Description of the measurement protocols

Dataset: Leaf gas exchange, leaf water potential, and reflectance measurements, BIONTE, Brazil, 2023

Authors: Julien Lamour, Daisy Souza, Bruno Gimenez, Regison Oliveira, Kim Ely, Débora Plácido, Valdiek Menezes, Alistair Rogers

Contacts: jlamour.sci@gmail.com, alistair@lbl.gov, brunoolivagimenez@gmail.com

Plant material

This dataset was measured near Manaus, Brazil, inside the BIONTE experiment plots T1B4SB3 and T4B2SB1 from 2023-08-15 to 2023-11-02. Measurements were made on trees selected along the trails that were created inside the two blocks (Table 1). We accessed the canopy top using an articulated boom lift (Genie Z 80-60). Thirty trees of different species were chosen for the measurements based on their wood density and putative nitrogen-fixing status. Three successive campaigns of measurements were made within the time period (August, September, and November) to repeat measurements on the same trees and test the effect of the drought and season on leaf traits. Additional leaf water potential measurements from 5 days between 2023-05-17 and 2023-08-23 are also included in the dataset.

Table 1: Description of the trees selected for this study. Heights were measured in August 2023 using a weighted tape measure. DBH is taken from the BIONTE inventory. Sap_Flow indicates whether the tree is fitted with a sap flow sensor.

TreeID	Species	Height (m)	DBH (cm)	Sap_Flow	Block
3X	Andira parviflora	24	24.5	YES	B4T1
Bellu	Bellucia imperialis	23	24.5	NO	B4T1
Cecropia	Cecropia sciadophylla	23.7	22.1	NO	B4T1
176	Duckeodendron cestroides	23	62.1	YES	B4T1
274	Endopleura uchi	29	45.9	YES	B2T4
116X	Eperua duckeana	24	30.5	YES	B2T4
276C	Eschweilera atropetiolata	20.3	19.9	NO	B4T1
280	Eschweilera coriacea	26	45.6	YES	B2T4
84X	Eschweilera truncata	22.5	19.8	YES	B2T4
324	Geissospermum argenteum	23	77.6	YES	B4T1
Inga	Inga paraensis	24	21.7	NO	B4T1
275E	Inga paraensis	24.3	26.7	YES	B4T1
319P	Inga splendens	30	22.9	YES	B4T1
317	Lecythis prancei	29	56.1	YES	B4T1

300X	Licania coriacea	23	21.3	YES	B2T4
317M	Miconia minutiflora	24	23.6	YES	B4T1
90XD	Miconia minutiflora	20.5	10.3	YES	B2T4
165MA	Ocotea floribunda	19.8	15.1	YES	B4T1
184J	Pourouma guianensis	23.5	33	YES	B4T1
186M	Pourouma velutina	22.9	27.1	YES	B4T1
115XA	Pourouma villosa	22	14.4	YES	B2T4
85XC	Pourouma villosa	20.5	17.3	YES	B2T4
431	Pouteria guianensis	31	58.6	NO	B4T1
185X	Protium tenuifolium	23	28	YES	B4T1
102X	Simarouba amara	25	25.5	YES	B2T4
Swartzia	Swartzia arborescens	22	27.7	YES	B4T1
420X	Swartzia panacoco	23	21	NO	B4T1
Tachi	Tachigali chrysophylla	40	58	NO	B4T1
176YC	Vismia guianensis	13.2	14.9	NO	B4T1
91XB	Xylopia spruceana	24.5	20.1	YES	B2T4

List of measurements and sample identification
This dataset contains gas exchange measurements, leaf spectroscopy (reflectance), and leaf water potential.

For the gas exchange measurements, the dataset comprises traditional photosynthetic response curves to CO_2 (A-C_i), light (A-Q), and dark-adapted respiration (R_{dark}) made on cut branches. The branches were cut predawn or at dawn and recut into water to restore hydraulic conductivity. Response curves and dak-adapted respiration were generally (but not always) measured consecutively on the same leaf, with a stabilization time between each measurement.

The dataset also includes steady-state gas exchange measurements made on attached top-of-canopy leaves with sufficient time for photosynthesis and stomata to acclimate (>= 20 minutes). We call these measurements “survey” measurements; they can be used to estimate the maximum carboxylation capacity of Rubisco using the one-point method (De Kauwe *et al.*, 2016; Burnett *et al.*, 2019). These measurements can also be used to estimate stomatal traits since we waited for the steady state of both the photosynthetic and conductance rates.

All the measured leaves have a unique identifier made of a 5-digit number (e.g. BNLXXXXX). This identifier can be used to identify measurements made on the same leaves (i.e. reflectance, LWP, and gas exchange measurements). The trees also have a unique ID within the BIONTE plots which is used to report information about their growth and mortality. This ID can be used to link measurements by other NGEE project teams or other teams on the site, such as sap flow.

Gas exchange measurements
We used 4 LICOR-6400XT gas exchange apparatus to conduct gas exchange measurements. The protocols for the A-C_i, AQ, R_{dark}, and survey methods are detailed below. They followed established and documented protocols that have been used in previous studies (e.g. Rogers *et al.*, 2019; Lamour *et al.*,

2021, 2023). Each day we followed the standard LI-COR 6400XT warm-up procedure and verifications in the morning prior to making measurements.

AQ curve protocol

We set the flow rate at 500 µmol s^{-1}, the light at a saturating irradiance (2000 µmol m^{-2} s^{-1}), CO2S = 420 µmol mol^{-1}, Tblock at ambient (or higher for dew point control if required). We waited for instrument stability and then matched. After we inserted the leaf and checked for a leak we monitored A and g_{sw} for a minimum of 20 minutes to ensure stability and acclimation to saturating light. After stability of A and g_{sw} was reached, the AQ curve was conducted with irradiance levels of 2000, 2000, 2000, 1800, 1500, 1250, 1000, 750, 600, 500, 375, 250, 180, 125, 75, 60, 50, 30, 20, 0 and 2000 µmol m^{-2} s^{-1}. Measurements were logged when the standard deviation of A was below 0.2 µmol m^{-2} s^{-1} and when the standard deviation of Qin was below 1 µmol m^{-2} s^{-1} over 20 seconds, with a wait time of 60 - 200 seconds.

A-C_i curve protocol

We set the flow rate at 500 µmol s^{-1}, the light at a saturating irradiance, and CO_2 reference at 435 µmol mol^{-1} and waited for stability of A and g_{sw}. We used an A-C_i autoprogram with CO_2 reference levels set at 435, 300, 250, 175, 125, 70, 50, 435, 435, 435, 475, 675, 800, 1000, 1400, 1800, and 435 µmol mol^{-1}. After each CO_2 level change, IRGAs were matched, then a log taken after stability was reached, measured as the standard deviation of CO2R < 0.75 ppm and A < 0.2 µmol m^{-2} s^{-1} over 20 seconds, with a wait time of 60 - 200 seconds.

Dark-adapted dark respiration protocol

Dark-adapted measurements were performed with a flow rate of 300 µmol s^{-1}, CO2R = 420 µmol mol^{-1}, light = off, LI-COR head covered by dark cloth. The data was logged automatically using the Autolog2 program (log every 5 sec, match every 10 minutes). After stabilization of A and g_{sw} was reached, and after a minimum of 30 minutes, we matched and continued to log data for 2.5 minutes, or more if necessary to achieve 20 points of stable data.

Survey protocol

We first selected the target leaf and positioned the LI-COR head. We measured the ambient air temperature (Tair °C), relative humidity (RH%), and wind speed (m/s) with a Kestrel portable weather station. We measured the leaf temperature with an Apogee InfraRed thermometer and the incident irradiance on the chosen leaf with the LI-COR quantum sensor positioned to represent the leaf orientation. We then set the environmental conditions on the LI-COR as: Tblock = measured air temperature; Qin = 1800, 400, 100 µmol m^{-2} s^{-1}. We then clamped the leaf and started an Auto program that logged gas exchange measurements every 5 seconds, and matched the IRGAs every ten minutes. We waited for stabilization of g_{sw} and A and at least 20 minutes. We waited at least 2.5 minutes after a match to achieve 20 points of stable data and then stopped the measurements. After completion of the measurements, we excised the leaf and sent it to the ground for further measurements. In addition to the measurements at 1800, 400 and 100 µmol m^{-2} s^{-1} we also performed dark measurements at Qin = 0 µmol m^{-2} s^{-1} with the light source off and a dark cloth covering the sensor head, following the dark adapted protocol.

Curation and fitting of the gas exchange measurements

Data and metadata follow the recommendations of the ESS-DIVE Leaf-level gas exchange reporting format (Ely et al, 2001) and we used this standard to define the variables, units and information within the files. Note that we provide the raw output files from the instruments as well as curated data. For the A-Ci curves, we also estimated the parameters of the FvCB model (Farquhar *et al.*, 1980) V_{cmax25} and J_{max25} using the f.fitting function of the LeafGasExchange package with the default photosynthetic parameters from the package (see package info: Lamour & Serbin 2021, and previous article Lamour *et al.*, 2021).

Leaf spectral reflectance measurements

All the leaves measured for gas exchange were subsequently measured for reflectance. All the reflectance measurements were made with an ASD FieldSpec3 attached to a Plant Probe. This spectrometer measures the reflectance from 350 nm to 2500 nm. One or several measurements were made on each leaf, depending on the size of the leaves, and averaged.

Leaf water potential

LWP measurements were made on the same leaves as the survey gas exchange measurements and as the A-C_i, AQ, and R_{dark} measurements. Just after the survey measurement, the leaves were cut and put into a plastic bag. The plastic bag was immediately sent to the ground where a second team measured the leaf water potential. LWP was also measured in some of the leaves from the cut branches after completion of the A-C_i, A-Q, and R_{dark} measurements. We used standard measurement practices for a Scholander-type pressure chamber, described briefly here. Before measurement, each leaf's petiole was cut again with a fresh razor blade to ensure hydration of the xylem at the cut. Leaves were then placed inside a pressure chamber (PMS Model 1000), and the compression gland was tightened around the petiole to a level where excess nitrogen would not escape when the system was pressurized. Pressure was slowly raised, while the operator observed the cut petiole segment for xylem sap forming on the cut. Once sufficient pressure had been reached to force xylem sap out of the petiole, the operator noted down the pressure reading and depressurized the system.

LWP measurements were also made on specific days without associated measurements (no gas exchange, reflectance, etc.). These measurements therefore do not have a SampleID. The TreeID is used to identify the trees.

References

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