Coping with branch excision when measuring leaf net photosynthetic rates in a lowland tropical forest

Verryckt Lore T. [1, *] , Van Langenhove Leandro [1], Ciais Philippe [2], Courtois Elodie A. [3], Vicca Sara [1], Peñuelas Josep [4], Stahl Clément [5], Coste Sabrina [5], Ellsworth David S. [6], Posada Juan M. [7], Obersteiner Michael [8], Chave Jérôme [9], Janssens Ivan A. [1]

[1] Department of Biology University of Antwerp Wilrijk ,Belgium
[2] Laboratoire des Sciences du Climat et de l'Environnement CEA-CNRS-UVSQ Gif-sur-Yvette ,France
[3] Laboratoire Ecologie, Évolution, Interactions des Systèmes Amazoniens (LEEISA) Université de Guyane CNRS IFREMER Cayenne, French Guiana
[4] CSIC Global Ecology CREAF-CEAB-CSIC-UAB Cerdanyola del Valles Barcelona, Spain
[5] UMR Ecofog, AgroParisTech, CNRS, Cirad INRA Université des Antilles Université de Guyane Kourou ,France
[6] Hawkesbury Institute for the Environment Western Sydney University Penrith NSW ,Australia
[7] Biology Department Faculty of Natural Sciences Universidad del Rosario Bogotá ,Colombia
[8] International Institute for Applied Systems Analysis (IIASA) Laxenburg, Austria
[9] UMR 5174 Laboratoire Evolution et Diversité Biologique Université Paul Sabatier CNRS Toulouse ,France

* Corresponding author : Lore T. Verryckt, email address : lore.verryckt@uantwerpen.be

Abstract :

Measuring leaf gas exchange from canopy leaves is fundamental for our understanding of photosynthesis and for a realistic representation of carbon uptake in vegetation models. Since canopy leaves are often difficult to reach, especially in tropical forests with emergent trees up to 60 m at remote places, canopy access techniques such as canopy cranes or towers have facilitated photosynthetic measurements. These structures are expensive and therefore not very common. As an alternative, branches are often cut to enable leaf gas exchange measurements. The effect of branch excision on leaf gas exchange rates should be minimized and quantified to evaluate possible bias. We compared light-saturated leaf net photosynthetic rates measured on excised and intact branches. We selected branches positioned at three canopy positions, estimated relative to the top of the canopy: upper sunlit foliage, middle canopy foliage, and lower canopy foliage. We studied the variation of the effects of branch excision and transport among branches at these different heights in the canopy. After excision and transport, light-saturated leaf net photosynthetic rates were close to zero for most leaves due to stomatal closure. However, when the branch had acclimated to its new environmental conditions—which took on average 20 min—light-saturated leaf net photosynthetic rates did not significantly differ between the excised and intact branches. We therefore conclude that branch excision does not affect the measurement of light-saturated leaf net photosynthesis, provided that the branch is recut under water and is allowed sufficient time to acclimate to its new environmental conditions.

1. INTRODUCTION

The measurement of leaf gas exchange is important for our understanding of photosynthesis (e.g. Dang, Margolis, Coyea, Sy & Collatz, 1997, Schaberg, Shane, Cali, Donnelly & Strimbeck, 1998, Domingues et al., 2010, Cavaleri, Reed, Smith & Wood, 2015, Heberling, Kichey, Decocq & Fridley, 2016), to understand processes of plant evolution and ecological strategies (Wright et al., 2004), and to improve the representation of carbon uptake in vegetation models (Rezende et al., 2016). In areas such as Amazonia, where the number of plants exceeds 14,000 about half being trees (Cardoso et al., 2017), the challenge of measuring leaf gas exchange across the region is enormous. Even at accessible field sites, reaching canopy leaves is difficult, because they are situated between 30 and 60 m above the ground (Perry, 1978).

Canopy cranes or towers have facilitated the in-situ measurement of leaf gas exchange in forest canopies, but these structures are rare (Basset, Horlyck & Wright, 2003, Nakamura et al., 2017). Therefore, branch excision is often necessary prior to leaf gas exchange measurement. The excision of branches using a single-rope climbing technique (Perry, 1978), pole pruners, or shotguns (e.g. Messier, Mcgill & Lechowicz, 2010) are different methods that can give access to leaves in the canopy. The effect of excision of branches on leaf gas exchange rates should therefore be minimised and quantified to evaluate possible bias.

Tropical studies measuring gas exchange on excised branches have reported different effects of excision on the leaf net photosynthetic rate (A_n). Some species are not affected (Santiago & Mulkey, 2003, Rowland et al.,2015), whereas for other species A_n decreases within a few minutes to several days (Santiago & Mulkey, 2003). The reported effects can be caused by alterations in the xylem stream after excision, influencing stomatal response (Sperry, Alder & Easttlack, 1993, Williamson & Milburn, 1995). Therefore, it has been advised that prior to

69 measurement, excised branches should be recut under water, to remove embolised xylem vessels

70 and to restore hydraulic conductivity for maintaining an adequate supply of water to the leaves.

71 Santiago and Mulkey (2003) and Rowland et al. (2015) studied upper-canopy, sunlit leaves.

72 However, several leaf physiological traits of tropical trees, such as A_{sat} and g_s, change with

73 height (Kenzo et al., 2015). The effect of excision might thus also vary with tree height. To the

74 best of our knowledge, vertical differences in the excision effect have not yet been investigated.

75 In this study, we therefore assessed whether the effect of excision varied with depth in the

76 canopy.

77   Measurement can be conducted at a nearby campsite (Rowland et al., 2015), or in a

78 laboratory further away (Ellsworth & Reich, 1993, Dang et al.,1997), implying different timings

79 between excision and measurement and different environmental conditions. The excised

80 branches are thus treated in different ways prior to leaf gas exchange measurements, even though

81 the studies cited tried to minimise the time between excision and the actual measurement and to

82 maintain the original light conditions for the excised branch.

83   We assessed the effects of branch excision and transport on leaf net photosynthetic rate at

84 light saturation (A_{sat}) and the variation of these effects amongst branches at different heights in

85 the canopy. We hypothesised that the effects of excision and transport would be smaller in the

86 canopy, because these branches are acclimated to darker conditions than the branches at the top

87 of the canopy. We assumed that they would be less stressed after excision and transport through

88 a dark forest. We therefore tested for a faster recovery of A_{sat} for branches lower in the canopy.

89 **2. METHODS**

90 **2.1   Study site**

91   This study was conducted at the Nouragues Research Station (4°02' N, 52°41' W), an old-

92   growth, tropical lowland forest site in French Guiana, South America. The climate is tropical

93   humid, with a dry season lasting from August to November and a short dry period in March.

94   Mean annual temperature is 26.3°C and mean annual rainfall is about 3000 mm (Bongers,

95   Charles-Dominique, Forget & Théry, 2001).

96   **2.2   Measurement of leaf gas exchange**

97   We selected seven trees (Table 1) belonging to four dominant families at this field site:

98   Fabaceae, Lecythidaceae, Malvaceae, and Burseraceae. A_{sat} (measured at 1300 µmol/m^2/s) and

99   stomatal conductance (g_s; µmol/m^2/s) were measured on mature leaves using an infrared gas

100 analyser (LI-6400XT, LI-COR, Lincoln, NE, USA). The measurements were carried out at three

101 canopy positions, estimated relative to the top of the canopy: upper sunlit foliage, middle canopy

102 foliage, and lower canopy foliage. We measured A_{sat} at each position for at least three leaves on

103 an intact branch attached to the tree, with the assistance of a tree climber. This 2 m-long branch

104 was subsequently excised, lowered gently from the canopy to a person who then immediately

105 recut the branch under water to reconstitute the water supply to the leaves, and transported

106 (within about ten minutes) to a nearby camp site in an opaque plastic bag to avoid further water

107 loss due to transpiration, where at least two leaves were measured on the excised branch. The

108 base of the branch always remained under water during transport. The branch was again recut

109 under water at the camp site to prevent from potential disruption of hydraulic conductivity that

110 may have arisen during transport. A_n at light saturation from these excised branches was

111 continuously logged. A_n was then defined as A_{sat} when A_n remained stable, i.e. did not vary more

112 than five percent for at least two minutes. Relative humidity was maintained as near ambient as

113 possible (70-85%), and the rate of air flow was 500 mL/min during these measurements, which

114   were carried out between 0930 h and 1500 h. The CO_2 level in the chamber was constant at 400

115   ppm, and the chamber temperature was controlled by maintaining the block temperature at 30 ±

116   0.2°C.

117   We tested an additional tree species at the camp site, *Eperua falcata*, with sunlit leaves

118   reachable from the ground without climbing. We tested the effect on A_{sat} of transporting the

119   excised branch in an opaque plastic bag. We continuously measured A_n at light saturation before

120   excision and continued measuring it after excision without transporting the branch in a plastic

121   bag. We also excised a branch, after measuring A_{sat}, and transported it similarly to the other

122   excised branches and again continuously measured A_n at light saturation to compare both

123   methods. These measurements were carried out between 1000 h and 1130 h.

124   **2.3   Data analyses**

125   The effect of excision on A_{sat} was tested with a paired t-test, comparing pre- and post-cutting

126   measurements for each leaf. We also tested if the slope of the linear regression between A_{sat} for

127   non-excised and excised branches differed from 1. This analysis was carried out for all data, but

128   also for upper, middle and lower canopy foliage separately. We analysed our A_{sat} data using a

129   two-way ANOVA, with excision (pre- or post-cutting), tree ID and canopy position (upper,

130   middle, lower foliage) as fixed factors. We specifically explored the two-way interactions

131   between these factors to assess the (interaction) effects of tree ID and canopy position on

132   excision. We used $P < 0.05$ to identify significant differences. Additionally we performed a

133   bootstrap analysis (10,000 replicates) for this two-way ANOVA for which we allowed

134   resampling within the same canopy position (upper, middle, lower foliage). We assumed that the

135   interaction effects would not be significant if the bias-corrected and accelerated (BCa) 95%

136   confidence intervals included zero. All analyses were performed in R 3.3.3 (R Core Team, 2017)

137   using the ggplot2 package (Wickham, 2009) for visualising the data.

138   **3. RESULTS**

139   **3.1   Effect of excision**

140   In general, A_{sat} did not differ significantly between the excised and non-excised branches (paired

141   t-test: $P = 0.10$), and the slope of the linear regression did not differ significantly from 1 ($P =$

142   0.21; Figure 1). When analysing the upper, middle and lower canopy foliage separately, the

143   intercept and the slope of the linear regression did not differ significantly from 0 ($P_{upper} = 0.79$,

144    $P_{middle} = 0.64$, $P_{lower} = 0.54$) and 1 ($P_{upper} = 0.79$, $P_{middle} = 0.86$, $P_{lower} = 0.19$), respectively. The

145   two-way ANOVA showed no interaction between excision and tree ($P = 0.59$) or canopy

146   position ($P = 0.55$). The interaction between tree ID and canopy position was significant ($P =$

147   0.03), with different responses of A_{sat} between tree IDs and canopy positions. Excision was not a

148   significant factor in the two-way ANOVA ($P = 0.49$), indicating no differences in A_{sat} before and

149   after excision. Individual differences between pre- and post-cutting measurements of A_{sat} are

150   shown in Figure S1. The bootstrap analysis showed a tendency of an excision effect for tree 7,

151   with BCa 95% confidence intervals approaching zero [0.144, 4.317], whereas it did not show any

152   effect for the other tree species, nor of canopy position, since these BCa 95% confidence

153   intervals all included zero. Intrinsic water-use efficiency (iWUE) did not differ significantly

154   between the excised and non-excised branches, with the slope of the linear regression not

155   differing significantly from 1 ($P = 0.66$; Figure S2).

156   **3.2   Effect of darkness in *E. falcata*: to bag or not to bag?**

157   Branch excision of the *E. falcata* tree in the camp had no effect on the continuously measured A_n

158   at light saturation (Figure 2a). When the excised branch was first transported in a plastic bag and

159   A_n at light saturation was measured 10 min after recutting the branch under water, A_n at light

160   saturation was more than 50 percent lower than the original value (Figure 2b). A_n at light

161   saturation increased again after about 30 minutes of stabilisation, and A_{sat} was similar to the

162   measurement on the intact branch in the tree.

3.3   Stabilisation time

164   A_n at light saturation after branch excision and transport to the camp was near zero (<1

165    $\mu mol/m^2/s$) for 65 percent of the leaves and decreased by 30-80 percent for the remainder of the

166   leaves. A_n increased over time after the leaf was placed in the leaf chamber and exposed to 1300

167    $\mu mol/m^2/s$ photosynthetically active radiation (Figure 3a), and A_{sat} did not differ significantly

168   from A_{sat} for the leaf measured on the intact branch in the tree (Figure 1). The time to

169   stabilisation across all trees averaged 22 min, but ranged between 6 and 44 min (Figure 3b,

170   Figure S3, Figure S4). The stabilisation time was longer for tree 7 than the other trees. The

171   stabilisation time was longer for upper canopy foliage (27 ± 9 min) compared to middle (22 ± 10

172   min) and lower (19 ± 7 min) canopy foliage (Figure 3c).

4. DISCUSSION

174   We quantified the effects of branch excision on A_{sat} as differences between A_{sat} measured on an

175   intact branch in the tree and A_{sat} measured on the same leaf after branch excision under water. A_n

176   at light saturation decreased soon after the branch was excised and transported to the camp site

177   (Figure 2b, Figure S5a). This immediate decrease in A_n at light saturation was caused by stomatal

178   closure (Figure 2b, Figure S5b), but the stomata reopened on average 22 min after the branch

8

179 was re-exposed to light (Figure 3b, c). A_n at light saturation, however, did not decrease in the

180 excised *E. falcata* branch kept in the light (Figure 2a), suggesting that the stomatal closure was

181 due to light limitation rather than dehydration caused by excision. This requires confirmation by

182 testing more tree species, because this was an unreplicated experiment. We only tested one

183 species that we kept at constant light while excising the branch.

184   The cut branches were transported from the canopy to the forest floor, where less than

185 two percent of the incoming light at the top of the canopy is typically available (Yoda, 1974,

186 Théry, 2001, Montgomery & Chazdon, 2002). We then cut the excised branch under water and

187 transported it in an opaque bag to the camp site, where we measured leaf gas exchange. Light is a

188 major determinant of stomatal conductance (Motzer, Munz, Küppers, Schmitt & Anhuf, 2005),

189 so both the dark conditions at the forest floor and the opaque bag likely induced stomatal closure.

190 Stomata respond quickly to changing light conditions (Roelfsema & Hedrich, 2005, Devireddy,

191 Zandalinas, Gómez-Cadenas, Blumwald & Mittler, 2018), allowing the plant to regulate its

192 water-use efficiency. Stomata of the leaves can thus be expected to close after excision due to

193 light limitation to maintain their water balance and to reopen when the leaves are re-exposed to

194 light in the leaf chamber. The recovery of stomatal conductance and A_n was faster for leaves

195 lower in the canopy and thus typically exposed to less light (Figure 3c), supporting our

196 hypothesis that branches lower in the canopy would recover faster after excision. The differences

197 in recovery time along a height gradient in the canopy might also be explained by the differences

198 in leaf water potential. Upper-canopy leaves typically have a lower water potential and the

199 branches at the top of the canopy might thus experience more stress when excised compared to

200 middle- and lower-canopy branches (Koch, Sillett, Jennings & Davis, 2004, Olson et al., 2018).

201 Although we did not measure the leaf water potentials, our data on stomatal conductance and

202 intrinsic water use efficiency suggest that this did not play a major role in our study.

203 A_{sat} did not differ significantly before and after excision at the camp site after acclimating

204 to the new light conditions in the leaf chamber. The immediate decrease in A_n at light saturation

205 was restored after, on average, 22 min, which is in contrast to other studies where excision had

206 no effect or a delayed decrease in A_{sat} was shown (Lange, Führer & Gebel, 1986, Meng & Arp,

207 1992, Ellsworth & Liu, 1994, Dang et al., 1997, Ambrose, Sillett & Dawson, 2009, Dusenge et

208 al., 2015, Rowland et al., 2015). Measurements of A_{sat} on sugar maple leaves, immediately after

209 excision and rehydration of the branch, were always within five percent of the rate measured on

210 intact branches (Ellsworth & Liu, 1994). Excision did not affect A_{sat} in *Pinus banksiana* or *Picea*

211 *mariana*, either immediately or during the next 14 h (Dang et al., 1997). Rowland et al. (2015)

212 reported that excision did not affect photosynthesis in tropical trees. Our branches were allowed

213 to stabilise in a canopy opening in full ambient irradiance for at least 30 min after recutting

214 underwater prior to the measurements. Dusenge et al. (2015) found no difference in V_{cmax}, the

215 maximum carboxylation rate, and only a small negative effect on J_{max}, the maximum electron

216 transport rate, for tropical trees ten minutes before and after excision. A_n in twigs of *Picea abies*

217 was constant for a minimum of 15 min after excision (Lange et al.,1986), and net photosynthesis

218 of *Picea rubens* twigs did not vary significantly within about six minutes after detachment

219 (Meng & Arp, 1992). A_{sat} did not decrease after excision in *Sequoia sempervirens* and *S.*

220 *giganteum* in California, USA (Ambrose et al., 2009). The leaves for all these studies were

221 exposed to full sunlight prior to measuring photosynthesis or were kept in the chamber under

222 saturating light conditions, in contrast to our study.

223   Santiago and Mulkey (2003) reported that some species retained more than 85 percent of

224   the photosynthetic rate for intact branches (i.e. pre-excision) 60 min after excision, whereas the

225   rates for other species were less than 40 percent of the rates for intact branches within three

226   minutes. The effect of excision can vary among species, and was shown to depend on the

227   production of latex or resin since these exudates might clog the xylem, reduce sap flow, and

228   thereby reduce foliar gas exchange (Santiago & Mulkey, 2003). Excision did not generally affect

229   A_{sat} in all seven of our species, although A_{sat} decreased significantly after excision for sunlit

230   upper-canopy foliage for tree 2 (*Tetragastris altissima*) and middle-canopy foliage for trees 3

231   (*Eschweilera coriacea*) and 7 (*E. falcata*) (Figure S1). A_{sat} may have decreased because the time

232   needed for the leaves to acclimate to the settings of light and humidity at the camp was not long

233   enough to reach A_{sat}. For tree 2 (*Tetragastris altissima*), belonging to the Burseraceae family that

234   is known to produce latex or resin (Plowden, Uhl & De Assis Oliveira, 2004, Weeks, Daly &

235   Simpson, 2005), the decrease in A_{sat} after excision for sunlit, upper-canopy leaves might have

236   been influenced by the resin produced by this species, although we did not observe any resin

237   production. No decrease in A_{sat} after excision was observed for middle and lower canopy leaves

238   of this tree. A_{sat} measured in the camp on the excised branch was larger than A_{sat} measured on the

239   intact branches for some trees (Figure S1), perhaps because xylem water potentials were higher

240   in the rehydrated excised branches than the intact branches (Comstock & Ehleringer, 1984).

241   Since there was no significant decrease in A_{sat}, we assume that there was no effect of excision on

242   leaf photosynthesis, although there is large variation among individually sampled leaves.

243   Additionally, given that we only sampled seven species, it is still recommended to test the effect

244   of excision before measuring leaf gas exchange for other species than those studied here or in

245   previous studies (e.g. Santiago & Mulkey, 2003, Ambrose et al., 2009, Dusenge et al., 2015,

246   Rowland et al., 2015).

247   We conclude that branch excision does not affect the measurement of A_{sat} when the

248   branch is recut under water after excision and the measurement is carried out for a sufficiently

249   long time (at least 20 minutes) after the branch has acclimated to its new environmental

250   conditions for reopening the stomata. Light is necessary to reopen the stomata when the branch is

251   transported in dark conditions, either on the forest floor or inside an opaque bag. This process

252   could be facilitated by transporting the excised branch in a bag containing LED lights, or the

253   branch could be exposed to sunlight before measurement.

265 **CONFLICT OF INTEREST**

266 The corresponding author confirms on behalf of all authors that there have been no involvements

267 that might raise the question of bias in the work reported or in the conclusions, implications, or

268 opinions stated.

269 **DATA AVAILABILITY STATEMENT**

270 The data that support the findings of this study are openly available in Dryad Digital Repository

271 at https://doi.org/10.5061/dryad.v9s4mw6rg.

272 **LITERATURE CITED**

273 AMBROSE, A. R., SILLETT, S. C., & DAWSON, T. E. (2009). Effects of tree height on branch

274   hydraulics, leaf structure and gas exchange in California redwoods. *Plant, Cell &*

275   *Environment, 32*(7), 743-757. doi:10.1111/j.1365-3040.2009.01950.x

276 BASSET, Y., HORLYCK, V., & WRIGHT, S. J. (2003). *Studying Forest Canopies from Above: The*

277   *International Canopy Crane Network*. Smithsonian Tropical Research Institute and

278   UNEP.

279 BONGERS, F., CHARLES-DOMINIQUE, P., FORGET, P.-M., & THERY, M. (2001). *Nouragues:*

280   *Dynamics and Plant-Animal Interactions in a Neotropical Rainforest*. Kluwer Academic

281   Publishers.

282 CARDOSO, D., SÄRKINEN, T., ALEXANDER, S., AMORIM, A. M., BITTRICH, V., CELIS, M., …

283   FORZZA, R. C. (2017). Amazon plant diversity revealed by a taxonomically verified

284   species list. *Proceedings of the National Academy of Sciences, 114*(40), 10695-10700.

285   doi:10.1073/pnas.1706756114

286 CAVALERI, M. A., REED, S. C., SMITH, W. K., & WOOD, T. E. (2015). Urgent need for warming

287   experiments in tropical forests. *Global Change Biology, 21*, 2111-2121.

288   doi:10.1111/gcb.12860

289 COMSTOCK, J., & EHLERINGER, J. (1984). Photosynthetic responses to slowly decreasing

290   leaf water potentials in *Encelia frutescens*. *Oecologia, 61*, 241-248.

291   doi:10.1007/BF00396767

292 DANG, Q. L., MARGOLIS, H. A., COYEA, M. R., SY, M., & COLLATZ, G. J. (1997). Regulation of

293   branch-level gas exchange of boreal trees: roles of shoot water potential and vapor

294   pressure difference. *Tree Physiology, 17*(8-9), 521-535. doi:10.1093/treephys/17.8-9.521

295   DEVIREDDY, A. R., ZANDALINAS, S. I., GÓMEZ-CADENAS, A., BLUMWALD, E., & MITTLER, R.

296   (2018). Coordinating the overall stomatal response of plants: Rapid leaf-to-leaf

297   communication during light stress. *Science Signaling, 11*(518), eaam9514.

298   doi:10.1126/scisignal.aam9514

299   DOMINGUES, T. F., MEIR, P., FELDPAUSCH, T. R., SAIZ, G., VEENENDAAL, E. M., SCHRODT, F., …

300   LLOYD, J. (2010). Co-limitation of photosynthetic capacity by nitrogen and phosphorus in

301   West Africa woodlands. *Plant, Cell & Environment, 33*, 959-980. doi:10.1111/j.1365-

302   3040.2010.02119.x

303   DUSENGE, M. E., WALLING, G., GÅRDESTEN, J., NYRONZIMA, F., ADOLFSSON, L., NSABIMANA, D.,

304   & UDDLING, J. (2015). Photosynthetic capacity of tropical montane tree species in

305   relation to leaf nutrients, successional strategy and growth temperature. *Oecologia, 117*,

306   1183-1194. doi:10.1007/s00442-015-3260-3

307   ELLSWORTH, D. S., & LIU, X. (1994). Photosynthesis and canopy nutrition of four sugar maple

308   forests on acid soils in northern Vermont. *Canadian Journal of Forest Research, 24*,

309   2118-2127. doi:10.1139/x94-272

310   ELLSWORTH, D. S., & REICH, P. B. (1993). Canopy structure and vertical patterns of

311   photosynthesis and related leaf traits in a deciduous forest. *Oecologia, 96*, 169-178.

312   doi:10.1007/BF00317729

313   HEBERLING, J. M., KICHEY, T., DECOCQ, G., & FRIDLEY, J. D. (2016). Plant functional shifts in

314   the invaded range: a test with reciprocal forest invaders of Europe and North America.

315   *Functional Ecology*, *30*(6), 875-884. doi:10.1111/1365-2435.12590

316 KENZO, T., INOUE, Y., YOSHIMURA, M., YAMASHITA, M., TANAKA-ODA, A., &

317   ICHIE, T. (2015). Height-related changes in leaf photosynthetic traits in diverse Bornean

318   tropical rain forest trees. *Oecologia, 177*, 191-202. doi:10.1007/s00442-014-3126-0

319 KOCH, G. W., SILLETT, S. C., JENNINGS, G. M., & DAVIS, S. D. (2004). The limits to tree

320   height. *Nature, 428*, 851-854. doi:10.1038/nature02417

321 LANGE, O. L., FÜHRER, G., & GEBEL, J. (1986). Rapid field determination of photosynthetic

322   capacity of cut spruce twigs (Picea abies) at saturating ambient CO2. *Trees, 1*, 70-77.

323   doi:10.1007/bf00197027

324 MENG, F.-R., & ARP, P. A. (1992). Net photosynthesis and stomatal conductance of red spruce

325   twigs before and after twig detachment. *Canadian Journal of Forest Research, 23*, 716-

326   721. doi:10.1139/x93-093

327 MESSIER, J., MCGILL, B. J., & LECHOWICZ, M. J. (2010). How do traits vary across ecological

328   scales? A case for trait-based ecology. *Ecology Letters*, *13*(7), 838-848.

329   doi:10.1111/j.1461-0248.2010.01476.x

330 MONTGOMERY, R., & CHAZDON, R. (2002). Light gradient partitioning by tropical tree seedlings

331   in the absence of canopy gaps. *Oecologia, 131*(2), 165-174. doi:10.1007/s00442-002-

332   0872-1

333 MOTZER, T., MUNZ, N., KÜPPERS, M., SCHMITT, D., & ANHUF, D. (2005). Stomatal conductance,

334   transpiration and sap flow of tropical montane rain forest trees in the southern Ecuadorian

335   Andes. *Tree Physiology*, *25*, 1283-1293. doi:10.1093/treephys/25.10.1283

336 NAKAMURA, A., KITCHING, R. L., CAO, M., CREEDY, T. J., FAYLE, T. M., FREIBERG, M., …

337   ASHTON, L. A. (2017). Forests and Their Canopies: Achievements and Horizons in

338   Canopy Science. *Trends in Ecology & Evolution, 32*(6), 438-451.

339   doi:10.1016/j.tree.2017.02.020

340 OLSON, M. E., SORIANO, D., ROSELL, J. A., ANFODILLO, T., DONOGHUE, M. J.,

341   EDWARDS, E. J., … MÉNDEZ-ALONZO, R. (2018). Plant height and hydraulic

342   vulnerability to drought and cold. *PNAS, 115*(29), 7551-7556.

343   doi:10.1073/pnas.1721728115

344 PERRY, D. R. 1978. A method of access into the crowns of emergent and canopy trees.

345   *Biotropica, 10*(2), 155-157. doi:10.2307/2388019

346 PLOWDEN, C., UHL, C., & DE ASSIS OLIVEIRA, F. (2004). Alipumilio (Diptera: Syrphidae)

347   fly association with Burseraceae tree resins in the eastern Brazilian Amazon. *Journal of*

348   *Natural History, 38*(15), 1941-1947. doi:10.1080/00222930310001618886

349 R CORE TEAM. 2017. *R: A Language and Environment for Statistical Computing*. R Foundation

350   for Statistical Computing, Vienna, Austria.

351 REZENDE, L. F. C., ARENQUE, B. C., AIDAR, S. T., MOURA, M. S. B., VON RANDOW, C.,

352   TOURIGNY, E., … OMETTO, J. P. H. B. (2016). Evolution and challenges of dynamic

353   global vegetation models for some aspects of plant physiology and elevated atmospheric

354   CO_2. *International Journal of Biometeorology, 60*(7), 945-955. doi:10.1007/s00484-015-

355   1087-6

356 ROELFSEMA, M. R., & HEDRICH, R.. 2005. In the light of stomatal opening: new insights into 'the

357   Watergate'. *New Phytologist, 167*, 665-691. doi:10.1111/j.1469-8137.2005.01460.x

358 ROWLAND, L., LOBO-DO-VALE, R. L., CHRISTOFFERSEN, B. O., MELEM, E. A., KRUIJT, B.,

359   VASCONCELOS, S. S., … MEIR, P. (2015). After more than a decade of soil moisture

360   deficit, tropical rainforest trees maintain photosynthetic capacity, despite increased leaf

361   respiration. *Global Change Biology, 21*(12), 4662-4672. doi:10.1111/gcb.13035

362 SANTIAGO, L. S., & MULKEY, S. S. (2003). A test of gas exchange measurements on excised

363   canopy branches of ten tropical tree species. *Photosynthetica, 41*(3), 343-347.

364   doi:10.1023/B:PHOT.0000015457.92479.eb

365 SCHABERG, P. G., SHANE, J. B., CALI, P. F., DONNELLY, J. R., & STRIMBECK, G. R. (1998).

366   Photosynthetic capacity of red spruce during winter. *Tree Physiology, 18*, 271-276.

367 SPERRY, J. S., ALDER, N. N., & EASTLACK, S. E. (1993). The Effect of Reduced Hydraulic

368   Conductance on Stomatal Conductance and Xylem Cavitation. *Journal of Experimental*

369   *Botany, 44*(6), 1075-1082. doi:10.1093/jxb/44.6.1075

370 THE PLANT LIST. 2013. Version 1.1. http://www.theplantlist.org/ (accessed November 2018).

371 THÉRY, M. (2001) Forest light and its influence on habitat selection. *Plant Ecology, 153*(1-2),

372   251-261. doi:10.1023/A:1017592631542

373 VERRYCKT, L. T., VAN LANGENHOVE, L., CIAIS, P., COURTOIS, E. A., VICCA, S.,

374   PEÑUELAS, J., … JANSSENS, I. A. (2020). Data from: Coping with branch excision

375   when measuring leaf net photosynthetic rates in a lowland tropical forest. Dryad Digital

376   Repository. doi:10.5061/dryad.v9s4mw6rg

377 WEEKS, A., DALY, D. C., & SIMPSON, B. B. (2005). The phylogenetic history and

378   biogeography of the frankincense and myrrh family (Burseraceae) based on nuclear and

379   chloroplast sequence data. *Molecular Phylogenetics and Evolution*, *35*, 85-101.

380   doi:10.1016/j.ympev.2004.12.021

381 WICKHAM, H. (2009). *ggplot2: Elegant Graphics for Data Analysis*. . Springer-Verlag New

382   York.

383 WILLIAMSON, V. G., & MILBURN, J. A. (1995). Cavitation events in cut stems kept in water:

384   implications for cut flower senescence. *Scientia Horticulturae, 64*(4), 219-232.

385   doi:10.1016/0304-4238(95)00842-X

386 WRIGHT, I. J., REICH, P. B., WESTOBY, M., ACKERLY, D. D., BARUCH, Z., BONGERS, F., …

387   VILLAR, R. (2004). The worldwide leaf economics spectrum. *Nature, 428*(6985), 821-

388   827. doi:10.1038/nature02403

389 YODA, K. (1974). Three-dimensional distribution of light intensity in a tropical rain forest of

390   West Malaysia. *Japanese Journal of Ecology, 24*(4), 247-254.

391   doi:10.18960/seitai.24.4_247

392

TABLES

394 *Table 1. Overview of the tree species selected for this study. The Plant List (2013) was used to validate the scientific*

395 *names and to confirm authorship. The diameter at breast height (DBH) and total height are given for each tree. The*

396 *heights of the upper, middle, and lower canopy foliage indicate the heights above the ground surface at which the*

397 *branches were sampled.*

Tree ID	Family	Species name	DBH (cm)	Total height (m)	Height upper foliage (m)	Height middle foliage (m)	Height lower foliage (m)
1	Malvaceae	*Sterculia cf. multiovula* E.L. Taylor	39.5	32	30	25	20
2	Burseraceae	*Tetragastris altissima* (Aubl.) Swart	47.4	30	27	23	20
3	Lecythidaceae	*Eschweilera coriacea* (DC.) S.A. Mori	53.2	38	36	32	25
4	Lecythidaceae	*Lecythis persistens* Sagot	47.4	42	42	34	29.5
5	Lecythidaceae	*Eschweilera coriacea* (DC.) SA. Mori	54.7	35	33	29	23
6	Fabaceae	*Inga* sp.	36.6	31	28	22	17.5
7	Fabaceae	*Eperua falcata* Aubl.	81.8	40	38	33	27

FIGURE LEGENDS

399   FIGURE 1 Leaf net photosynthetic rate at light saturation (A_{sat}; µmol/m^2/s) for non-excised

400   (intact A_{sat}) and excised (excised A_{sat}) branches (n = 21). Each point represents an average of 2-3

401   leaves for each canopy position and tree, with standard errors on the x- and y-axes. The slope of

402   the linear regression did not differ significantly from 1 ($P = 0.63$). The black line represents the

403   1:1 line and the grey shading shows the 95% confidence interval.

404   FIGURE 2 Leaf net photosynthetic rate (A_n; µmol/m^2/s) at light saturation and stomatal

405   conductance (g_s; µmol/m^2/s) in *Eperua falcata* as functions of time of day, during an

406   unreplicated experiment to test the effect of transporting the excised branch in an opaque plastic

407   bag on A_{sat}. (A) A_n was continuously measured before and after excision of the branch. The solid

408   black line represents the time of excision. (B) A_n was measured on the attached branch. The

409   excised branch was transported in an opaque plastic bag, and A_n was again continuously

410   measured after 10 min until A_n had stabilised and differed by less than five percent from the

411   original values before excision. The dashed line represents A_n before excision.

412   FIGURE 3 (A) Actual data of a representative response of continuously measured leaf net

413   photosynthetic rate (A_n) at light saturation after excision and transport of the branch. Time 0

414   corresponds to the placement of the leaf in the gas exchange chamber. (B) Average stabilisation

415   time for the seven trees. The error bars are the standard errors of the mean. No data are available

416   for tree 5, because we did not log A_n continuously. (C) Average stabilisation time for branches at

417   different positions in the canopy (U, upper; M, middle; L, lower) for the seven trees. The error

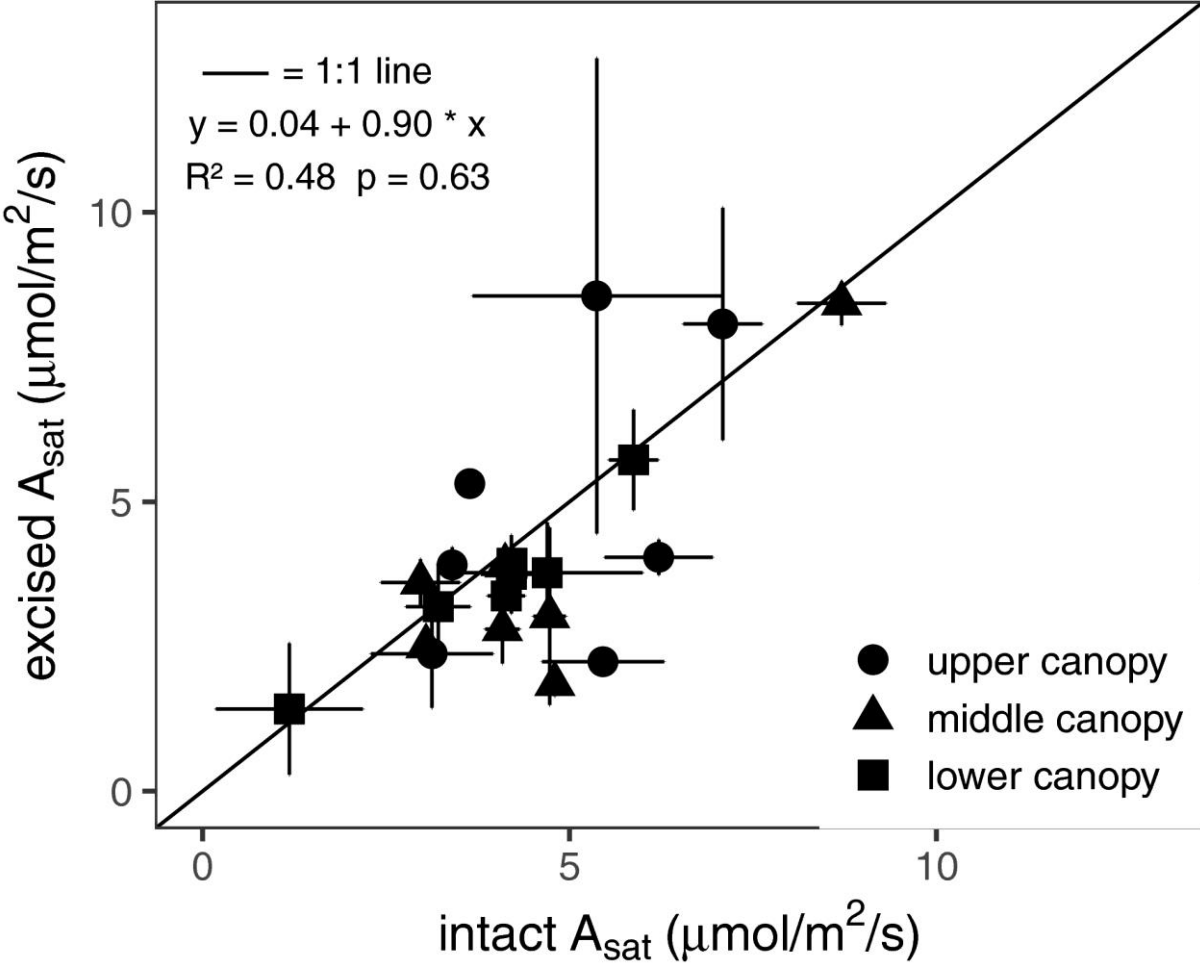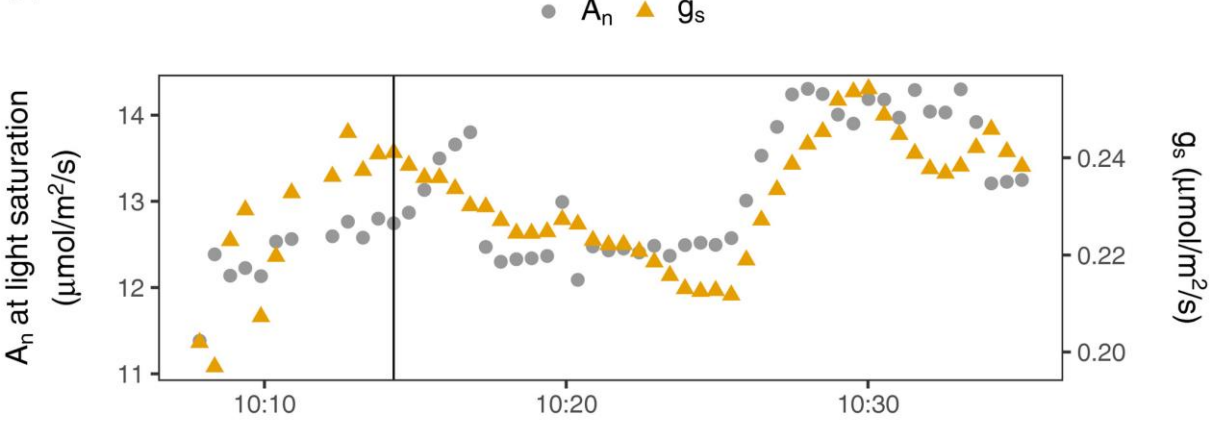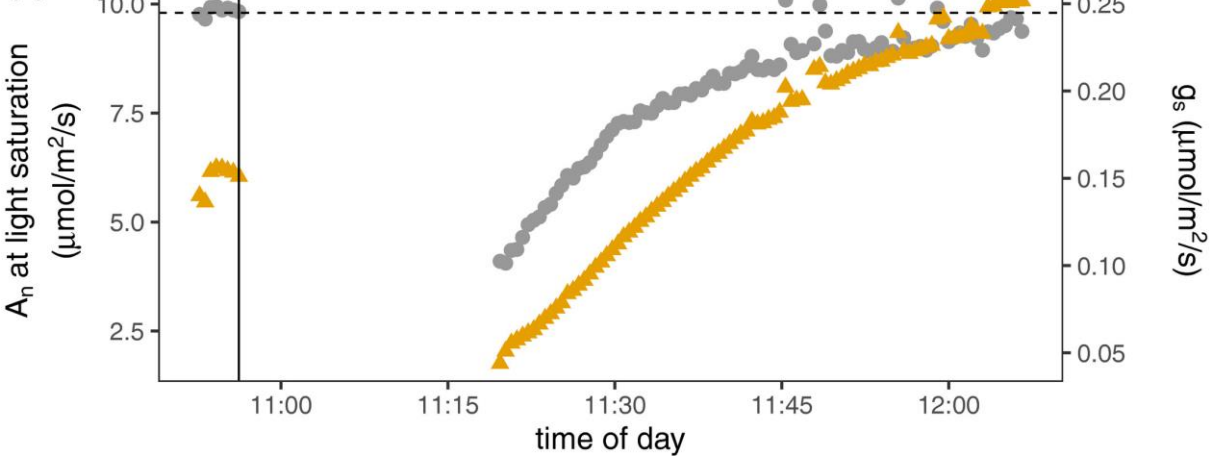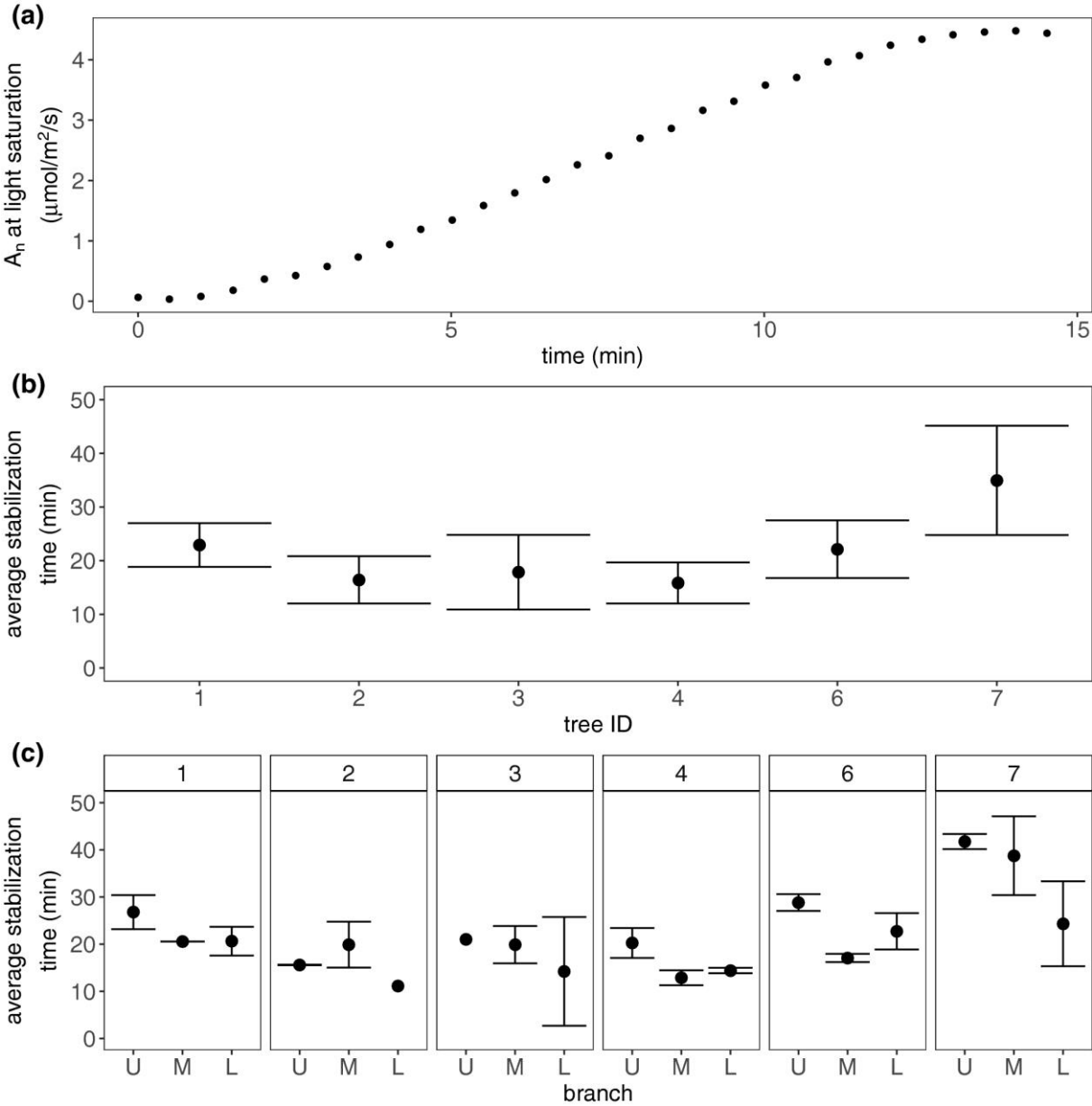418   bars are the standard errors of the mean.

Figure 1

Figure 2

Figure 3

SUPPORTING INFORMATION

Article title: Coping with branch excision when measuring leaf net photosynthetic rates in a lowland tropical forest

Authors: Lore T. Verryckt[1], Leandro Van Langenhove[1], Philippe Ciais[2], Elodie A. Courtois[3], Sara Vicca[1], Josep Peñuelas[4], Clément Stahl[5], Sabrina Coste[6], David S. Ellsworth[7], Juan M. Posada[8], Michael Obersteiner[9], Jérôme Chave[10], Ivan A. Janssens[1]

The following Supporting Information is available for this article:

FIGURE S1 Box and whisker plots of the replicates of leaf net photosynthetic rate at light saturation (A_{sat}; µmol m^{-2} s^{-1}) compared between attached (tree) and excised (camp) branches for (a) upper sunlit foliage, (b) middle canopy foliage, and (c) lower canopy foliage.

FIGURE S2 Intrinsic water-use efficiency (iWUE; µmol CO_2 mol^{-1} H_2O) compared between non-excised (tree iWUE) and excised (camp iWUE) branches (n = 21).

FIGURE S3 Actual data of continuously measured leaf net photosynthetic rate (A_n) at light saturation after excision and transport of the branch.

FIGURE S4 Histogram of the stabilisation times (min) for six trees.

FIGURE S5 Leaf net photosynthetic rate (A_n) at light saturation and stomatal conductance (g_s) compared between the start of the continuously logged leaf gas exchange measurements (time 0) and when A_n remained stable.
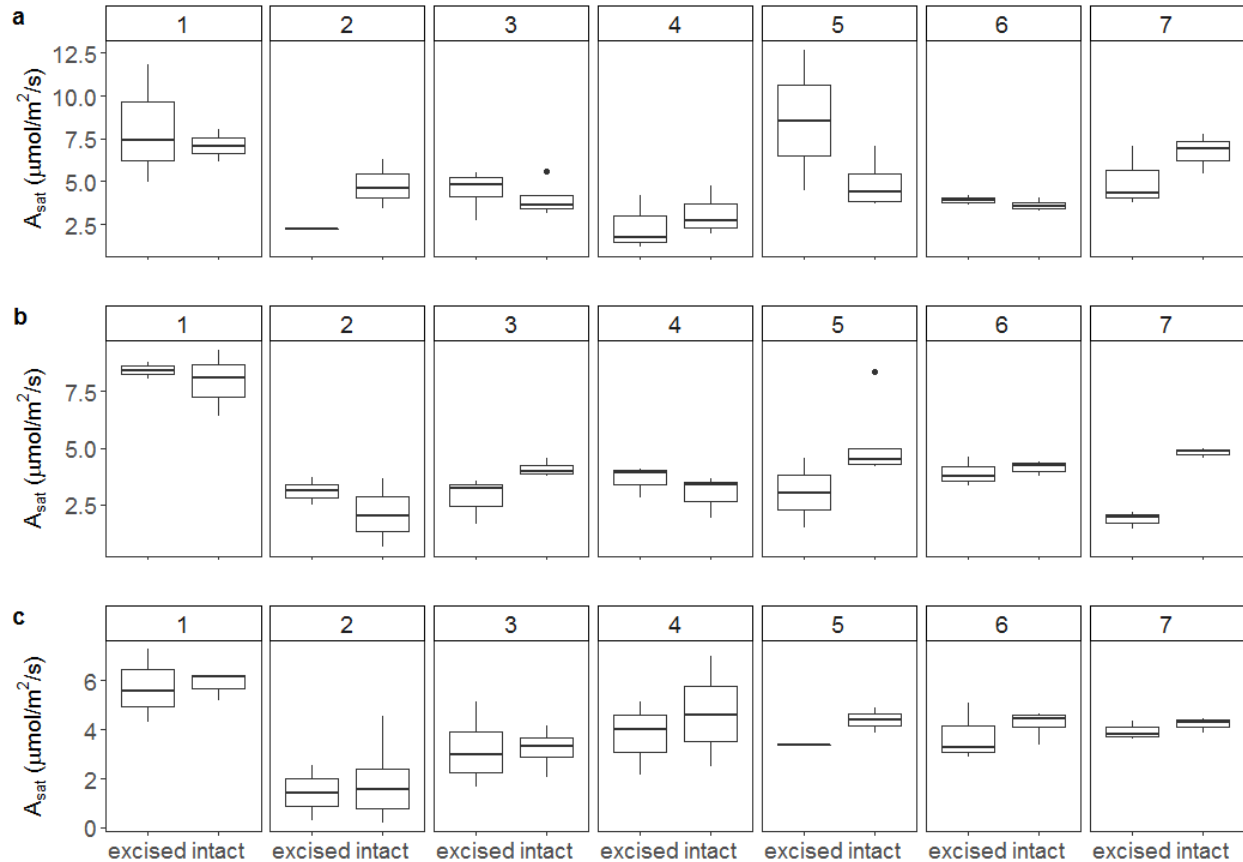
Fig. S1 *Box and whisker plots of the replicates of leaf net photosynthetic rate at light saturation (A_{sat}; $\mu mol/m^2/s$) compared between attached (intact) and excised (excised) branches for (A) upper sunlit foliage, (B) middle canopy foliage, and (C) lower canopy foliage. A two-way ANOVA showed no interaction between excision and tree ($P = 0.59$) or canopy position ($P = 0.55$). The numbers above the plots are the tree IDs (see Table 1). The height of the box is the interquartile range, the horizontal bar is the median, the whiskers extend to 1.5 times the first or third quartile, the circles are outliers.*
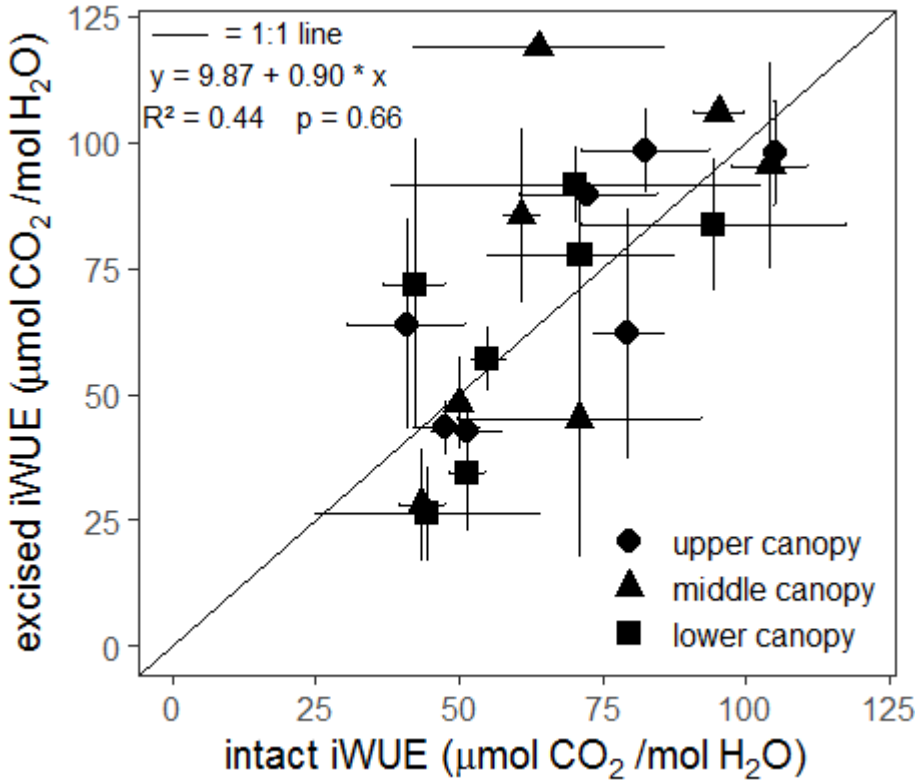
Fig. S2 *Intrinsic water-use efficiency (iWUE; μmol CO_2/mol H_2O) compared between non-excised (intact iWUE) and excised (excised iWUE) branches (n = 21). iWUE was calculated as leaf net photosynthetic rate at light saturation (A_{sat}) divided by stomatal conductance measured at A_{sat}. Each point represents an average of 2-3 leaves for each canopy position for each tree, with standard errors on the x- and y-axes. The circles, triangles, and squares represent upper, middle, and lower canopy branches, respectively. The branches were cut (y-axis), so iWUE for the excised branches may not be identical to iWUE for intact branches. Excision, however, did not significantly affect iWUE; the slope of the linear regression did not differ significantly from 1 (P = 0.66). The black line represents the 1:1 line.*
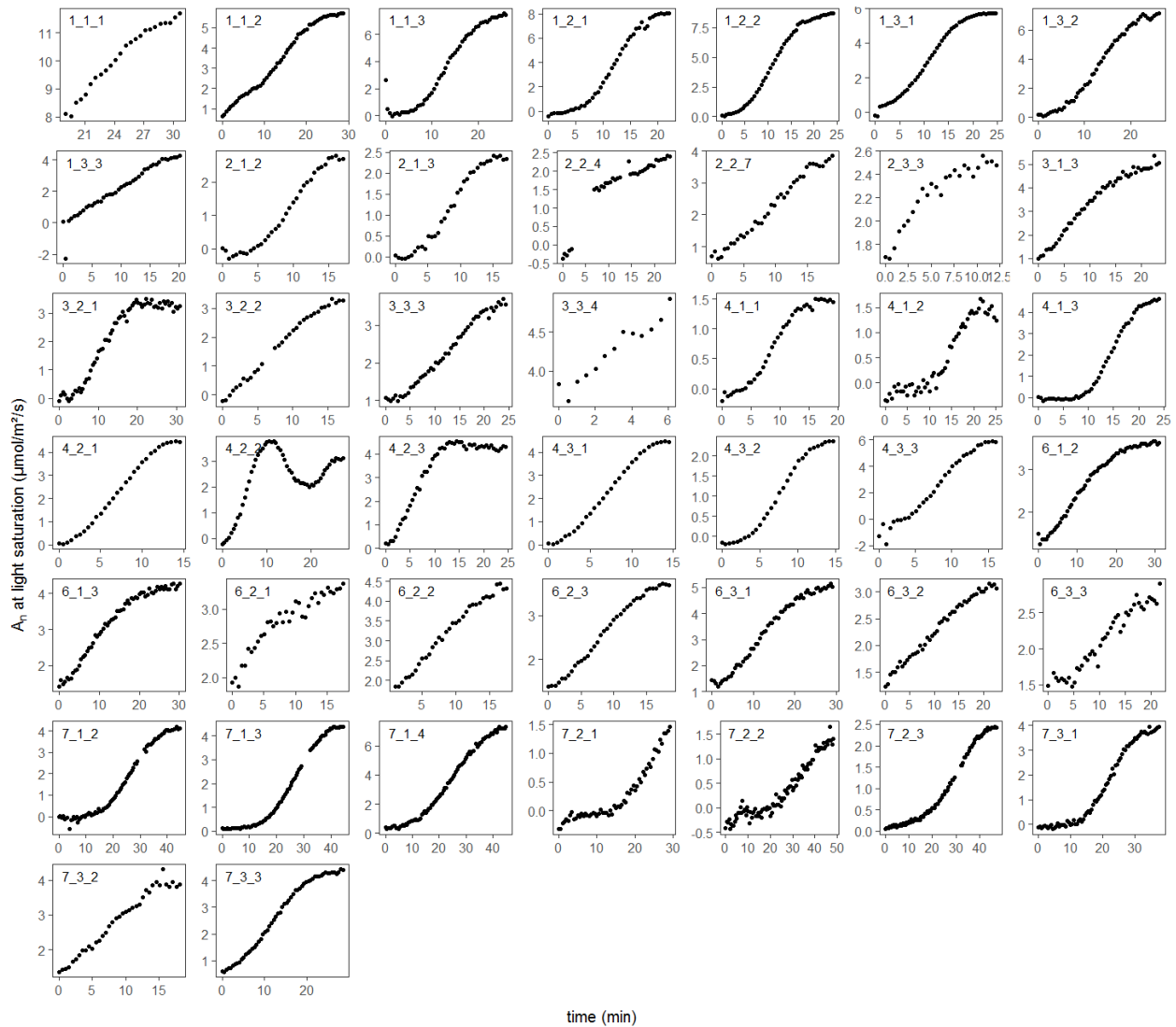
Fig. S3 *Actual data of continuously measured leaf net photosynthetic rate (A_n; μmol/m²/s) at light saturation after excision and transport of the branch. Time 0 corresponds to the placement of the leaf in the gas exchange chamber. The ID number of each plot refers to the tree number (1-7; no data available for tree 5 since we did not log A_n continuously), followed by the canopy height (1 = upper canopy, 2 = middle canopy, 3 = lower canopy) and the number of the replicate.*
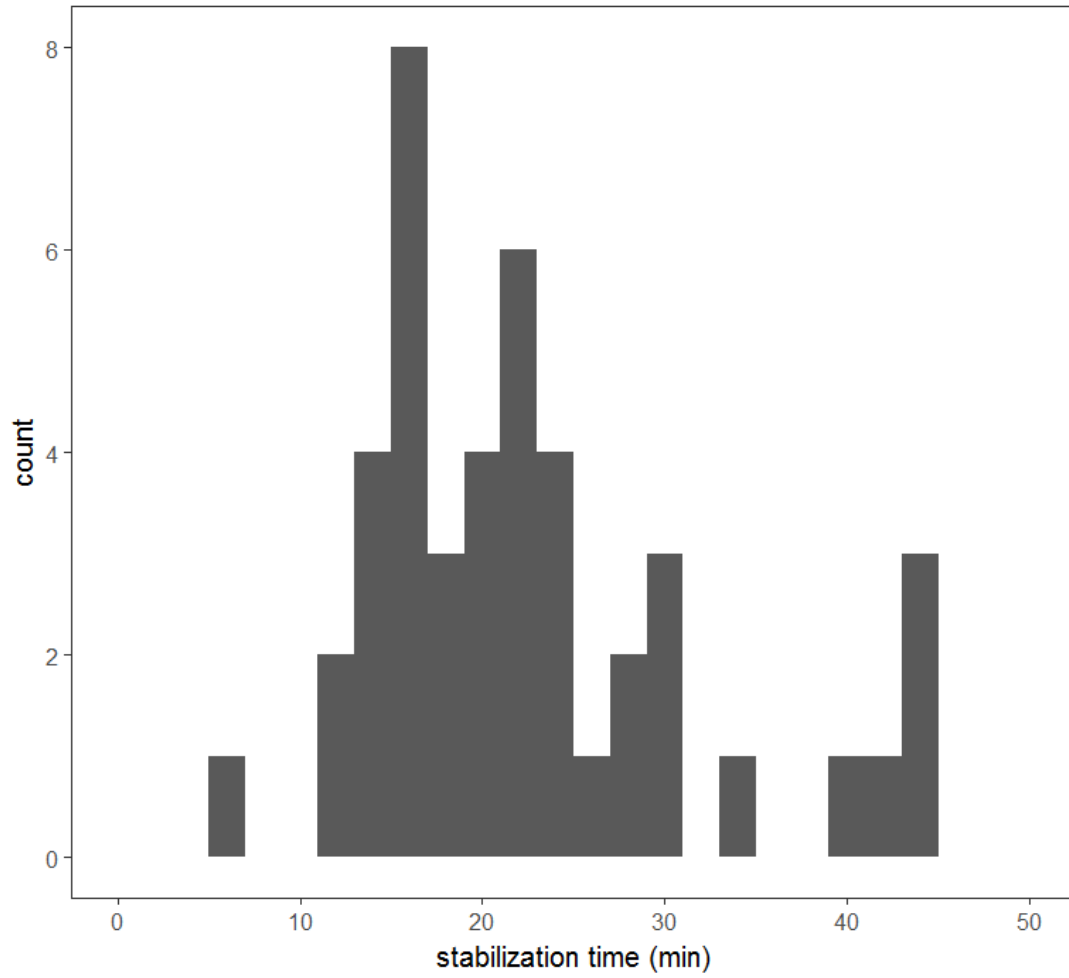
Fig. S4 *Histogram of the stabilization times (min) for six trees. No data are available for tree 5, because we did not log A_n continuously.*
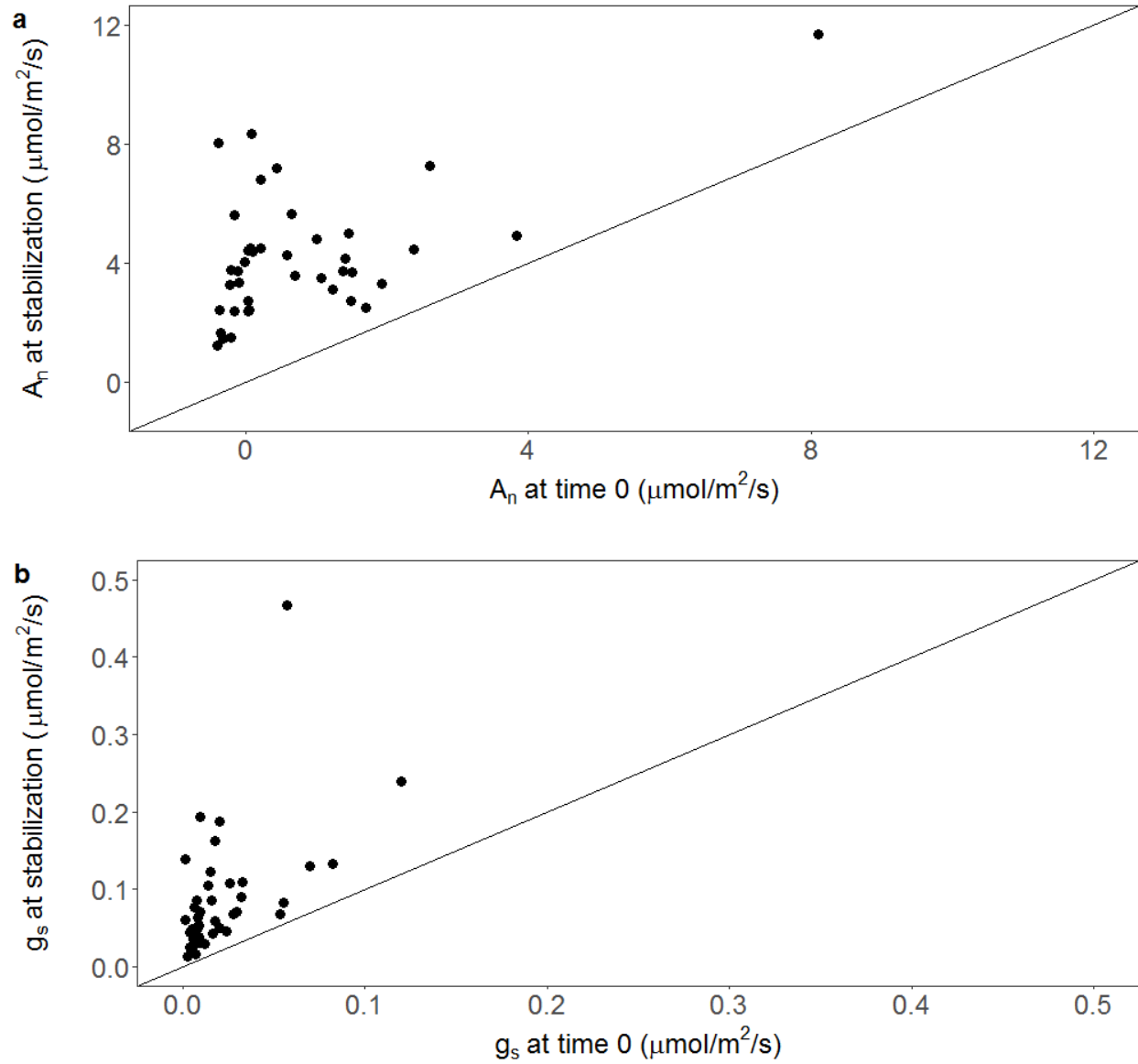
***Fig. S5** (A) Leaf net photosynthetic rate (A_n) at light saturation and (B) stomatal conductance (g_s) measured at the start of the continuously logged leaf gas exchange measurements (time 0) and when A_n remained stable (at stabilisation, i.e. A_n did not vary by more than five percent for at least two minutes).*