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Method Article

Development of a lightweight, portable, waterproof, and low power stem respiration system for trees ☆

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ABSTRACT

Stem respiration is a quantitatively important, but poorly understood component of ecosystem carbon cycling in terrestrial ecosystems. However, a dynamic stem gas exchange system for quantifying real-time stem carbon dioxide (CO_2) efflux (E_s) is not commercially available resulting in limited observations based on the static method where air is recirculated through a stem enclosure. The static method has limited temporal resolution, suffers from condensation issues, requires a leak-free enclosure, which is often difficult to verify in the field, and requires physically removing the chamber or flushing it with ambient air before starting each measurement.

- With the goal of improving our quantitative understanding of biophysical, physiological, biochemical, and environmental factors that influence diurnal E_s patterns, here we present a custom system for quantifying real-time stem E_s in remote tropical forests.
- The system is low cost, lightweight, and waterproof with low power requirements (1.2-2.4 W) for real-time monitoring of stem E_s using a 3D printed dynamic stem chamber and a 12V car battery. The design offers control over the flow rate through the stem chamber, eliminates the need for a pump to introduce air into the chamber, and water condensation issues by removing water vapor prior to CO_2 analysis.
- Following a simple CO_2 infrared gas analyzer (IRGA) calibration and match procedure with a 400-ppm standard, we quantified diurnal E_s observations over a 24-hours period during the summer growing season from an ash tree (*Fraxinus sp.)* in Fort Collins, Colorado. The results are consistent with previous laboratory and field studies that show E_s can be suppressed during the day relative to the night.

Specifications Table

Subject area:	Environmental Science
More specific subject area:	Tree respiration
Name of your method:	Real-time Stem CO_2 efflux system for trees
Name and reference of original method:	N.A.
Resource availability:	Please see Table 1

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Method details

Importance of autotrophic respiration in the global carbon cycle

Autotrophic aerobic respiration is the controlled oxidation of photosynthetically fixed carbon by plants resulting in the consumption of molecular oxygen (O_2) and the production of carbon dioxide (CO_2). In non-photosynthetic tissues, aerobic respiration is a major cellular source of usable chemical energy (ATP), reducing power (NADH), and source of carbon skeletons needed in numerous physiological processes including maintenance of existing tissues, growth and development, reproduction, defensive and signaling processes during responses to abiotic and biotic stress, and senescence processes [1]. Despite the high rates of CO_2 photo-assimilation in leaves, aerobic respiration in all plant tissues (and photorespiration in leaves during the day) leads to a large fraction of assimilated carbon returning to the atmosphere as CO_2. While highly uncertain, autotrophic respiration of terrestrial ecosystems represents a major atmospheric source of CO_2 with an annual global source estimated between 4 to 7 times that of anthropogenic fossil fuel combustion [2]. In dynamic vegetation models, autotrophic respiration is often calculated as the sum of leaf, stem, and root respiration [3]. While environmental and biological influences over leaf respiration during the day and night are becoming increasingly common across biomes globally due to the availability of numerous commercial dynamic leaf gas exchange systems [4], limited observations of dynamic stem gas exchange have been reported, likely constrained by a lack of commercial sensors. Respired CO_2 in tree stems can diffuse to the atmosphere driven by the concentration gradient between the inner bark and ambient air [5,6]. This mechanism is known as stem CO_2 efflux (E_s, μmol m^{-2} s^{-1}) and is estimated to represent a large but uncertain fraction of total autotrophic respiration of trees [7].

Static versus dynamic methods of stem E_s quantification

Limited studies, most of which have employed a commercial system designed for soil respiration and adapted to stems, have utilized the static technique to estimate E_s. For this method, air inside a stem chamber is recirculated through an IRGA for CO_2 concentration measurements. The rate of CO_2 accumulation over time is then used to estimate E_s. This static method was primarily adapted to stems from the use of existing commercial soil respiration systems [7]. Environmental variables impacting soil respiration within forested ecosystems are generally considered to change slowly throughout the day with air pressure control to minimize pressure related artifacts deemed more important than fast and continuous flux measurements [8]. While having the advantage of simplicity due to the need for only a single IRGA for CO_2, the static method suffers from numerous issues that limit its potential value as a tool in dynamic E_s studies and the influence of biological and environmental variables. As E_s is not directly measured, but instead estimated from the slope of [CO_2] versus time, very high CO_2 concentrations (thousands of ppm CO_2) rapidly build up inside the enclosure, reducing the CO_2 concentration gradient between the inner bark and ambient air [9]. This in turn reduces the CO_2 efflux and can therefore lead to underestimates of CO_2 efflux rates. Moreover, the method assumes a complete leak free enclosure where ambient air is prevented from entering the chamber by sealing the chamber to the stem with various glues [10]. However, small leaks, which are difficult to detect and quantify in the field, reduce the rate at which CO_2 accumulates in the stem chamber, and quickly become more significant with time as the CO_2 concentration inside the chamber rapidly increases above ambient air levels. Moreover, after each measurement period lasting 5-30 min, the stem enclosure must be removed from the stem to reintroduce ambient air. Alternatively, the chambers must be rapidly flushed with ambient air just prior to each E_S measurement, increasing the complexity. Thus, stem respiration measurements using the static method typically require manual installation and deinstallation for each measurement point. This leads to poor time resolution making the method generally unable to resolve potentially large diurnal patterns in E_S as well as fast dynamics on the time scales of < 15 min associated changes in sap velocity and incoming sunlight during the passing of clouds [7], for example. In addition, high humidity environments are often encountered near the base of trees where most stem E_S observations have been reported, with stem transpiration often leading to significant condensation inside the CO_2 Infrared Gas Analyzers (IRGAs). As IRGAs do not function under saturating humidity conditions, a complete loss of data is often encountered when condensation occurs, especially if E_S measurements are sequentially performed over time. In summary, static chambers suffer from a number of issues including high humidity and condensation issues, requires a rigorously leak-free enclosure which is difficult to verify in the field, quickly generate a greatly altered CO_2 stem atmosphere that can lead to errors in determining E_S by greatly altering stem-atmosphere concentration gradients, and the requirement to flush the enclosure with ambient air before starting each measurement, increasing complexity and constraining the time resolution of E_S observations.

The lack of a low-cost commercially available system for monitoring real-time stem E_S under challenging field conditions precludes a comprehensive analysis of the dependence of diurnal stem E_S on biophysical (wood density, sap wood volume, bark thickness), physiological (e.g. growth, net photosynthesis, transpiration, and aerobic respiration rates), biochemical (volatile organic compound metabolism, nutrient and respiratory substrates and pathways), and environmental (temperature, light, moisture availability) factors. To overcome these limitations, here we present the development of a low cost, lightweight, waterproof system with low power requirements (0.1-0.2 A at 12V) for real-time monitoring of stem E_S using a custom 3D printed dynamic stem chamber, dual IRGAs for continuous ambient and stem air CO_2 concentration observations, and a car battery. The disadvantages of this system are the requirement for accurate and constant flow control through the chamber, continuous water removal and CO_2 measurements of both the reference and stem enclosure air using two distinct IRGAs that are regularly “matched” such that any measured difference between the ambient air and stem CO_2 concentrations (ΔCO_2) can be attributed to respiratory activities of the stem. The design offers control over the flow rate through the stem chamber, minimizes complexity by eliminating the need for a pump to introduce air into the chamber, and water condensation issues by removing H_2O vapor prior to CO_2 analysis. Following a simple calibration procedure with

Table 1
Lists of materials used for the construction of the Portable Stem Respiration system.

Part Name	Supplier Name, Country, Website	Model number	Quantity
Water proof case	Pelican Products Inc., USA, www.pelican.com	1535 Case: Interior (20.39 in x 11.20 in x 7.21 in)	1
Carbon Dioxide gas analyzer	Li-Cor BioSciences, USA, www.licor.com	Li-820	2
Gelman 1 Micron Filter Assembly	Li-Cor BioSciences, USA, www.licor.com	9967-008	2
Bev-o-Line tubing (1/4" x 50′)	Li-Cor BioSciences, USA, www.licor.com	1/4" x 50′	1
1/4" quick connect union	Li-Cor BioSciences, USA, www.licor.com	300-03123	2
1/4" quick connect needle valve	Li-Cor BioSciences, USA, www.licor.com	300-10471	1
Water vapor scrub tube assembly	Li-Cor BioSciences, USA, www.licor.com	9960-093	2
Indicating dririte	W A Hammond Dririte Co LTD, USA, www.dririte.com	10-20 mesh, 5 lbs	1
Air pump	Delaman, www.amazon.com	12V DC Mini Diaphram Pump	1
1/4" stainless steel tee	Swagelok, www,swagelok.com	SS-400-3	2
1/4" Swagelok Bulkhead union	Swagelok, www,swagelok.com	SS-400-61	2
USB mouse	Various	N.A.	1
USB to micro-USB cable	Various	N.A.	1
USB hub (4 ports)	Various	N.A.	1
USB silicon rollable keyboard	SUNGWOO HIGHTECH, USA, www.swhitech.com/eng	N.A.	1
12 VDC mini-pc with fan	GMK electronic design GmbkH, Germany, www.gmk-electronic-design.de	GMK Mini PC, NucBox Windows	
10 Mini Computer with Intel	1		
battery powered portable monitor	UPERFECT,China, https://www.uperfectmonitor.com/	15.6" monitor/battery with mini HDMI	1
micro HDMI to HDMI cable	Various	N.A.	1
3D printed Stem Chamber	N.A.	3D Files for printing: Tree_Chamber_280.sat and Tree_Chamber_500.sat	1
Gas flow meter	SKC LTD, USA, www.skcltd.com	Chek-mate flowmeter, 0.50 L/min	1

a 400 ppm CO_2 standard at the beginning of each weekly measurement campaign, we show that the system shows low IRGA drift over time ($\Delta CO_2 < 10$ ppm) is highly sensitive to stem CO_2 efflux (observed ΔCO_2 ranging from 60-1,000 ppm). We demonstrate the practical use of the system in Colorado by quantifying E_S for 24-hours and relating the resulting flux to air temperature.

Design, installation and operation of the portable stem respiration system

The list of items used in the construction of the portable stem respiration system are shown in Table 1. A batch of 10 custom 3D stem chamber (polyethylene terephthalate glycol, PEG) were printed using a 3D printer at Lawrence Berkeley National Laboratory using the CAD file included as a free supplementary file: Tree_Chamber_280.sat. To create a decent seal between the stem chamber base and the stem, a 1/2" thick rectangular foam rectangle was cut to the interior dimensions and glued to the inside base of the stem chamber using silicon sealant. $\frac{1}{4}$" quick connect union fittings were then attached onto the stem $\frac{1}{4}$" inlet and outlet port for quick connections to tubing. Following lightly cleaning the surface of the stem are to be measured with a brush one day prior to measurements, the stem chamber was placed with the foam gaskets towards the stem and was secured using two cinch straps (Fig. 1). Adjacent to the tree and installed in the inverted position with the mouth at the same height as the stem chamber, an inverted 10 Gallon ambient air buffer is installed on a vertical support structure.

All other items were installed and configured in a waterproof and breathable pelican case with an integrated gas-exchange valve to equilibrate air pressure inside and outside of the case (See Table 1 for complete list of material items inside the case). The monitor was mounted to the inside lid and the electrical components, pump, fittings, water vapor traps, particle filters, CO_2 IRGAs, and gas sample tubing and fittings were installed inside of the case on top of the bottom foam layer. All power was supplied externally using a 12 VDC battery and distributed to the PC, pump, and two CO_2 IRGAs inside the case using a parallel circuit. In addition to the integral 2 A fast blow glass fuses protecting each of the CO_2 IRGAs internally, the 12 VDC circuit is protected from an overcurrent with a 5 A fast blow glass fuse. To prepare the system for operation, the case is first opened, and fresh Dri-rite is placed in the two water vapor traps which are then carefully resealed (Fig. 2a). Following this, the two $\frac{1}{4}$" caps on the outside of the pelican case protecting the ambient and stem air inlets are removed and connected to the appropriate length of $\frac{1}{4}$" sample tubing to reach from 1) the air inlet on the case to the stem chamber air outlet and 2) from the ambient air inlet on the case to inside the ambient air buffer. Note, keeping both tubing segments the same length ensures that a similar air flow rate is established through the stem and ambient IRGAs. Following this, the power to the main unit is switched on, which automatically turns on the air sample pump and the two IRGAs. The mini-PC is then switched on and communication is established with the ambient air and stem air IRGAs via USB communication cables. The air flow rate entering the ambient air and stem air $\frac{1}{4}$" sample tubing is then measured using the 0-500 mL/min flow meter. The air flow rate is adjusted through both ambient and stem air tubing together using the manual valve just upstream of the pump. Opening this valve decreases the flow rate through the IRGAs while closing this valve increases it. The valve is adjusted such that 80-100 ml/min is maintained through both ambient and stem IRGAs. The valve is then locked to ensure the flow is held constant throughout the duration of the stem respiration experiment (24 hours).

Fig. 1. Design and installation of stem chamber used for continuous observations of Es. CAD image showing a. Left, b. Back, c. Top, and d. 3D view, e. Example installation of stem chamber onto a stem using two cinch straps on a tropical tree in the Brazilian Amazon. Note the grey silicon and plastic cap above the stem enclosure used to prevent water from entering the stem chamber during rainstorms.

Fig. 2. Preparation of dynamic stem respiration system for its first operation in a controlled laboratory environment. **a**. Opening the case and switching the system on powered by a 12 VDC external battery. **b**. Connecting a 10 L Tedlar bag sample with 400 ppm CO_2 for calibration and match procedure prior to each 24-hour measurement period.

Match and calibration procedure and stem CO_2 efflux measurements

Once the desired flow rates are achieved, the delay time for each of the IRGAs should be separately determined by briefly blowing near the ambient air sample and stem tubing and recording the time required to observe the peak in CO_2 concentration on the monitor. Note, the delay with 100 ml/min air flow through each of the sample tubes was determined to be < 3 min due to the dead volume of the system (mainly the water trap). The stem respiration system is then calibrated and matched prior to installation onto a

tree and logging CO_2 concentrations on the mini-PC. The calibration and match procedure can be performed in the lab or field using a 10 L Tedlar gas sample bag with 400 ppm CO_2. A $\frac{1}{4}$" stainless steel tee fitting is used to connect both the ambient air and stem air to the opened Tedlar bag containing the 400-ppm standard (Fig. 2b). Note that if both ambient and stem air IRGAs are flowing at 100 ml/min (200 ml/min total flow), then the standard will run out in 50 min. However, the calibration/match procedure was found to take 10-15 min following initiation. Note that this time is recommended to fully replace the air in the tubing, water vapor traps, and IRGAs with the 400 ppm calibration air sample. Once CO_2 concentrations in each of the two IRGAs reaches steady state, record the offset from 400 ppm (should be less than 5 ppm) and initiate a point calibration of each IRGA with the stated concentration of 400 ppm (the CO_2 concentration in the standard). Following each IRGA calibration, the two sample tubes can then be re-installed on the sample and ambient air inlets on the back of the case. The other end of the gas sample tubes are then connected to the outlet of the stem chamber (stem air sample) and inserted and secured in the ambient air reservoir (ambient air sample). A third $\frac{1}{4}$" tube is also inserted and secured in the ambient air reservoir and connected to the ambient air inlet on the stem chamber.

CO_2 efflux measurement is then initiated by recording average CO_2 concentrations every 30-60 seconds on both ambient air and stem air IRGAs. Once measurements are initiated, the monitor is switched off with the mini-PC continuing to collect CO_2 data. The case can then be closed and left for continuous operation until the Dri-rite needs replacing (24 hours in warm humid environments like tropical forests). Following completion of the measurements the following day, once the case is re-opened, the data logging is stopped and stored files are transferred to a USB drive. Following this, the system is transported to the next tree to be studied, followed by a new match/calibration procedure as necessary. However, we found that even after continuous measurements on 3-7 different tree species during one week, the 400 ppm calibration/match procedure showed a low drift of the IRGAs with the CO_2 offset determined by weekly calibrations < 5 ppm. Following data collection, the stem CO_2 efflux rates were determined from 15-minute averages of the ambient air and stem air CO_2 concentration time series. Stem CO_2 efflux rates (E_s, μmol m^{-2} s^{-1}) every 15 minutes were calculated according to equation 1 where F is the flow rate of ambient air through the stem chamber: (0.1 L min^{-1}), ΔCO_2 (ppm) is the difference in CO_2 concentration between the stem air and ambient air, and A is the enclosed stem area of 9.95E-3 m^2 (15.3 cm x 6.5 cm).

$$E_S\ (\mu\text{mol}\ m^{-2}s^{-1}) = F \times \frac{1\ min}{60\ s} \times \frac{1\mu mol}{22.4\mu L} \times \frac{\Delta CO_2}{A} \quad (1)$$

Validation of lightweight, portable, waterproof, and low power dynamic stem respiration system for trees

In this section, we report a field test of the simple, low cost, waterproof, and portable stem respiration system for continuous observations of tree stem CO_2 efflux from custom 3D printed dynamic stem gas exchange chambers. This system, which is enclosed in a waterproof pelican case, includes two Infrared Gas Analyzers (IRGAs) that continuously measure CO_2 concentrations from the ambient air reservoir near the stem and air exiting the gas exchange stem chamber installed at breast height. Prior to installing the system on the stem in the field, the calibration and match procedure was conducted as described in the previous section.

We collected data from an Ash tree, *Fraxinus sp.,* in Colorado, USA during the summer of 2021 to validate the new method for determining real time stem E_s rates. The Ash tree genus is widespread and grows across much of Europe, Asia, and North America. The tree was estimated at 10 meters in height with a 70-80 cm diameter. The study was conducted in a suburban neighborhood in Fort Collins, Colorado, USA. The site receives an average annual precipitation of 409 mm with a low of 10 mm in January and a high of 61 mm in May. The soil is an Acidic Haplustalfs series which consists of fine-loamy very deep, well-drained soils. Raw CO_2 concentration data from the ambient and stem air IRGAs was recorded in real-time with a 1-minute logging frequency on the mini-PC starting at 8:00 AM on 22-July-2022. One delimited text file for the ambient air and stem air CO_2 concentration time series data was downloaded at the end of the 24 hour experiment. In addition, air temperature, which largely determines the magnitude of plant transpiration though its strong influence over the vapor pressure deficit (VPD) was also obtained for relations with stem E_s data. Air temperature was collected roughly 5 miles away at the Fort Collins Weather Station. In addition, stem temperature measurements were taken manually with a hand-held thermal imaging system (Flir-E5) for comparison with air temperature. All CO_2 and temperature data were averaged every 15 minutes prior to plotting and correlation analysis.

The results show that continuous positive gradient in CO_2 (ΔCO_2) was maintained by the stem emissions during both the day and night (Fig. 3a). Ambient air CO_2 varied throughout the 24-hour period reaching a maximum in the early morning pre-dawn period on 23-July-2021. Stem air CO_2 also varied substantially throughout the 24-hour period reaching a maximum near mid-night on 22-July-2021. Ambient air CO_2 stayed at least 61 ppm below stem CO_2 at all times during the 24-hour period with a maximum gradient occurring just prior to midnight on 22-July-22. When Equation 1 was used to calculate the stem CO_2 efflux rates (E_s, μmol $m^{-2}s^{-1}$) every 15 minutes, a diurnal trend was observed with E_s reaching higher values during the night and suppressed values during the day. E_s reached a maximum value of just prior to midnight on 22-July-22 of 6.8 μmol $m^{-2}s^{-1}$ (Fig. 3b). In contrast, air temperature, and also likely tree transpiration, peaked around 2:00 PM in the afternoon. Moreover, when plotted versus air temperature, a negative relationship was observed with decreasing E_s with increasing temperature (Fig. 4). These observations are consistent with previous studies on diurnal E_s patterns of field trees which showed a similar magnitude of E_s as well as a suppression during the daytime relative to the nighttime [11].

Although mitochondrial respiration is known to increase with temperature [12], recent studies have shown that daytime E_s is suppressed during the day relative to the night [7,11, 13]. However, the biological and physical mechanisms that give rise to E_s suppression is under discussion and includes mechanisms like enhanced CO_2 storage [14,15], transport of CO_2 in the transpiration stream [16], suppression of stem mitochondrial respiration under reduced day-time stem turgor pressure [17], enhanced night-time

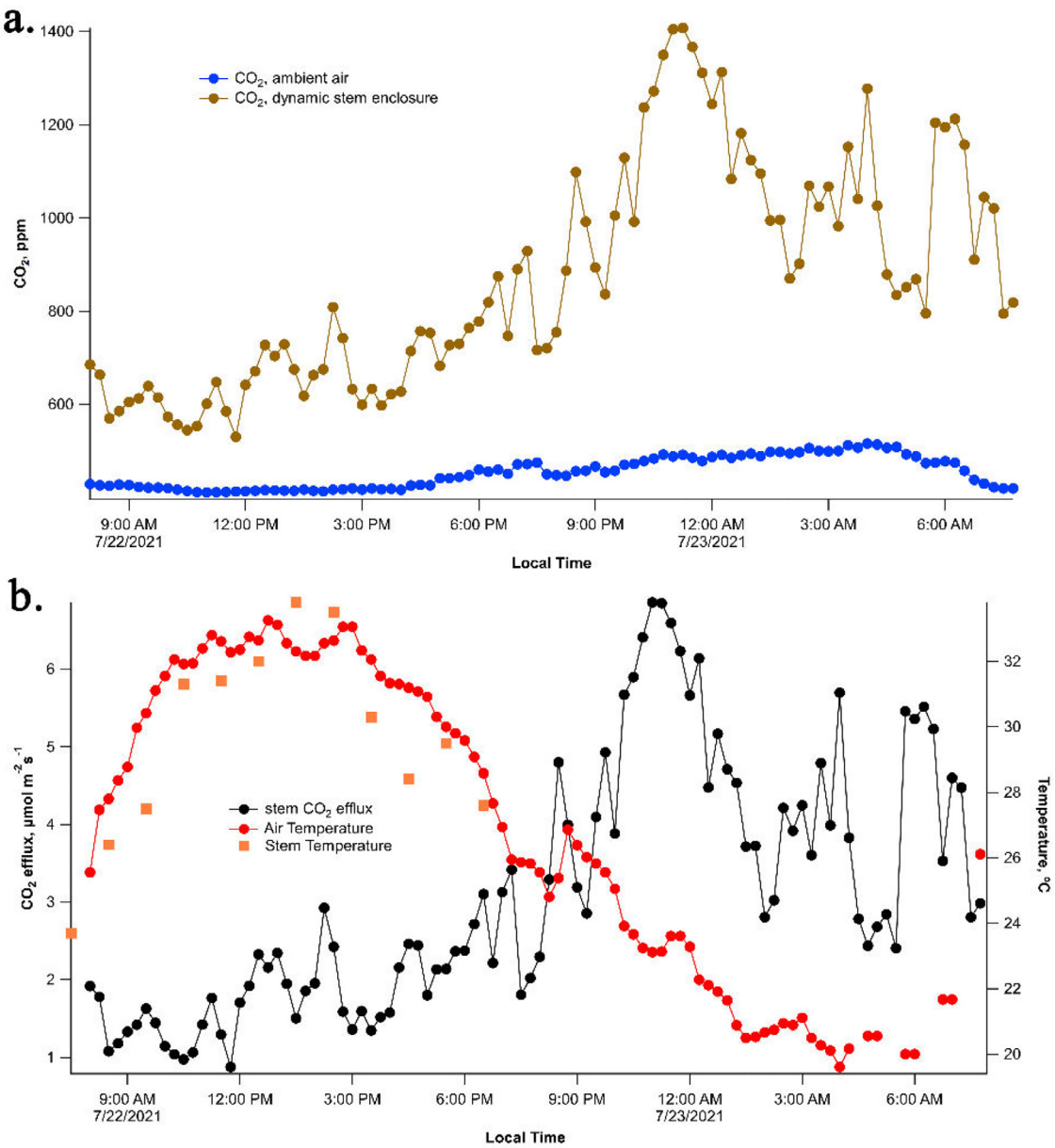

Fig. 3. Diurnal CO_2 **a**. concentrations in ambient air and stem chamber air and **b**. stem E_s flux together with air and stem temperature from an Ash tree at breast height in Fort Collins, CO, USA.

growth rates [18], and stem CO_2 re-assimilation via both light dependent photosynthesis in green tissues [13] and light-independent fixation via phosphoenylpyruvate carboxylase (PEP) as a part of anaplerotic metabolism [19]. For example in a recent study, daytime E_s suppression was observed on young poplar trees growing in a greenhouse and this was attributed to temperature-dependent increases in xylem transport of locally respired CO_2 and lowered turgor pressure that constrained mitochondrial respiration [20]. Thus, in order to verify daytime E_s suppression in other species, determine biological and environmental conditions where it does not occur, and discriminate between these mechanisms, the dynamic stem CO_2 efflux system presented here should be of high value to the research community.

Concluding remarks

In order to field test the portable dynamic stem CO_2 efflux system in a remote forested region of the world under heavy rain conditions, we deployed the system to Manaus, Brazil during the 2022 rainy season. Although the results of the diurnal stem E_s measurements will be presented and discussed in a future research article once data collection is completed, the results demonstrate

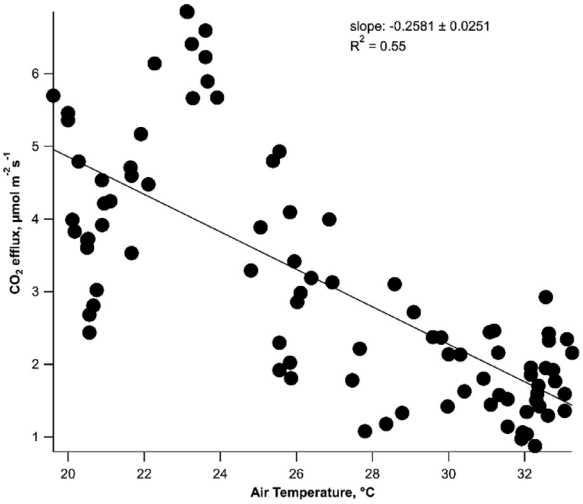

Fig. 4. Scatter plot and linear regression between E_s flux and air temperature from an Ash tree at breast height in Fort Collins, CO, USA.

Fig. 5. Masters student Edson Augusto from the National Institute for Amazon Research (INPA) in Manaus, Brazil setting up a diurnal E_s data collection from a canopy tree in a remote central Amazon rainforest ecosystem.

that the system is capable of running off of a charged car battery for many weeks. Moreover, despite heavy rains in the remote field location, with the case closed and the system wrapped in a ground tarp, continuous CO_2 efflux observations were collected in hyper diverse forest transects as well as remote locations far away from a power source (Fig. 5). We conclude that the system will be of great use in tropical carbon cycle research with the goal of understanding the biological and environmental influences on diurnal and seasonal E_s patterns in diverse tropical forests.

Ethics statements

No participant data was collected during the testing of the portable, waterproof, stem respiration system.

Supplementary Material

The following computer aided drafting (CAD) file (Tree_Chamber_280.sat) used to print the 3D stem chambers used in this study can be downloaded and used free of charge as a supplementary document. Please cite this paper when using this design.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Kolby Jardine: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration. **Edson Augusto:** Investigation, Writing – original draft, Formal analysis. **Sienna D. Levine:** Investigation, Writing – original draft. **Aatish Sunder:** Validation, Resources, Writing – review & editing, Visualization. **Suman Som:** Resources, Writing – review & editing. **Jeffrey Chambers:** Methodology, Validation, Investigation, Project administration, Funding acquisition.

Data Availability

Data will be made available on request.

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