

## **Materials and methods summary for BCI spectroscopy and Vcmax observations from October 2022.**

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### **Site**

We collected data from Barro Colorado Island, Panama (BCI; 9°9' N, 79°50' W), part of the Barro Colorado Nature Monument managed by the Smithsonian Tropical Research Institute (STRI). BCI is a semi-deciduous lowland moist tropical forest, characterized by an annual rainfall of around 2600 mm and a distinct dry season from January to April (<100 mm month<sup>-1</sup>), and maintains an average temperature of 26°C. Approximately 12% of tree species exceeding 10 m in height are facultatively or obligately deciduous (Leigh, 1999).

A 40-m tower is situated near a 50-ha Forest Global Earth Observatory (ForestGEO) inventory plot within BCI and is equipped with sensors that measure meteorological data (i.e. incoming solar radiation, relative humidity, air temperature, air pressure, wind speed and direction, and precipitation) at half-hourly increments, as well as eddy covariance fluxes to estimate gross primary productivity, latent heat and sensible heat (see Detto and Pacala (2022) and Figure 1 for details). Dr. Matteo Detto (Princeton University) is the point of contact for BCI eddy covariance tower data.

Near the tower, there is a 50 hectare inventory plot that was established in 1981 and is now part of the ForestGEO network (see [ForestGEO web page about BCI](#)).

### **Species and sampling design**

In October 2022, we sampled sun-exposed branches from 26 individual trees using two distinct methods. First, we used a pole pruner to collect branches from within reach of the ground near the flux tower (within and adjacent to the northeast corner of the 50-ha plot), targeting trees with direct sunlight exposure at heights up to approximately 18 meters. We also flew an unmanned aerial vehicle (UAV), the DJI Matrice 600 PRO (DJI, Nanshan, Shenzhen, China), equipped with a robotic DeLeaves canopy sampling saw arm (Outreach Robotics, Quebec, Canada) to sample branches from the top of the canopy, in an area around the southern part of the BCI basecamp. The choice of sampling area for UAV collection was dictated by sufficiently large drone takeoff zones (the clearings within the ForestGEO inventory plot were too small for our drone to takeoff and land with the robotic arm accessory). The 26 trees we sampled were

identified by botanists on BCI, and each represented a different species. Collectively, the sampled species include phenological diversity, including evergreen (no leaf shedding occurs), facultatively Deciduous (leaf shedding depends on local microclimate and access to water), obligately deciduous (leaf shedding is more pronounced during entire dry season), and brevi-deciduous (leaf shedding occurs briefly). Dr. Joe Wright (Smithsonian Tropical Research Institute) has data on tree phenology at BCI.

The sequence of data collection was branch collection followed by prompt re-cutting, carrying branches to the gas exchange system for measurement (generally 5-30 minutes), A/Ci curves (generally 30-60 minutes), carrying branches to the lab (generally 10-60 minutes). We transported branches back to the lab in water (typically a dry bag filled with water). Then finally leaf reflectance measurements (typically within 90 minutes of arrival in the lab). Sometimes when multiple branches were collected, there were additional lags before a gas exchange system or spectrometer became available to measure a given branch. All paired gas exchange and reflectance spectra measurements were conducted the same day.

### **A-Ci curves**

To measure leaf gas exchange, we collected branches of approximately 1 meter in length using either a pole pruner or UAV. Immediately following collection, branches were immersed in water, and re-cut underwater (7 to 10 cm of the branch base was removed) to restore xylem water continuity, minimizing the risk of stomatal closure. Using LI-6400XT and LI-6800 systems (Bluestem OS v2.0, Li-Cor Biosciences, Nebraska, USA), we measured gas-exchange on one leaf per branch (and therefore per tree), selecting healthy, mature leaves verified as photosynthetically active by our monitoring of stomatal conductance, CO<sub>2</sub> assimilation, and transpiration rates. Each leaf was acclimated within a 6 cm<sup>2</sup> chamber area for approximately 15 minutes before measurements commenced. Throughout the gas-exchange measurements, we maintained constant conditions during leaf acclimation to the chamber: light intensity at 2,000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , a leaf temperature of 31°C, and chamber [CO<sub>2</sub>] at 400 ppm. When using the LI-6800 we controlled vapor pressure deficit (VPD) between 1.5 kPa to 2.0 kPa, and when using the LI-6400XT, we kept relative humidity between 50 and 55%. Following acclimation, all chamber conditions were kept constant, except for CO<sub>2</sub> concentration, which was adjusted in reference steps of 400, 300, 200, 100, 50, 400, 600, 800, 1000, 1200, and 1500 ppm.

### **Reflectance spectra protocols**

We collected leaf reflectance spectra from the same leaf and same spot on the leaf where gas exchange measurements were performed. In addition to the gas exchange spot, when possible we also measured 2 other spots on the leaf (when not possible due to small leaf size or small area of intact lamina, we measured the original spot 2 additional times), resulting in 3

spectral measurements per leaf. We also measured 3-4 additional leaves per branch (each with 3 measurements per leaf) to better represent the variability within an individual crown of each tree. We used a FieldSpec 3 Spectroradiometer (Analytical Spectral Devices (ASD), Boulder, CO, USA) to measure spectral radiance across visible (VIS), near-infrared (NIR), and short-wave infrared (SWIR) wavelengths from 350 to 2500 nm, with sampling intervals of 1.4 nm and 2 nm for the ranges 350-1000 nm and 1000-2500 nm, respectively. Spectrometer specifications included a full-width-half-maximum (FWHM) of 3 nm at 700 nm, and 10 nm at both 1400 nm and 2100 nm. The ASD FieldSpec 3 was coupled to a leaf probe of the same brand via optical fiber bundle. Leaves were secured within the leaf probe, and each spot was measured with an optimized 1-second integration time to mitigate potential heat effects on data quality. The leaf probe had a built-in, swappable white reference and dark reference, which we used for instrument calibration during measurements (approximately every 15 minutes we performed an optimization, white reference and dark reference). All spectra measurements were made indoors in a lab at the BCI field station. To reduce the impacts of overlap mismatch between spectrometer sensors, we let our instrument warm up for at least 30 minutes every day prior to use.

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**Citation for draft manuscript with A/Ci curves:**

Working title: Model simulations reveal variation in species-specific contributions to GPP related to leaf function, seasonality, and climatic events in a moist tropical forest. Authors: Kelvin Acebron, Loren P. Albert, Emmelia J. Braun, Petya K. Campbell, Matteo Detto, Maquelle N. Garcia, Egor Prikaziuk, Sabrina E. Russo, Charles D. Southwick, Christiaan van der Tol, Vicente Vazquez, and Sean M. McMahon.