PROTOCOL

Isabelle Maréchaux, Patrick Heuret, Ninon Besson, Sabrina Coste, Clément Stahl, Ilona Clocher, Nicolas Barbier
2024

Site

The leaf sampling was carried out between June 2022 and March 2023 at the Paracou field site located near Sinnamary in French Guiana (5.274 N, 52.928 W) and managed by the Centre International de Recherche Agronomique (CIRAD, https://paracou.cirad.fr/). The climate at Paracou is classified as tropical, with an average temperature of 26°C and an annual rainfall of 3100 mm. The dry season occurs from mid-August to mid-November with usually less than 100 mm rainfall per month. Sampling was carried out within the footprint of the eddy flux tower, Guyaflux (Bonal *et al.* 2008), and within old-growth permanent forest plots, where all trees above 10 cm in diameter at breast height were identified and spatially localized. More than 590 species and subspecies have been identified at the Paracou field station (Derroire *et al*. 2022).

Species and leaf sampling

Eight species with contrasting leaf phenological strategies were selected. For each species, two to three individual trees whose crown reached the canopy top and could be delineated and identified within aerial RGB images were selected (Ball *et al.* 2023). Each of the 22 selected canopy trees were sampled several times during the sampling period. Each time, the tree was climbed between ca 9 am and 1 pm using the single-rope technique (Anderson *et al.* 2015) and a top canopy branch exposed to the sun was cut by the climbers and sent to the ground. The branches were then recut under water and exposed to full light in an open area for leaf gas exchange measurements. For each branch, gas exchange measurements were performed on one to three leaves per cohort of leaves. Leaf cohorts were identified by delineating growth units along the axes using morpho-anatomical markers (such as cataphyll scars, series of short internodes, reduced leaf size, acrotonic branching; refer to Nicolini *et al.* 2012 for an example).

Gas exchange measurements

Gas exchange measurements were made with a LICOR 6800 (LI-COR, Lincoln, NE, USA) equipped with a 6 cm^2 leaf chamber (6800-01A). Curves of net CO_2 assimilation (A) response to intercellular CO_2 concentration (C_i) were built using the Dynamic Assimilation Technique (DAT) protocol (Saathoff & Welles 2021; Tejera-Nieves *et al.* 2024). Each day, we followed the standard warm-up procedure and verifications prior to measurements, and matched the IRGAs between two consecutive curves (Busch *et al.* 2024). Measurements were performed between ca 9:30 am and 2 pm.

The incident leaf irradiance inside the chamber was set at 1200 µmol m^{-2} s^{-1}. The air temperature inside the chamber was set close to the ambient air temperature, typically between 27°C and 32°C. The initial CO_2 concentration of the air entering the chamber was set at 415 ppm with a flow rate of generally 650 µmol s^{-1}, except for some species (e.g. *Pouteria eugenifolia*), which typically required a lower flow rate of 500 µmol s^{-1}. We waited a minimum of 10 minutes, and longer if needed to reach a steady state for the photosynthesis rate and conductance before launching the DAT. The DAT protocol started from a CO_2 concentration of 10 µmol CO_2 mol^{-1} with a CO_2 concentration ramp speed of 100 µmol CO2 mol^{-1} min^{-1}.

Leaf optical properties

Leaf reflectance and transmittance were measured in the lab after a full night of branch rehydration in water and under opaque plastic bags. We used a Jaz handheld spectrometer with a SpectroClip-TR probe with reflectance and transmittance integrating spheres (Ocean Optics Inc., Dunedin, FL, USA). The active illuminated area of the SpectroClip was 5 mm in diameter. A Tungsten-halogen light source provided a spectral range of 400 to 1100 nm with a 0.46 nm spectral resolution. A WS-1 diffuse reflectance standard, with reflectivity >98%, was used as white reference (Ocean Optics, Dunedin, FL, USA).

Leaf mass per area and elemental composition

Once scanned, the leaves were dried at 65°C for at least three days. Finally, their dry mass was measured with a digital scale at a 0.0001 g precision (Mettler Toledo, Switzerland) and their leaf mass per surface area (LMA) was calculated using the fresh leaf area (Epson perfection V800; Epson America Inc., Long Beach, CA, USA) and its dry mass. Leaf elemental composition was measured by the SILVATECH lab (Nancy, France) with the Unicube elemental analyzer (Elementar, Langenselbold, Germany).

Leaf phenological stage

RGB drone imagery acquired every 15 days from October 2020 using UAV allowed us to monitor changes in crown status of canopy trees over time, and in particular to document phenological events such as leaf shedding and flushing (bud burst and crown greening). Using this information, we were able to estimate leaf age based on their position within growth units along the axes. For each species, we plotted LMA as a function of leaf age, and classify leaves younger than the age at which the increase of LMA slows down as “young”. By default, leaves classified as "old" are those located on the penultimate growth unit, which is generally formed during the previous year (except in cases of polycyclism where multiple growth units elongate within the same year). Leaves referred to as “mature” are those in between.

Acknowledgments

This work was supported by funding from ANR (the French National Research Agency) under the "Investissements d’avenir" program with the references ANR-16-IDEX-0006, ANR-10-LABX-25-01 (Labex CEBA, project Phenobs). We thank SILVATECH (Silvatech, INRAE, 2018. Structural and functional analysis of tree and wood Facility, doi: 10.15454/1.5572400113627854E12) from UMR 1434 SILVA, 1136 IAM, 1138 BEF and 4370 EA LERMAB from the research center INRAE Grand-Est Nancy for its contribution to leaf elemental composition measurements. SILVATECH facility is supported by the French National Research Agency through the Laboratory of Excellence ARBRE (ANR-11-LABX-0002-01). We also thank Jocelyn Cazal, Alpha, Joryan Octavie for support in climbing trees, Louise Bonhomme for assistance in measurements in the field and the lab, Grégoire Vincent and James Ball for substantial contribution to the UAV-based phenological monitoring and selection of sampled individuals. We thank Geraldine Derroire and Laetitia Proux for permission to use the facilities at Paracou. Paracou ForestResearch Station in French Guiana is managed and supported by CIRAD, UMR EcoFoG (https://paracou.cirad.fr/).

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