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

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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.

Keywords : branch cutting, canopy physiology, French Guiana, gas exchange, photosynthesis, rainforest, stomatal conductance

46 **1. INTRODUCTION**

47 The measurement of leaf gas exchange is important for our understanding of photosynthesis (e.g.
48 Dang, Margolis, Coyea, Sy & Collatz, 1997, Schaberg, Shane, Cali, Donnelly & Strimbeck,
49 1998, Domingues et al., 2010, Cavaleri, Reed, Smith & Wood, 2015, Heberling, Kichey, Decocq
50 & Fridley, 2016), to understand processes of plant evolution and ecological strategies (Wright et
51 al., 2004), and to improve the representation of carbon uptake in vegetation models (Rezende et
52 al., 2016). In areas such as Amazonia, where the number of plants exceeds 14,000 about half
53 being trees (Cardoso et al., 2017), the challenge of measuring leaf gas exchange across the region
54 is enormous. Even at accessible field sites, reaching canopy leaves is difficult, because they are
55 situated between 30 and 60 m above the ground (Perry, 1978).

56 Canopy cranes or towers have facilitated the in-situ measurement of leaf gas exchange in
57 forest canopies, but these structures are rare (Basset, Horlyck & Wright, 2003, Nakamura et al.,
58 2017). Therefore, branch excision is often necessary prior to leaf gas exchange measurement.
59 The excision of branches using a single-rope climbing technique (Perry, 1978), pole pruners, or
60 shotguns (e.g. Messier, McGill & Lechowicz, 2010) are different methods that can give access to
61 leaves in the canopy. The effect of excision of branches on leaf gas exchange rates should
62 therefore be minimised and quantified to evaluate possible bias.

63 Tropical studies measuring gas exchange on excised branches have reported different
64 effects of excision on the leaf net photosynthetic rate (A_n). Some species are not affected
65 (Santiago & Mulkey, 2003, Rowland et al., 2015), whereas for other species A_n decreases within
66 a few minutes to several days (Santiago & Mulkey, 2003). The reported effects can be caused by
67 alterations in the xylem stream after excision, influencing stomatal response (Sperry, Alder &
68 Easttack, 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 $\mu\text{mol}/\text{m}^2/\text{s}$) and
99 stomatal conductance (g_s ; $\mu\text{mol}/\text{m}^2/\text{s}$) 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 CO₂ 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 ($P_{\text{upper}} = 0.79,$
144 $P_{\text{middle}} = 0.64, P_{\text{lower}} = 0.54$) and 1 ($P_{\text{upper}} = 0.79, P_{\text{middle}} = 0.86, P_{\text{lower}} = 0.19$), 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.

163 **3.3 Stabilisation time**

164 A_n at light saturation after branch excision and transport to the camp was near zero (<1
165 $\mu\text{mol}/\text{m}^2/\text{s}$) 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 $\mu\text{mol}/\text{m}^2/\text{s}$ 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).

173 **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

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.

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264 for helping us with the field measurements.

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>.

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392

393 **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	<i>Sterculia cf. multiovula</i> E.L. Taylor	39.5	32	30	25	20
2	Burseraceae	<i>Tetragastris altissima</i> (Aubl.) Swart	47.4	30	27	23	20
3	Lecythidaceae	<i>Eschweilera coriacea</i> (DC.) S.A. Mori	53.2	38	36	32	25
4	Lecythidaceae	<i>Lecythis persistens</i> Sagot	47.4	42	42	34	29.5
5	Lecythidaceae	<i>Eschweilera coriacea</i> (DC.) SA. Mori	54.7	35	33	29	23
6	Fabaceae	<i>Inga</i> sp.	36.6	31	28	22	17.5
7	Fabaceae	<i>Eperua falcata</i> Aubl.	81.8	40	38	33	27

398 **FIGURE LEGENDS**

399 FIGURE 1 Leaf net photosynthetic rate at light saturation (A_{sat} ; $\mu\text{mol}/\text{m}^2/\text{s}$) 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 ; $\mu\text{mol}/\text{m}^2/\text{s}$) at light saturation and stomatal
405 conductance (g_s ; $\mu\text{mol}/\text{m}^2/\text{s}$) 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

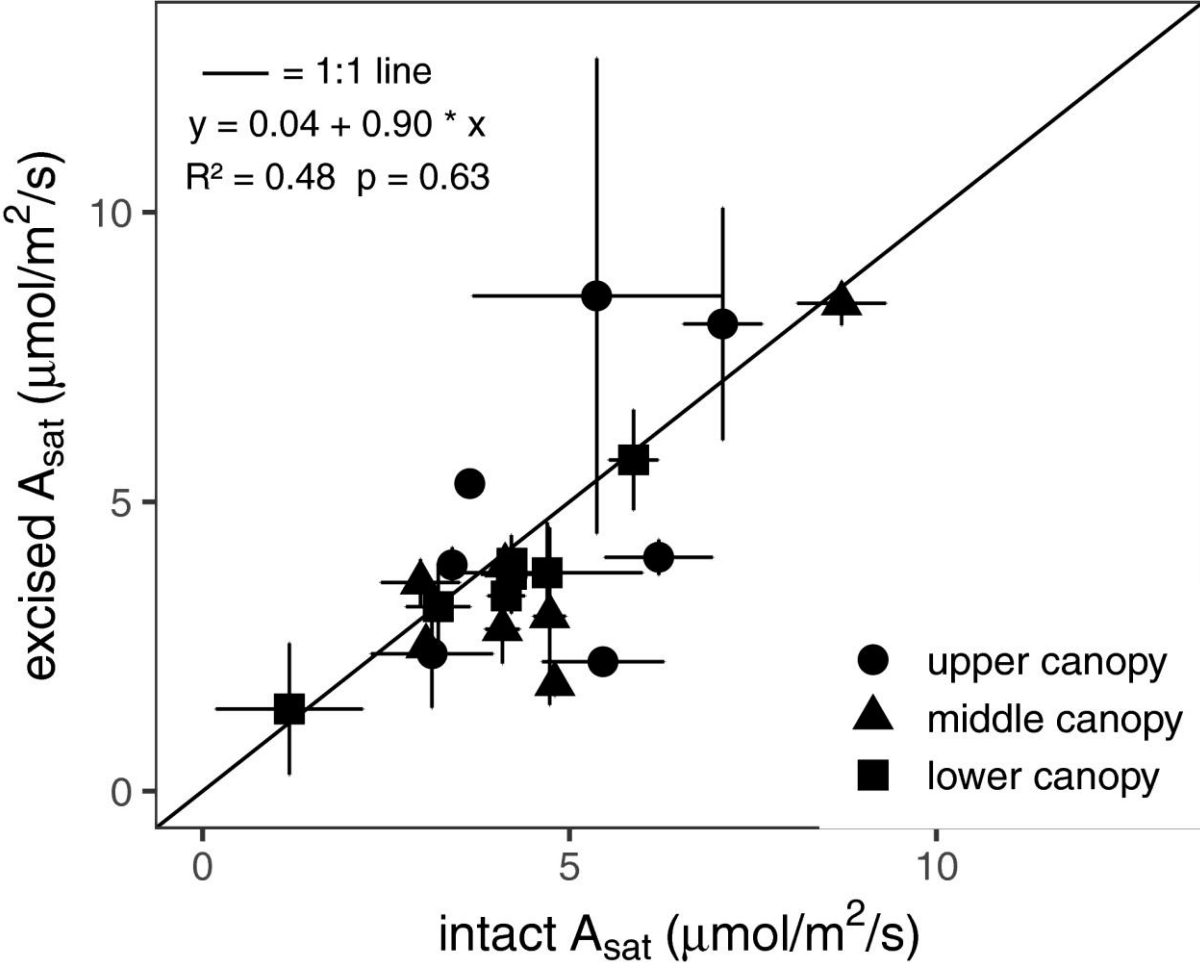
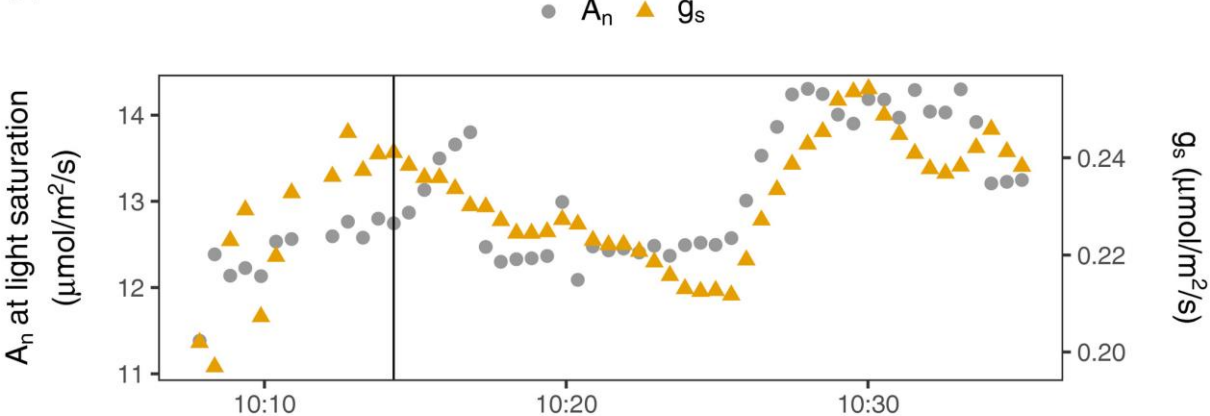


Figure 2

(a)



(b)

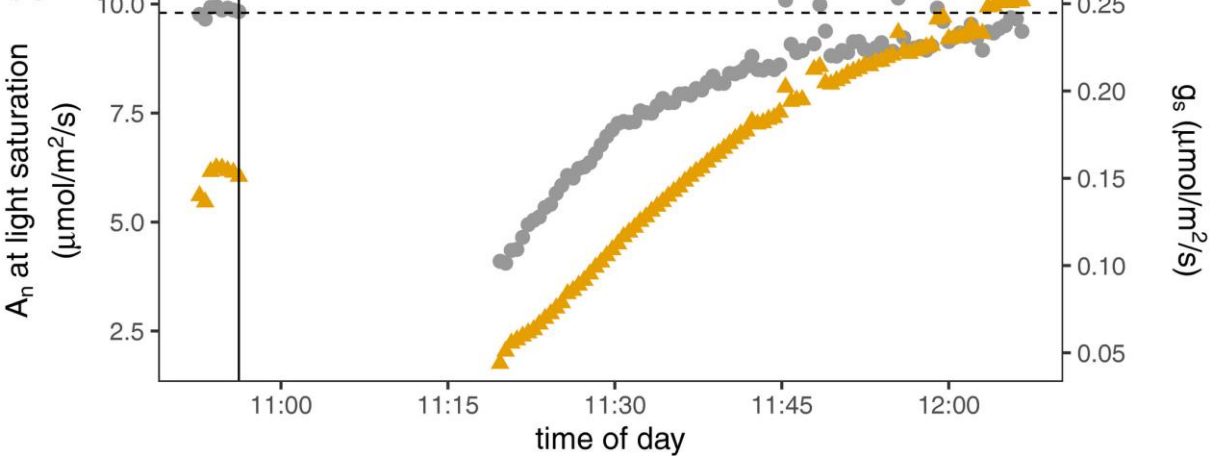
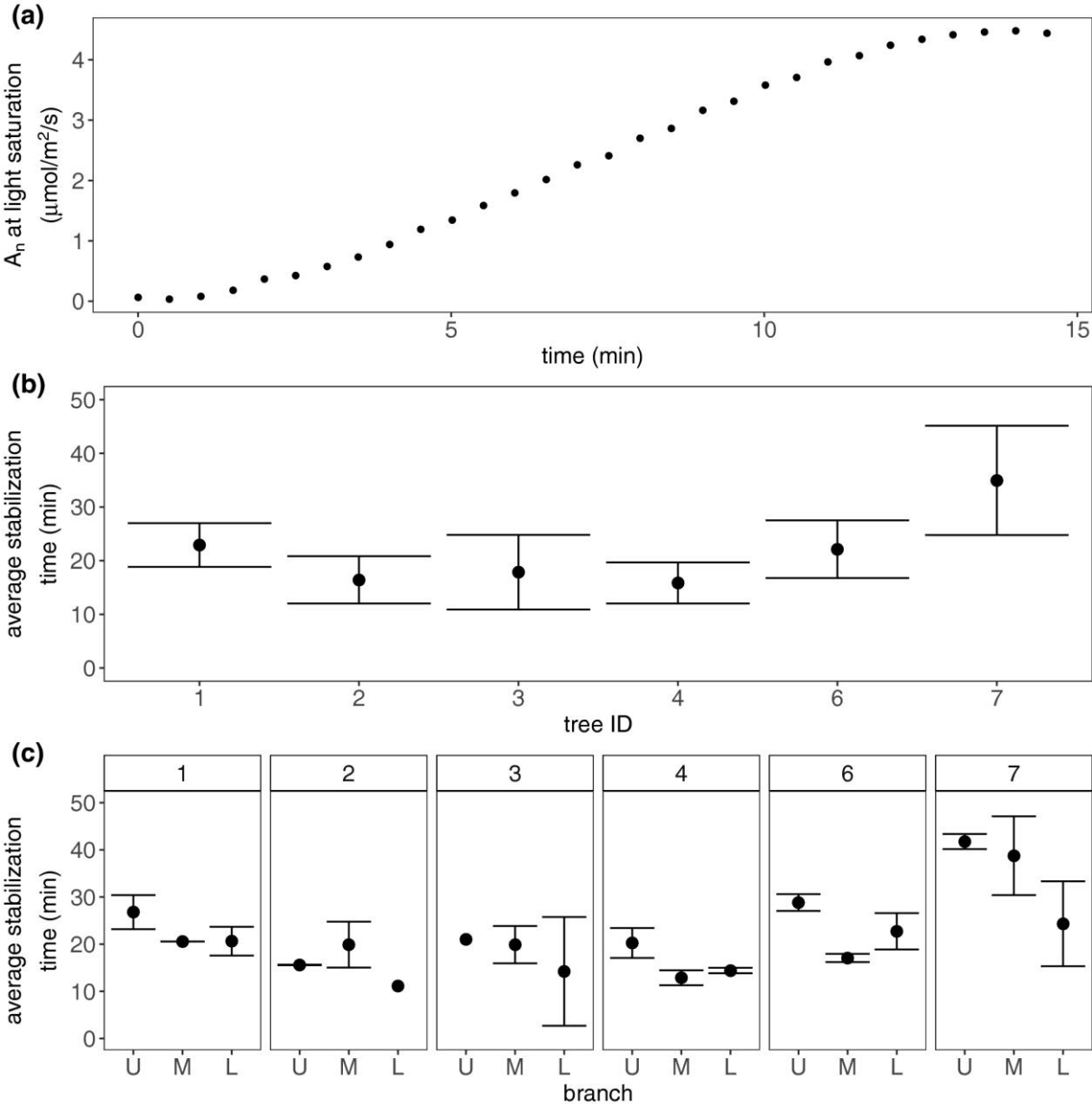


Figure 3



SUPPORTING INFORMATION

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

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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} ; $\mu\text{mol m}^{-2} \text{s}^{-1}$) 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; $\mu\text{mol CO}_2 \text{mol}^{-1} \text{H}_2\text{O}$) 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.

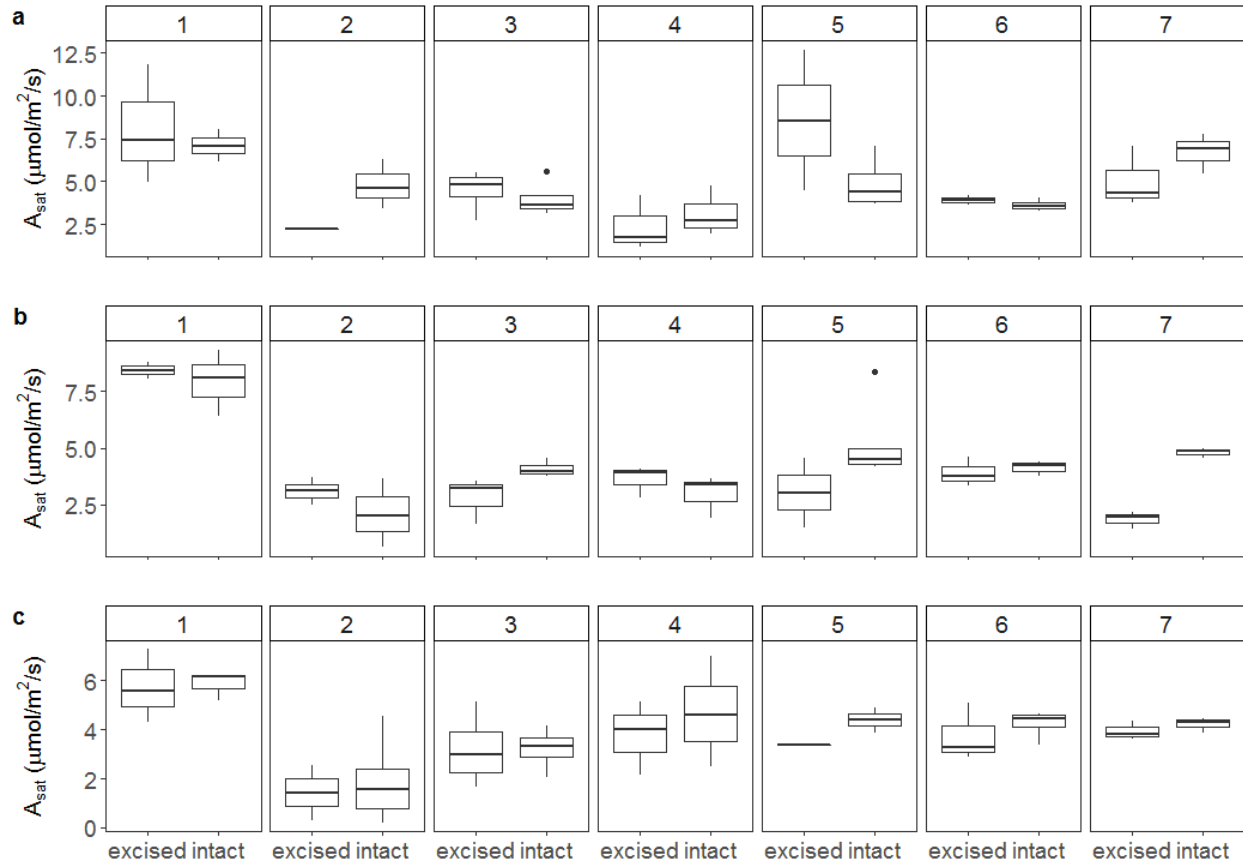


Fig. S1 Box and whisker plots of the replicates of leaf net photosynthetic rate at light saturation (A_{sat} ; $\mu\text{mol}/\text{m}^2/\text{s}$) compared between attached (intact) and excised (excised) branches for (A) upper sunlit foliage, (B) middle canopy foliage, and (C) lower canopy foliage. 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.

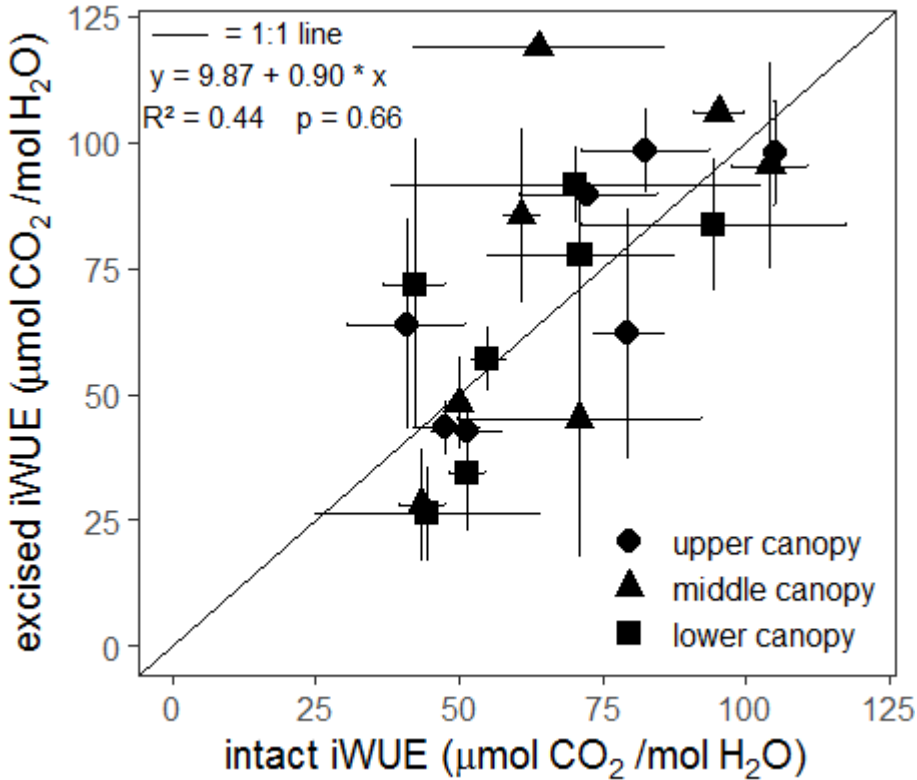


Fig. S2 Intrinsic water-use efficiency (iWUE; $\mu\text{mol CO}_2 / \text{mol H}_2\text{O}$) compared between non-excised (intact iWUE) and excised (excised iWUE) branches ($n = 21$). iWUE was calculated as leaf net photosynthetic rate at light saturation (A_{sat}) divided by stomatal conductance measured 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.

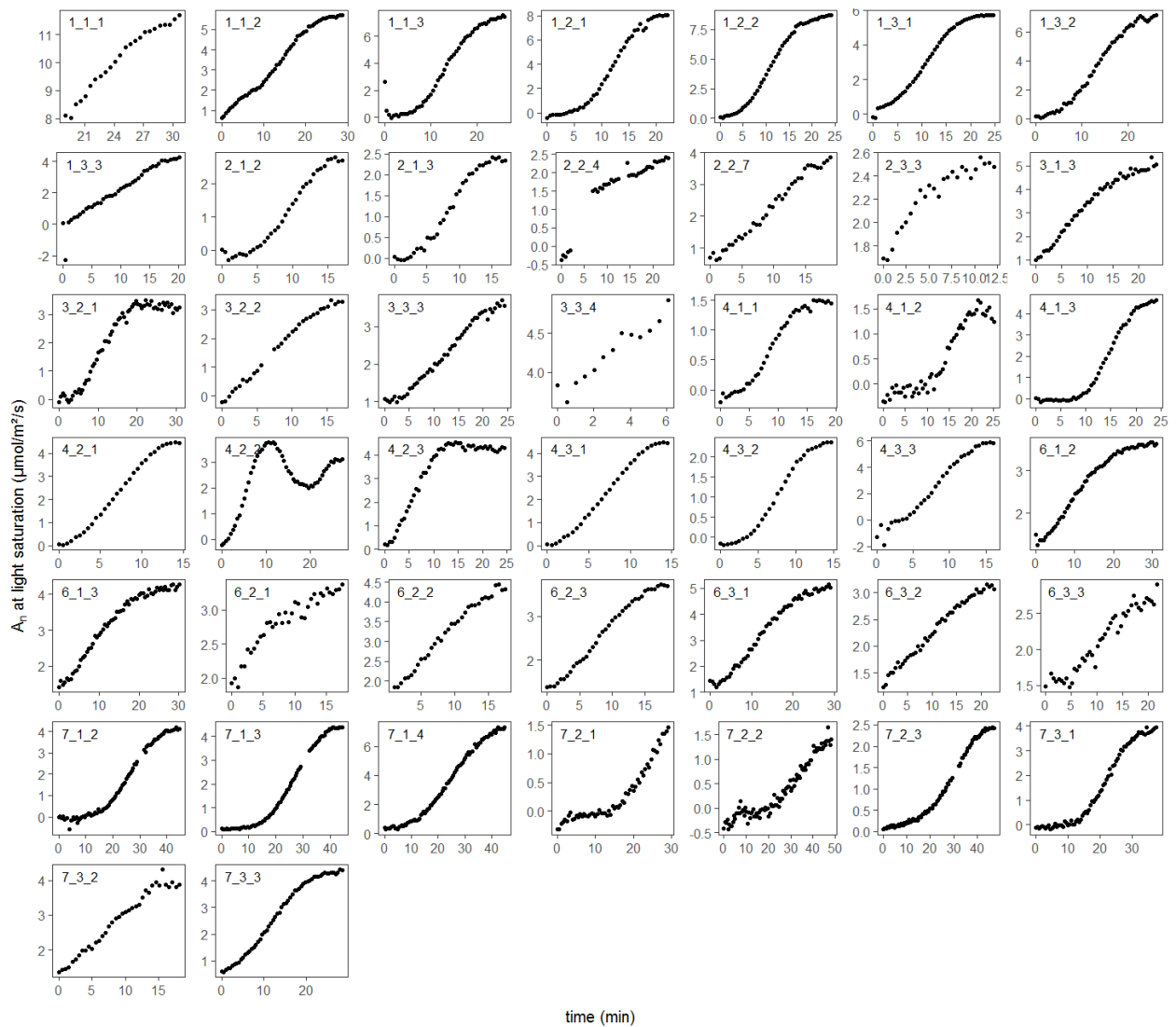


Fig. S3 Actual data of continuously measured leaf net photosynthetic rate (A_n ; $\mu\text{mol}/\text{m}^2/\text{s}$) at light saturation after excision and transport of the branch. Time 0 corresponds to the placement of the leaf in the gas exchange chamber. The ID number of each plot refers to the tree 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.

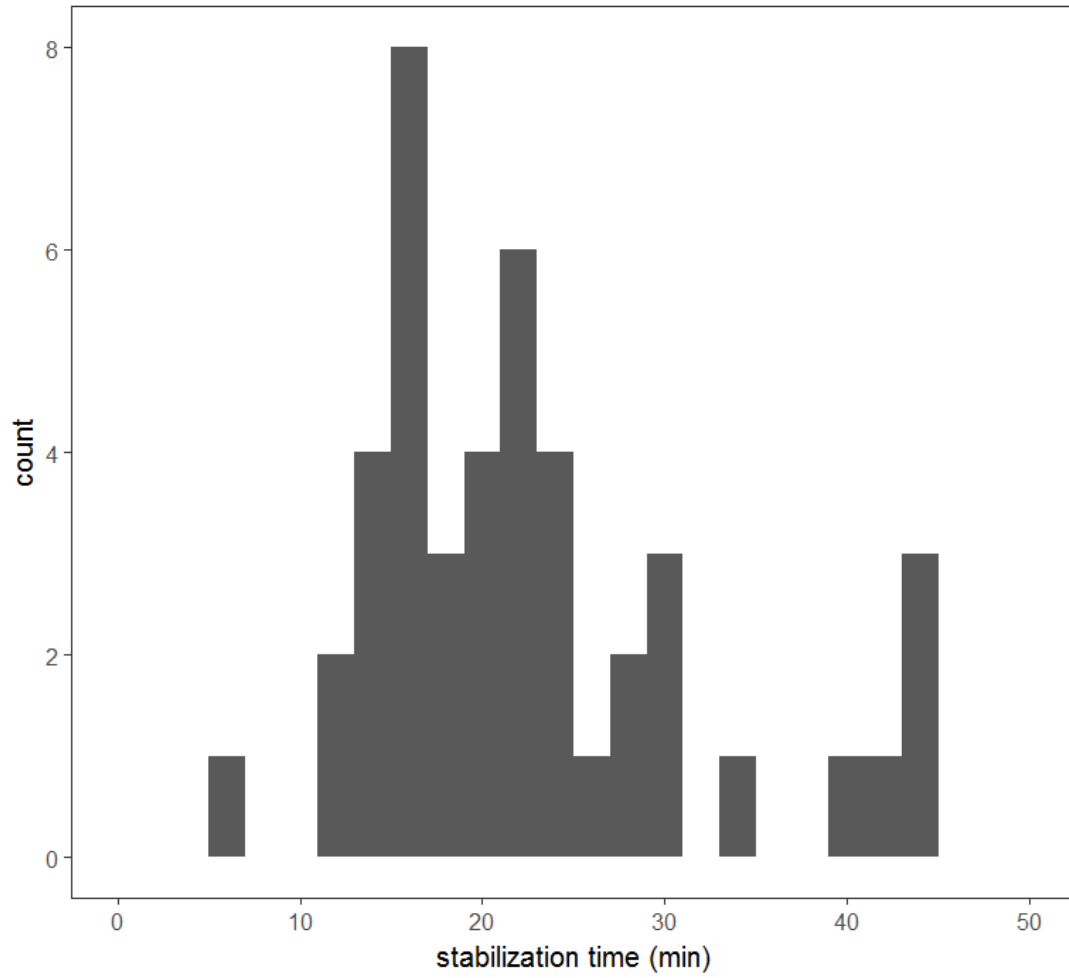


Fig. S4 Histogram of the stabilization times (min) for six trees. No data are available for tree 5, because we did not log A_n continuously.

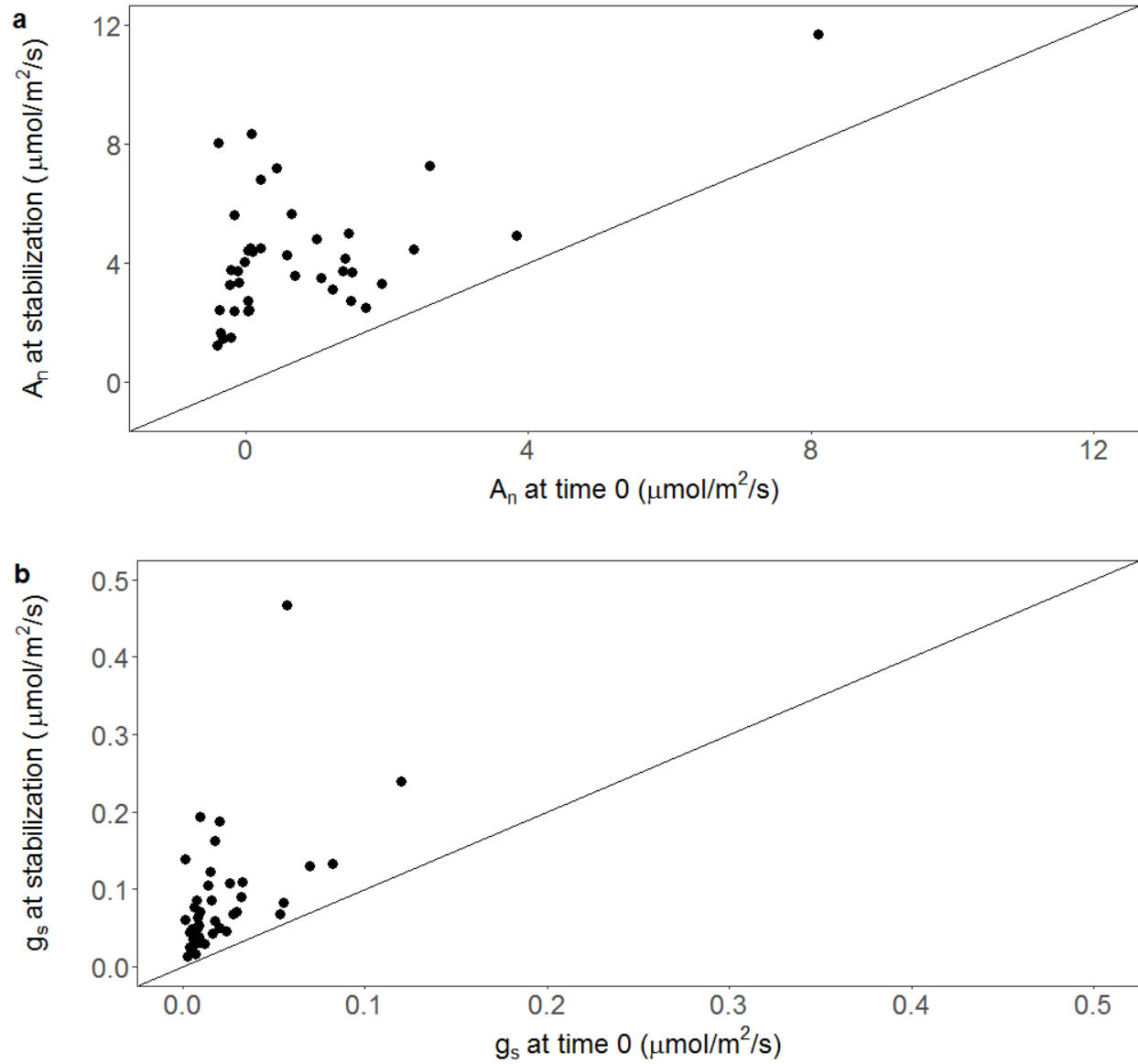


Fig. S5 (A) Leaf net photosynthetic rate (A_n) at light saturation and (B) stomatal conductance (g_s) measured at the start of the continuously logged leaf gas exchange measurements (time 0) and when A_n remained stable (at stabilisation, i.e. A_n did not vary by more than five percent for at least two minutes).