

1 **Note and Comments**

2

3 **Title:**

4 Reduction of photosynthesis before midday depression occurred: leaf photosynthesis of

5 *Fagus crenata* in relation to canopy position and a number of days after rainfall.

6

7 **Authors:**

8 Kohei Koyama<sup>1\*</sup>, Kihachiro Kikuzawa<sup>1</sup>

9

10 \*Corresponding author.

11

12 <sup>1</sup> Laboratory of Plant Ecology, Ishikawa Prefectural University, Suematsu, Nonoichi,

13 Ishikawa, 921-8836, Japan

14

15 (\*) Email address: [kkoyama@ishikawa-pu.ac.jp](mailto:kkoyama@ishikawa-pu.ac.jp)

## 16 **Abstract**

17 We investigated an effect of canopy position and a number of days after rainfall on  
18 reduction of photosynthetic rate in a *Fagus crenata* forest in summer 2008, during days  
19 when midday depression was not apparent. We compared in-situ photosynthetic rate and  
20 photosynthetic rate that was calculated by photosynthetic light response curves  
21 measured in the morning. The ratio, in-situ photosynthesis divided by the  
22 curve-estimated value, declined towards the end of each day for the upper leaves, but  
23 not for the lower leaves. Total photosynthesis was reduced only for the upper leaves by  
24 12% during five days after the rainfall.

25

26 **Key words:** Photosynthesis; stomatal conductance; net primary production; temperate  
27 forest; *Fagus crenata* Blume.

28

## 29 **Introduction**

30 Temperate forests are carbon sinks against climate change (Granier et al. 2000; 2002;  
31 Luysaert et al. 2008; Saigusa et al. 2008; Kato and Tang 2008; Ito 2008; 2010; Koyama  
32 & Kikuzawa 2010a; Joo et al. 2011). The “big leaf model” extended single-leaf  
33 responses (Farquhar 1989) into whole-plant responses under the same scheme, and  
34 proposed simple relations predicting ecosystem carbon gain based on an optimization  
35 hypothesis (Sellers et al. 1992; Dewar et al. 1996; Haxeltine and Prentice 1996;

36 Terashima et al. 2005; Koyama and Kikuzawa 2009; 2010b). Recently, the big leaf  
37 model was validated even under fluctuating light intensity, both theoretically (Koyama  
38 and Kikuzawa 2010b), and empirically (Rosati and DeJong 2003; Rosati et al. 2004;  
39 Posada et al. 2009; Koyama and Kikuzawa 2010b). However, there have also been  
40 countless results showing that the optimization was not always realized (Field 1983;  
41 Hirose and Werger 1987; Meir et al. 2002; Posada et al. 2009). One possible explanation  
42 for this discrepancy is that photosynthesis is not only limited by light, but also by other  
43 factors (Mooney and Gulmon 1979; Meir et al. 2002; Niinemets and Valladares 2004).  
44 Even when light is plentiful, both stomatal and non-stomatal limitation plays important  
45 roles for reducing in-situ photosynthetic rate for trees (Ishida et al. 1999a; Muraoka et al.  
46 2000; Niinemets et al. 2004; Valladares and Pearcy 2002; Misson et al. 2010). In this  
47 study, we will demonstrate that photosynthesis of upper leaves on *Fagus crenata* forest  
48 was not solely determined by light, but also by stomatal limitation.

49         Leaf stomatal conductance is controlled by many factors. External environment  
50 such as air humidity (Jarvis 1976; Tenhunen et al. 1987; Ball et al. 1987; Harley and  
51 Tenhunen 1991; Leuning 1995; see review by Damour et al. 2010) controls stomata via  
52 increment of transpiration (Mott and Parkhurst 1991; Mott and Peak 2010). However,  
53 Damour et al. (2010) concluded that in addition to those leaf-level responses, including  
54 an effect of soil water condition is needed. Tazaki et al (1980) reported that upper leaves  
55 of mulberry tree showed no midday depression in the second day after the last rainfall,

56 but apparent depression occurred in the eighth day. Nakai et al. (2010) also reported in  
57 the experiment of *Salix gracilistyla* cuttings, that the longer the drought treatment, the  
58 more stomatal limitation on photosynthesis. However, those studies did not investigate  
59 the difference of stomatal limitation between canopy positions.

60         Within a single canopy, a limiting factor for photosynthesis may differ among  
61 different positions. Firstly, leaves from different positions within a canopy themselves  
62 differ morphologically, chemically, anatomically and physiologically. This is observed  
63 in *Fagus crenata* (Uemura et al. 2000; Yamasaki and Kikuzawa 2003; Iio et al. 2005;  
64 2009; Koyama and Kikuzawa 2010ab), other temperate deciduous trees (Niinemets  
65 1995; 2010; Koyama and Kikuzawa 2010b; Yoshimura 2010) and temperate evergreens  
66 (Hozumi and Kirita 1972; Katahata et al. 2007). Also, leaves on higher position may  
67 have smaller water reserves (Jarvis 1976) and / or longer water pathway from root  
68 (Ryan et al. 2006). Hence, it is expected that a degree of stomatal limitation of  
69 photosynthesis should differ among different canopy positions. However, in most of the  
70 previous studies, the effect of canopy position was not investigated (cf. Tazaki et al.  
71 1980; Zotz and Winter 1996; Muraoka et al. 2000; Ishida et al. 1999a; 2000; Valladares  
72 et al. 2008; Misson et al. 2010; Nakai et al. 2010). For other studies which investigated  
73 different canopy positions, the temporal pattern in relation to soil water conditions was  
74 not investigated (cf. Muraoka and Koizumi 2005; Iio et al. 2009). To date, knowledge  
75 on the interaction between soil water condition and canopy position is very limited (cf.

76 Niinemets et al. 2004). Regarding the above argument, it is necessary to simultaneously  
77 investigate both of the above two factors (Niinemets and Valladares 2004). The aim of  
78 this study is to elucidate spatial and temporal pattern of efficiency of photosynthetic rate  
79 under field condition in a Japanese beech forest (*Fagus crenata* Blume). We tested the  
80 following two hypotheses. (1) Daytime reduction of photosynthesis was apparent for the  
81 upper leaves, but not for the lower leaves. (2) This reduction was interactively affected  
82 by the soil water condition. There will be no apparent reduction in daily photosynthetic  
83 rate just after a rainfall, but will be a significant reduction when the number of days  
84 after the last rainfall increased.

85

## 86 **Methods**

### 87 Species and Site

88 *Fagus crenata* Blume is a late-successional deciduous canopy tree distributed in  
89 mountainous regions in Japan (Matsui et al. 2004). The site was a 15-year-old plantation  
90 in the Ishikawa Prefectural Forest Experiment Station (36°25'N, 136°38'E, elevation  
91 220 m), which is located at the foot of Mt. Hakusan. At the plantation, 130 trees were  
92 planted in 1995 in the area of 171 m<sup>2</sup>. Those trees were grown by seeds from Mt.  
93 Hakusan (Jiro Kodani, personal communication), and hence they should belong to the  
94 Japan Sea coast haplotypes (cf. Okaura and Harada 2002). The stand height and the  
95 mean DBH were around 6 m and 4.7 cm in 2008, respectively. The canopy was closed

96 and there was almost no vegetation on the forest floor.

97           The site climate was one of the typical Japan Sea coast regions, characterized  
98 by heavy snowfall in winter. The measurement was conducted from 27 July to 1 August  
99 2008, when the summer rainy season was finished. Mean annual temperature and mean  
100 annual precipitation are 13.0 °C and 2438 mm, respectively (2003 – 2007). Mean  
101 temperature in August was 25.0 °C (2003 – 2007), being the hottest month. However, in  
102 August there usually was no severe drought in that region, and rainfall typically occurs  
103 at least in every one week (Koyama K, personal obs.). Mean monthly precipitation was  
104 184 mm (2003 – 2007), which was slightly lower than the mean monthly precipitation  
105 (203 mm). The mean temperature and total precipitation in 2008 was 12.8 °C and 2229  
106 mm, respectively. The mean temperature and the total precipitation in August 2008 was  
107 24.2 °C and 182 mm, respectively, being not greatly different from the normal years.  
108 The above data were from the Annual Report of the Ishikawa Prefectural Forest  
109 Experiment Station (2003 – 2008).

110

111   Photosynthesis measurement

112   A total of 91 mm rainfall was observed within two days in 27 and 28 July 2008 at the  
113 site (data from the Ishikawa Prefectural Forest Experiment Station, by courtesy of Dr.  
114 Jiro Kodani). There was no rain during successive measurement days as confirmed by a  
115 plastic funnel-top container set above the canopy. The measurements were conducted on

116 the 1st, the 3rd and the 5th day after the last rainfall on 28 July. We used two portable  
117 photosynthesis systems (LI-6400, LI-COR, Lincoln, USA), with CO<sub>2</sub> supply inside the  
118 chambers kept at 350 ppm. Four leaves on the upper part of the canopy (i.e. “the upper  
119 leaves”) were selected from one branch at the height of 5.7 m. Those were accessed by a  
120 scaffolding tower. Another four leaves on the lower canopy (i.e. “the lower leaves”)  
121 were selected from one branch on an adjacent tree at the height of 1.5 m, as there were  
122 no accessible lower leaves on the same tree. For each position (the upper and lower),  
123 incident photosynthetically active photon flux density (PPFD) were measured for seven  
124 times with 1 - 2 hours intervals during daytime (8:30 - 16:00) with a quantum sensor  
125 (IKS-27, KOITO Kogyo, Yokohama, Japan) placed above the branch of those leaves.  
126 Hence, the angle of each leaf was not considered in this study. Net photosynthetic rates  
127 of those intact leaves at each moment were measured by the two LI-6400s under the  
128 PPFD of the same intensity just recorded on those leaves on each occasion, which were  
129 supplied by the LED light sources (LI-6400-02B). On each occasion, the PPFDs were  
130 kept constant until the equilibration of the leaves (typically, 20 - 40 min). Leaf  
131 conductance to H<sub>2</sub>O ( $g$ ) ( $\text{mol m}^{-2} \text{s}^{-1}$ ), leaf temperature, and vapor pressure deficit based  
132 on leaf temperature (VPD) (kPa) were simultaneously calculated by the LI-6400s. In the  
133 following, we will regard  $g$  as equivalent to leaf stomatal conductance, assuming that  
134 leaf boundary layer resistance was negligible. Immediately after each occasion of the  
135 photosynthetic measurement, dark respiration rate was measured with zero light.

136 We simultaneously measured one photosynthetic light response curve for each  
137 of the same sample leaves with the same LI-6400s. Those measurements were  
138 conducted from 9:30 to 11:30 a.m., intermittently between the in-situ photosynthesis  
139 measurements described above. For the lower leaves, all the four leaves were measured  
140 on the 1st day. For the upper leaves, as we could not finish the measurement on the 1st  
141 day, each two of the four leaves were measured on the 1st and the 3rd day. On each  
142 measurement, the leaves were firstly induced by  $\text{PPFD} = 1500 \mu\text{mol m}^{-2} \text{s}^{-1}$  until  
143 equilibration. Then, PPFD was changed from higher to lower (2000, 1500, 1000, 750,  
144 500, 250, 125, 63, 32 and  $0 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) with the LEDs. On each occasion of the  
145 change, PPFD was kept constant until the equilibration of the leaves. During those  
146 light-response measurements, leaf temperatures were not controlled and ranged  $28.6^\circ\text{C}$   
147  $- 33.2^\circ\text{C}$  and  $28.1^\circ\text{C} - 31.9^\circ\text{C}$  for the upper and the lower leaves, respectively.

148

149 Data analysis

150 Diurnal course of in-situ gross photosynthetic rate ( $P_{\text{g\_mes}}$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) for each leaf  
151 was calculated as the sum of net photosynthesis and dark respiration rate at each  
152 moment. We set another open-sky PPFD sensor set above the canopy during the  
153 measurement days, and we set  $P_{\text{g\_mes}}$  at the dawns and the sunsets as both zeros (i.e.  
154 when the open-sky PPFD became zero) on each day. Daily photosynthesis for each leaf  
155 was estimated by interpolations of those  $P_{\text{g\_mes}}$  values.

156 We alternatively calculated hypothetical diurnal courses of gross  
157 photosynthetic rate, estimated solely by the diurnal change of the light intensity. Each  
158 net photosynthetic rate during the light-response measurement (from PPFD 0 to 2000  
159  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , see above) were converted to gross photosynthetic rate by adding dark  
160 respiration rate (PPFD = 0). Then, non-rectangular hyperbola (Marshall and Biscoe  
161 1980) was fitted by KaleidaGraph 4 (Synergy Software, Reading, USA) ( $r^2 > 0.997$ ):

162

$$163 \quad P_{g_{cv}} = \frac{\Phi I + P_{\max} - \sqrt{(\Phi I + P_{\max})^2 - 4\theta\Phi I P_{\max}}}{2\theta} \quad (\mu\text{mol m}^{-2} \text{s}^{-1}) \quad \text{Eq. 1}$$

164

165 In Eq. 1,  $P_{g_{cv}}$  ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) indicates a curve-estimated gross photosynthetic rate at  
166 each incident PPFD intensity ( $I$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ).  $P_{\max}$  indicates maximum gross  
167 photosynthetic rate of that leaf when  $I$  approaches infinity. The other two parameters,  $\phi$   
168 ( $\mu\text{mol } \mu\text{mol}^{-1}$ ) and  $\theta$  (dimensionless) indicate initial slope and convexity, respectively.  
169 The curve-estimated photosynthetic rate ( $P_{g_{cv}}$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) at each time was estimated  
170 by substituting the same incident PPFD as was used in the in-situ photosynthetic rate  
171 described above into Eq.1 with each different set of parameters  $P_{\max}$ ,  $\phi$  and  $\theta$  for each  
172 different leaf. We used each single set of parameters for each leaf, such that same  
173 photosynthetic light response curves were assumed throughout the five measurement  
174 days. In other words, we hypothetically fixed conditions of the leaves at the time of

175 light-response curve measurements, and only PPFD was changed as the same way as  
176 the actual diurnal courses.

177

178 Efficiency of photosynthesis

179 Actual photosynthetic rate ( $P_{g\_mes}$ ) should be under the effect of diurnal change of both

180 light intensity and all the other factors. In contrast, hypothetical  $P_{g\_cv}$  should be

181 independent of diurnal change of the factors other than light. Then, the ratio between the

182 above two values ( $P_{g\_mes} / P_{g\_cv}$ ) will be interpreted as diurnal change of efficiency of

183 photosynthesis, as affected by the non-light limitation. This ratio should be reduced,

184 when in-situ photosynthesis at that moment ( $P_{g\_mes}$ ) was reduced by non-light factors.

185 Note that  $P_{g\_cv}$  (and hence  $P_{g\_mes} / P_{g\_cv}$  as well) also depends on the non-light factors at

186 the time of the measurement of the photosynthetic light response curve, but was

187 independent of “diurnal change” of non-light factors, as we fixed each leaf at one

188 particular time of the day. Because of this,  $P_{g\_mes} / P_{g\_cv}$  can be higher than unity, when

189 photosynthesis was limited less compared with the time of the measurement of

190 photosynthetic light response curves.

191

192 Statistics

193 Linear regressions and Pearson’s correlation coefficients ( $r$ ) were calculated by SPSS

194 13.0J for Windows (SPSS Japan Inc, Tokyo, Japan).

## 195 Results

196 Upper leaves had higher maximum gross photosynthetic rate ( $P_{g\_max}$ ) and dark  
197 respiration rate (Table 1), which is consistent with the previous studies (Iio et al. 2005;  
198 Koyama and Kikuzawa 2010b). Diurnal course of in situ gas exchange rate ( $P_{g\_mes}$ )  
199 basically followed incident PPFD both for the upper and the lower leaves, except in the  
200 afternoon on the fifth day (Fig. 1a). There were no apparent “midday” depressions for  
201 the both positions. The only visible difference was found in the upper leaves on the 5th  
202 day, in which afternoon photosynthetic rate was apparently lower than that in the  
203 morning. Diurnal course of VPD and leaf temperature showed similar pattern as that of  
204 PPFDs (Fig. 1c). Leaf conductance to  $H_2O$  (g) tended to decline towards the end of each  
205 day (Fig. 1b). This trend was evident on all the days for the upper leaves, but only on  
206 the 5th day for the lower leaves. The actual in-situ daily gross photosynthetic rate  
207 averaged over the three measurement days was 0.35 and 0.042 ( $\text{mol m}^{-2} \text{day}^{-1}$ ) for the  
208 upper and lower leaves, respectively.

209 Although “midday depression” was not apparent, a clear difference was  
210 detected between canopy positions when we compare  $P_{g\_mes} / P_{g\_cv}$  (Fig. 2). For the  
211 upper leaves  $P_{g\_mes} / P_{g\_cv}$  linearly declined towards the end of each day. In contrast, for  
212 the lower leaves  $P_{g\_mes} / P_{g\_cv}$  only fluctuated around the mean value for all the days.  
213 The slope of the decline of the upper leaves became steeper (i.e. more negative), as the  
214 number of days after the last rain increased (Fig. 3). The  $P_{g\_mes} / P_{g\_cv}$  were strongly and

215 positively correlated with leaf conductance to H<sub>2</sub>O for the upper leaves, but there were  
216 no consistent relationships for the lower leaves (Fig. 4).

217

## 218 **Discussion**

219 We found a clear difference between canopy positions; only the upper leaves showed  
220 declining trends in photosynthetic efficiency (Fig. 2). We estimated the percentage loss  
221 of daily gross photosynthetic rate due to this decline as follows. When  $P_{g\_mes} / P_{g\_cv}$  of  
222 one particular time (say, 15:00) on one day was 0.85, while  $P_{g\_mes} / P_{g\_cv}$  of the earliest  
223 morning within that day was 1.1 (see Fig. 2 upper), we calculated the ratio  $1.1 / 0.85 (=$   
224  $1.3)$ . It means that leaves in the earliest morning can conduct 1.3 times more  
225 photosynthesis than the actual leaf exists at 15:00 under the same PPFD observed at  
226 15:00. Then, we calculated product of this ratio and  $P_{g\_mes}$  on each time on each day. It  
227 is the estimation of hypothetical photosynthetic rate conducted by a leaf, being “fixed”  
228 at the condition of that in the earliest morning, whilst PPFD on that leaf was changed as  
229 the same way as the actual diurnal course. Finally, we calculated potential gross daily  
230 photosynthetic rate by the interpolation of those values as the same manner as the above  
231 actual ones. Compared with those potential values, actual gross daily photosynthetic  
232 rates were 12% and 1% less for the upper and the lower leaves, respectively. Hence, the  
233 reduction was modest for the upper leaves, and did not exist for the lower leaves. Those  
234 results supported the hypothesis that photosynthesis is constrained more greatly for the

235 upper leaves than the lower leaves (Jarvis 1976; Niinemets et al. 2004).

236           Among the upper leaves,  $P_{g\_mes} / P_{g\_cv}$  decreased towards the end of each day.

237 This result supported Jarvis (1976), who suggested that water reserves within branches

238 or stems were gradually exhausted in upper canopies towards the end of each day. This

239 diurnal decline became steeper, as the number of days after the rainfall increased (Fig.

240 3). Then, the visible reduction of photosynthetic rate was eventually observed in the

241 afternoon on the fifth day (Fig. 1a). Those results are consistent with the study of a

242 mulberry tree (Tazaki et al. 1980), which showed that midday depression was not

243 evident until the second day after the last rainfall, but it was apparent from one week

244 after the rainfall. Those results were consistent with the classical scheme of the

245 periodical decline of soil water potential from morning to sunset within each day, with

246 the absolute water availability decreases as days after the last rain proceeds (Slatyer

247 1967). Kikuzawa et al. (2004) reported 46.5% reduction of daily photosynthesis for the

248 sun leaves of *Alnus sieboldiana*, when the apparent midday depression occurred. Our

249 results (12% loss without visible midday depression) suggests that a reduction of

250 photosynthesis does not abruptly rises up to 46% when an apparent midday depression

251 is observed, but it gradually increases from the onset of a rainless period. This indicates

252 that there may be no threshold condition for a midday depression. Hence, our results

253 depicted more general pattern than that of Tazaki et al. (1980), since it showed reduction

254 of photosynthesis before “midday depression” was apparent.

255 Stomatal limitation was expected to be higher for the upper leaves, which were  
256 exposed to high light environment (Niinemets and Valladares 2004). In addition, leaves  
257 on the higher position may have smaller water reserves (Jarvis 1976), longer water  
258 pathway from root (Ryan et al. 2006) and, specifically for tall trees, less water potential  
259 due to gravitation (Ishii et al. 2008; Nabeshima and Hiura 2008). Another mechanism  
260 suggested that sunlit upper leaves may have more water supplies by a selective  
261 investment of water into favorable sunlit leaves (Sprugel et al. 2002). In this study (6 m  
262 height), the gravitational effect should be negligible. However, we could not separate  
263 each of those confounding effects, as we selected upper and lower leaves from different  
264 individuals. Hence, before generalizing our results to other forests, mechanistic  
265 measurements including investigation of water supply on each position is needed to  
266 separate those factors.

267 We did not measured photoinhibition. Actual reduction is caused by both  
268 stomatal limitation and photoinhibition (Ishida et al. 1999abc; 2000; 2001; Muraoka et  
269 al. 2000; Werner et al. 2001; Valladares and Pearcy 2002; Yamazaki et al. 2007; Misson  
270 et al. 2010). Uemura et al. (2005) discussed that the reduction of photosynthesis in  
271 another *Fagus crenata* forest was due to photoinhibition, rather than stomatal closure.  
272 Our results showed that at least some part of reduction of photosynthesis was related to  
273 stomatal closure, which did not violate the conclusion by those studies.

274 We used LED light-sources, such that an effect of heat loading (Ishida et al.

275 2000; Uemura et al. 2005; Iio et al. 2009; Vogel 2009) may not have been properly  
276 evaluated. Although our method has a clear disadvantage in these points, it has another  
277 merit than those using natural sunlight. Diurnal course of incident photosynthetic rate  
278 did not show a visible midday depression on the 1st day (Fig 1a), which is consistent  
279 with Tazaki et al. (1980). However, when we calculated the  $P_{g\_mes} / P_{g\_cv}$ , the reduction  
280 became apparent from the 1st day (Fig. 2). Hence, the reduction was detected only when  
281 we evaluated the ratio of the two kind of photosynthetic rates,  $P_{g\_mes}$  and  $P_{g\_cv}$ , both of  
282 which were measured by the same LEDs, so that were readily comparable.

283         The study period was during the hottest season of one year on one particular  
284 ecotype of *Fagus crenata*. However, patterns of photosynthetic limitation should vary  
285 among seasons or years (Ishida et al. 1999b; Valladares et al. 2008; Misson et al. 2010).  
286 Generally, responses to environment differ among species (Turner et al. 1984; Ishida et  
287 al. 1999a; Uemura et al. 2000; 2005; Turnbull et al. 2002; Oguchi et al. 2005; Valladares  
288 et al. 2008; Kitaoka et al. 2009; Kamiyama et al. 2010), among ecotypes of *Fagus*  
289 *crenata* (Yamazaki et al. 2007; Tateishi et al. 2010), and between microhabitats  
290 (Koyama and Kikuzawa 2008; Nagano et al. 2009). A behavior of a plant can be  
291 considered as a summation of simple basic principles and those situation-dependent  
292 factors (Koyama and Kikuzawa 2009). Simple theories and complex factors are not  
293 mutually exclusive, but are compensatory for each other.

294 **Acknowledgements**

295 We thank Tomoyo Nakano and Hiroyuki Moriyama for fieldwork assistances. Jiro Kodani gave us  
296 useful comments. This study received financial support from Ministry of Education, Science, Sports  
297 and Culture of Japan (grant #20370014) and from Research Fund of Ishikawa Prefectural University.

298

## 299 **References**

300 Ball JT, Woodrow IE, Berry JA (1987) A model predicting stomatal conductance and its contribution to  
301 the control of photosynthesis under different environmental conditions. In: Biggins I (ed) Progress in  
302 photosynthesis research, vol IV.5. Nijhoff, Dordrecht, pp 221-224

303 Cowan IR, Farquhar GD (1977) Stomatal function in relation to leaf metabolism and environment. Symp  
304 Soc Exp Biol 31:471-505

305 Damour G, Simonneau T, Cochard H, Urban L (2010) An overview of models of stomatal conductance at  
306 the leaf level. Plant Cell Environ 33:1419-1438

307 Dewar RC (1996) The correlation between plant growth and intercepted radiation: an interpretation in  
308 terms of optimal plant nitrogen content. Ann Bot 78:125–36

309 Farquhar GD (1989) Models of integrated photosynthesis of cells and leaves. Phil Trans Roy Soc Lond B  
310 323:357-367

311 Field C (1983) Allocating leaf nitrogen for the maximization of carbon gain: Leaf age as a control on the  
312 allocation program. Oecologia 56:341-347

313 Granier A, Ceschia E, Damesin C, Dufrière E, Epron D, Gross P, Lebaube S, Le Dantec V, Le Goff N,  
314 Lemoine D, Lucot E, Ottorini JM, Pontailler JY, Saugier B (2000) The carbon balance of a young Beech

315 forest. *Funct Ecol* 14:312-325

316 Granier A, Pilegaard K, Jensen NO (2002) Similar net ecosystem exchange of beech stands located in  
317 France and Denmark. *Agric For Meteorol* 114:75–82

318 Harley PC, Tenhunen JD (1991) Modeling the photosynthetic response of C3 leaves to environmental  
319 factors. In: Boote KJ, Loomis RS (eds) *Modeling crop photosynthesis, from biochemistry to canopy*,  
320 CSSA, Madison, pp 17-19

321 Haxeltine A, Prentice IC (1996) A general model for the light-use efficiency of primary production. *Funct*  
322 *Ecol* 10:551–561

323 Hirose T, Werger MJA (1987) Maximizing daily canopy photosynthesis with respect to the leaf nitrogen  
324 allocation pattern in the canopy. *Oecologia* 72:520–526

325 Hozumi K, Kirita H (1970) Estimation of the rate of total photosynthesis in forest canopies. *Bot Mag*  
326 *Tokyo* 83:144-151

327 Iio A, Fukasawa H, Nose Y, Kato S, Kakubari Y (2005) Vertical, horizontal and azimuthal variations in  
328 leaf photosynthetic characteristics within a *Fagus crenata* crown in relation to light acclimation. *Tree*  
329 *Physiol* 25:533-544

330 Iio A, Fukasawa H, Nose Y, Naramoto M, Mizunaga H, Kakubari Y (2009) Within-branch heterogeneity  
331 of the light environment and leaf temperature in a *Fagus crenata* crown and its significance for  
332 photosynthesis calculations. *Trees* 23:1053-1064

333 Ishida A, Nakano T, Matsumoto Y, Sakoda M, Ang LH. (1999a) Diurnal changes in leaf gas exchange and  
334 chlorophyll fluorescence in tropical tree species with contrasting light requirements. *Ecol Res* 14:77-88

335 Ishida A, Toma T, Marjenah (1999b) Limitation of leaf carbon gain by stomatal and photochemical  
336 processes in the top canopy of *Macaranga conifera*, a tropical pioneer tree. *Tree Physiol* 19:467-473

337 Ishida A, Toma T, Marjenah (1999c) Leaf gas exchange and chlorophyll fluorescence in relation to leaf  
338 angle, azimuth, and canopy position in the tropical pioneer tree, *Macaranga conifera*. *Tree Physiol*  
339 19:117-124

340 Ishida A, Toma T, Ghazali DI, Marjenah (2000) In situ study of the effects of elevated temperature on  
341 photoinhibition in climax and pioneer species. In: Guhardja E, Fatawi M, Sutisna M, Mori T, Ohta S  
342 (eds) *Rainforest Ecosystems of East Kalimantan: El Niño, Drought, Fire and Human Impacts*.  
343 *Ecological Studies* 140. Springer, Tokyo, pp 269–280

344 Ishida A, Nakano T, Uemura A, Yamashita N, Tanabe H, Koike N (2001) Light-use properties in two  
345 sun-adapted shrubs with contrasting canopy structures. *Tree Physiol* 21:497–504

346 Ishikawa Prefectural Forest Experiment Station (2008) *Ishikawaken Ringyo Shikenjyo Gyomu hokoku*,  
347 year 2003 – 2007 (online files downloaded in 2010)

348 Ishii HT, Jennings GM, Sillett SC, Koch GW (2008) Hydrostatic constraints on morphological  
349 exploitation of light in tall *Sequoia sempervirens* trees. *Oecologia* 156:751-763

350 Ito A (2008) The regional carbon budget of East Asia simulated with a terrestrial ecosystem model and  
351 validated using AsiaFlux data. *Agric For Meteorol* 148:738–747

352 Ito A (2010) Changing ecophysiological processes and carbon budget in East Asian ecosystems under  
353 near-future changes in climate: implications for long-term monitoring from a process-based model.  
354 *Journal of Plant Research* 123:577-588

- 355 Jarvis PG (1976) The interpretation of the variations in leaf water potential and stomatal conductance  
356 found in canopies in the field. *Phil Trans Roy Soc Lond B* 273:593–610
- 357 Joo SJ, Park MS, Kim GS, Lee CS (2011) CO<sub>2</sub> flux in a cool-temperate deciduous forest (*Quercus*  
358 *mongolica*) of Mt. Nam in Seoul, Korea. *J Ecol Field Biol* 34:95-106
- 359 Kamiyama C, Oikawa S, Kubo T, Hikosaka K (2010) Light interception in species with different  
360 functional groups coexisting in moorland plant communities. *Oecologia* 164:591-599
- 361 Katahata S, Naramoto M, Kakubari Y, Mukai Y (2007) Seasonal changes in photosynthesis and nitrogen  
362 allocation in leaves of different ages in evergreen understory shrub *Daphniphyllum humile*. *Trees*  
363 21:619–629
- 364 Kato T, Tang Y (2008) Spatial variability and major controlling factors of CO<sub>2</sub> sink strength in Asian  
365 terrestrial ecosystems: evidence from eddy covariance data. *Glob Change Biol* 14:1–16
- 366 Katul G, Manzoni S, Palmroth S, Oren R (2010) A stomatal optimization theory to describe the effects of  
367 atmospheric CO<sub>2</sub> on leaf photosynthesis and transpiration. *Ann Bot* 105:431-442
- 368 Kikuzawa K, Shirakawa H, Suzuki M, Umeki K (2004) Mean labor time of a leaf. *Ecol Res* 19:365-374
- 369 Kitaoka S, Watanabe Y, Koike T (2009) The effects of cleared larch canopy and nitrogen supply on gas  
370 exchange and leaf traits in deciduous broad-leaved tree seedlings. *Tree Physiol* 29:1503-1511
- 371 Koyama K, Kikuzawa K (2008) Intraspecific variation in leaf life span for the semi-evergreen liana  
372 *Akebia trifoliata* is caused by both seasonal and aseasonal factors in a temperate forest. *J Ecol Field Biol*  
373 31: 207-211
- 374 Koyama K, Kikuzawa K (2009) Is whole-plant photosynthetic rate proportional to leaf area? A test of

375 scalings and a logistic equation by leaf demography census. *Am Nat* 173:640-649

376 Koyama K, Kikuzawa K (2010a) Can we estimate forest gross primary production from leaf lifespan? A  
377 test in a young *Fagus crenata* forest. *J Ecol Field Biol* 33:253-260. DOI: 10.5141/JEFB.2010.33.3.253

378 Koyama K, Kikuzawa K (2010b). Geometrical similarity analysis of photosynthetic light response curves,  
379 light saturation and light use efficiency. *Oecologia* 163:53-63. DOI 10.1007/s00442-010-1638-9

380 Leuning R (1995) A critical appraisal of combined stomatal-photosynthesis model for C<sub>3</sub> plants. *Plant*  
381 *Cell Environ* 18:339-355

382 Luysaert S, Schulze ED, Börner A, Knohl A, Hessenmöller D, Law BE, Ciais P, Grace J (2008)  
383 Old-growth forests as global carbon sinks. *Nature* 455:213-215

384 Marshall B, Biscoe PV (1980) A model for C<sub>3</sub> leaves describing the dependence of net photosynthesis on  
385 irradiance. I. Derivation. *J Exp Bot* 31:29-39

386 Matsui T, Yagihashi T, Nakaya T, Tanaka N, Taoda H (2004) Climatic controls on distribution of *Fagus*  
387 *crenata* forests in Japan. *J Veg Sci* 15:57-66. DOI: 10.1111/j.1654-1103.2004.tb02237.x

388 Meir P, Kruijt B, Broadmeadow M, Barbosa E, Kull O, Carswell F, Nobre A, Jarvis PG (2002)  
389 Acclimation of photosynthetic capacity to irradiance in tree canopies in relation to leaf nitrogen  
390 concentration and leaf mass per unit area. *Plant Cell Environ* 25:343-357

391 Misson L, Limousin J-M, Rodriguez R, Letts MG (2010) Leaf physiological responses to extreme  
392 droughts in Mediterranean *Quercus ilex* forest. *Plant Cell Environ* 33:1898-1910

393 Mooney HA, Gulmon SL (1979) Environmental and evolutionary constraints on the photosynthetic  
394 characteristics of higher plants. In: Solbrig OT, Jain S, Johnson GB, Raven PH (eds) *Topics in plant*

395 population biology. Columbia University Press, New York, pp 316–337

396 Mott KA, Parkhurst DF (1991) Stomatal responses to humidity in air and helox. *Plant Cell Environ*

397 14:509-515

398 Mott KA, Peak D (2010) Stomatal responses to humidity and temperature in darkness. *Plant Cell Environ*

399 33:1084-1090

400 Muraoka H, Koizumi H (2005) Photosynthetic and structural characteristics of canopy and shrub trees in

401 a cool-temperate deciduous broadleaved forest: implication to the ecosystem carbon gain. *Agric For*

402 *Meteor* 134:39-59.

403 Muraoka H, Tang Y, Terashima I, Koizumi H, Washitani I (2000) Contributions of diffusional limitation,

404 photoinhibition and photorespiration to midday depression of photosynthesis in *Arisaema heterophyllum*

405 in the natural high light. *Plant Cell Environ* 23:235–250

406 Nabeshima E, Hiura T (2008) Size-dependency in hydraulic and photosynthetic properties of three *Acer*

407 species having different maximum sizes. *Ecol Res* 23:281-288

408 Nagano S, Nakano T, Hikosaka K, Maruta E (2009) Needle traits of an evergreen, coniferous shrub

409 growing at wind-exposed and protected sites in a mountain region: does *Pinus pumila* produce needles

410 with greater mass per area under wind-stress conditions? *Plant Biol* 11:94-100

411 Nakai A, Yurugi Y, Kisanuki H (2010) Stress responses in *Salix gracilistyla* cuttings subjected to

412 repetitive alternate flooding and drought. *Trees* 24:1087–1095

413 Niinemets Ü (1995) Distribution of foliar carbon and nitrogen across the canopy of *Fagus sylvatica*:

414 adaptation to a vertical light gradient. *Acta Oecol* 16:525-541.

415 Niinemets Ü (2010) A review of light interception in plant stands from leaf to canopy in different plant  
416 functional types and in species with varying shade tolerance. *Ecol Res* 25:693-714

417 Niinemets Ü, Valladares F (2004) Photosynthetic acclimation to simultaneous and interacting  
418 environmental stresses along natural light gradients: optimality and constraints. *Plant Biol* 6:254–268

419 Niinemets Ü, Sonninen E, Tobias M (2004) Canopy gradients in leaf intercellular CO<sub>2</sub> mole fractions  
420 revisited: interactions between leaf irradiance and water stress need consideration. *Plant Cell Environ*  
421 27:569-583

422 Oguchi R, Hikosaka K, Hirose T (2005) Leaf anatomy as a constraint for photosynthetic acclimation:  
423 differential responses in leaf anatomy to increasing growth irradiance among three deciduous trees. *Plant*  
424 *Cell Environ* 28:916–927

425 Okaura T, Harada K (2002) Phylogeographical structure revealed by chloroplast DNA variation in  
426 Japanese Beech (*Fagus crenata* Blume). *Heredity* 88:322–329

427 Posada JM, Lechowicz MJ, Kitajima K (2009) Optimal photosynthetic use of light by tropical tree crowns  
428 achieved by adjustment of individual leaf angles and nitrogen content. *Ann Bot* 103:795-805

429 Rosati A, DeJong TM (2003) Estimating photosynthetic radiation use efficiency using incident light and  
430 photosynthesis of individual leaves. *Ann Bot* 91:869-877

431 Rosati A, Metcalf SG, Lampinen BD (2004) A simple method to estimate photosynthetic radiation use  
432 efficiency of canopies. *Ann Bot* 93:567-574

433 Ryan M, Phillips N, Bond B (2006) The hydraulic limitation hypothesis revisited. *Plant, Cell & Environ*  
434 29:367-381

- 435 Saigusa N, Yamamoto S, Hirata R, Ohtani Y, Ide R, Asanuma J, Gamo M, Hirano T, Kondo H, Kosugi Y,  
436 Li S-G, Nakai Y, Takagi K, Tani M, Wang H (2008) Temporal and spatial variations in the seasonal  
437 patterns of CO<sub>2</sub> flux in boreal, temperate, and tropical forests in East Asia. *Agric For Meteorol*  
438 148:700–713
- 439 Sellers PJ, Berry JA, Collatz GJ, Field CB, Hall FG (1992) Canopy reflectance, photosynthesis, and  
440 transpiration. III. A reanalysis using improved leaf models and a new canopy integration scheme.  
441 *Remote Sens Environ* 42:187-216
- 442 Slatyer RO (1967) *Plant-water relationships*. Academic Press, London.
- 443 Tateishi M, Kumagai T, Suyama Y, Hiura T (2010) Differences in transpiration characteristics of Japanese  
444 beech trees, *Fagus crenata*, in Japan. *Tree Physiol* 30:748-760
- 445 Tazaki T, Ishihara K, Ushijima T (1980) Influence of water stress on the photosynthesis and productivity  
446 of plants in humid areas. In: Turner NC, Krammer PJ (eds) *Adaptation of plants to water and high*  
447 *temperature stress*. Wiley, New York, pp 309-321
- 448 Tenhunen JD, Pearcy RW, Lange OL (1987) Diurnal variations in leaf conductance and gas exchange in  
449 natural environments. In: Zeiger E, Farquhar GD, Cowan IR (eds) *Stomatal function*. Stanford  
450 University Press, California, pp 323-351
- 451 Terashima I, Araya T, Miyazawa S-I, Sone K, Yano S (2005) Construction and maintenance of the  
452 optimal photosynthetic systems of the leaf, herbaceous plant and tree: an eco-developmental treatise.  
453 *Ann Bot* 95:507-519
- 454 Turner NC, Schulze E-D, Gollan T. (1984) The responses of stomata and leaf gas exchange to vapour

455 pressure deficits and soil water content. I. Species comparison at high soil water contents. *Oecologia*  
456 63:338-342.

457 Uemura A, Ishida A, Nakano T, Terashima I, Tanabe H, Matsumoto Y (2000) Acclimation of leaf  
458 characteristics of *Fagus* species to previous-year and current-year solar irradiances. *Tree Physiol*  
459 20:945-951

460 Uemura A, Ishida A, Matsumoto Y (2005) Simulated seasonal changes of CO<sub>2</sub> and H<sub>2</sub>O exchange at the  
461 top canopies of two *Fagus* trees in a winter-deciduous forest, Japan. *For Ecol Manag* 212:230-242

462 Valladares F, Pearcy RW (2002) Drought can be more critical in the shade than in the sun: a field study of  
463 carbon gain and photo-inhibition in a Californian shrub during a dry El Niño year. *Plant Cell Environ*  
464 25:749-759

465 Valladares F, Zaragoza-Castells J, Sánchez-Gómez D, Matesanz S, Alonso B, Portsmouth A, Delgado A,  
466 Atkin OK (2008) Is shade beneficial for Mediterranean shrubs experiencing periods of extreme drought  
467 and late-winter frosts? *Ann Bot* 102:923-933

468 Vogel S (2009) Leaves in the lowest and highest winds: temperature, force and shape. *New Phytol* 183:  
469 13-26

470 Werner C, Ryel RJ, Correia O, Beyschlag W (2001) Effects of photoinhibition on whole-plant carbon gain  
471 assessed with a photosynthesis model. *Plant, Cell, Environ* 24:27-40

472 Yamasaki M, Kikuzawa K (2003) Temporal and spatial variations in leaf herbivory within a canopy of  
473 *Fagus crenata*. *Oecologia* 137:226-232

474 Yamazaki J, Yoda E, Takahashi A, Sonoike K, Maruta E (2007) Pacific Ocean and Japan Sea ecotypes of

- 475 Japanese beech (*Fagus crenata*) differ in photosystem responses to continuous high light. *Tree Physiol*  
476 27:961–968
- 477 Yoshimura K (2010) Irradiance heterogeneity within crown affects photosynthetic capacity and nitrogen  
478 distribution of leaves in *Cedrela sinensis*. *Plant Cell Environ* 33:750-758
- 479 Zotz G, Winter K (1996) Diel patterns of CO<sub>2</sub> exchange in rainforest canopy plants. In: Mulkey SS,  
480 Chazdon RL, Smith AP (eds.) *Tropical Forest Plant Ecophysiology*. Chapman & Hall, New York, pp  
481 89-113

482 **Table 1** The photosynthetic light response curve parameters and dark respiration rate  
 483 ( $R_d$ ) at the time of the curve measurement.

484	Upper	Lower	Units
485 $P_{\max}$	13.7 <sup>a</sup>	3.8 <sup>b</sup>	$\mu\text{mol m}^{-2} \text{s}^{-1}$
486 $\phi$	0.055	0.049	$\text{mol mol}^{-1}$
487 $\theta$	0.21	0.55	
488 $R_d$	1.8 <sup>c</sup>	0.2 <sup>d</sup>	$\mu\text{mol m}^{-2} \text{s}^{-1}$

490 Different uppercase letters: (a, b)  $p < 0.01$  and (c, d)  $p < 0.05$  ( $t$  - test).

491

#### 492 **Figure Legends (Figs. 1 - 4)**

493 **Fig. 1** Diurnal courses of the parameters. The left, the middle and the right column  
 494 shows the 1st, the 3rd and the 5th day after the last rain in 28 July 2008, respectively.  
 495 For each parameter but PPFD (one sensor), the mean value of the four leaves on each  
 496 position are respectively shown. The error bars indicate the unbiased estimate of the  
 497 standard deviation.

498 **a** (*Closed circles*) in situ gross photosynthetic rate of the leaves ( $P_{g\_mes}$ ). (*Open circles*)  
 499 PPFD on the branch. **b** Leaf conductance to  $\text{H}_2\text{O}$  ( $g$ ). **c** (*Open boxes*) leaf temperature.  
 500 (*Open circles*) vapor pressure deficit based on the leaf temperature (VPD).

501

502 **Fig. 2** Diurnal course of  $P_{g\_mes} / P_{g\_cv}$ , the ratio between in-situ photosynthetic rate on  
503 each time and photosynthetic rate estimated by photosynthetic light response curve,  
504 which was measured at one particular time during measurement days for each leaf. Each  
505 *open circle* and its error bars indicate mean value and its unbiased standard deviation,  
506 respectively, for the four leaves on each canopy position. Solid lines indicate significant  
507 linear regressions (\* $p < 0.05$ , \*\*  $p < 0.01$ ):

508

509 (1st day)  $P_{g\_mes} / P_{g\_cv} = 1.3 - 2.2 \times 10^{-2}$  Time of day ( $h$ ).

510 (3rd day)  $P_{g\_mes} / P_{g\_cv} = 1.4 - 2.9 \times 10^{-2} h$ .

511 (5th day)  $P_{g\_mes} / P_{g\_cv} = 1.4 - 4.1 \times 10^{-2} h$ .

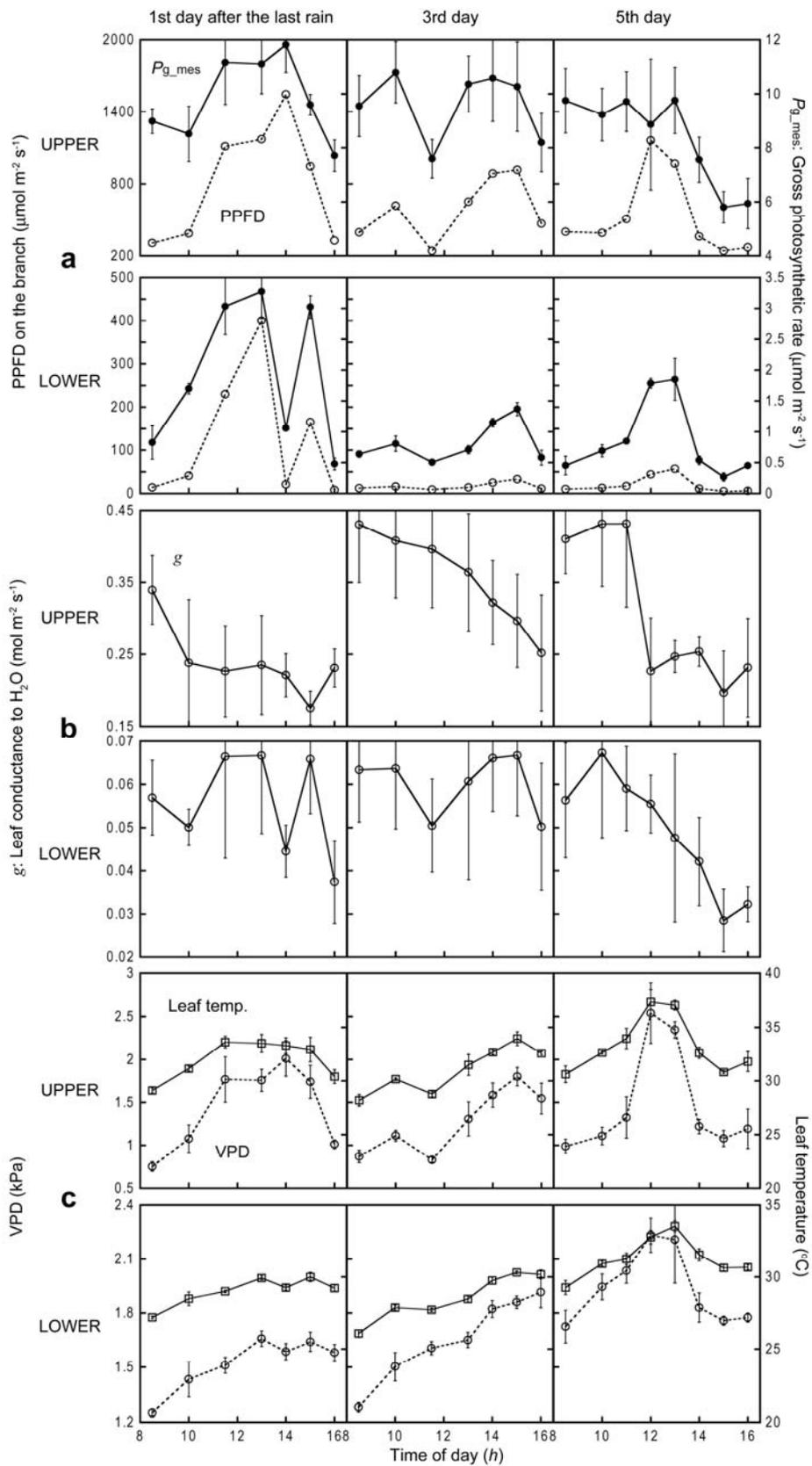
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513 **Fig. 3** The slopes of the diurnal decline of  $P_{g\_mes} / P_{g\_cv}$  (i.e. the slopes of the linear  
514 regressions for the upper leaves shown in Fig. 2) in relation to number of days after the  
515 last rain.

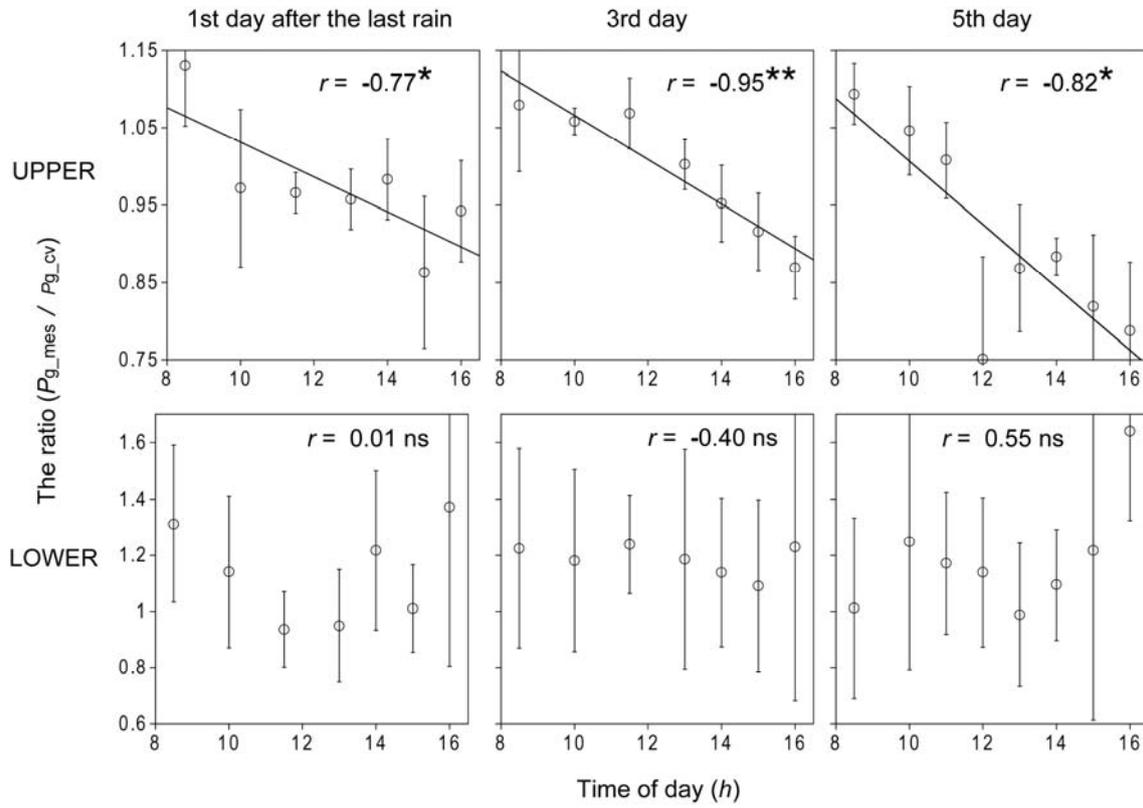
516

517 **Fig. 4**  $P_{g\_mes} / P_{g\_cv}$  in relation to  $g$ . Each *open circle* and its error bar indicate mean  
518 value and standard deviation, respectively, for the four leaves in each position.  
519 Pearson's correlation coefficients ( $r$ ) were shown (\*  $p < 0.05$ , \*\*\*  $p < 0.001$ ).

520



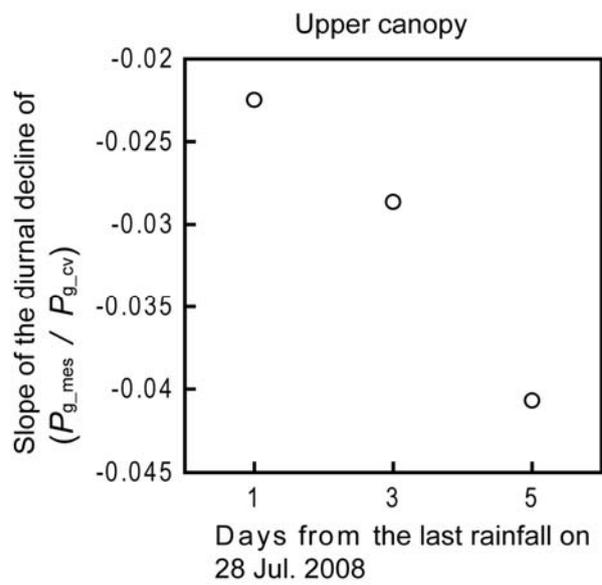
524 Fig. 2



525

526

527 Fig. 3



528

529

