Verryckt, Lore T.; Van Langenhove, Leandro; Ciais, Philippe; [et al.]. «Coping with branch excision when measuring leaf net photosynthetic rates in a lowland tropical forest». Biotropica, vol. 52, issue 4 (July 2020), p. 608-615. DOI 10.1111/btp.12774

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

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]

[1] Department of Biology, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium; [2] Laboratoire des Sciences du Climat et de l'Environnement, CEA-CNRS-UVSQ, Gif-sur-Yvette, 91191, France; [3] Laboratoire Ecologie, évolution, interactions des systèmes amazoniens (LEEISA), Université de Guyane, CNRS, IFREMER, 97300 Cayenne, French Guiana; [4] CSIC, Global Ecology CREAF-CEAB-CSIC-UAB, Cerdanyola del Valles, 08193 Barcelona, Spain; [5] INRA, UMR Ecofog, AgroParisTech, CNRS, Cirad, Université des Antilles, Université de Guyane, 97310 Kourou, France; [6] Université de Guyane, UMR Ecofog, AgroParisTech, CNRS, Cirad, INRA, Université des Antilles, 97310 Kourou, France; [7] Hawkesbury Institute for the Environment, Western Sydney University, Locked Bag 1797, Penrith, NSW 2751, Australia; [8] Programa de Biología, Universidad del Rosario, Cr. 24 No. 63C-69, 111221 Bogotá, D.C., Colombia; [9] International Institute for Applied Systems Analysis (IIASA), Laxenburg, Austria; [10] UMR 5174 Laboratoire Evolution et Diversité Biologique, Université Paul Sabatier, CNRS, Toulouse, France

Author for correspondence:

L. T. Verryckt

Email: lore.verryckt@uantwerpen.be

Received:________________; Revised:________________Accepted:________________

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 meters 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 minimised 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 amongst 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 minutes –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

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 measurement, excised branches should be recut under water, to remove embolised xylem vessels and to restore hydraulic conductivity for maintaining an adequate supply of water to the leaves. Santiago and Mulkey (2003) and Rowland et al. (2015) studied upper-canopy, sunlit leaves. However, several leaf physiological traits of tropical trees, such as A_{sat} and g_s, change with height (Kenzo et al., 2015). The effect of excision might thus also vary with tree height. To the best of our knowledge, vertical differences in the excision effect have not yet been investigated. In this study, we therefore assessed whether the effect of excision varied with depth in the canopy.

Measurement can be conducted at a nearby campsite (Rowland et al., 2015), or in a laboratory further away (Ellsworth & Reich, 1993, Dang et al.,1997), implying different timings between excision and measurement and different environmental conditions. The excised branches are thus treated in different ways prior to leaf gas exchange measurements, even though the studies cited tried to minimise the time between excision and the actual measurement and to maintain the original light conditions for the excised branch.

We assessed the effects of branch excision and transport on leaf net photosynthetic rate at light saturation (A_{sat}) and the variation of these effects amongst branches at different heights in the canopy. We hypothesised that the effects of excision and transport would be smaller in the canopy, because these branches are acclimated to darker conditions than the branches at the top of the canopy. We assumed that they would be less stressed after excision and transport through a dark forest. We therefore tested for a faster recovery of A_{sat} for branches lower in the canopy.

2. METHODS

2.1 Study site

This study was conducted at the Nouragues Research Station (4°02’ N, 52°41’ W), an old-growth, tropical lowland forest site in French Guiana, South America. The climate is tropical humid, with a dry season lasting from August to November and a short dry period in March. Mean annual temperature is 26.3°C and mean annual rainfall is about 3000 mm (Bongers, Charles-Dominique, Forget & Théry, 2001).

2.2 Measurement of leaf gas exchange

We selected seven trees (Table 1) belonging to four dominant families at this field site: Fabaceae, Lecythidaceae, Malvaceae, and Burseraceae. A_{sat} (measured at 1300 µmol/m^2/s) and stomatal conductance (g_s; µmol/m^2/s) were measured on mature leaves using an infrared gas analyser (LI-6400XT, LI-COR, Lincoln, NE, USA). The measurements were carried out at three canopy positions, estimated relative to the top of the canopy: upper sunlit foliage, middle canopy foliage, and lower canopy foliage. We measured A_{sat} at each position for at least three leaves on an intact branch attached to the tree, with the assistance of a tree climber. This 2 m-long branch was subsequently excised, lowered gently from the canopy to a person who then immediately recut the branch under water to reconstitute the water supply to the leaves, and transported (within about ten minutes) to a nearby camp site in an opaque plastic bag to avoid further water loss due to transpiration, where at least two leaves were measured on the excised branch. The base of the branch always remained under water during transport. The branch was again recut under water at the camp site to prevent from potential disruption of hydraulic conductivity that may have arisen during transport. A_n at light saturation from these excised branches was continuously logged. A_n was then defined as A_{sat} when A_n remained stable, i.e. did not vary more than five percent for at least two minutes. Relative humidity was maintained as near ambient as possible (70-85%), and the rate of air flow was 500 mL/min during these measurements, which were carried out between 0930 h and 1500 h. The CO_2 level in the chamber was constant at 400 ppm, and the chamber temperature was controlled by maintaining the block temperature at 30 ± 0.2°C.

We tested an additional tree species at the camp site, *Eperua falcata*, with sunlit leaves reachable from the ground without climbing. We tested the effect on A_{sat} of transporting the excised branch in an opaque plastic bag. We continuously measured A_n at light saturation before excision and continued measuring it after excision without transporting the branch in a plastic bag. We also excised a branch, after measuring A_{sat}, and transported it similarly to the other excised branches and again continuously measured A_n at light saturation to compare both methods. These measurements were carried out between 1000 h and 1130 h.

2.3 Data analyses

The effect of excision on A_{sat} was tested with a paired t-test, comparing pre- and post-cutting measurements for each leaf. We also tested if the slope of the linear regression between A_{sat} for non-excised and excised branches differed from 1. This analysis was carried out for all data, but also for upper, middle and lower canopy foliage separately. We analysed our A_{sat} data using a two-way ANOVA, with excision (pre- or post-cutting), tree ID and canopy position (upper, middle, lower foliage) as fixed factors. We specifically explored the two-way interactions between these factors to assess the (interaction) effects of tree ID and canopy position on excision. We used $P < 0.05$ to identify significant differences. Additionally we performed a bootstrap analysis (10,000 replicates) for this two-way ANOVA for which we allowed resampling within the same canopy position (upper, middle, lower foliage). We assumed that the interaction effects would not be significant if the bias-corrected and accelerated (BCa) 95%

confidence intervals included zero. All analyses were performed in R 3.3.3 (R Core Team, 2017) using the ggplot2 package (Wickham, 2009) for visualising the data.

3. RESULTS

3.1 Effect of excision

In general, A_{sat} did not differ significantly between the excised and non-excised branches (paired t-test: $P = 0.10$), and the slope of the linear regression did not differ significantly from 1 ($P = 0.21$; Figure 1). When analysing the upper, middle and lower canopy foliage separately, the intercept and the slope of the linear regression did not differ significantly from 0 ($P_{upper} = 0.79$, $P_{middle} = 0.64$, $P_{lower} = 0.54$) and 1 ($P_{upper} = 0.79$, $P_{middle} = 0.86$, $P_{lower} = 0.19$), respectively. The two-way ANOVA showed no interaction between excision and tree ($P = 0.59$) or canopy position ($P = 0.55$). The interaction between tree ID and canopy position was significant ($P = 0.03$), with different responses of A_{sat} between tree IDs and canopy positions. Excision was not a significant factor in the two-way ANOVA ($P = 0.49$), indicating no differences in A_{sat} before and after excision. Individual differences between pre- and post-cutting measurements of A_{sat} are shown in Figure S1. The bootstrap analysis showed a tendency of an excision effect for tree 7, with BCa 95% confidence intervals approaching zero [0.144, 4.317], whereas it did not show any effect for the other tree species, nor of canopy position, since these BCa 95% confidence intervals all included zero. Intrinsic water-use efficiency (iWUE) did not differ significantly between the excised and non-excised branches, with the slope of the linear regression not differing significantly from 1 ($P = 0.66$; Figure S2).

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

Branch excision of the *E. falcata* tree in the camp had no effect on the continuously measured A_n at light saturation (Figure 2a). When the excised branch was first transported in a plastic bag and A_n at light saturation was measured 10 min after recutting the branch under water, A_n at light saturation was more than 50 percent lower than the original value (Figure 2b). A_n at light saturation increased again after about 30 minutes of stabilisation, and A_{sat} was similar to the measurement on the intact branch in the tree.

3.3 Stabilisation time

A_n at light saturation after branch excision and transport to the camp was near zero (<1 μmol/m^2/s) for 65 percent of the leaves and decreased by 30-80 percent for the remainder of the leaves. A_n increased over time after the leaf was placed in the leaf chamber and exposed to 1300 μmol/m^2/s photosynthetically active radiation (Figure 3a), and A_{sat} did not differ significantly from A_{sat} for the leaf measured on the intact branch in the tree (Figure 1). The time to stabilisation across all trees averaged 22 min, but ranged between 6 and 44 min (Figure 3b, Figure S3, Figure S4). The stabilisation time was longer for tree 7 than the other trees. The stabilisation time was longer for upper canopy foliage (27 ± 9 min) compared to middle (22 ± 10 min) and lower (19 ± 7 min) canopy foliage (Figure 3c).

4. DISCUSSION

We quantified the effects of branch excision on A_{sat} as differences between A_{sat} measured on an intact branch in the tree and A_{sat} measured on the same leaf after branch excision under water. A_n at light saturation decreased soon after the branch was excised and transported to the camp site (Figure 2b, Figure S5a). This immediate decrease in A_n at light saturation was caused by stomatal closure (Figure 2b, Figure S5b), but the stomata reopened on average 22 min after the branch was re-exposed to light (Figure 3b, c). A_n at light saturation, however, did not decrease in the excised *E. falcata* branch kept in the light (Figure 2a), suggesting that the stomatal closure was due to light limitation rather than dehydration caused by excision. This requires confirmation by testing more tree species, because this was an unreplicated experiment. We only tested one species that we kept at constant light while excising the branch.

The cut branches were transported from the canopy to the forest floor, where less than two percent of the incoming light at the top of the canopy is typically available (Yoda, 1974, Théry, 2001, Montgomery & Chazdon, 2002). We then cut the excised branch under water and transported it in an opaque bag to the camp site, where we measured leaf gas exchange. Light is a major determinant of stomatal conductance (Motzer, Munz, Küppers, Schmitt & Anhuf, 2005), so both the dark conditions at the forest floor and the opaque bag likely induced stomatal closure. Stomata respond quickly to changing light conditions (Roelfsema & Hedrich, 2005, Devireddy, Zandalinas, Gómez-Cadenas, Blumwald & Mittler, 2018), allowing the plant to regulate its water-use efficiency. Stomata of the leaves can thus be expected to close after excision due to light limitation to maintain their water balance and to reopen when the leaves are re-exposed to light in the leaf chamber. The recovery of stomatal conductance and A_n was faster for leaves lower in the canopy and thus typically exposed to less light (Figure 3c), supporting our hypothesis that branches lower in the canopy would recover faster after excision. The differences in recovery time along a height gradient in the canopy might also be explained by the differences in leaf water potential. Upper-canopy leaves typically have a lower water potential and the branches at the top of the canopy might thus experience more stress when excised compared to middle- and lower-canopy branches (Koch, Sillett, Jennings & Davis, 2004, Olson et al., 2018).

Although we did not measure the leaf water potentials, our data on stomatal conductance and intrinsic water use efficiency suggest that this did not play a major role in our study.

A_{sat} did not differ significantly before and after excision at the camp site after acclimating to the new light conditions in the leaf chamber. The immediate decrease in A_n at light saturation was restored after, on average, 22 min, which is in contrast to other studies where excision had no effect or a delayed decrease in A_{sat} was shown (Lange, Führer & Gebel, 1986, Meng & Arp, 1992, Ellsworth & Liu, 1994, Dang et al., 1997, Ambrose, Sillett & Dawson, 2009, Dusenge et al., 2015, Rowland et al., 2015). Measurements of A_{sat} on sugar maple leaves, immediately after excision and rehydration of the branch, were always within five percent of the rate measured on intact branches (Ellsworth & Liu, 1994). Excision did not affect A_{sat} in *Pinus banksiana* or *Picea mariana*, either immediately or during the next 14 h (Dang et al., 1997). Rowland et al. (2015) reported that excision did not affect photosynthesis in tropical trees. Our branches were allowed to stabilise in a canopy opening in full ambient irradiance for at least 30 min after recutting underwater prior to the measurements. Dusenge et al. (2015) found no difference in V_{cmax}, the maximum carboxylation rate, and only a small negative effect on J_{max}, the maximum electron transport rate, for tropical trees ten minutes before and after excision. A_n in twigs of *Picea abies* was constant for a minimum of 15 min after excision (Lange et al.,1986), and net photosynthesis of *Picea rubens* twigs did not vary significantly within about six minutes after detachment (Meng & Arp, 1992). A_{sat} did not decrease after excision in *Sequoia sempervirens* and *S. giganteum* in California, USA (Ambrose et al., 2009). The leaves for all these studies were exposed to full sunlight prior to measuring photosynthesis or were kept in the chamber under saturating light conditions, in contrast to our study.

Santiago and Mulkey (2003) reported that some species retained more than 85 percent of the photosynthetic rate for intact branches (i.e. pre-excision) 60 min after excision, whereas the rates for other species were less than 40 percent of the rates for intact branches within three minutes. The effect of excision can vary among species, and was shown to depend on the production of latex or resin since these exudates might clog the xylem, reduce sap flow, and thereby reduce foliar gas exchange (Santiago & Mulkey, 2003). Excision did not generally affect A_{sat} in all seven of our species, although A_{sat} decreased significantly after excision for sunlit upper-canopy foliage for tree 2 (*Tetragastris altissima*) and middle-canopy foliage for trees 3 (*Eschweilera coriacea*) and 7 (*E. falcata*) (Figure S1). A_{sat} may have decreased because the time needed for the leaves to acclimate to the settings of light and humidity at the camp was not long enough to reach A_{sat}. For tree 2 (*Tetragastris altissima*), belonging to the Burseraceae family that is known to produce latex or resin (Plowden, Uhl & De Assis Oliveira, 2004, Weeks, Daly & Simpson, 2005), the decrease in A_{sat} after excision for sunlit, upper-canopy leaves might have been influenced by the resin produced by this species, although we did not observe any resin production. No decrease in A_{sat} after excision was observed for middle and lower canopy leaves of this tree. A_{sat} measured in the camp on the excised branch was larger than A_{sat} measured on the intact branches for some trees (Figure S1), perhaps because xylem water potentials were higher in the rehydrated excised branches than the intact branches (Comstock & Ehleringer, 1984). Since there was no significant decrease in A_{sat}, we assume that there was no effect of excision on leaf photosynthesis, although there is large variation among individually sampled leaves. Additionally, given that we only sampled seven species, it is still recommended to test the effect of excision before measuring leaf gas exchange for other species than those studied here or in previous studies (e.g. Santiago & Mulkey, 2003, Ambrose et al., 2009, Dusenge et al., 2015, Rowland et al., 2015).

We conclude that branch excision does not affect the measurement of A_{sat} when the branch is recut under water after excision and the measurement is carried out for a sufficiently long time (at least 20 minutes) after the branch has acclimated to its new environmental conditions for reopening the stomata. Light is necessary to reopen the stomata when the branch is transported in dark conditions, either on the forest floor or inside an opaque bag. This process could be facilitated by transporting the excised branch in a bag containing LED lights, or the branch could be exposed to sunlight before measurement.

ACKNOWLEDGEMENTS

L. T. Verryckt is funded by a PhD fellowship from the Research Foundation Flanders (FWO). I. A. Janssens acknowledges support from the European Research Council Synergy Grant; ERC-2013-SyG-610028 IMBALANCE-P. We thank the staff of the Nouragues Natural Reserve and The Nouragues Ecological Research Station, supported by USR mixte LEEISA (CNRS; Cayenne, French Guiana). The research station has benefited from financial support by French Investissement d’Avenir programs managed by the ANR (AnaEE-France ANR-11-INBS-0001; Labex CEBA ANR-10-LABX-25-01).

We are grateful to Stefan van Beveren, Valentine Alt, Jean-Loup Touchard and Anthony Percevaux for climbing the trees to collect the selected branches. We thank the rest of the team for helping us with the field measurements.

CONFLICT OF INTEREST

The corresponding author confirms on behalf of all authors that there have been no involvements that might raise the question of bias in the work reported or in the conclusions, implications, or opinions stated.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Dryad Digital Repository at https://doi.org/10.5061/dryad.v9s4mw6rg.

LITERATURE CITED

AMBROSE, A. R., SILLETT, S. C., & DAWSON, T. E. (2009). Effects of tree height on branch hydraulics, leaf structure and gas exchange in California redwoods. *Plant, Cell & Environment, 32*(7), 743-757. doi:10.1111/j.1365-3040.2009.01950.x

BASSET, Y., HORLYCK, V., & WRIGHT, S. J. (2003). *Studying Forest Canopies from Above: The International Canopy Crane Network*. Smithsonian Tropical Research Institute and UNEP.

BONGERS, F., CHARLES-DOMINIQUE, P., FORGET, P.-M., & THERY, M. (2001). *Nouragues: Dynamics and Plant-Animal Interactions in a Neotropical Rainforest*. Kluwer Academic Publishers.

CARDOSO, D., SÄRKINEN, T., ALEXANDER, S., AMORIM, A. M., BITTRICH, V., CELIS, M., … FORZZA, R. C. (2017). Amazon plant diversity revealed by a taxonomically verified species list. *Proceedings of the National Academy of Sciences, 114*(40), 10695-10700. doi:10.1073/pnas.1706756114

CAVALERI, M. A., REED, S. C., SMITH, W. K., & WOOD, T. E. (2015). Urgent need for warming experiments in tropical forests. *Global Change Biology, 21*, 2111-2121. doi:10.1111/gcb.12860

COMSTOCK, J., & EHLERINGER, J. (1984). Photosynthetic responses to slowly decreasing leaf water potentials in *Encelia frutescens*. *Oecologia, 61*, 241-248. doi:10.1007/BF00396767

DANG, Q. L., MARGOLIS, H. A., COYEA, M. R., SY, M., & COLLATZ, G. J. (1997). Regulation of branch-level gas exchange of boreal trees: roles of shoot water potential and vapor pressure difference. *Tree Physiology, 17*(8-9), 521-535. doi:10.1093/treephys/17.8-9.521

DEVIREDDY, A. R., ZANDALINAS, S. I., GÓMEZ-CADENAS, A., BLUMWALD, E., & MITTLER, R. (2018). Coordinating the overall stomatal response of plants: Rapid leaf-to-leaf communication during light stress. *Science Signaling, 11*(518), eaam9514. doi:10.1126/scisignal.aam9514

DOMINGUES, T. F., MEIR, P., FELDPAUSCH, T. R., SAIZ, G., VEENENDAAL, E. M., SCHRODT, F., … LLOYD, J. (2010). Co-limitation of photosynthetic capacity by nitrogen and phosphorus in West Africa woodlands. *Plant, Cell & Environment, 33*, 959-980. doi:10.1111/j.1365-3040.2010.02119.x

DUSENGE, M. E., WALLING, G., GÅRDESTEN, J., NYRONZIMA, F., ADOLFSSON, L., NSABIMANA, D., & UDDLING, J. (2015). Photosynthetic capacity of tropical montane tree species in relation to leaf nutrients, successional strategy and growth temperature. *Oecologia, 117*, 1183-1194. doi:10.1007/s00442-015-3260-3

ELLSWORTH, D. S., & LIU, X. (1994). Photosynthesis and canopy nutrition of four sugar maple forests on acid soils in northern Vermont. *Canadian Journal of Forest Research, 24,* 2118-2127. doi:10.1139/x94-272

ELLSWORTH, D. S., & REICH, P. B. (1993). Canopy structure and vertical patterns of photosynthesis and related leaf traits in a deciduous forest. *Oecologia, 96*, 169-178. doi:10.1007/BF00317729

HEBERLING, J. M., KICHEY, T., DECOCQ, G., & FRIDLEY, J. D. (2016). Plant functional shifts in the invaded range: a test with reciprocal forest invaders of Europe and North America. *Functional Ecology, 30*(6), 875-884. doi:10.1111/1365-2435.12590

KENZO, T., INOUE, Y., YOSHIMURA, M., YAMASHITA, M., TANAKA-ODA, A., & ICHIE, T. (2015). Height-related changes in leaf photosynthetic traits in diverse Bornean tropical rain forest trees. *Oecologia, 177*, 191-202. doi:10.1007/s00442-014-3126-0

KOCH, G. W., SILLETT, S. C., JENNINGS, G. M., & DAVIS, S. D. (2004). The limits to tree height. *Nature, 428*, 851-854. doi:10.1038/nature02417

LANGE, O. L., FÜHRER, G., & GEBEL, J. (1986). Rapid field determination of photosynthetic capacity of cut spruce twigs (Picea abies) at saturating ambient CO2. *Trees, 1*, 70-77. doi:10.1007/bf00197027

MENG, F.-R., & ARP, P. A. (1992). Net photosynthesis and stomatal conductance of red spruce twigs before and after twig detachment. *Canadian Journal of Forest Research, 23*, 716-721. doi:10.1139/x93-093

MESSIER, J., MCGILL, B. J., & LECHOWICZ, M. J. (2010). How do traits vary across ecological scales? A case for trait-based ecology. *Ecology Letters, 13*(7), 838-848. doi:10.1111/j.1461-0248.2010.01476.x

MONTGOMERY, R., & CHAZDON, R. (2002). Light gradient partitioning by tropical tree seedlings in the absence of canopy gaps. *Oecologia, 131*(2), 165-174. doi:10.1007/s00442-002-0872-1

MOTZER, T., MUNZ, N., KÜPPERS, M., SCHMITT, D., & ANHUF, D. (2005). Stomatal conductance, transpiration and sap flow of tropical montane rain forest trees in the southern Ecuadorian Andes. *Tree Physiology, 25*, 1283-1293. doi:10.1093/treephys/25.10.1283

NAKAMURA, A., KITCHING, R. L., CAO, M., CREEDY, T. J., FAYLE, T. M., FREIBERG, M., … ASHTON, L. A. (2017). Forests and Their Canopies: Achievements and Horizons in

Canopy Science. *Trends in Ecology & Evolution, 32*(6), 438-451. doi:10.1016/j.tree.2017.02.020

OLSON, M. E., SORIANO, D., ROSELL, J. A., ANFODILLO, T., DONOGHUE, M. J., EDWARDS, E. J., … MÉNDEZ-ALONZO, R. (2018). Plant height and hydraulic vulnerability to drought and cold. *PNAS, 115*(29), 7551-7556. doi:10.1073/pnas.1721728115

PERRY, D. R. 1978. A method of access into the crowns of emergent and canopy trees. *Biotropica, 10*(2), 155-157. doi:10.2307/2388019

PLOWDEN, C., UHL, C., & DE ASSIS OLIVEIRA, F. (2004). Alipumilio (Diptera: Syrphidae) fly association with Burseraceae tree resins in the eastern Brazilian Amazon. *Journal of Natural History, 38*(15), 1941-1947. doi:10.1080/00222930310001618886

R CORE TEAM. 2017. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

REZENDE, L. F. C., ARENQUE, B. C., AIDAR, S. T., MOURA, M. S. B., VON RANDOW, C., TOURIGNY, E., … OMETTO, J. P. H. B. (2016). Evolution and challenges of dynamic global vegetation models for some aspects of plant physiology and elevated atmospheric CO2. *International Journal of Biometeorology, 60*(7), 945-955. doi:10.1007/s00484-015-1087-6

ROELFSEMA, M. R., & HEDRICH, R.. 2005. In the light of stomatal opening: new insights into 'the Watergate'. *New Phytologist, 167*, 665-691. doi:10.1111/j.1469-8137.2005.01460.x

ROWLAND, L., LOBO-DO-VALE, R. L., CHRISTOFFERSEN, B. O., MELEM, E. A., KRUIJT, B., VASCONCELOS, S. S., … MEIR, P. (2015). After more than a decade of soil moisture deficit, tropical rainforest trees maintain photosynthetic capacity, despite increased leaf respiration. *Global Change Biology, 21*(12), 4662-4672. doi:10.1111/gcb.13035

SANTIAGO, L. S., & MULKEY, S. S. (2003). A test of gas exchange measurements on excised canopy branches of ten tropical tree species. *Photosynthetica*, *41*(3), 343-347. doi:10.1023/B:PHOT.0000015457.92479.eb

SCHABERG, P. G., SHANE, J. B., CALI, P. F., DONNELLY, J. R., & STRIMBECK, G. R. (1998). Photosynthetic capacity of red spruce during winter. *Tree Physiology, 18,* 271-276.

SPERRY, J. S., ALDER, N. N., & EASTLACK, S. E. (1993). The Effect of Reduced Hydraulic Conductance on Stomatal Conductance and Xylem Cavitation. *Journal of Experimental Botany*, *44*(6), 1075-1082. doi:10.1093/jxb/44.6.1075

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

THÉRY, M. (2001) Forest light and its influence on habitat selection. *Plant Ecology, 153*(1-2), 251-261. doi:10.1023/A:1017592631542

VERRYCKT, L. T., VAN LANGENHOVE, L., CIAIS, P., COURTOIS, E. A., VICCA, S., PEÑUELAS, J., … JANSSENS, I. A. (2020). Data from: Coping with branch excision when measuring leaf net photosynthetic rates in a lowland tropical forest. Dryad Digital Repository. doi:10.5061/dryad.v9s4mw6rg

WEEKS, A., DALY, D. C., & SIMPSON, B. B. (2005). The phylogenetic history and biogeography of the frankincense and myrrh family (Burseraceae) based on nuclear and chloroplast sequence data. *Molecular Phylogenetics and Evolution, 35*, 85-101. doi:10.1016/j.ympev.2004.12.021

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

WILLIAMSON, V. G., & MILBURN, J. A. (1995). Cavitation events in cut stems kept in water: implications for cut flower senescence. *Scientia Horticulturae, 64*(4), 219-232. doi:10.1016/0304-4238(95)00842-X

WRIGHT, I. J., REICH, P. B., WESTOBY, M., ACKERLY, D. D., BARUCH, Z., BONGERS, F., … VILLAR, R. (2004). The worldwide leaf economics spectrum. *Nature, 428*(6985), 821-827. doi:10.1038/nature02403

YODA, K. (1974). Three-dimensional distribution of light intensity in a tropical rain forest of West Malaysia. *Japanese Journal of Ecology*, *24*(4), 247-254. doi:10.18960/seitai.24.4_247

TABLES

Table 1. Overview of the tree species selected for this study. The Plant List (2013) was used to validate the scientific names and to confirm authorship. The diameter at breast height (DBH) and total height are given for each tree. The heights of the upper, middle, and lower canopy foliage indicate the heights above the ground surface at which the 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

FIGURE 1 Leaf net photosynthetic rate at light saturation (A_{sat}; µmol/m^2/s) for non-excised (intact A_{sat}) and excised (excised A_{sat}) branches (n = 21). Each point represents an average of 2-3 leaves for each canopy position and tree, with standard errors on the x- and y-axes. The slope of the linear regression did not differ significantly from 1 ($P = 0.63$). The black line represents the 1:1 line and the grey shading shows the 95% confidence interval.

FIGURE 2 Leaf net photosynthetic rate (A_{n}; µmol/m^2/s) at light saturation and stomatal conductance (g_{s}; µmol/m^2/s) in *Eperua falcata* as functions of time of day, during an unreplicated experiment to test the effect of transporting the excised branch in an opaque plastic bag on A_{sat}. (A) A_{n} was continuously measured before and after excision of the branch. The solid black line represents the time of excision. (B) A_{n} was measured on the attached branch. The excised branch was transported in an opaque plastic bag, and A_{n} was again continuously measured after 10 min until A_{n} had stabilised and differed by less than five percent from the original values before excision. The dashed line represents A_{n} before excision.

FIGURE 3 (A) Actual data of a representative response of continuously measured leaf net photosynthetic rate (A_{n}) at light saturation after excision and transport of the branch. Time 0 corresponds to the placement of the leaf in the gas exchange chamber. (B) Average stabilisation time for the seven trees. The error bars are the standard errors of the mean. No data are available for tree 5, because we did not log A_{n} continuously. (C) Average stabilisation time for branches at different positions in the canopy (U, upper; M, middle; L, lower) for the seven trees. The error bars are the standard errors of the mean.