RESEARCH ARTICLE

Measuring Photosynthesis of Entire Tree Crowns and Pulse Label Trees in Large Closed-Chamber with $^{13}CO_2$ in the Field: Design and Testing

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Abstract

Measuring the photosynthesis of entire tree crowns and pulse labelling trees with $^{13}CO_2$ are valuable approaches to study carbon acquisition, transfer, and allocation; however, it is challenging for trees in the field. The objective was to develop and field test large chambers (35 - 45 m³) that enclosed the entire crown of a tree, provided a reliable estimate of tree crown photosynthesis, and ensured efficient $^{13}CO_2$ labelling. The chambers, made of transparent polyethylene film pulled tightly over a frame, were equipped with an air conditioner, fans, and air blowers. Air temperature, relative humidity, and photosynthetic photon flux density were measured outside and inside each chamber. Six of 4-year-old rubber trees (*Hevea brasiliensis*) were pulsed-labelled with 18 L of $^{13}CO_2$ in June and October 2016. The mean air temperature inside the chambers was 1.2 °C higher and the relative humidity 8 % lower than the outside air. The crown photosynthesis, calculated from the decrease in the CO_2 concentration inside the chamber, was in the range 140 - 249 µmol s^{-1} and was significantly related to photosynthetic photon flux density, total leaf area of the tree, and average net CO_2 assimilation at leaf level. The labelling efficiency, estimated as the ratio of the amount of ^{13}C recovered in the foliage immediately after labelling divided by the amount of ^{13}C delivered to the tree, was in the range 43 - 68 %. The designed chamber was suitable to estimate crown photosynthesis and perform $^{13}CO_2$ pulse labelling of 5-m-tall trees in the field.

Keywords: $^{13}CO_2$ labelling, *Hevea brasiliensis*, Carbon stable isotope, Rubber trees, Whole crown net CO_2 exchange rate

Introduction

Leaf gas exchange of plants determines their biomass production but also their carbon and water balance in interaction with climate and is crucial to assess and forecast the effects of climate changes on plant productivity and resilience [1,2]. Although photosynthesis is rather easy to measure at the leaf level, or at the whole plant level for small plants, this is more difficult for the whole crown of trees. Measurements of photosynthesis on single leaves must be extrapolated to the whole tree crown level because measuring all the leaves individually would be extremely difficult. Such extrapolations are based on individual-leaf gas exchange data up-scaled with more or less complex representations of the crown, from simple "big leaf" models to 3D architectural ones [3]. However, such models are seldom validated by actual measurements of whole tree photosynthesis, while crown photosynthesis is influenced by the crown

architecture (leaf angle and position, self-shading), the presence of several leaf cohorts, and neighborhood effects of adjacent crowns [3-5]. However, while gas exchanges between the forest canopy and the atmosphere can be measured at the ecosystem level using the eddy-covariance method [6,7], such fluxes include both the tree component and that of the understory and soil. In addition, the fluxes measured by eddy-covariance integrate large spatial areas of several tens of hectares, which makes it practically impossible to compare genotypes or either horticultural or silvicultural practices.

Once carbon has been assimilated by leaf photosynthesis, its distribution within the tree determines the maintenance and growth of different compartments, such as leaves, branches, roots, or fruits and their ability to store carbohydrates to ensure their functioning during periods when direct assimilation is not sufficient, for example during periods of defoliation in deciduous species [8,9]. Pulse labelling the foliage with $^{13}CO_2$ makes it possible to trace the fate and dynamics of the labelled assimilates into the whole plant, its different organs, and metabolites [10-12]. Because ^{13}C is not radioactive it poses no safety issues and can be easily used in field experiments.

Based on past experience [13-15], the main challenges for measuring crown photosynthesis and pulse-labelling trees are the size of the chamber that can enclose the whole tree crown and the climate control inside the chamber. One of the 1st attempts to measure whole tree photosynthesis was on young apple trees several decades ago [16]; subsequently, other systems were developed, but limited to young, small trees [17-20] or to short-term measurements [21-23] that excluded the possibility to perform $^{13}CO_2$ labelling. The size of the chamber is crucial for trees planted at low density, such as rubber tree or fruit trees, because they generally develop large crowns, requiring chambers of several tens of cubic meters. In addition, climate control is crucial in tropical conditions because the temperature inside a closed chamber rises rapidly without proper control. Thus, the current study aimed to develop and test a system including a large closed-chamber to measure photosynthesis of an entire tree crown and pulse label the tree with $^{13}CO_2$ in a rubber plantation in Eastern Thailand.

Materials and methods

Study site

The research was conducted in a rubber plantation (*Hevea brasiliensis,* clone RRIT 408) at the Chachoengsao Rubber Research Centre (CRRC), Rubber Authority of Thailand, Chachoengsao province (13°34′ N, 101°27′E, 69 m elevation) in eastern Thailand. In this area, the reported mean annual air temperature and cumulative rainfall are 27.1 °C and 1,247 mm, respectively, with a dry season from December to April [24]. The soil is a sandy-clay-loam soil belonging to the Kabin Buri series [25]. Trees were spaced 7 m in rows 2.5 m apart and were 4 years old. In total, 7 trees were selected for this experiment in June and October 2016 (**Table 1**).

Table 1 Growth characteristics of 6 trees pulse-labelled with $^{13}CO_2$ in June (#1–#3) and in October (#4 - #6) and non-labelled tree (#7).

Tree	#1	#2	#3	#4	#5	#6	#7
Girth at 1.8 m (cm)	19.5	21.0	20.1	22.5	23.5	21.0	19.0
Tree height (m)	6.0	6.0	5.7	5.1	5.3	6.3	5.5
Crown wide (m)	4.2	4.0	3.8	3.8	4.2	4.0	3.7
Total leaf area (m^2)	20.0	20.0	22.5	22.8	26.9	21.3	-
Total leaf biomass (kg)	2.5	2.3	2.5	3.0	2.8	2.6	-

Design of large chamber to measure tree crown CO$_2$ exchange rates and pulse-label trees with $^{13}CO_2$

The whole crown of the tree was enclosed in a chamber attached to scaffolds surrounding the tree (**Figures 1** and **2**) using a design adapted from previous studies on eucalypt and beech trees [14,15]. The chamber, made of 150 µm-thick transparent polyethylene film, consisted of 4 lateral walls (3.0 - 3.8 m in height, depending on the size of the crown) and a top that was heat-sealed to the lateral walls. The chamber's total volume was in the range 35 - 45 m^3. Immediately before the measurement, the crown was covered by the chamber, which was pulled tightly over the chamber support frame made from 1.3 cm diameter steel pipe. The pipes were cut to the desired lengths and connected to each other forming a cubic shape. The size of the chamber support frame was adjusted to fit the size of each crown. The floor of the chamber (12 m^2)

was made of 4 plywood sheets (8 mm thick) that were cut out in 1 corner to accommodate the trunk. The floor was supported by 1.3 cm diameter steel pipes arranged in a square shape and covered by a sheet of transparent polyethylene film which was sealed with the chamber walls using clips and duct tape.

Figure 1 Overview of the large closed-chamber designed to field measure photosynthesis of whole tree crowns and pulse label trees with $^{13}CO_2$, installed on a rubber tree.
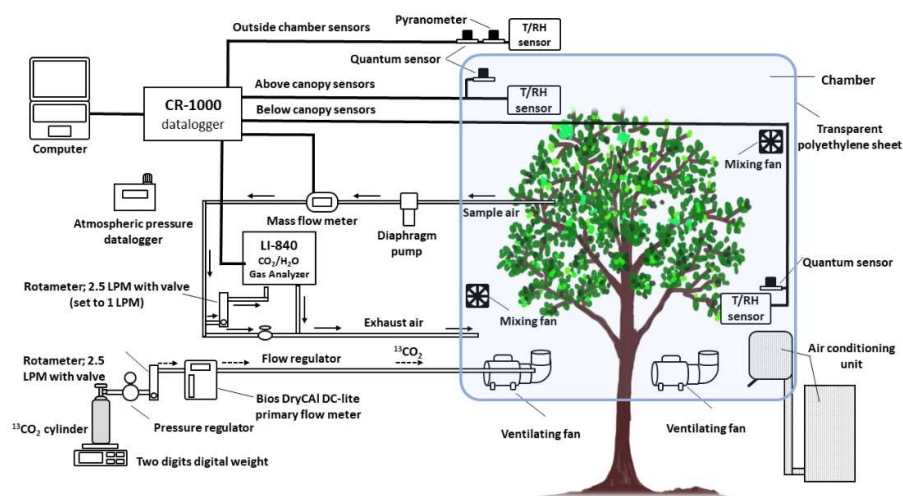

Figure 2 Schematic diagram of overview of large closed-chamber designed for field measurement of photosynthesis of whole tree crown and to pulse label tree with $^{13}CO_2$.

Chamber made of 150 μm-thick transparent polyethylene film and attached to scaffolds surrounding the tree excluded for clarity (see **Figure 1**). Air cooling ensured by air conditioning unit and air mixing using 2 axial fans and 2 air blowers inside chamber. Sensors measuring air temperature, humidity, and photosynthetic photon flux density installed inside and outside the chamber and connected to datalogger. CO_2 concentration inside the chamber measured using infrared gas analyzer. $^{13}CO_2$ from gas cylinder injected into labelling chamber just above air blower, using rotameter and primary air flow meter. A split-type air conditioner (TRANE; 36,000 BTU/h; WI, USA), powered by a 25 kVA single phase diesel generator, was used to limit the increase in air temperature inside the chamber compared to that of the outside air and to avoid water condensation. The outdoor unit was fixed to a frame located next to the chamber at the height of its floor and the indoor unit was placed inside the chamber. In addition, 2 axial fans and 2 air blowers were placed inside the chamber to ensure the air was well mixed inside the chamber. Air temperature and the relative humidity (RH) were measured with 1 probe outside the chamber and 2

probes inside the chamber (HMP 50, Vaisala, Helsinki, Finland). The PPFD was measured outside and inside the chamber (LI-190 R; LI-COR Industries; Lincoln, NE, USA). Microclimatic data were stored every minute using a data logger (CR 1000; Campbell Scientific Inc.; Logan, UT, USA). Moving the entire chamber, including the scaffolding frame and air conditioning unit, from tree to tree took a few hours, allowing measurement of 1 tree per day at least, with replications for statistical purposes.

Measurements of tree crown CO_2 exchange rates

Whole-canopy gas exchange measurements were performed on 6 rubber trees on June 25, 26 and 27 and on October 5, 6, and 7, early and late, respectively, in the rainy season in 2016. After closing the chamber, the decrease in CO_2 concentration inside the chamber due to the photosynthesis of the crown leaves was monitored for 20 min in June and for 10 - 15 min in October before starting $^{13}CO_2$ injection (see below). The CO_2 concentration was measured using an infrared gas analyzer (LI-840; LI-COR Industries; Lincoln, NE, USA) and the values were stored on the datalogger every minute. One additional rubber tree was measured on June 14 for 82 min until the concentration in the chamber decreased from ambient to below 100 µmol mol^{-1}. This tree was not labelled with $^{13}CO_2$.

The slope of the decrease in CO_2 concentration was used to calculate net crown CO_2 exchange rates (P_{crown}, in µmol s^{-1}) based on Eq. (1):

$$P_{crown} = \frac{\Delta[CO_2]}{\Delta t} \frac{V \times P_{atm}}{R \times (T_{air} + 273.15)} \tag{1}$$

where $\frac{\Delta[CO_2]}{\Delta t}$ is the slope of the linear variations in CO_2 concentrations over time, V is the system volume (chamber, tubes, and analyzer, with the latter 2 being negligible, in m^3), T_{air} is the air temperature (°C), R is the ideal gas constant (8.314 J K^{-1} mol^{-1}), and P_{atm} is the atmospheric pressure in Pa measured using an absolute barometric pressure sensor (BMP 280; Bosch Sensortec; Reutlingen, Germany).

Pulse labelling trees with $^{13}CO_2$

Pulse labelling was performed on 3 trees in June, 2016 and 3 trees in October, 2016 by injecting $^{13}CO_2$ in the chamber just after of the crown CO_2 exchange measurements had been completed. Almost-pure $^{13}CO_2$ (99.299 %; Cambridge Isotope Laboratory Inc.; Andover, MA, USA) was constantly injected into the labelling chamber using a primary air flow meter (DryCal DC-Lite; BIOS International Corporation; Butler, NJ, USA) at a rate adjusted to balance the estimated net rate of CO_2 consumption by crown photosynthesis and for 45 - 70 min to deliver approximately the same amount of $^{13}CO_2$ to each labelled tree (18 L of $^{13}CO_2$ per tree, equivalent to approximately 9.3 g of ^{13}C or 32 g of $^{13}CO_2$). The masses of $^{13}CO_2$ injected into the chamber were confirmed each time by weighing the gas cylinder just before and after labelling. The $^{13}CO_2$ was delivered close to an air blower to facilitate mixing. After the injection period, the chamber remained closed for an additional 15 min for the tree to assimilate part of the remaining $^{13}CO_2$ in the labelling chamber. Then, the chamber was opened and removed. Notably, the CO_2 concentration in the chamber could not be measured during the labelling because the gas analyzer was designed for measuring $^{12}CO_2$ and had low sensitivity to $^{13}CO_2$.

Leaf photosynthesis

Net CO_2 assimilation was measured using a portable gas exchange analyzer (Li6400XT; LI-COR Lincoln, NE, USA) with the photon flux density set at 1,400 µmol m^{-2} s^{-1}; the CO_2 concentration inside the chamber was 390 µmol mol^{-1} (SD = 5), the leaf-to-air vapor pressure difference was 2.0 kPa (SD = 0.4), and the leaf temperature was 32 °C (SD = 2). Net CO_2 assimilation was measured on 16 leaflets on each tree, selected at 4 intercardinal positions (north-east, north-west, south-west, and south-east), 2 heights (the upper and lower halves of the crown) and 2 leaf whorls (1st and 2nd whorls).

Leaf sampling and ^{13}C analyses

Leaves were collected from each tree before labelling (2 samples) and immediately after labelling (4 samples). Each sample consisted of 8 leaves (with each leaf comprising 3 large leaflets), collected from 8 different positions in the crown (in the lower and upper crown sections in the same 4 intercardinal positions as mentioned earlier).

The leaf samples were stored in a chilled box and transported to the laboratory where leaf area was measured using a leaf area meter (LI-3100C; LI-COR; USA). The samples were dried in a microwave oven at 800 W for 2 min, weighed and then ground into fine powder using a ball-grinder (MM 400; Retsch,

Haan, Germany). *The leaf mass per area (LMA) was calculated as the ratio of leaf dry mass to leaf area of the sampled leaves* to estimate total leaf biomass of tree crowns.

The ^{13}C analyses were performed by placing tin capsules containing 1 mg of dry leaf powder in an elemental analyzer coupled to a continuous flow isotope ratio mass spectrometer (vario ISOTOPE cube coupled to the IsoPrime 100; IsoPrime Ltd; Cheadle, UK).

The ^{13}C atom fraction, $x(^{13}C)$, was calculated from the carbon isotope composition ($\delta^{13}C$), expressed relative to the isotope ratio of the Vienna Pee Dee Belemnite standard ($R_{VPDB} = 0.0111802$). The excess ^{13}C atom fraction, $x^E(^{13}C)$, was calculated as the difference between $x(^{13}C)$ of leaves collected after and before labelling, multiplied by the carbon content of the leaves (C) and the leaf mass (M), using Eqs. (2) and (3):

$$x(^{13}C) = \frac{(\delta^{13}C+1) \times R_{VPDB}}{[(\delta^{13}C+1) \times R_{VPDB}]+1} \tag{2}$$

$$x^E(^{13}C) = \sum \left(x(^{13}C)_{lab} - x(^{13}C)_{unl} \right) \times C \times M \tag{3}$$

Crown leaf area and mass

The periodic pattern of rubber shoot development [26,27] gives the branches a sub-verticillate arrangement (whorl). In a given clone, the number of leaves per whorl is rather stable, resulting in a good correlation between the number of whorls counted by sight and the leaf area of a tree. Therefore, the total leaf area (A_L) was estimated using the equation proposed by Srisondee [28], which makes it possible to predict the leaf area with excellent accuracy. They reported a root mean squared error of 3.8 ($R^2 = 0.95$) when the leaf areas measured on 38 trees were compared to the predicted values using Eq. (4):

$$A_L = 0.01948 \times C_{170}^{1.298} \times F_{>3}^{0.5042} \tag{4}$$

where $F_{>3}$ is the number of leaf whorls with more than 3 leaves and C_{170} is the trunk girth at 1.7 m from the ground. Total leaf biomass was estimated from A_L using LMA of leaf samples collected for ^{13}C analysis.

Statistical analyses

All calculations and statistical analyses were performed using the statistical software R 4.1.0 software [29]. Linear models (based on the "lm" function in R) were used to estimate the initial slopes of the decrease in CO_2 concentration with time in the chamber. Significant differences in crown photosynthesis and excess ^{13}C in leaves between June and October were assessed using an analysis of variance (based on the "aov" function in R). A linear mixed-effects model with trees as the random effect was used to test for differences in the isotope composition of leaves between June and October (based on the "lmer" function in lme4 package in R). The effects of position in the crown and the orientation or whorl on leaf photosynthesis were assessed using linear mixed-effects models with trees as the random effect. Linear regression between crown photosynthesis and either photosynthetic photon flux density, total leaf area, or the average net CO_2 assimilation of leaves were calculated based on the "lm" function.

Results and discussion

Environmental condition in chamber

The light transmission in the photosynthetically active range of radiation (PAR) of the polyethylene film used for the chamber walls was, as expected for this type of material, over 90 % of incoming photosynthetic photon flux density (PPFD, **Figure 3**). In addition, polyethylene is known to have only little impact on the spectral composition of the PAR passing through the film [17]. The air temperature (T_{air}) in the chambers was on average 1.2 °C above the temperature measured outside the chamber, with a maximum observed positive deviation of 3 °C during the approximately 90 min the chambers were closed (**Figure 3**). This indicated that the power of the air-conditioning system was strong enough to maintain the ambient temperature in large, unshaded crown chambers in tropical conditions. However, the counterpart of this efficiency was a fairly substantial drop in RH, which also showed rapid variations of large amplitudes due to the intermittent operation of the air conditioner (**Figure 3**). If, on average, the decrease in RH was limited to 8 %, the maximum drop during a cooling cycle could temporarily exceed 30 %. The main limitation of this system is that although the temperature was properly controlled, the air RH showed large fluctuations.

The performance can be improved in the future by adding an ultrasonic mist generator controlled by a humidity probe.
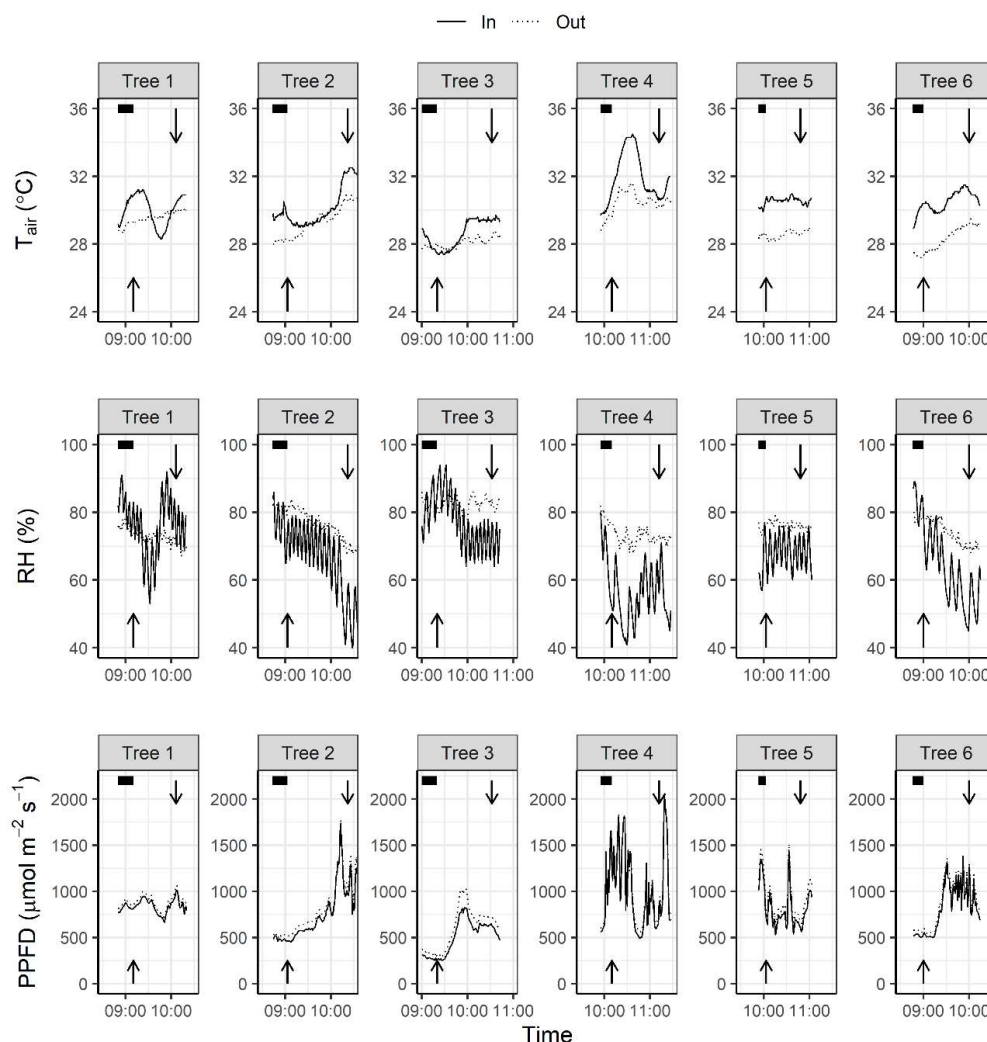

Figure 3 Air temperature (T_{air}), relative humidity (RH), and photosynthetic photon flux density (PPFD) recorded inside (solid lines) and outside (dotted lines) closed chamber. Trees #1 - #3 measured in June and trees #4 - #6 were measured in October. Black horizontal bar at top of each panel indicates when crown CO_2 exchange rate was calculated from decrease in CO_2 concentration in chamber. Two vertical arrows indicate beginning and end of $^{13}CO_2$ injection.

Response of crown photosynthesis to decreased CO₂ concentration

The decrease in CO_2 concentration for the tree that was not labelled with $^{13}CO_2$ was monitored for 82 min until the concentration in the chamber decreased from near ambient (385 µmol mol^{-1}) to below 100 µmol mol^{-1} (**Figure 4(A)**). During the measurement, T_{air}, RH and PPFD averaged 30 °C (SD = 1.3), 51 % (SD = 11) and 1,330 µmol m^{-2} s^{-1} (SD = 630), respectively. The decrease in CO_2 concentration in the chamber was not linear and was best predicted by a 3rd-degree polynomial function, with its 1st derivative being the slope ($\frac{\Delta[CO_2]}{\Delta t}$) which can be used in Eq. (1) to calculate crown photosynthesis. Crown photosynthesis decreased when the CO_2 concentration decreased in the chamber, as expected for a C3 plant (**Figure 4(B)**). The CO_2 compensation concentration when the net crown photosynthesis reached 0 (when gross photosynthesis and respiration were balanced) was 70 µmol mol^{-1}. This value was in the upper range

of typical values for leaves of C3 plants, which was expected because the crown also included branches that add additional CO_2 loss through their respiration [30].
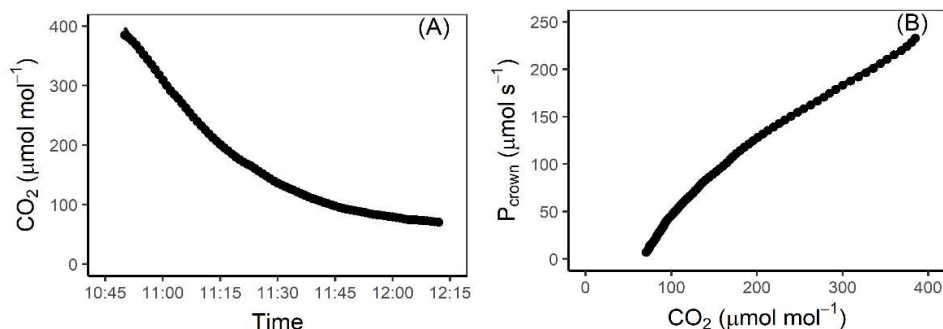

Figure 4 (A) Decrease in CO_2 concentration in chamber for 82 min after closure until concentration decreased from ambient to below 100 μmol mol^{-1}. (B) 1st derivative of 3rd-degree polynomial function fitted to decrease in CO_2 concentration and used to calculate crown photosynthesis (P_{crown}) based on Eq. (1). Measurement made on June 14 on tree (#7 in **Table 1**) not labelled with $^{13}CO_2$.

Variations of crown photosynthesis among trees at near ambient CO₂ concentration

The decrease in CO_2 concentration was monitored for 10 - 20 min on 6 trees that were labelled with $^{13}CO_2$ immediately after (**Figure 5**). The initial linear slope of the decrease of CO_2 concentration was used to calculate crown photosynthesis.

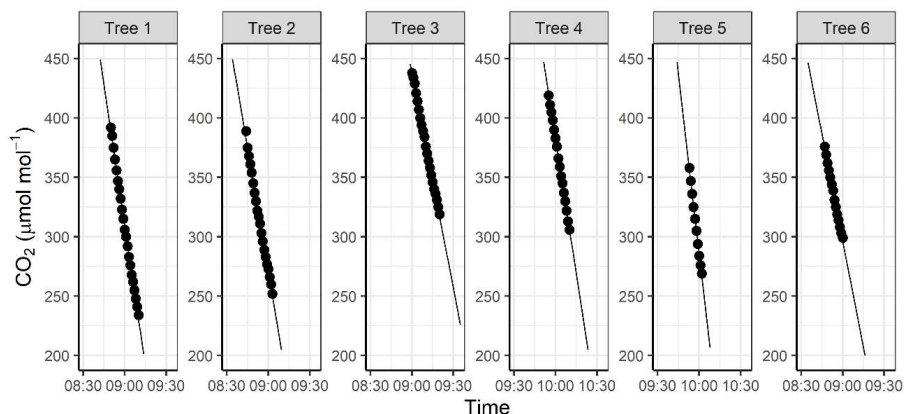**Figure 5** Decrease in CO_2 concentration in chamber for 10 - 20 min after closure and before injecting $^{13}CO_2$. Closed circles are measurements and solid lines are adjusted linear relationships between time and CO_2 concentrations. Trees #1 - #3 measured in June and trees #4 - #6 measured in October.

On average, crown photosynthesis was 183 μmol s^{-1} with a mean photon flux density during all measurements of 700 μmol m^{-2} s^{-1}. With a tree spacing of 7 m in rows 2.5 m apart, this was equivalent to 10.5 μmol m^{-2} s^{-1} on a ground area basis. Eddy flux tower estimates of gross primary production (GPP) at the same light intensity in a nearby 27-year-old rubber plantations was about 20 μmol m^{-2} s^{-1} from May to October [7]. Two reasons could explain this twice as high value. First, net crown photosynthesis is the balance between gross leaf photosynthesis and crown respiration (leaves and branches), while respiration is not included in the GPP estimate. Second, the trees in the 27-year-old plantation were much taller (more than 20 m) and had a completely closed canopy with a maximum leaf area index of 6.2 during the rainy season [7], whereas canopy cover was only about 70 % of the ground area in the current experiment. Crown photosynthesis varied between the 6 trees, but no significant differences were found between trees measured in June and those measured in October (P = 0.18). The variation of crown photosynthesis was well explained by the differences in the average photon flux density between the different dates of measurement, as reflected by significant positive relationships (R^2 = 0.84 and P = 0.01 with PPFD measured

outside the chamber, **Figure 6(A)**; $R^2 = 0.78$ and $P = 0.02$ with PPFD measured inside the chamber). Crown photosynthesis was not related to the RH inside the chamber ($P = 0.14$), but unexpectedly was related to RH measured outside the chamber ($R^2 = 0.76$ and $P = 0.03$), which could be explained by the strong negative correlation between photon flux density and relative humidity ($R = -0.88$, $P = 0.02$). In other words, a lower RH was recorded when conditions during the measurement were very sunny than when there was intermittent cloud cover. Although the effect of photon flux density was dominant, there was also a positive trend between crown photosynthesis and whole tree leaf area ($R^2 = 0.53$ and $P = 0.09$, **Figure 6(B)**).
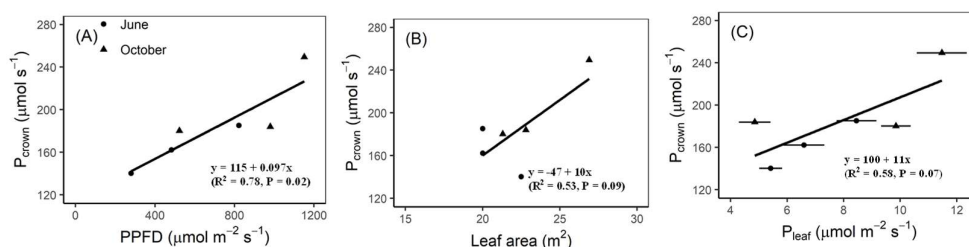

Figure 6 Relationship between crown photosynthesis (P_{crown}) and (A), photosynthetic photon flux density (PPFD), (B) total leaf area, and (C) average net CO_2 assimilation of 16 leaves for each tree (P_{leaf}; horizontal bars represent standard errors of mean). Circles show trees measured in June and triangles show trees measured in October. Linear regression lines are shown in each panel.

Variations of leaf photosynthesis between and within tree crowns, and relationship with crown photosynthesis

Crown photosynthesis was also positively related to mean leaf photosynthesis ($R^2 = 0.58$ and $P = 0.07$, **Figure 6(C)**). Mean leaf photosynthesis exhibited large differences between the 6 tree crowns as shown above (**Figure 6(C)**), in the range 4.9 - 11.4 μmol m^{-2} s^{-1}. These values were in agreement with measurements conducted on seedlings and mature rubber trees [31-35]. Within-crown variations were less pronounced than between-crown variations. Neither the position in the crown (upper or lower half of the crown), nor the orientation (north-east, north-west, south-west, or south-east), nor the whorl (1st or 2nd whorls) significantly influenced leaf photosynthesis ($p > 0.1$). This last result was in agreement with the lack of variation in the leaf nitrogen content per unit area with leaf age [33].

^{13}C recovered in leaves

The total amounts of ^{13}C recovered in tree leaves, $x^E\left(^{13}C\right)$, were in the range 4.0 - 6.3 g tree^{-1} (**Table 2**), representing 43 - 68 % of the $^{13}CO_2$ injected in the chamber. Not all the injected $^{13}CO_2$ was supposed to be recovered in the foliage, because it was diluted in the pre-existing $^{12}CO_2$ in the chamber (330 ppm on average) and not all the $^{13}CO_2$ was assimilated by leaves before the chamber was opened. As mentioned above, it was not possible to measure the $^{13}CO_2$ concentration in the chamber because of the low sensitivity of the gas analyzer to $^{13}CO_2$. However, based on the rates of injection of $^{13}CO_2$ and crown photosynthesis measured before injecting $^{13}CO_2$, the remaining mass of ^{13}C in the chamber at the opening time was estimated at 3.2 ± 0.3 g. Thus, the total amounts of ^{13}C recovered in tree leaves accounted for 89 % of the assimilated $^{13}CO_2$ on average after accounting for the mass of ^{13}C remaining in the chamber. The fact that 43 - 68 % of the injected $^{13}CO_2$ was recovered in the leaves after the end of labelling highlighted the effectiveness of the design of the chamber and the labelling protocol.

Table 2 Isotope composition (δ^{13}C) of leaves collected immediately after labelling (mean ± standard error for 4 composite leaf samples for each tree) and excess ^{13}C in whole foliage of trees, $x^E\left(^{13}C\right)$, for 3 trees labelled in June and 3 trees labelled in October. Numbers in bracket represent efficiency of labelling, calculated as ratio between $x^E\left(^{13}C\right)$ and the amount of ^{13}C injected in chamber. Differences between June and October tested using mixed-effect model with tree as random effect for δ^{13}C and linear model for $x^E\left(^{13}C\right)$.

Month	Tree	δ^{13}C (‰)	$x^E\left(^{13}C\right)$ (g tree^{-1})
June	#1	432 ± 23	6.2 (68 %)
	#2	377 ± 11	5.0 (54 %)
	#3	443 ±1 0	6.3 (69 %)
October	#4	237 ± 19	4.1 (44 %)
	#5	307 ± 11	4.7 (51 %)
	#6	272 ± 7	4.0 (43 %)
Month effect	F-values	25.7	10.3
	p-values	0.007	0.03

F-values are the ratio of the between-group to the within-group variances of the linear model and the *p*-values are the probability of obtaining these F-values if the difference between the 2 months are not significant.

The total amounts of ^{13}C recovered from the tree leaves, $x^E\left(^{13}C\right)$, were significantly higher in June than in October (5.8 ± 0.4 and 4.3 ± 0.2 g tree^{-1}, respectively, n = 6, P = 0.03), despite no significant differences in crown photosynthesis. The amounts of ^{13}C recovered in the tree leaves represented 63 % of the ^{13}C injected in the chamber in June on average, and 46 % in October. The reason for this difference remains unknown; however, seasonal differences in ^{13}C losses by either respiration or emission of volatile organic compounds cannot be excluded. Rubber trees are indeed strong emitters of isoprene and monoterpenes, especially in the wet season [36,37].

Conclusions

The large crown chamber designed in this study was successfully tested on 5 - 6 m tall rubber trees in the field, both to measure reliable crown photosynthesis and to achieve effective pulse-labelling of trees with ^{13}CO$_2$. This design is suitable for further studies on other tree species of interest in horticulture or silviculture, especially for addressing whole tree carbon balance and carbon allocation to specific sink organs or functions, such as fruit maturation, latex regeneration, and storage of sugars. In addition, crown photosynthesis datasets provide valuable information for testing and validating functional 3-D models of trees.

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