the greenhouse (up to 24%)
32 was correctly predicted at the single-plant scale. Light interception largely varied
33 between maize lines that differed on leaf angles (nearly stable between experiments)
34 and area (highly variable between experiments). Estimated RUEs varied between
35 maize lines but were similar in two experiments with contrasting incident light. They
36 closely correlated with measured gas exchanges.
- 37 • The methods proposed here identified reproducible traits that might be used in
38 further field studies, thereby opening the way for large-scale genetic analyses of the
39 components of plant performance.

40

41 **Key words:** environmental characterization, high-throughput phenotyping, maize, light
42 interception, radiation-use efficiency, architecture

43

44 Introduction

45 Understanding the genetic controls of biomass production and yield is a major challenge in
46 the context of climate change (Murchie *et al.*, 2009; Zhu *et al.*, 2010; Reynolds *et al.*, 2012).
47 Yield (Y) can be dissected as a function of incident light (PPFD), the fraction of light
48 intercepted by the crop (ϵ), the efficiency of the conversion of light into biomass, also called
49 radiation-use efficiency (RUE,(Monteith, 1977)) and the partitioning of biomass to yield
50 (harvest index, HI):

$$51 \quad Y = HI \times \sum_{i=1}^n PPF D_i \times \epsilon_i \times RUE_i, \quad (1)$$

52 where n is the duration of crop growth (d), $PPFD_i$, ϵ_i and RUE_i are the incident light, the
53 fraction of intercepted light and RUE on the i^{th} day. Y can be genetically improved by
54 increasing any of the terms of Eq. 1. Whereas HI has been one of the main determinants for
55 improving yields in wheat during the 20th century (Calderini *et al.*, 1995; Sayre *et al.*, 1997),
56 there is probably little avenue for further improvements in most crops (Austin *et al.*, 1980;
57 Foulkes *et al.*, 2011; Reynolds *et al.*, 2012). The remaining terms of Eq. 1, ϵ and RUE, are
58 directly related to light capture and photosynthetic efficiency at canopy level (Zhu *et al.*,
59 2010; Reynolds *et al.*, 2012). The genetic variability of leaf area development has a high
60 effect on light interception at early stages of the plant cycle (Hay & Porter, 2006; Murchie *et al.*,
61 2009). Changes in canopy architecture also affect interception *via* genotypes with erect
62 leaves that decrease light saturation at the top of the canopy and allow better penetration
63 of light, thereby reducing the proportion of leaf area experiencing low light (Long *et al.*,
64 2006; Zhu *et al.*, 2010; Reynolds *et al.*, 2012). Studies comparing the relationship between
65 the genetic variabilities of leaf architecture and light interception at the intraspecific level
66 have been limited to a small number of genotypes, with contrasting conclusions (Louarn *et al.*
67 *et al.*, 2008; Hammer *et al.*, 2009; Moreau *et al.*, 2012).

68 Estimations of RUE are usually based on consecutive and destructive sampling of
69 aboveground biomass over time, which is not feasible for the large number of genotypes
70 involved in genetic analyses (Sinclair & Muchow, 1999). Gas-exchange measurements are
71 also difficult to tackle at high throughput and are usually limited to measurements at the
72 single-leaf level (Albrizio & Steduto, 2005). A phenotyping platform allowing measurements
73 of 3D plant architecture and estimates of plant biomass with a time definition of one day

74 offers new possibilities to estimate light interception and RUE of hundreds of genotypes,
75 together with their responses to environmental conditions. However, no method is currently
76 proposed because of technical difficulties.

77 - The spatial variability of incident light can be up to 30% within a greenhouse (Stanhill *et al.*,
78 1973; Kozai & Kimura, 1977; Brien *et al.*, 2013) or a growth chamber (Granier *et al.*, 2006).
79 Conditions also vary between experiments, in greenhouses because of climatic conditions
80 and in growth chambers because of differences between chambers (Massonnet *et al.*,
81 2010). A genetic analysis of plant performance therefore requires a precise evaluation of
82 the PPFD (diffuse and direct) available to each plant of the greenhouse or growth chamber
83 during each experiment.

84 - Light interception can be derived from 3D plant architecture, combined with estimates of
85 the direction of sunbeams and with the proportion of direct vs. diffuse light (Sinoquet *et al.*,
86 2001). Platform experiments present a difficulty compared with the field, namely that
87 they often harbour composite canopies in which each plant is surrounded by plants of
88 another genotype. Hence, it is necessary to distinguish the light interception by each plant
89 to dissect the genetic variabilities of ϵ and RUE.

90 The objective of this paper was to develop a non-invasive, automatized and accurate
91 procedure to derive light interception and radiation-use efficiency in high-throughput
92 phenotyping platforms. To our knowledge, we present here the first methods for estimating
93 the local PPFD received by each individual plant and for estimating light interception and
94 RUE at a throughput of thousands of plants. We have tested whether RUE derived from the
95 methods presented here is stable between experiments and related to leaf gas-exchange
96 measurements.

97

98

100 ***The PHENOARCH phenotyping platform***

101 The PHENOARCH platform (<http://bioweb.supagro.inra.fr/phenoarch>), hosted at the M3P,
102 Montpellier Plant Phenotyping Platforms (<https://www6.montpellier.inra.fr/lepse/M3P>), is
103 based on a PhenoWare™ system (PhenoWare™, Lyon, France) composed of a conveyor belt
104 structure of 28 lanes carrying 60 carts with one pot each (i.e. 1680 pots), plus a conveyor
105 belt system that feeds the imaging or the watering units. The imaging unit is composed of
106 two cabins with 3D image acquisition involving top and side RGB cameras (Grasshopper3,
107 Point Grey Research, Richmond, BC, Canada) equipped with 12.5-75mm TV zoom lens
108 (Pentax, Ricoh Imaging, France) and LED illumination (5050 - 6500K colour temperature).
109 Five watering units are composed of weighing terminals (ST-Ex, Bizerba, Balingen, Germany)
110 and high-precision pumps (520U, Watson Marlow, Wilmington, MA, USA). Circulation of
111 plants through conveyors, image acquisition and irrigation management are controlled by an
112 industrial open automation system based on PC Control technology (Beckhoff CX 2020,
113 Beckhoff Automation, Verl, Germany) that allows localization in real time of every pot in the
114 platform and individually programming pot displacements. Imaging and watering routines
115 are sequentially performed every day. Plants are then moved back to the same positions and
116 orientation, so plant position in respect to neighbours is conserved throughout the
117 experiment. PHENOARCH has held experiments with different species including cereals
118 (maize (*Zea mays* L.), wheat, rice, sorghum) (Sciara *et al.*, 2015), grapevine (Coupel-Ledru *et*
119 *al.*, 2014) and apple trees (Lopez *et al.*, 2015). The plant density can be adapted for each
120 species, from 13 plants m⁻² in the default setting with 1680 plants to double densities for
121 smaller plants or half densities for small trees or adult maize plants. Experiments performed
122 until mid-2014 used a Lemnatec technology (LemnaTec, Wüerselen, Germany) that was then
123 replaced by the methods presented above.

124 Micro-meteorological conditions are constantly monitored at six positions in the greenhouse
125 at the top of the plant canopy. Air temperature and humidity are measured every minute
126 (HMP45C, Vaisala Oy, Helsinki, Finland), together with PPF (SKP215, Skye Instruments,
127 Powys, UK). The temperature of the meristematic zone of eight plants distributed in the
128 greenhouse is measured with a fine copper-constantan thermocouple (0.2 mm diameter)
129 located between the sheaths of two leaves located at meristem height. Air vapour pressure

130 difference (VPD) is estimated at each time step as the difference in water vapour pressure
131 between saturation at air temperature and the current vapour pressure in the air. All data of
132 air/meristem temperature, PPFD and relative humidity are averaged and recorded every 15
133 min (NI CompactRio, National Instruments, Austin, TX, USA) and stored in the PHIS database
134 (<http://web.supagro.inra.fr/phis>).

135 ***Spatial variability of incident light in the greenhouse***

136 Daily incident PPFD over each plant of the platform ($PPFD_{(xy)}$) was estimated by combining a
137 2D map of light transmission and the outside PPFD ($PPFD_{ext}$) measured every 15 min with a
138 sensor placed on the greenhouse roof (SKS 1110, Skye Instruments, Powys, UK). Maps of the
139 fraction of transmitted direct (T_{dir}) and diffuse (T_{dif}) light were calculated every hour of each
140 day of the year by using 169 hemispherical images of the greenhouse using a digital camera
141 (Nikon Coolpix 4500, Nikon, Melville, NY, USA) fitted with a fisheye lens with a 183° field of
142 view (Nikon FC-E8 Fisheye Converter, Nikon). Images were taken every m^2 in the
143 greenhouse, at 0.4, 1 and 1.5 m high, i.e. at heights representing the top of canopies of
144 different species and phenological stages. Only data at 1m height are presented here. The
145 camera and lens were placed vertically (checked with a spirit level) and the geographical
146 North was referenced. Hemispherical images were analysed using the Ilastik 1.1.8 software
147 (Sommer *et al.*, 2011) (Supporting Information Fig. S1, Table S1). Calculation of sun paths,
148 transmitted direct and diffuse radiation was then performed using standard astronomical
149 formulae using R scripts (R_Core_Team, 2015) available as an open application (Supporting
150 Information Methods S1). The refraction in the glass of the greenhouse changed the angle of
151 sun beams by 0 to 36° for incident angles up to 60°. It returned to its original value in air so
152 refraction resulted in a translation of sunbeams by 0 to 2 cm, and was therefore considered
153 as negligible. The amount of transmitted direct radiation was computed every hour as a
154 function of solar position, calculated from the daily time-course of sun path, in relation to
155 the gap fraction at each position along the sun path (i.e. fraction of the image without
156 greenhouse structure or lamps, Fig. 1). The amount of transmitted diffuse PPFD was
157 calculated using a standard overcast sky (SOC) diffuse model (Moon & Spencer, 1942), in
158 which diffuse radiation flux varies with zenith angle and then depends on the gap fraction of
159 the greenhouse. Transmissions of direct and diffuse light were further corrected by the

160 transmittance coefficient of light through glass, measured using a spectroradiometer
161 (HR4000, Ocean Optics, Dunedin, FL, USA).

162 The amount of PPFD reaching each of the XY positions within the greenhouse ($PPFD_{(x,y)}$) on a
163 given day (i) was calculated as:

$$164 \quad PPFD_{(x,y)i} = \sum_{j=0}^d f_{d,j} \times PPFD_{ext,j} \times T_{dir(x,y)j} + (1 - f_{d,j}) \times PPFD_{ext,j} \times T_{dif(x,y)} \quad (2)$$

165 Where f_d is the fraction of direct light and $(1 - f_d)$ is the fraction of diffuse light from incoming
166 external global radiation corresponding to the j^{th} hour and d the duration of daylight,
167 calculated according equations detailed in (Spitters *et al.*, 1986).

168 ***Image analysis and reconstruction of plant architecture***

169 RGB colour images (2056 x 2454) from thirteen views (twelve side views from 30° rotational
170 difference and one top view) were captured daily for each plant during the night. Images
171 were captured while the plant was slowly rotating using a brushless motor. Top and side
172 cameras were calibrated using reference objects in order to convert pixels into mm^2 . Plant
173 pixels from each image were segmented from those of the background with HSV
174 thresholding using OpenCV libraries (Open Source Computer Vision Library:
175 <http://opencv.org>). A 3D representation of each plant of the platform was obtained using a
176 silhouette-carving algorithm. Plant skeletons were extracted from binarised 2D RGB images
177 using the thinning algorithm of (Zhang & Suen, 1984), implemented in ImageJ (Rasband,
178 1997-2014). Skeletons were further processed with the 'Analyse Skeleton' ImageJ plugin
179 ([Arganda-Carreras et al., 2010](#)) to obtain crossings and the endpoints of the different
180 branches of the skeleton. These points were used to navigate through the skeleton image
181 and segment it into 50-pixels-long elementary lines. The angle of each elementary segment
182 with the vertical was computed as the absolute value of the arctangent between z- and x-
183 coordinates of segments endpoints. All data, namely raw and processed images together
184 with metadata were stored in the PHIS database (<http://web.supagro.inra.fr/phis/>).

185 ***Light interception and radiation-use efficiency***

186 Daily light interception was estimated for each plant of the platform by using the functional-
187 structural RATP (radiation absorption, transpiration and photosynthesis) plant model
188 (Sinoquet *et al.*, 2001) available under OpenAlea platform
189 (<http://openalea.gforge.inria.fr/dokuwiki/doku.php>) (Pradal *et al.*, 2008; Pradal *et al.*, 2015).

190 Briefly, the canopy was split into cubic voxels of 0.2 m, characterized each by the density of
191 leaf area and the leaf angle distribution calculated from the 3D virtual representations of the
192 neighbouring plants. Density of leaf area was calculated as the cumulative area of all leaf
193 segments in the voxel, regardless of the plant they originated from. The calculated mean leaf
194 angle in a voxel was calculated as the mean of angles of all leaf segments in the considered
195 voxel.

196 For each voxel, intercepted PPFd was calculated every day from a sample of 46 beam angles,
197 with a cumulative value equal to the incident PPFd at the corresponding x y position in the
198 greenhouse (PPFD_(xy)). For each direction, beam extinction was computed by applying Beer's
199 law within the sequence of intersected cells by each beam. The daily PPFd intercepted by
200 each plant was obtained by cumulating the PPFd interception for each voxel weighed by the
201 relative contribution of the considered plant to the voxel area. Radiation-use efficiency was
202 then estimated as the slope of plant biomass production to cumulative intercepted PPFd.

203 ***Leaf gas exchange measurements***

204 A portable open gas exchange system (LI-COR 6400XT, LI-COR Inc., Lincoln, NE, USA) was
205 used to measure leaf gas exchange in youngest fully expanded leaf blades in a set of eight
206 maize lines. The net CO₂ assimilation rate (A_N), stomatal conductance (g_s) of those leaves
207 were measured inside the greenhouse from 10:00 to 14:00 (solar time) at 1500 $\mu\text{mol photon}$
208 $\text{m}^{-2} \text{s}^{-1}$ of PPFd, a leaf temperature of 28°C, a leaf-to-air vapour pressure deficit of about 1.2-
209 1.5 kPa and an ambient CO₂ of 400 $\mu\text{mol mol}^{-1}$.

210 ***Plant growth***

211 The leaf area and the fresh plant weight of individual plants were estimated from images
212 taken in 13 directions. Briefly, pixels extracted from RGB images were converted into fresh
213 plant weight and leaf area using linear models derived from regression of data from
214 multiple side view images and destructive measurements performed at different
215 phenological stages, from 5 to 14 appeared leaves (i.e. from 15 to 50 days at 20°C after
216 emergence). The resulting conversion was accurate and unbiased (Supporting Information
217 Fig. S2). The time courses of leaf area or fresh plant weight were then fitted individually to
218 the three-parameter Gompertz function,

$$219 \quad y = a \times e^{-e^{(b-cx)}} \quad (3)$$

220 using appropriate R scripts (R_Core_Team, 2015). Time courses were expressed as a function
221 of equivalent days at 20°C (Parent *et al.*, 2010). At the end of the experiment, shoots of all
222 plants were harvested and total plant biomass was measured.

223 **Genetic material and growth conditions**

224 The techniques presented above were tested in two experiments carried out in autumn (Exp.
225 1) and winter-spring (Exp. 2), with markedly different incident PPFD. Two panels were
226 studied involving 60 and 200 maize (*Zea mays* L.) lines for Exp. 1 and Exp. 2, respectively. A
227 common set of 23 maize inbred lines with tropical origin was grown in both experiments,
228 chosen for maximising the genetic and phenotypic variabilities. Plants were grown in
229 polyvinyl chloride (PVC) 9 L pots (0.19 m diameter, 0.4 m high) filled with a 30:70 (v/v)
230 mixture of a clay and organic compost. Three seeds per pot were sown at 0.025m depth and
231 thinned to one per pot when leaf three emerged. In each of the experiments two levels of
232 soil water content were experienced; (i) retention capacity (WW, soil water potential of -
233 0.05 MPa) and (ii) mild water deficit (WD, soil water potential of -0.5 MPa) by compensating
234 transpired water three times per day via individual measurements of each plant. The weight
235 of water in each pot was calculated at the beginning of the experiment from the weight of
236 soil and measured soil water content. It was then maintained at a constant value by
237 considering that the weight loss between two time-points was due to transpiration plus soil
238 evaporation, after correction for the change in plant fresh weight every day (Eq. 3). Each line
239 was replicated 5 and 7 times for the WW and WD treatments, respectively in Exp. 1, whereas
240 each line was replicate 4 times in Exp. 2. Greenhouse temperature was maintained at 25 ±
241 3°C during the day and 20°C during the night. Supplemental light was provided either during
242 day time when external solar radiation dropped below 300 W m⁻² or to extend photoperiod
243 using 400 W HPS Plantastar lamps (OSRAM, Munich, Germany) with 0.4 lamps m⁻². The
244 resulting photoperiod was 12/12h day/night. The amount of light supplied by lamps was
245 taken into account in the calculations of local PPFD.

246 **Statistical analyses**

247 Two-way analyses of variance (ANOVA) were performed using the lm procedure
248 (R_Core_Team, 2015) to calculate the effects of experiment and genotype. Broad-sense
249 heritability (h^2) was calculated as:

$$250 \quad h^2 = \sigma_G^2 / (\sigma_G^2 + \sigma_e^2/r) \quad (4)$$

251 Where σ^2_G is the genotypic variance, σ^2_e is the residual variance, and r is the number of plant
252 replicates per genotype. All statistical tests and graphs were performed using R 3.1.3
253 (R_Core_Team, 2015).

254 **Results**

255 ***Estimating the spatial variability of local available light for each plant of the platform***

256 We have modelled the fraction of direct and diffuse light reaching each plant every hour of
257 each day of the year based on 169 hemispherical images taken at different x-y positions of
258 the greenhouse (1 image m^{-2}), which capture the obstacles to light (e.g. beams of the
259 greenhouse, lamps or cabins, Fig. 1). For each image, the daily time-course of sun path was
260 simulated based on the latitude, day of year and time of day, as presented in Fig. 1a-d for
261 summer and winter solstices and spring and autumn equinoxes. Sun paths occupied more
262 central positions in the image and were longer during summer compared to winter because
263 of the changes of solar position with the vertical. They crossed the structures of the
264 greenhouse for a fraction of the day and reached plants (path superimposed on the sky)
265 otherwise. The proportion of transmitted direct PPFD was estimated from the gap fraction
266 over the sun path (frequency for a light beam to cross the structure in the absence of
267 obstacle), weighed for light intensity and displacement rate along the sun path at each time-
268 step (Supporting Information, Methods S1). Light transmission through the greenhouse glass
269 was also estimated, resulting in a transmittance coefficient (k_g) of 0.76.

270 Daily transmission of direct light was calculated every day for each of the 1680 positions in
271 the greenhouse, resulting in large spatial and temporal variations. The duration of peaks of
272 transmission was shorter in winter than in summer solstices, whereas the maximum value
273 was close to 60% in all cases (Fig. 1e-h). The time course of transmission also changed with
274 position in the greenhouse (Fig. 1e-l), with a spatial variability that was greater in winter,
275 with up to 30% differences between locations, compared to 14% in summer. The x-y position
276 with maximum transmission itself changed with time of year. Integrated over one day, these
277 data resulted in a map of direct transmission for each day of the year (Fig. 2i-l, Supporting
278 Information Video S1). The transmission of diffuse radiation was also calculated for each
279 position of the greenhouse from the same hemispherical images, resulting in a spatial
280 variation of 11.9% (Fig. 2b). Whereas transmission of direct light maps was calculated each

281 day of the year (Fig. 2a), the transmission of diffuse light only depends of the greenhouse
282 structure so the map presented in Fig 2b is independent of days of the year. The total
283 amount of direct plus diffuse PPFD reaching each location in the greenhouse was finally
284 estimated using Eq. 2 (Fig. 2).

285 The method presented above can be extended to any other greenhouse provided that
286 images are available (Supplementary Information Methods S1). It was tested by comparing
287 the simulated PPFD at six locations in the greenhouse with PPFD measured at the same
288 locations. Measured PPFD cumulated over one week showed an appreciable spatial
289 variability (24%, from 173 to 220 mol m⁻²), which was adequately accounted for by the
290 simulated PPFD at the same positions (Fig. 3a). The daily time courses of observed vs
291 simulated PPFD averaged over the six positions in the greenhouse were also compared on
292 two days with high and low PPFD values, respectively. Fig. 3b shows that observed and
293 simulated time courses closely matched, so the cumulative PPFD values estimated with the
294 methods presented above was not biased.

295 ***Estimating leaf angles over time***

296 The side plant image containing maximum information for the quantification of leaf angles
297 was chosen for each plant and day as that containing most leaves (Fig. 4a). To that end, we
298 have used the binarised top view of the plant on which we performed a reduced major axis
299 regression, allowing us to choose the side image with the best angle (Fig. 4b). This image was
300 then segmented and skeletonized (Fig. 4cd). The endpoints of the different branches of plant
301 skeletons (red circles) were used to navigate through the skeleton and to dissect it into 50-
302 pixels-long elementary segments (Fig. 4f). The orientation of each elementary segment was
303 then computed, thereby allowing calculation of angles for each segment. The mean leaf
304 angle was then computed either for a whole plant or in each voxel, as shown in Fig. 4e for
305 the plant presented in Fig 4a-e. An example of this procedure is shown in Figure 4g where
306 mean leaf angles clearly differed between three maize lines showing either sub-horizontal
307 angles (close to 0) or more erect architectures. Angles corresponding to each line remained
308 essentially stable with phenological stages as illustrated in Figure 4h.

309 ***Calculating daily light interception by each plant of the greenhouse***

310 The composite canopy in the greenhouse (i.e. with neighbouring plants belonging to
311 different lines) was reconstructed from individual 3D point cloud representations of each

312 plant, and split into in a grid of voxels for calculating light interception using the RATP model.
313 For each plant, actual plant leaf area and plant leaf angle distribution were uniformly
314 distributed among the 3D point cloud. Figure 5 represents a schematic diagram of how the
315 RATP modelling inputs were generated:

- 316 - First, reconstructed 3D plants were positioned according to their actual coordinates in the
317 greenhouse to obtain a 3D point cloud representing the canopy (Fig 5a).
- 318 - Second, a 3D grid with cubic voxels of 0.20 m side was fit to the canopy bounding box and
319 filled using positions, leaf angles and leaf areas associated to each point of the 3D canopy
320 (Fig. 5a). Each voxel may thus include leaves of several neighbouring plants, with a range of
321 angular distribution. Fig. 5b represents the whole greenhouse discretized in this way,
322 where represented volumes are proportional to the leaf area in the corresponding voxel
323 and the colour represents the dominant leaf angle.
- 324 - Fourth, intercepted PPF_D was calculated every day in each voxel from local PPF_{D(xy)i}
325 obtained with light maps. The calculation began with upper voxels, so the light reaching
326 voxels at lower positions depended on both local PPF_D and on transmission through upper
327 voxels. The amount of PPF_D intercepted by each voxel was then partitioned between
328 neighbouring plants as a proportion of leaf area of each plant in the considered voxel.
- 329 - Finally, the daily PPF_D intercepted by each plant was computed by cumulating PPF_D
330 intercepted by this plant by each voxel.

331 An example of reconstructed architecture of ten neighbouring plants and of calculated
332 intercepted PPF_D is presented in Fig. 6 at three times of the plant cycle (20, 35 and 50 d after
333 sowing). Leaves increasingly interacted with each other on days 35 onwards (Fig. 6a-c).
334 Calculated intercepted light (Fig 6d-f) increased with time, with an increasing variability
335 between plants. The model captured (i) the effect of architectural characteristics of each
336 plant on days 20 and 35 (see the comparison between plants 6, 7 and 10 with low angles
337 with horizontal, vs. plant 2 with higher angle), (ii) the competition between plants on day 50,
338 in which tall plants such as plant 2 had a much higher light interception compared with
339 smaller, dominated plants such as plant 3. Hence, light interception on days 20 and 35 might
340 be considered as similar to those of plants in a canopy with a single genotype, while that on
341 day 50 largely depended on the spatial distribution of different genotypes in the
342 greenhouse.

343 ***The suite of methods presented here allowed unifying experiments carried out in different***
344 ***seasons, with a common ranking of genotypes for radiation-use efficiency.***

345 We have tested the interest of the method in an experiment with 200 maize lines and by
346 comparing a set of 23 maize lines in two experiments. The latter were performed at
347 contrasting incident PPFs in order to compare values of RUE. Overall, plants accumulated
348 more biomass in winter/spring compared to autumn (75%, Fig. 7a), with a correlation
349 between genotype performance in autumn and spring, although the ranking of genotypes
350 slightly changed between experiments (genotype x environment interaction, Fig. 7a inset).
351 Light interception also clearly differed between experiments, largely due to a difference in
352 leaf area (55% difference, not shown). In contrast, the relation between intercepted light
353 and biomass was common to both experiments (Fig. 7e). Indeed, the difference in biomass
354 between experiments was entirely accounted for by the difference in intercepted light (12.5
355 and 23 mol plant⁻¹ in autumn and winter/spring, respectively, Fig. 7b), attributable to higher
356 leaf area development and higher amount of incident light. Hence, the mean RUE of the
357 canopy (slope of the regression between biomass and intercepted PPF) was common to
358 both experiments. Furthermore, RUEs measured on individual lines closely correlated
359 between the two experiments (Fig. 7f inset), with neither significant effects of experiment
360 nor genotype x experiment interaction. The genotypic difference in RUE was significant,
361 ranging from 7.0 to 11.1 g FW mol⁻¹. Hence, the methods presented here allowed dissecting
362 the differences between two experiments with large differences in biomass into (i) genotypic
363 traits that did not differ between experiments but had a high genetic variability, namely leaf
364 angle and radiation-use efficiency, (ii) environmental differences, essentially incident light,
365 that affected both biomass and leaf area, thereby generating the large differences that were
366 observed between experiments, (iii) plant traits that differed between experiments due to
367 environmental variables, in particular leaf growth.

368 We have extended our analysis in Exp. 2b to 1600 plants of 200 genotypes, subjected or not
369 to a mild water deficit (Fig. 8). Overall, both biomass and light interception were affected by
370 water deficit, with a high genetic variability in both cases. Estimated RUE had a large genetic
371 variability (from 6.0 to 12 g FW mol⁻¹) and was affected by water deficit by 12% in average.
372 Hence, the change in biomass with water deficit was related to both intercepted light due to

373 a lower leaf area and to decreased RUE. In both watering scenarios biomass and RUE
374 displayed high heretabilities ($0.7 < h^2 < 0.8$).

375 Finally, we have checked whether the observed differences in RUE between genotypes were
376 related to gas-exchange measurements. This was performed in the 8 genotypes with highest
377 contrasts in RUE in Exp. 1 under two contrasting water conditions. Tight correlations were
378 observed between whole-plant RUE values and single-leaf net photosynthesis ($r^2 = 0.54$, $P =$
379 0.001) and stomatal conductance ($r^2 = 0.61$, $P < 0.001$) (Fig. 9), suggesting that RUE estimated
380 at high throughput with the suite of methods presented here could be a surrogate for gas-
381 exchange measurements and vice versa.

382

383 Discussion

384 ***A characterization of the light received by each plant based on a model rather than by*** 385 ***increasing sensor number***

386 Consistent with intuitive observations of shaded zones in a greenhouse, our study shows
387 that steep gradients of light availability occur over distances smaller than 1m, resulting in
388 differences in incident PPFD by up to 10% between neighbouring plants. Hence, we confirm
389 the large spatial variability of light in greenhouses (Stanhill *et al.*, 1973; Kozai & Kimura,
390 1977), but also provide a method for mapping it with high spatial resolution. If characterized
391 directly with sensors, this mapping would need at least one sensor per m², i.e. hundreds of
392 sensors technically difficult to maintain and calibrate. The method presented here avoids
393 this problem by accurately simulating the incident light received by each plant at any time of
394 the year. It has the advantage of having a spatial definition of tens of centimetres and of
395 estimating the respective amounts of direct and diffuse PPFD received by plants, which can
396 bias the calculation of intercepted light because of the high proportion of diffuse light in
397 greenhouses (Sinclair *et al.*, 1992; Sinclair & Muchow, 1999). Finally, this method is rapid
398 (the time for taking the 169 images was 4 hours, and the computing time was less than 2
399 hours). It can be used in any greenhouse regardless of the presence of a phenotyping
400 platform, and is valid as long as the structures of the greenhouse are not changed, and as
401 obstacles to light do not change with time of the year (e.g. a deciduous tree near the
402 greenhouse).

403 A striking result of our study was the relatively low fraction of total daily transmitted light
404 (ca. 30%) compared with other studies that state light transmissions ranging between 0.46
405 and 0.84% (Niinemets & Keenan, 2012). This discrepancy is probably due to the fact that
406 most of studies measuring light transmission in greenhouses report values based on midday
407 measurements with sun beams close to the vertical, thereby overestimating transmission
408 (Niinemets & Keenan, 2012) .

409 ***Dissecting biomass accumulation allows identification of components with high*** 410 ***repeatability and genetic variation.***

411 We have shown that coupling a 3D reconstruction method to a structure-function model
412 together with a fine characterisation of environmental conditions allows estimation of light

413 interception and RUE of thousands of plants with good heritabilities. The suite of methods
414 presented here allowed dissection of biomass into (i) traits that are repeatable between
415 experiments such as RUE or leaf angles, which have a large genetic variability (Mickelson *et*
416 *al.*, 2002; Tian *et al.*, 2011; [Ku *et al.*, 2012](#)) and can therefore be considered as intrinsic to
417 each genotypes, (ii) traits that are highly dependent on environmental conditions such as the
418 change with time of intercepted light.

419 In the results presented in this work, RUE values ranged from 7.0 to 11 g FW mol⁻¹. If
420 expressed in a dry weight basis (considering an average water content of 90%) and per unit
421 of light energy (MJ), our estimates range from 3 to 5 g DW MJ⁻¹. These RUE values, although
422 relatively high compared with field measurements, are often reported in plants grown in
423 controlled environments (Hammer & Vanderlip, 1989). This high estimates of RUE can be
424 related to the high proportion of diffuse radiation inherent to greenhouses or chamber
425 structures ([Sinclair *et al.*, 1992](#); [Sinclair & Muchow, 1999](#)). In addition, the lower daily
426 incident radiation in the greenhouse compared with the field may induce high values of RUE
427 through a greater photosynthetic efficiency and compensation mechanisms (Baille *et al.*,
428 2006). RUE values observed in this work are in accordance with other studies in maize if
429 expressed in a dry weight basis ([Otegui *et al.*, 1995](#); [Lindquist *et al.*, 2005](#); [Louarn *et al.*,](#)
430 [2008](#); [Rattalino Edreira & Otegui, 2012](#)). Proper measurements of plant dry biomass would
431 be needed to compare with available field data. Furthermore, partitioning of biomass to
432 roots and maintenance costs associated to respiration can be an important source of
433 variation in RUE. Such measurements are not easily compatible with the throughput of 1680
434 plants presented in this study, so only the resulting RUEs can be estimated at this
435 throughput.

436

437 Although genetic improvement of RUE has been suggested as a way to increase yield
438 potential in major crops ([Zhu *et al.*, 2010](#); [Reynolds *et al.*, 2011](#)), few studies have explored
439 its genetic variability probably given to the technical difficulties to study this trait ([Acreche *et*](#)
440 [al., 2009; \[Narayanan *et al.*, 2013\]\(#\); \[Koester *et al.*, 2016\]\(#\)\). Other works have shown the
441 potential of 3D reconstruction methods coupled to light distribution algorithms \(radiosity or
442 ray tracing\) to asses photosynthesis in plant canopies \(\[Prieto *et al.*, 2012\]\(#\); \[Song *et al.*, 2013\]\(#\);
443 \[Pound *et al.*, 2014\]\(#\); \[Burgess *et al.*, 2015\]\(#\)\). These methods rely on highly-realistic surface-](#)

444 based plant reconstructions which require high number of images (ca. 35-65 per plant in
445 complex canopies) or plant digitisations that are difficult to automatize in the context of
446 high-throughput phenotyping. Conversely, our method, uses 3D point clouds that require
447 less reconstruction steps (i.e. binarisation and projection), is easy to automatize with
448 standard image analysis library (openCV), and can be obtained with a limited set of images
449 (from 3 to 12). Although precision in reconstruction is lower compared with the techniques
450 described above, our method can accommodate a certain level of error in the 3D
451 reconstructed plant provided that leaf area estimates are precise enough. Indeed, accurate
452 estimates of the total plant leaf area are easy to obtain from a set of binarised 2D images
453 (Supporting Information Fig. S2). ([Golzarian et al., 2011](#); [Hartmann et al., 2011](#)). Another
454 advantage of RATP is that uses a statistical approach that avoids difficulties relating to
455 relative positions of leaves belonging to neighbouring plants. Indeed, the respective
456 positions of leaves may change from one day to another. This is the main reason why we
457 have adopted a probabilistic approach with the RATP model, rather than an explicit
458 description of beam intersecting virtual leaves.

459 The facts that RUE was highly heritable, repeatable between experiments with different
460 incident light (but similar soil water or nutrient status), and correlated with gas-exchange
461 measurements suggests that our measurement of RUE can have a great value for exploring
462 the genetic variability of a surrogate of canopy photosynthesis at high-throughput in large
463 collections of genotypes, which is a topic of growing interest ([Slattery & Ort, 2015](#); [Koester
464 et al., 2016](#)). This method may also allow exploring the change in RUE with environmental
465 conditions by subjecting collections of genotypes to a range of water or nitrogen status,
466 known to largely affect RUE ([Teixeira et al., 2014](#)), of CO₂ concentration ([Hui et al., 2001](#);
467 [Sakai et al., 2006](#)), or of temperature ([Louarn et al., 2008](#)). Finally, using the genotypic values
468 of RUE estimated here in a crop model will allow examining its consequences on yield of a
469 large number of genotypes in a variety of climatic scenarios ([Boote et al., 2013](#); [Parent &
470 Tardieu, 2014](#)).

471 However, we are aware of several methodological difficulties associated with the approach
472 presented here. The first of them is that RUE is the result of a calculation that takes light
473 interception into account. Hence, any error in the estimation of intercepted light results in
474 an error in RUE. The method has been tested successfully in plants with relatively simple

475 architecture such as maize, but serious errors in the calculation of light interception can
476 occur in plants with complex architecture such as rapeseed, with a high level of occlusion
477 (i.e. in which many leaves are hidden by other leaves). The choice of voxel size can be
478 associated with inaccurate results (Combes *et al.*, 2008) and needs to be adjusted depending
479 on the species and the target variable of study. Indeed, it results from an optimization
480 between (i) an adequate representation of gaps in an open canopy and (ii) conforming to
481 Beer-Lambert assumptions within the voxel.

482

483 Other difficulties are associated with methodological choices. If the primary objective is to
484 analyse the genetic control of leaf growth and plant architecture, one tends to use images
485 taken during the night to minimize the change with time of leaf angles or shape due to
486 epinasty (Greenham *et al.*, 2015) or leaf rolling (Hay *et al.*, 2000; Sirault *et al.*, 2015),
487 especially under water deficit. However, this choice can bias the calculation of light
488 interception. Conversely, the use of day-time measurements results in more accurate
489 estimation of light interception but decrease the heritability of measurements of leaf area or
490 angles due to leaf movements or rolling during the day. In the same way, a full
491 characterization of light interception would require that plants are organized in micro-
492 canopies of about 10 plants sharing a common genotype. However, this considerably
493 decreases the number of genotypes studied per experiment, thereby impeding genome-
494 wide association studies that require at least 250-300 genotypes (Beavis, 1998; Malosetti *et*
495 *al.*, 2013). We show here that working with single plants surrounded by plants of different
496 genotypes can provide good results until plants of different genotypes shade each other (Fig.
497 7), but some traits such as the vertical distribution of light interception in the canopy cannot
498 be analysed with this design, although it can have an appreciable effect on light interception
499 (Moreau *et al.*, 2012; Sadras *et al.*, 2012). None of these points question the method itself,
500 but rather the protocol of the experiments using the method.

501 Whereas the method for light mapping can be easily applied in any greenhouse regardless of
502 the presence of a phenotyping platform, the light interception routine is only accessible to
503 platforms equipped with 3D imaging of individual plants, thereby limiting its diffusion. Its
504 main interest is to fill a gap in photosynthesis research, namely the high throughput
505 estimation of light interception and RUE in view of genetic analyses, rather than to be widely
506 distributed in hundreds of platforms.

507

508

509 **Conclusion**

510 We believe that the suite of methods proposed here may have a significant impact on future
511 studies of canopy photosynthesis because of it is compatible with the necessary throughput
512 for genetic analyses and because it allows dissecting the genetic variability of biomass
513 accumulation into different traits that have each their genetic architecture. Field-estimations
514 of intercepted light based on field-based imaging or spectral techniques (Comar *et al.*, 2012;
515 Sankaran *et al.*, 2015) will still be necessary, but they can be combined with genotypic values
516 of RUE and leaf angles estimated in the platform, thereby avoiding the time-consuming step
517 of sequential destructive measurements of plant biomass.

518

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529 **Author Contributions**

530 L. C-B., F.T. and C.W. planned and designed the research, L. C-B., B.S performed experiments,
531 L. C-B., C.F. and N.B. analysed data and L. C-B., C.F. and F.T. wrote the manuscript.

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720

721 **Figure Captions**

722 **Figure 1.** Hemispheric images of the greenhouse seen from below at a given x-y position,
723 superimposed to the sun paths (yellow lines) during (a) winter and (c) summer solstices and
724 (b) spring and (d) autumn equinoxes. Time courses of the fraction of transmitted direct light
725 at three different positions (red, green and black lines) in the greenhouse at four different
726 dates (e-h). Grey dashed lines represent the average greenhouse transmission value. Maps
727 of the fraction of transmitted direct light in the greenhouse at the same dates (i-l). Black, red
728 and green dots represent the three studied positions in the greenhouse. The black arrow
729 represents the geographical North.

730 **Figure 2.** Schematic representation of the method for estimating the local PPFD reaching
731 each x-y position in the greenhouse using daily direct light maps (a), a diffuse light map (b)
732 and the local amount of PPFD (direct + diffuse) light map (c).

733 **Figure 3.** Comparison of measured and estimated available PPFD. (a) Bar plots represent the
734 comparison between measured and estimated weekly PPFD at each of the six positions in
735 the greenhouse equipped with light sensors. (b) Time courses of measured and estimated
736 PPFD with an hourly basis at the positions of the six light sensors.

737 **Figure 4.** Step-by step method to extract mean leaf angles from a multi-view set of *Zea mays*
738 plant images (a), side image selection from top image (b), binarization (c), skeletonization
739 (d), identification of 50-pixel elements and calculation of angles for each element (e,f). The
740 output of calculations is presented for three maize lines with contrasting architectures. (g)
741 Time course of mean leaf angle as a function of thermal time ($d_{20^{\circ}\text{C}}$) after sowing, autumn
742 experiment (h). Values are the mean \pm SD of 5 replicates.

743 **Figure 5.** Canopy structure *Zea mays* plants in the greenhouse superimposed to a three-
744 dimensional array of voxel $0.20\text{ m} \times 0.20\text{ m} \times 0.20\text{ m}$ (a). 3D representation of the grid
745 corresponding to the 1680 plant in the greenhouse (b). Each volume represents a voxel, with
746 a size proportional to the leaf area inside the voxel and a colour representing the dominant
747 leaf angle class. Dark blue 60° , pale blue 53° , green 47° , orange 42° , red 36° .

748 **Figure 6.** 3D representation of 10 contiguous *Zea mays* plants in the greenhouse at 20 (a), 35
749 (b) and 50 (c) days after sowing. (d ,e, f) Bar plots representing daily light interception per
750 plant obtained with the RATP model for the plants depicted in Figures a, b and c.

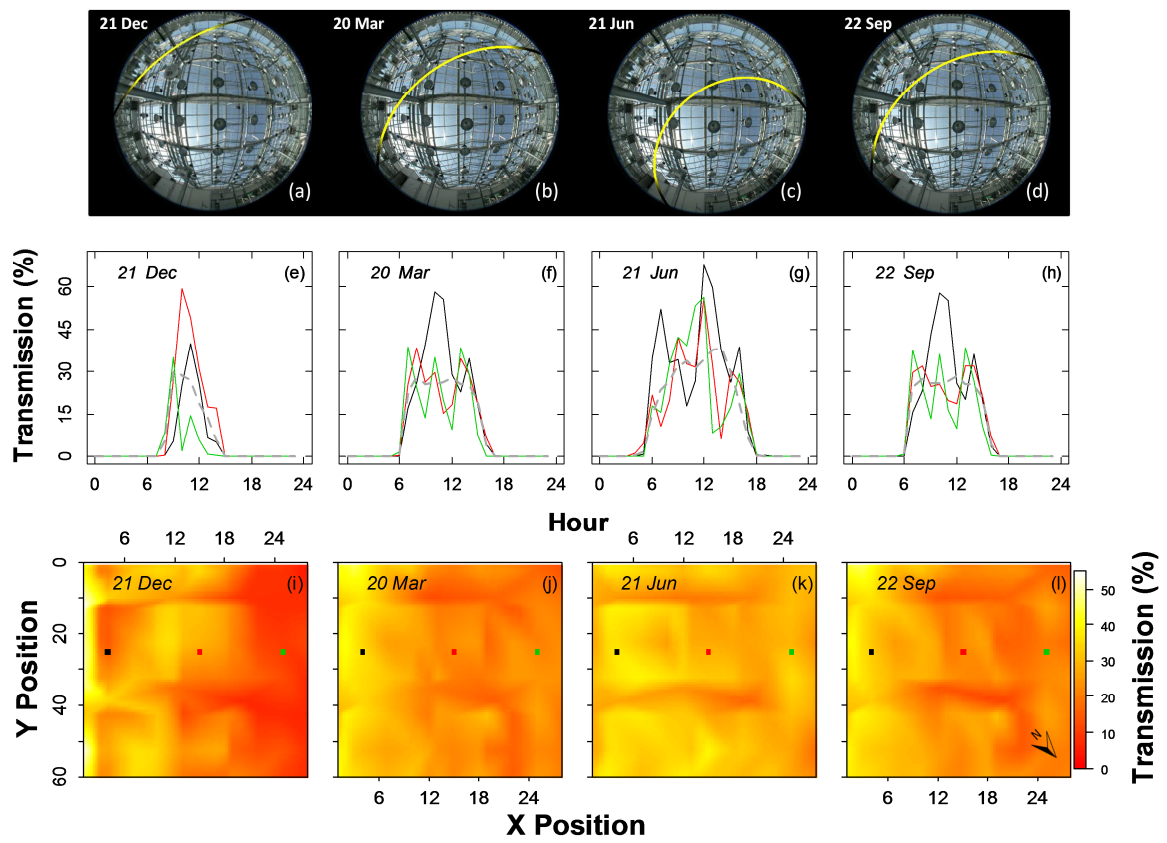
751 **Figure 7.** Time courses of biomass accumulation (a) and intercepted PPFD (c), and biomass
752 accumulation of *Zea mays* as a function of intercepted PPFD (d) in two experiments in
753 autumn and winter-spring. Insets in panels a, c and e present the comparison of biomass (b),
754 intercepted PPFD (d) and RUE (f) between experiments. Values are the mean \pm SD of 115 and
755 92 replicates for autumn and winter experiments, respectively.

756 **Figure 8.** Histograms showing variation in cumulated intercepted PPFD (a) and biomass (b)
757 per plant in *Zea mays* plants growing in Exp 2. The relationship between total intercepted
758 PPFD per plant and total cumulated biomass (c). Red and blue symbols / bars refer to water-
759 deficit and well-watered conditions. Each point represents a plant (n= 1600 (200 genotypes x
760 2 water scenarios x 4 repetitions))

761 **Figure 9.** Relationship between radiation-use efficiency (RUE) and leaf net photosynthesis (a)
762 and leaf stomatal conductance (b) in eight genotypes of *Zea mays* with contrasting RUEs
763 grown under well-water (open symbols) and water-deficit conditions (grey symbols). Each
764 point represents the mean \pm SE of 3 replicates.

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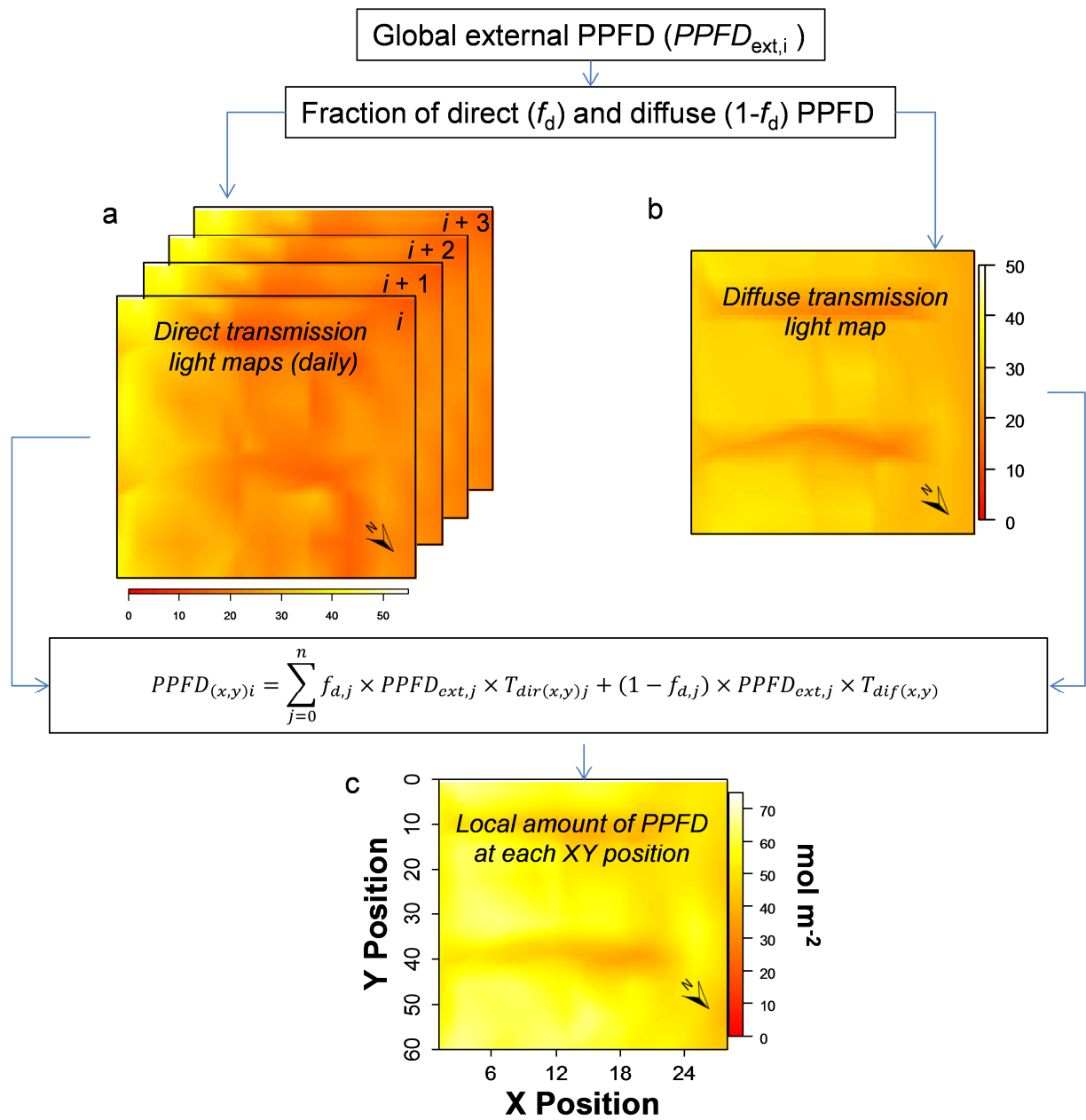
766 Fig. 1



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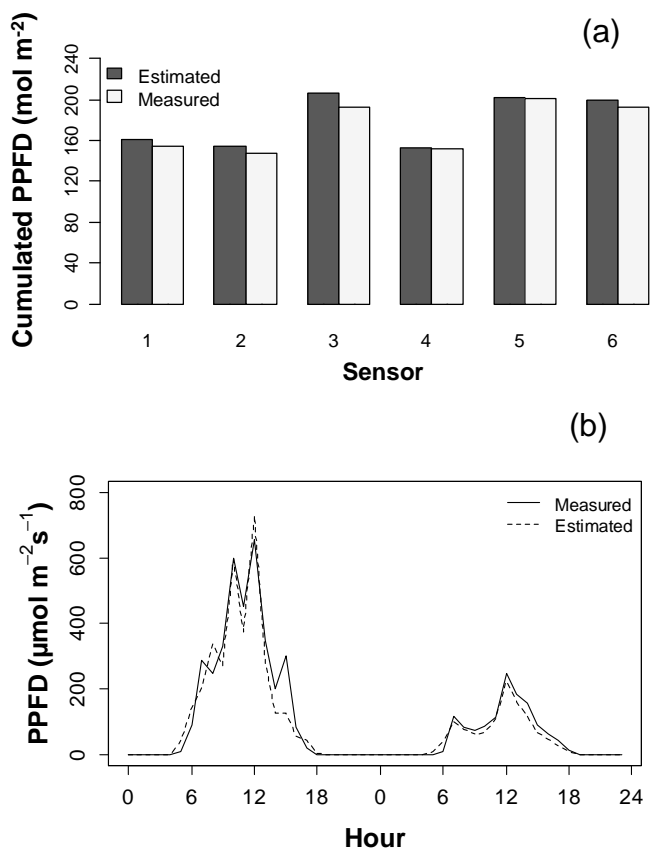
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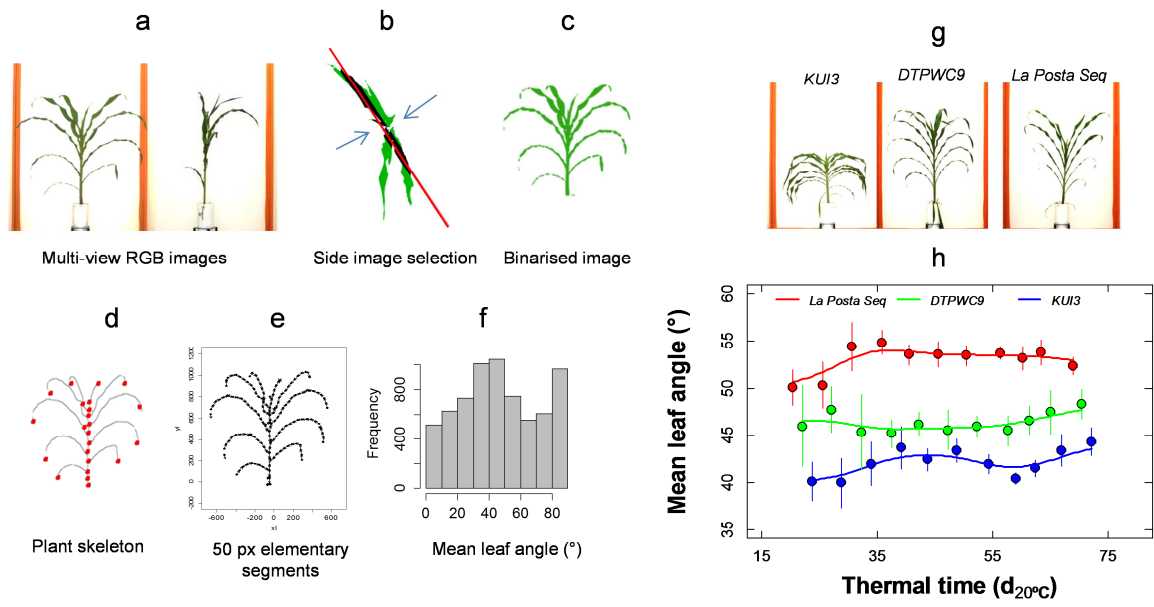
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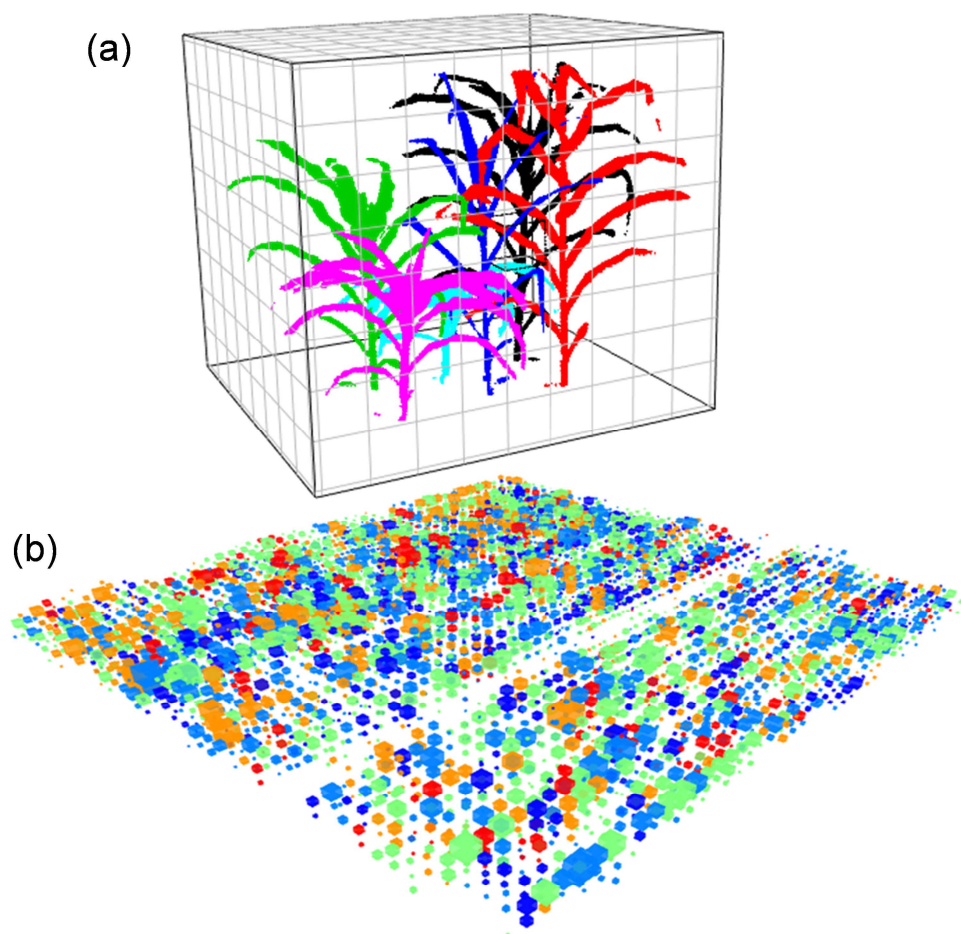


777 Fig. 4



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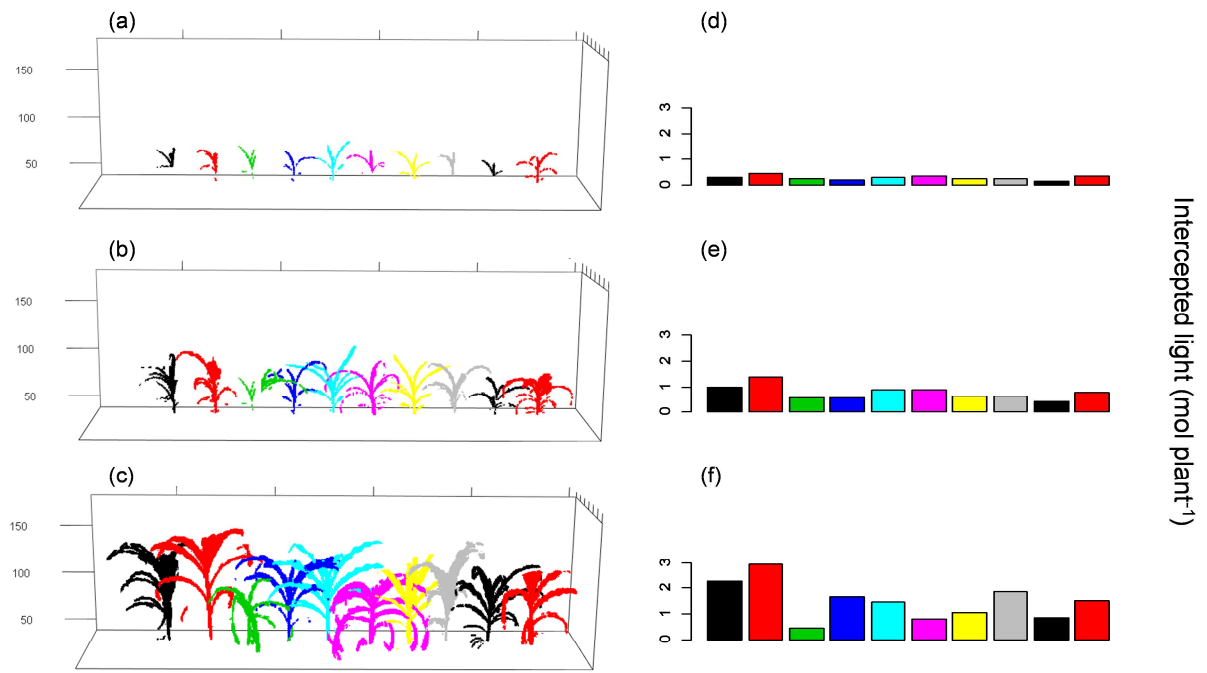
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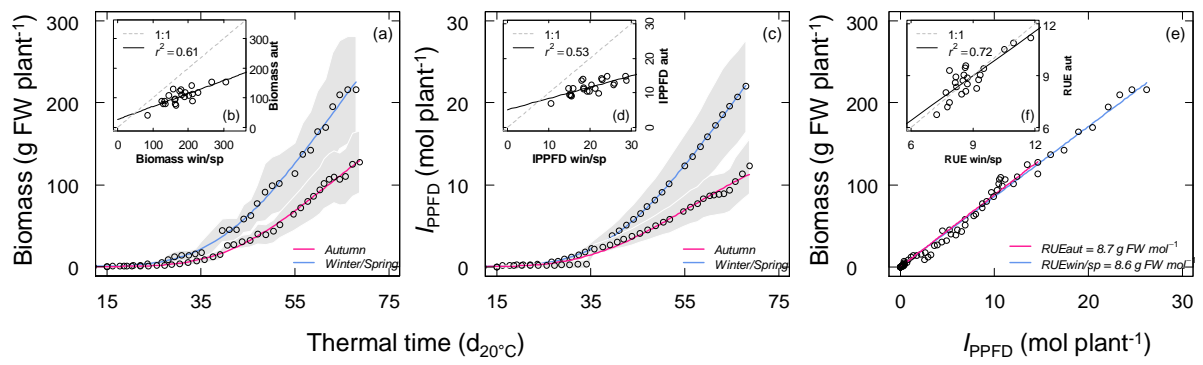
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783 Fig.6

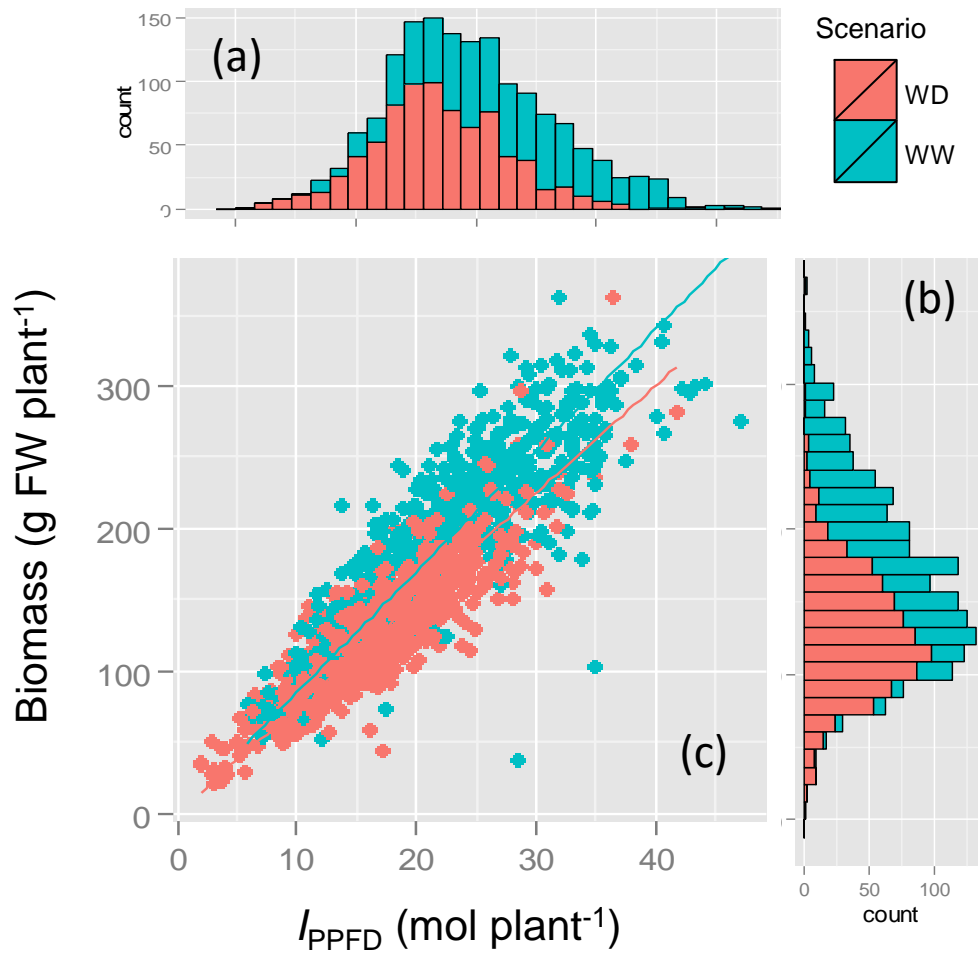


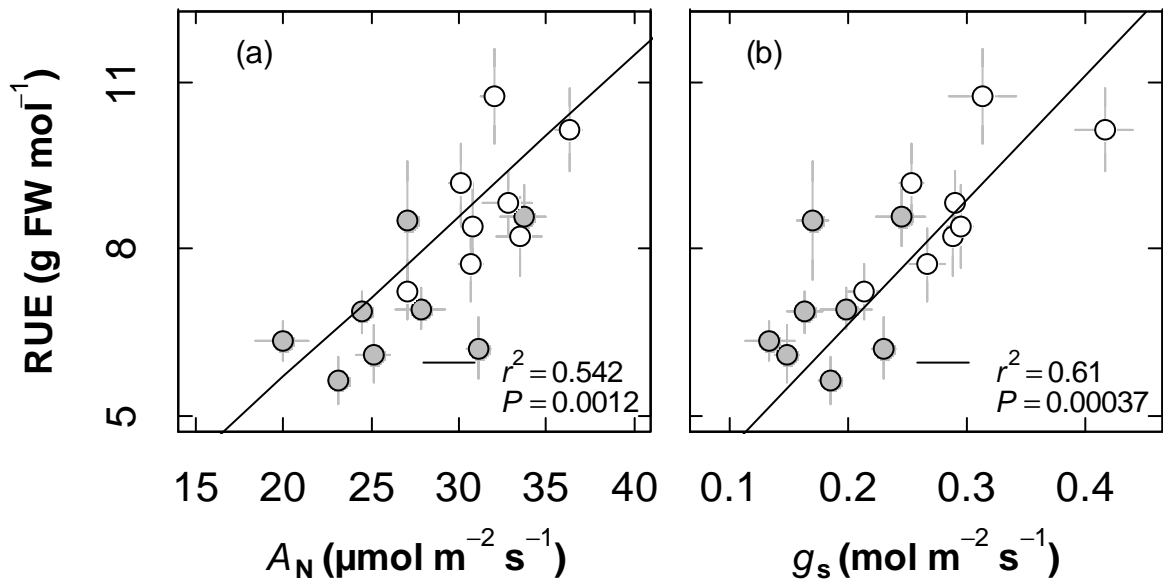
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785 Fig.7



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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1. Pipeline analysis of greenhouse hemispherical images.

Fig. S2. Comparison between measured and predicted leaf area and plant biomass.

Methods S1. Shiny App for Sun Paths and Light transmission calculation.

Table S1. Detailed list of software and packages used in this study.

Video S1. Direct light transmission over a year at the different positions in the greenhouse.

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