



## Methods paper

# A new automated stem CO<sub>2</sub> efflux chamber based on industrial ultra-low-cost sensors

Johannes Brändle<sup>1,2</sup> and Norbert Kunert<sup>1,2,3</sup>

<sup>1</sup>Conservation Ecology Center, Smithsonian Conservation Biology Institute, ForestGEO, 1500 Remount RD, Front Royal, 22630, VA, USA; <sup>2</sup>Center for Tropical Forest Science-Forest Global Earth Observatory, Smithsonian Tropical Research Institute, Apartado 0843-03092, Panamá, Republic of Panamá <sup>3</sup>Corresponding author (KunertN@si.edu, Braendle.J@gmx.de)

Received July 1, 2019; accepted October 2, 2019; handling Editor Sari Palmroth

**Tree autotrophic respiratory processes, especially stem respiration or stem CO<sub>2</sub> efflux ( $E_{\text{stem}}$ ), are important components of the forest carbon budget. Despite efforts to investigate the controlling processes of  $E_{\text{stem}}$  in recent years, a considerable lack in our knowledge remains on the abiotic and biotic drivers affecting  $E_{\text{stem}}$  dynamics. It has been strongly advocated that long-term measurements would shed light onto those processes. The expensive scientific instruments needed to measure gas exchange have prevented  $E_{\text{stem}}$  measurements from being applied on a larger temporal and spatial scale. Here, we present an automated closed dynamic chamber system based on inexpensive and industrially broadly applied CO<sub>2</sub> sensors, reducing the costs for the sensing system to a minimum. The CO<sub>2</sub> sensor was cross-calibrated with a commonly used gas exchange system in the laboratory and in the field, and we found very good accordance of these sensors. We tested the system under harsh tropical climatic conditions, characterized by heavy tropical rainfall events, extreme humidity and temperatures, in a moist lowland forest in Malaysia. We recorded  $E_{\text{stem}}$  of three *Dyera costulata* (Miq.) trees with our prototype over various days. The variation of  $E_{\text{stem}}$  was large among the three tree individuals and varied by 7.5-fold. However, clear diurnal changes in  $E_{\text{stem}}$  were present in all three tree individuals. One tree showed high diurnal variation in  $E_{\text{stem}}$ , and the relationship between  $E_{\text{stem}}$  and temperature was characterized by a strong hysteresis. The large variations found within one single tree species highlight the importance of continuous measurement to quantify ecosystem carbon fluxes.**

**Keywords:** Arduino, autotrophic respiration, ecosystem respiration, high temporal resolution data, long-term measurements, microcontroller.

## Introduction

In forest ecosystems, a great fraction of the assimilated carbon by trees is respired back to the atmosphere through autotrophic respiration. Autotrophic respiration releases roughly 50–70% of the photosynthetic products back to the atmosphere (e.g., DeLucia et al. 2007). Studies in the tropics have shown that this biome is at the lower end of the range of carbon use efficiency (CUE) and that up to 70% of the gross primary productions ends up in autotrophic respiration (Chambers et al. 2004, Fernández-Martínez et al. 2014). Woody components release ~20% of the total autotrophic respiration (Chambers et al.

2004, Malhi 2012). However, correct scaling of CO<sub>2</sub> fluxes from different tree organs to ecosystem level or even to individual tree level remains challenging—mainly due to insufficient and nonrepresentative measurement techniques (Katayama et al. 2014). Most measurements in ecological studies, especially in the tropics, follow the assumption that stem CO<sub>2</sub> efflux ( $E_{\text{stem}}$ ,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) follows some general rules, e.g., that  $E_{\text{stem}}$  depends largely on differences in temperature. In general, the temperature inside a tropical forest stand is very constant, and thus  $E_{\text{stem}}$  is assumed to not vary a great deal throughout the day (Chambers et al. 2004, Katayama et al.

2016). Accordingly, most studies reduce their sampling effort and only measure  $E_{\text{stem}}$  once a month over a couple of minutes and scale those measurements to annual estimates (Chambers et al. 2004, Robertson et al. 2010).

Recent studies on tropical trees showed a large diurnal variation of  $E_{\text{stem}}$  (Kunert and Mercado Cardenas 2012, Kunert and Edinger 2015) similar to diurnal patterns in temperate and boreal trees (e.g., Teskey and McGuire 2002, Gansert and Burgdorf 2005, Aubrey and Teskey 2009). In contrast, Rowland et al. (2018) found only limited diurnal changes in  $E_{\text{stem}}$  in tropical tree species. Further, vertical measurements on a tree in the Amazon revealed that there can be large differences in  $E_{\text{stem}}$  even within a tree individual (Kunert 2018). Long-term measurements on different tree organs (coarse roots, lower and upper trunk, branches) of Norway spruce and European beech trees confirmed such large differences in  $E_{\text{stem}}$  of different organs (Kuptz et al. 2011). Another study states that measurements along tree height revealed only negligible vertical variation in  $E_{\text{stem}}$ ; however, the study highlights that this can differ among species and sizes (Katayama et al. 2019).

Several studies on temperate trees have tried to explain the large diurnal variation of  $E_{\text{stem}}$  and attribute those diurnal changes partly to internal transport processes of dissolved  $\text{CO}_2$  and/or re-fixation of  $\text{CO}_2$  (Teskey and McGuire 2002; Wittmann et al. 2005, Bloemen et al. 2013a, 2013b, 2013c). Hence, the actual meaning of  $E_{\text{stem}}$  is not clear, and the source of the  $\text{CO}_2$  emitting out of the stem is not yet resolved (for review see Teskey et al. 2008, Trumbore et al. 2013). Accordingly,  $E_{\text{stem}}$  is very hard to predict, and values for ecosystem-level estimates of autotrophic stem respiration derived from short-term measurements need to be regarded with a certain skepticism (Kunert and Mercado Cardenas 2012). These studies recommend continuous measurement of  $E_{\text{stem}}$  over longer periods. Continuous measurement assures that deviations from the commonly assumed temperature–respiration relationship can be seen in the measurements (e.g., Kunert and Mercado Cardenas 2012). This means that all trees in a study need to be equipped with a  $\text{CO}_2$  sensing system at the same time that automatically record  $E_{\text{stem}}$  values for a longer period. Continuous measurements in most existing studies were conducted on only a few trees, most likely due to the very expensive equipment needed (e.g., see also Etzold et al. 2013, Salomón et al. 2016). Ryan et al. (1995) and Lavigne et al. (1996) used manifold controlled automated  $E_{\text{stem}}$  measurements significantly reducing the costs by using one  $\text{CO}_2$  gas analyzer for various trees, but this approach has its spatial limitations. For example, in diverse tropical forests, species replicates are scattered over large areas; hence, independent and very energy-efficient systems are needed. An optimal experimental setup would be to equip trees with respiration chambers at different heights at the same time, as demonstrated by Ceschia

et al. (2002) and Tarvainen et al. (2014, 2018). Such a proposed experimental setup would increase the costs multiple times, but an affordable and reliable system would allow a significant increase in the number of long-term data collected in many ecosystems.

Summarizing, long-term studies on  $E_{\text{stem}}$  are still rare, especially on tropical trees. It seems plausible that one of the problems in measuring  $E_{\text{stem}}$  on a larger scale is the high price of  $\text{CO}_2$  sensing systems.  $\text{CO}_2$  gas analyzers from well-established providers cost usually a couple of thousands of US dollars (Harmon et al. 2015). In addition, a lot of maintenance and manpower are needed when measuring  $\text{CO}_2$  fluxes in a reasonable temporal and spatial resolution. However, there have been large advances in the development of low-cost, small data-logging devices and industrially used sensor techniques. Those components make it possible to build affordable setups to measure almost any kind of ecological and plant physiological parameter at high resolution and with the necessary replicates. For example, low-cost  $\text{CO}_2$  sensors have been applied successfully to measure soil  $\text{CO}_2$  flux in a tropical forest in Costa Rica (Harmon et al. 2015). Where the earlier study invested about 600 US dollars per respiration chamber, we developed an ultra-low-cost system, for less than 70 US dollars (excluding the power source), able to measure  $E_{\text{stem}}$  for more than 3 days without any maintenance needed and delivering data of similar quality to conventional approaches of automated chamber systems.

Here, we want to present an affordable system that can be adapted to measure constantly any kind of  $\text{CO}_2$  efflux in high temporal resolution. We constructed a closed dynamic chamber system (hereafter CDC) consisting of an industrial ultra-low-cost  $\text{CO}_2$  gas sensor and a microcontroller for data recording. We validated the sensors in the laboratory and in the field with a high precision  $\text{CO}_2$  sensing system, and tested the functionality on three tropical trees in Malaysia. We present our first results of continuous fully automated measurements of  $E_{\text{stem}}$ . The data provided show a large diurnal variation of  $E_{\text{stem}}$  and indicate a significant deviation even among different individuals of the same species, highlighting the importance of applying long-term measurements in the field.

## Materials and methods

### Description of the chamber

We designed a CDC to measure stem surface  $\text{CO}_2$  effluxes associated with stem respiration and  $\text{CO}_2$  diffusion out of the tree stem. A summary of how to measure  $E_{\text{stem}}$  can be found in Chambers et al. (2004) or Marthews et al. (2012). We used an industrial  $\text{CO}_2$  sensor (Intelligent Infrared Carbon Dioxide Module, MH-Z14A; Winsen Electronics Technology Co., Ltd, Zhengzhou China) and a microcontroller (Arduino Pro Mini, Arduino, Ivrea, Italy) with an external data-logger-shield

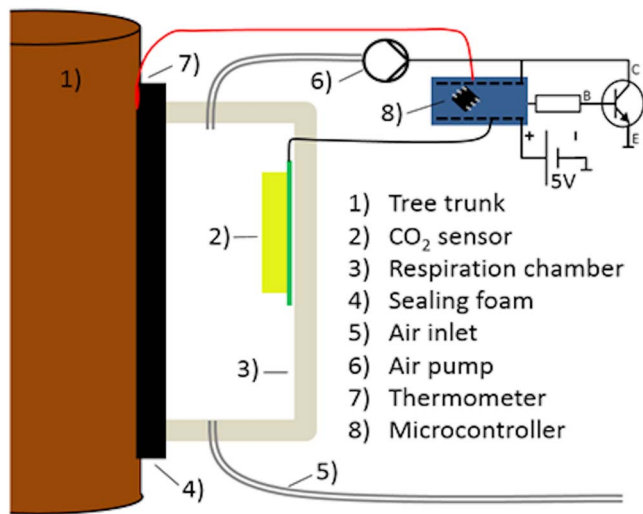


Figure 1. Schematic diagram for ultra-low-cost sensing systems based on an Intelligent Infrared Carbon Dioxide Module. The microcontroller is equipped with a real-time clock and SD card logging shield to record the data with a timestamp (data-logging-shield not indicated in the diagram).

equipped with a secure digital flash memory (SD) card slot and real-time clock (Data-Logger-Shield ID8122, Deek-Robot<sup>®</sup>, Shenzhen, China) for data recording. The CO<sub>2</sub> sensor had a measuring range of 400 ~ 5000 ppm (the accuracy is  $\pm 50$  ppm + 3% of reading value according to the manufacturer) and was glued directly into a plastic chamber (inner dimension: 175 × 40 × 32 mm; thickness: 10 mm) of white, opaque polyethylene that served as the respiration chamber (Figure 1). We chose a chamber volume of 195 ml (excluding the sensor volume) because different studies on tropical trees used similar sized chambers between 125 and 250 ml without an internal fan as used, e.g., in large soil efflux chambers (e.g., Chambers et al. 2004, Kunert 2018, Muhr et al. 2018). The respiration chamber was cinched on the tree stems with tie-down straps (Figure 2) at breast height (1.3 m) and sealed on to the bark with flexible closed porous cellular foam (EPDM-Quality, Reiff Technische Produkte GMBH, Reutlingen, Germany). The whole system was operated with a commercial 5 V power bank (Xiaomi Mi 2, 20,000 mAh, Xiaomi Corporation, Hong Kong, China). The power bank could be replaced within seconds when necessary due to a USB interface cable for power. We divided the half-hourly measuring cycle in a 15-min 'respiration' period and a 15-min 'flushing' period. In the first 15 min, we measured the increase of CO<sub>2</sub> over time in the respiration chamber (1-s record interval). After the respiration period, the chamber was flushed with ambient air for 15 min with a small air pump (70 ml/min, Pollin Electronics, Pförring, Germany). The air pump sucked out the CO<sub>2</sub> enriched air from the chamber and took up ambient air through the air inlet (Figure 1). We chose a relatively long measuring cycle because the sensor has a delay time of 120 s (see datasheet of manufacturer) and to ensure that the small



Figure 2. Photograph of the CDC system installed on an *Acer pseudo-platanus* tree during validation campaign with an Li-8100 system. On top is the power bank, in the middle the plastic box with the electronic components (microcontroller and pump) and below the chamber with the sensor inside.

pump was able to flush the entire chamber. For pump control, we used a transistor (Diotec Transistor (BJT) BC337-25 TO-92 1 NPN, Conrad Electronic SE, Hirschau, Germany) emitter circuit in which the base pin was connected to the microcontroller. The air inlet consisted of a 60 cm long tubing with an inner diameter of 2 mm (aquarium tubing, TOOGOO<sup>®</sup>, Shenzhen City, China). Due to the small diameter and the length of the tubing, we assumed that there was no air exchange with the ambient air during the respiration period and when the pump was off. Similar open-ended tubing is used commonly on lids of soil respiration chambers to compensate for the pressure during lid installation. However, we tested if there was any exchange of air during the measurement cycle by breathing around the open end of the air inlet. We could not detect any increase of CO<sub>2</sub> inside the chamber due to this test. We installed a thermistor (3950 NTC, Thermistor, Adafruit, New York, NY, USA) between the bark and the foam to record bark surface temperature (Figure 1, see also below in text). Rough cost estimation of

Table 1. Cost estimation of components used for the CDC system

Components	Price (in US \$)	Brand/description
CO <sub>2</sub> sensor	<30.00	Winsen/MH-Z14A NDIR CO <sub>2</sub> sensor
Microcontroller	<2.00	TZT/Pro Mini Module Atmega328
Air pump	<5.00	JQB031 (70 ml/min)
Tubing	<3.00	TOOGOO®
Transistor	<1.00	Diotec/BC337-25
Data-logger-shield	<5.00	Deek-Robot®/Logging shield
Voltage converter for air pump	<5.00	MissBirdler/I2C ICC 5 V-3.3 V
Resistors	<1.00	—
Thermocouple	<5.00	Adafruit/10 K Precision Epoxy Thermistor-3,950 NTC
Wiring and breadboard	<10.00	—
5 V USB powerbank	<34.00	Xiaomi Mi 2 Powerbank (20,000 mAh)
Total	<101.00	

the (electrical) components used in the CDC system is given in Table 1. With some solder skills, a student can construct a CDC system within 8 h.

#### Calculation of $E_{stem}$

For analysis, we extracted ~300 s of measurements of the increase in CO<sub>2</sub> from each respiration cycle and fitted a linear regression. CO<sub>2</sub> concentration against time was checked for linearity for each measurement. Linear regression coefficient of determination ( $r^2$ ) was > 0.9 for each measurement. The slope of the linear regression was used as a function of time to determine  $E_{stem}$ , which was corrected for atmospheric pressure and temperature by using Eq. (1) as follows:

$$E_{stem} = \left( \frac{PV}{RTA} \right) \frac{dC}{dt}$$

where P is the standard barometric pressure (Pa), V the volume of the chamber (195 ml, accounting for space of sensor installation), R the universal gas constant, T the bark surface temperature of a tree (K), A the projected area (70 cm<sup>2</sup>) on tree surface of the chamber, and  $dC/dt$  the increase of CO<sub>2</sub> inside the chamber in time. We protected our chamber with a plastic foil and a reflective foil to prevent any thermal influence of direct sunlight. To allow good ventilation, the foil was sealed to the tree stem only above the chambers and was left open at the bottom to allow good ventilation of the tree stem. We checked our chambers for leaks prior to and after the experiment by exposing the sealings of the chamber to extreme CO<sub>2</sub> concentrations and simultaneously monitored the output of our devices for a very high increase in CO<sub>2</sub>. When no increase was observed, we assumed there were no leakages in our systems.

#### Sensor calibration and field verification

We verified our CO<sub>2</sub> sensor by comparing measurements of respiratory activity with a portable gas exchange fluorescence system (GFS-3000, Heinz Walz GmbH, Effeltrich, Germany). Therefore, we placed the MH-Z14A sensor of our CDC in an

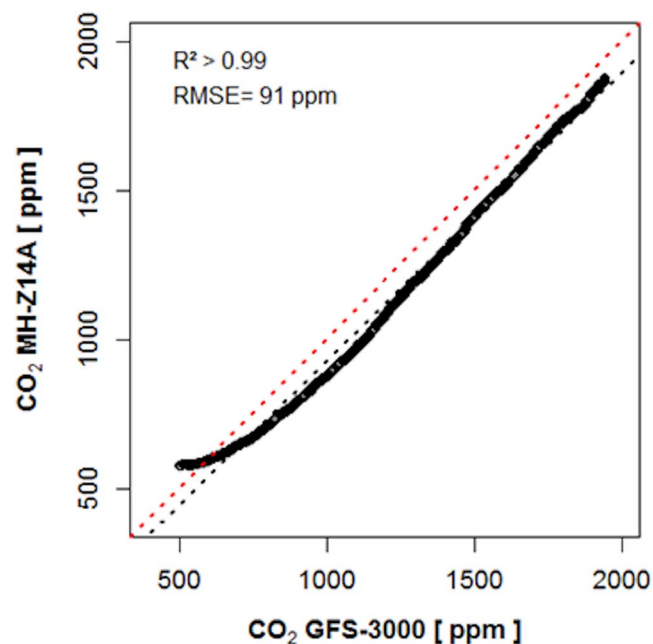


Figure 3. Comparisons of the MH-Z14A sensor with the GFS-3000. The dashed light grey line is the 1:1 line, the dashed dark grey line is the linear regression between the output of the two sensors.

air-tight sealed cuvette which was connected to the GFS-3000 system (Figure 3). We used GFS-3000 to increase CO<sub>2</sub> levels inside the cuvette from 500 to 2000 ppm (~ 1 ppm sec<sup>-1</sup>) over several minutes. Both devices recorded the constantly increasing CO<sub>2</sub> concentration every 1 s.

In a second test, we validated our CDC system in the field by comparing measurements with a commercial system. As a commercial system, we used a portable infrared gas analyzer (Li-8 100; Li-cor Inc., Lincoln, NE, USA) which is often used for stem respiration measurements (e.g., Etzold 2013). Therefore, two identical respiration chambers (same chambers were used as described in the Description of the chamber section) were installed on an emergent *Acer pseudoplatanus* (L.) (Sapindaceae) tree. The chambers were attached directly

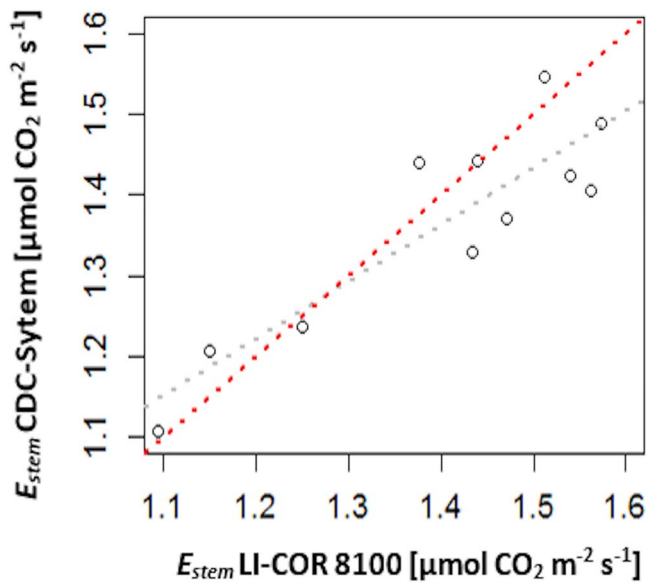


Figure 4. Comparisons of the CDC system with the Li-8100. The dashed darker line is the 1:1 line, the gray dashed line is the linear regression between the output of the two sensors (regression:  $y = 0.71x + 0.37$ ,  $r^2 = 0.77$ ,  $P < 0.001$ ).

above each other in a horizontal position. We connected the inlet and outlet of one chamber with inlet and outlet ports of the Li-8100, operating in closed mode. The other chamber was used for our CDC system. The test was carried out on 3 July in the Black forest area (950 m above sea level) by continuously measuring stem respiration. We performed 11 measurements with both the CDC and the Li-8100 system. Therefore, the chambers were manually flushed with ambient air, using the integrated pumps of the two systems. All measurements were done between 10:00 and 21:00 h. After the chambers were flushed with ambient air, the increase in CO<sub>2</sub> concentration was measured for at least  $\sim 10$  min. For the Li-8100 system, the increase of CO<sub>2</sub> was calculated using a linear fit over  $\sim 300$  gas concentration measurements, starting directly after a 'respiration' cycle was initiated. For the CDC system, the CO<sub>2</sub> release was calculated using a linear fit over  $\sim 300$  gas concentration measurements, integrating a time offset due to the response time of the sensor (see description of the sensor above).  $E_{\text{stem}}$  was calculated for both systems as described in the section before.

### Field test

In a third experiment, we tested the CDC under harsh tropical field conditions. The field test was performed at three trees at the forest edge around the Pasoh field station in Malaysia (2°58'08.0"N, 102°17'49.0"E). We tested our CDC on three individuals of the common species *Dyera costulata* (Miq.) (Apocynaceae) in different measuring campaigns on 20, 23, and 27 October 2018. We had only one prototype of the CDC system, thus we could not test the system on different trees

at the same time. The three *Dyera costulata* trees (DYERCO1, DYERCO2, and DYERCO3) were of similar size (diameter at breast height 32, 28.5 and 29.5 cm, respectively) but differed in stand structure. DYERCO1 was located in an open space directly on the grassland of the research station with several meters distance (at least  $>10$  m in each direction) from the forest edge, whereas DYERCO2 and DYERCO3 were directly connected to the forest edge. DYERCO2 was surrounded by forest from two sides (south and west), whereas DYERCO3 on one side only (west). The distance between DYERCO2 and DYERCO3 was small ( $\sim 10$  m), whereas DYERCO1 was several meters ( $\sim 50$  m) away. Each individual was measured once for at least 24 h. The prototype of the CDC was always cinched on the north side of a tree stem. Installation of the system usually took a few minutes when carried out by two people. We let the system run until the power bank was exhausted (usually after 3 days using a commercial power bank with a capacity of 20,000 mAh). As described above, the setup was protected with a plastic foil and a reflective foil to prevent it from direct rain and to reduce any thermal influence of direct sunlight. The foil was sealed to the tree stem only above the CDC and was left open at the bottom to allow good ventilation of the tree stem. We checked for leaks prior and after each measurement period by closing the air pump inlet and forcing an under pressure at the air inlet. When pressure stayed stable, we assumed that the chamber was air-tight. We calculated  $E_{\text{stem}}$  as described before.

### Results

Sensor validation test in the laboratory showed that the response of MH-Z14A sensor had a strong correlation with the output of the GFS-3000 (root mean square error (RMSE) = 91 ppm). After a delay, the output of MH-Z14A sensor matched that from the GFS-3000 precisely, except for an offset of  $\sim 40$  ppm (Figure 3). It is worth mentioning that the MH-Z14A was not calibrated before the validation test, although such a function does exist. As we were interested in the relative change of CO<sub>2</sub> over time and not the absolute change, we decided not to calibrate for the offset.

The sensor validation test in the field on an *Acer pseudoplatanus* tree showed that our CDC had a good correlation with the output of the Li-8100 (Figure 4). Overall, the temporal patterns of the two devices were similar. All measurements of both devices showed a linear slope with a correlation coefficient  $>0.98$ . The mean CDC system efflux rate was 3% lower than that from the Li-8100 (mean  $\pm$  standard deviation,  $1.36 \pm 0.13 \mu\text{mol m}^{-2} \text{s}^{-1}$  and  $1.40 \pm 0.16 \mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively). The diurnal pattern for efflux from the measured tree was similar for both systems, meaning that if the Li-8100 measured higher efflux rates in the afternoon, then the CDC also did the same. In one measuring cycle, the CDC system efflux rates were notably lower than those of the Li-8100. Specifically,

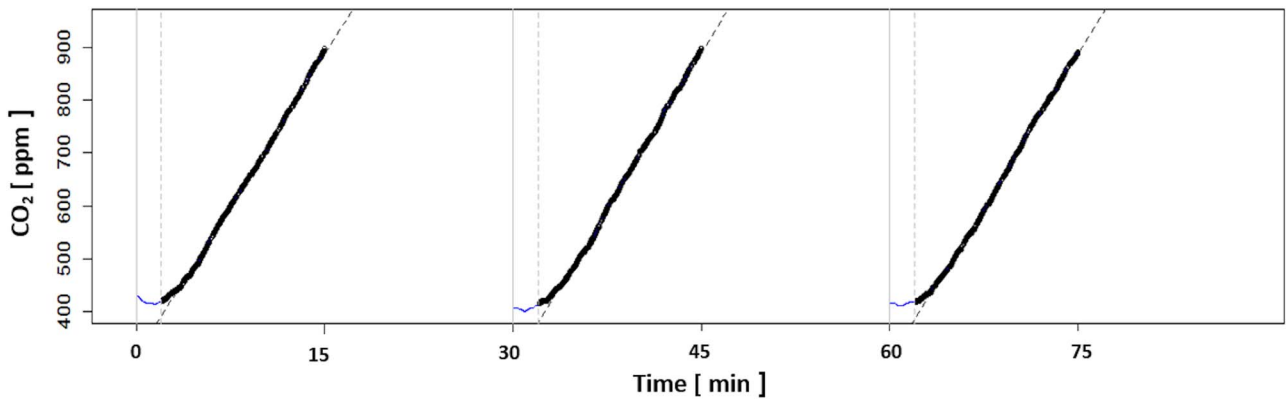


Figure 5. Example of three measure periods on a *Dyera costulata* tree (DYERCO2) on 23 October from 15:00 to 16:30 h. Vertical gray solid lines indicate starting points of measurement cycles. Black solid line indicate duration of sensors response time. Vertical gray dash lines indicate the end of sensors response time. Black points representing raw data output of CDC over the 15-minute measure cycles. Black dash lines representing regression lines for each measurement period. All regression analyses showed  $r^2 > 0.9$ .

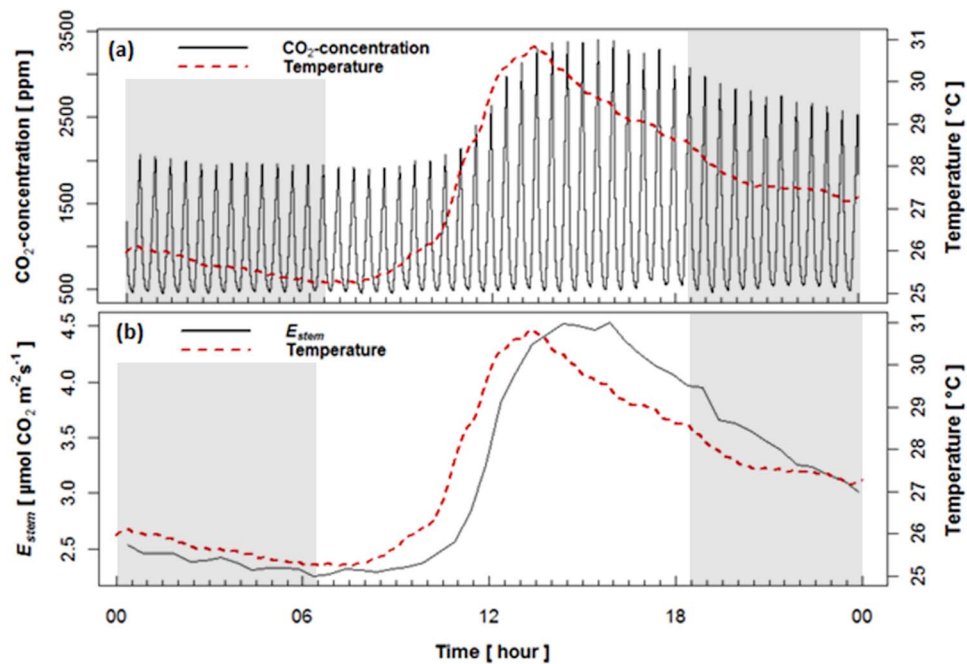


Figure 6. Diurnal measurements in the field application on a *Dyera costulata* tree (DYERCO3) in the Pasoh Forest Reserve in Malaysia. The raw data output of the CDC (a); raw data translated into  $E_{\text{stem}}$  and the bark surface temperature (b). Night times are indicated by shaded areas.

the estimated CDC system efflux rates in this measuring cycle were about 11% lower than the respective Li-8100 rates, whereas in the other measure cycles, sensor outputs matched with each other precisely (<10% deviation).

Our setup worked well under difficult field conditions, including exposure to high humidity and high temperatures, and heavy rainfall events in the tropics. CO<sub>2</sub> concentration against time was checked for linearity for each measurement cycle, and  $r^2$  for each cycle was > 0.9 (an example is given in Figure 5). The CO<sub>2</sub> concentration in the chamber increased rapidly from about 500 ppm to a maximum during the 15-min 'respiration' period

and constantly decreased to about 500 ppm during the 15-min 'flushing' period (Figure 6a). There were clear variations in  $E_{\text{stem}}$  between the measured trees (Figure 7a). DYERCO3 showed the highest variation in  $E_{\text{stem}}$ , ranging from 2.3 to 4.5  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , throughout the day.  $E_{\text{stem}}$  of DYERCO3 was relatively stable in the morning, peaked in the early afternoon and decreased continuously until nighttime. This change in  $E_{\text{stem}}$  coincided, although delayed, with the increase of bark temperature (compare Figure 6b). Accordingly,  $E_{\text{stem}}$  of this tree did not scale linearly with temperature and was characterized by a distinct hysteresis. Bark temperature differed slightly between the

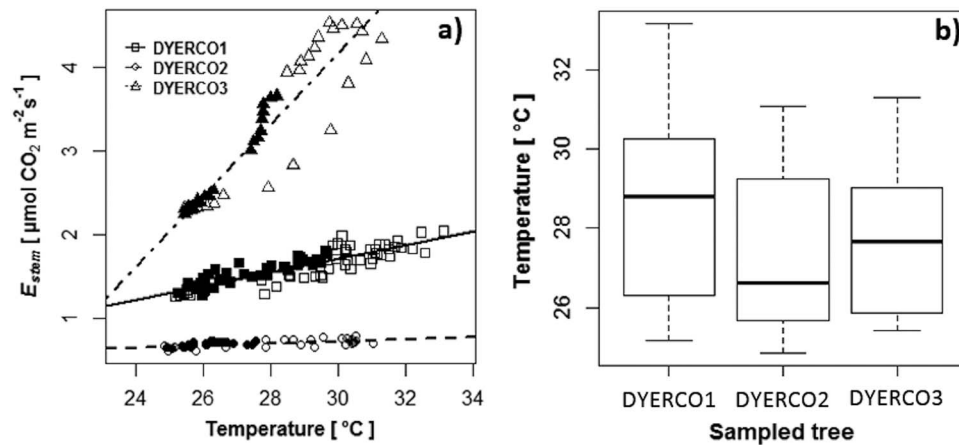


Figure 7. (a) Linear relationships between  $E_{stem}$  and temperature during diurnal measurements of the three *Dyera costulata* trees. (regression DYERCO1:  $y = 0.082x - 0.734$ ,  $r^2 = 0.78$ ,  $P > 0.01$  (solid line); regression DYERCO2:  $y = 0.012x + 0.357$ ,  $r^2 = 0.38$ ,  $P > 0.01$  (dashed line); regression DYERCO3:  $y = 0.427x - 8.621$ ,  $r^2 = 0.86$ ,  $P > 0.01$  (dotdash line)). Black symbols represent measurements at nighttime (19:00 to 07:00 h). (b) Comparison of measured bark temperature of the three *D. costulata* trees during measurement campaign of 24 h.

three measurement campaigns (Figure 7b). DYERCO1 showed the highest variation in bark temperature, with the highest temperatures throughout measurement campaigns, whereas on DYERCO2 (lowest variation) and on DYERCO3, diel changes in bark temperature were less evident.

## Discussion

We present a very cheap and precise system to measure  $E_{stem}$  with a high temporal resolution. The system was run without any technical issues in the field over the entire study period of approximately 2 weeks in the tropics. During this time, it was exposed to extreme humidity and temperature, and worked stably during days with heavy tropical rainfall events. Our design can easily be modified to measure a wide range of different ecosystem CO<sub>2</sub> fluxes, e.g., a modified design could be used to measure soil respiration or even nighttime respiration of leaves. Furthermore, our system is based on a programmable microcontroller. This means that other electrical components (e.g., dendrometer, sap flow sensor, or WIFI-Module for remote communication) can be easily integrated. In addition, our device is light-weight (<1 kg), easy to install, power-efficient, and can be powered with any commercial power bank (with disabled automatic shut off) or other 5 V power sources for at least 3 full days (estimated with a capacity of 20 Ah at 3.6 V). Therefore, our device can be applied at remote locations that are difficult to access or where no constant electrical power source is available. Further, its low weight makes it easy to transport compared with other gas analyzers, which are usually heavy and in addition require heavy batteries. Last, the unproblematic installation allows installation of the device in higher parts of the tree stem or in the tree canopy. However, we are aware that our system could have limitations, e.g., our design seems not

to be useful for very short measurements (e.g., measurements < 5 min).

Our first results from the sensor validation in the laboratory and in the field suggest that the sensors deliver reliable data and could be an alternative for the application in environmental science. We are convinced that our system is a valuable and urgently needed tool to continuously measure  $E_{stem}$  on a larger spatial and temporal scale. This could help to tackle the long-standing question of how  $E_{stem}$  varies within tree communities and within the same tree individual (Asao et al. 2015, Kunert 2018, Katayama et al. 2019). Further, this approach can be used to quantify autotrophic respiration with a higher and thus more precise resolution than the conventional method of using punctual monthly measurements (as described in Chambers et al. 2004). This will, in turn, improve the values for ecosystem carbon models and help to understand changes in carbon cycling with global climate change.

The need for such long-term measurements is underlined by our observation of large variations of  $E_{stem}$  among the three trees we measured. Our values for  $E_{stem}$  were between 0.6 and 4.5  $\mu\text{mol m}^{-2} \text{ s}^{-1}$ . Other studies like Chambers et al. (2004) give an average value of  $E_{stem}$  of 0.6  $\mu\text{mol m}^{-2} \text{ s}^{-1}$  across a large variety of trees species in the Amazon. However, Chambers et al. (2004) do not account for the diurnal variation of  $E_{stem}$ . Those low values (on average 1.00  $\mu\text{mol m}^{-2} \text{ s}^{-1}$  regardless of season) are confirmed by a similar study by Rowland et al. (2018). In this study, they follow the assumption of only limited diurnal changes in  $E_{stem}$ . However, there is a significant data gap in their continuous  $E_{stem}$  measurements. Data points during the important midday hours when the largest variations in  $E_{stem}$  occur are missing. In contrast, continuous measurements in a neotropical *Hymenolobium pulcherrimum* tree and various

mango trees in an orchard in Manaus (Kunert and Mercado Cardenas 2012, Kunert and Edinger 2015, respectively) resulted in significant diurnal changes in  $E_{\text{stem}}$ . For example, in this one *Hymenolobium* tree, daytime values of  $6.5 \mu\text{mol m}^{-2} \text{s}^{-1}$  were observed, whereas  $E_{\text{stem}}$  was only  $4.0 \mu\text{mol m}^{-2} \text{s}^{-1}$  at nighttime. In an old-growth forest in the Central Amazon,  $E_{\text{stem}}$  was  $2.9 \mu\text{mol m}^{-2} \text{s}^{-1}$  on average, slightly lower, in a *Scleronema micranthum* tree, but also showed significant variation within a diurnal cycle (Kunert 2018). Such large day night differences in tropical ecosystems were found by Levy et al. (1999), who report  $6.0 \mu\text{mol m}^{-2} \text{s}^{-1}$  during the day and  $3.5 \mu\text{mol m}^{-2} \text{s}^{-1}$  during the night in a *Combretum micranthum* shrub in an African shrub. Further, one tree (DYERCO3) showed the typical diel hysteresis between  $E_{\text{stem}}$  and stem temperature, which has often been reported for other trees in many studies (Ryan et al. 1995, Maier 2001, Damesin et al. 2002, Kunert and Mercado Cardenas 2012). Internal transport processes of dissolved  $\text{CO}_2$  are often made responsible for the expression of the hysteresis (Kunert and Edinger 2015).

In summary, we present a new and affordable system to measure  $E_{\text{stem}}$ . The system works well under a variety of environmental conditions (temperate and tropical ecosystems). We are aware that our system has limitations, e.g., it might not be suitable for all applications. For example, for longer flow pathways, real-time sensors response or a moisture correction is needed. Our results suggest that a high variation in  $E_{\text{stem}}$  can be detected when  $E_{\text{stem}}$  is measured continuously. We are convinced that this raises the justified question of the representativeness of most measurements in ecological studies that follow the assumption of very low diurnal changes in  $E_{\text{stem}}$ . Our approach with very affordable  $\text{CO}_2$  sensors will facilitate the urgently needed long-term and large-scale data collection, and thus help to shed light onto the missing links in our knowledge about carbon cycling in forest ecosystems.

## Acknowledgments

We would like to thank the Forest Research Institute Malaysia for giving us access to the Pasoh field site to test our equipment under tropical conditions. Further, we like to thank Jürgen Kreuzwieser for lab space and the GFS-3000 for calibrating our sensors. We would also like to thank the staff from the Chair of Silviculture at the University of Freiburg, for letting us use their Li-8100. We thank four anonymous reviewers for their valuable suggestions on how to improve the article. We would like to thank an additional native speaker and one reviewer for editing the final version of the article.

## Authors' contributions

N.K. initiated the study. J.B. designed and constructed the prototype of the chamber and wrote the Arduino sketch. J.B. and N.K. designed a second prototype for the application in the

field and experimented in the field. J.B. and N.K. analyzed and interpreted the data, and wrote the article together.

## Conflicts of interest statement

The authors declare no conflict of interest.

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