# Methodological Details for GSTI Dataset Kyaw et al. (2024)

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## Overview

- This dataset includes much of the leaf spectral and leaf morphological data used for our paper published in *Remote Sensing* (Kyaw et al. 2024) but also includes some data that we did not report; gas exchange parameters being the most notable.
- Our study sought to determine how well spectral data (in combination with air temperature measurements) can be used to diagnose heat stress in trees that are abundant in eastern North American cities.
- Our site was the campus of the University of Maryland – Baltimore County, which we selected because it provided access to regionally important tree species, with each experiencing a range in the environmental conditions common in cities (e.g., the amount of paved surface, turf, etc. surrounding trees).
- The study included nine tree species, with six individuals for all species but *Quercus rubra*, for which there were two (Table 1 in Kyaw et al. 2024, $n$ = 51 trees in total).

## Field sampling approach

- Monthly field campaigns took place from July - October 2022, each spanning 2-3 days. Almost all trees were visited once per campaign ($n$ = 202 visits in total).
- Tree visits occurred during afternoon hours to maximize the heat stress experienced by trees. Visits entailed cutting a branch segment (usually 40-50 cm long) from the sun-exposed side of a tree's crown, submerging the branch's cut end in water, recutting, and swiftly transporting the branch segment to a central location.
- Leaf spectral reflectance and leaf physiological parameters were then measured immediately using leaves that were similar with respect to size, developmental stage, etc. Methodological details are below, though they focus on gas exchange measurements. Additional details on spectral reflectance measurements can be found in Kyaw et al. (2024).
- Spectral reflectance was typically measured from each of three selected leaves using an SVC HR-1024i with an LC-RP Pro leaf clip (Spectra Vista Corporation, Poughkeepsie, NY, USA). The number of leaves measured sometimes varied and multiple leaves sometimes had to be placed next to each other to cover the field of view. A white reference measurement was made with the LC-RP Pro's spectralon panel prior to measurements from each tree. Averaged spectral reflectance curves were exported from the instrument, and these averages therefore appear in our dataset.

- An additional leaf from the branch segment was weighed fresh then sealed in a plastic bag. Leaf area and dry mass were later measured, making it possible to determine leaf water content (LWC) and leaf mass per area (LMA).
- An LI-6800 (LI-COR Biosciences, Lincoln, NE, USA) was used to measure several gas exchange and fluorescence parameters simultaneous with spectral reflectance. This was done with an additional/analogous leaf that remained attached to the branch segment. Measurements included net carbon assimilation ($A_{net}$), transpiration (E), and stomatal conductance to water ($g_{sw}$). Note that all of these are used to calculate the intercellular $CO_2$ concentration ($C_i$).

## Details of physiological measurements

- We attempted to record A-$C_i$ curves throughout the July campaign and initially during the August campaign. We did this using the dynamic assimilation technique (Saathoff and Welles 2021), but the approach was only intermittently successful (details to follow). We therefore switched to recording a single set of values (i.e., "survey" measurements) using the standard steady-state approach.
- A multi-phase flash was given to each measured leaf to induce fluorescence (and thereby determine electron transport rate, qP, etc.). Whether the gas exchange procedure entailed recording A-$C_i$ curves or survey measurements, fluorescence pulses were made with reference $CO_2$ ($CO_{2R}$) at 400 ppm. This yielded extraneous gas exchange data points in A-$C_i$ curves that were later removed (details below).
- For the sake of consistency and parsimony, we opted to submit single pairs of $A_{net}$ and $C_i$ values from each tree visit to the GSTI database, even when A-$C_i$ curves were successful. This way the single point method could be used to determine $V_{cmax}$ in all cases.
- Key settings on the LI-6800 were as follows:
    - $CO_{2R}$ (for survey measurements): 400 ppm
    - $T_{leaf}$: 35° C in July and August, 25° C in September, 20° C in October
    - PPFD: 2000 μmol $m^{-2}$ $s^{-1}$
    - Flow: 700 μmol $s^{-1}$
    - $VPD_{leaf}$: 1.5 kPa (note that this was often not achieved)
- Plots of $A_{net}$ vs. $C_i$ were of highly variable quality, with low quality typically manifesting as curves that did not increase monotonically. However, plots of $A_{net}$ vs. $CO_{2R}$ did increase monotonically even in these cases, and otherwise appeared as expected. Given that computations of $C_i$ are strongly influenced by $g_{sw}$ and E (equations provided here), we took the discrepancy to mean that problems measuring water loss (E and $g_{sw}$) had frequently rendered $C_i$ estimates inaccurate. Later measurements of E and $g_{sw}$ (during survey measurements) confirmed that they were often much less stable than net carbon assimilation ($A_{net}$) was.

- We attribute the challenges of measuring water loss primarily to the high heat (~35° C) and low humidity (<50%) of the ambient environment; leaf vapor pressure deficits often exceeded 3 kPa. Regardless, recorded values of E and $g_{sw}$ were sometimes negative or, when they were positive but unrealistically small, $C_i$ was unrealistically large (>350 ppm).
- Although we measured dark respiration ($R_d$) only intermittently during July and August campaigns, we measured it consistently during September and October campaigns. Our GSTI dataset therefore contains many more data points from those later months.
- $R_d$ was measured after turning off the light source and waiting for $A_{net}$ to achieve steady state, which usually took 2-3 min.

## Extraction of best-available gas exchange data

- Here we describe the procedures used to identify and extract reliable values of $A_{net}$ and $C_i$ for use in single point estimates of $V_{cmax}$. They were applied to the raw data using a script in R 4.2.2 (R Project for Statistical Computing, Vienna, Austria) for maximal consistency.
- In cases where dynamic A-$C_i$ curves had been attempted, we first isolated the data with $CO_{2R}$ near 400 ppm (specifically 350-450 ppm). As a precaution, we further required that records had PPFD (a.k.a. $Q_{in}$) > 1990, $A_{net}$ > 0, and $C_i$ > 0. In the majority of cases, this left 10-15 records that followed an increasing trend, but with ~4-6 outliers. In a minority of cases, no trend was apparent. Subsequent steps were intended to isolate data that followed the increasing trend (if any) and to remove outliers.
- Records clearly influenced by fluorescence pulses typically had some $A_{net}$ values far greater or smaller than those measured during the $CO_2$ ramp of the A-$C_i$ curve process (i.e., gross outliers). Because these outliers occurred when $CO_{2R}$ was very close to 400 ppm, we removed all records with $A_{net}$ below those measured at minimal $CO_{2R}$ (~350 ppm) or greater than those measured at maximal $CO_{2R}$ (~450 ppm).
- We next ran a linear regression (of $A_{net}$ vs. $C_i$) and identified modest outliers, specifically those with standardized residuals > 2. We then refitted the regression. If the final slope was positive and $R^2$ > 0.9, we retained data from the curve. Otherwise, data from that tree visit were excluded from the final dataset (this was the majority).
- Means of parameter values from remaining data records were compiled into a single dataset, along with those measured at steady state.
- Records were filtered such that only those meeting the following criteria were retained:
    - $A_{net}$ > 0 (except for measurements of $R_d$)
    - E and $g_{sw}$ > 0
    - $C_i$ < 300 and > 100 ppm
    - $VPD_{leaf}$ < 4 kPa

## Final notes

- The final gas exchange dataset contains 94 data rows, 17 of which come from A-$C_i$ curves and 77 of which come from survey measurements. There are also 65 measurements of $R_d$.
- We conducted additional campaigns in 2023. Although a dataset for 2023 has not yet been compiled for GSTI (as of December 2024), it followed nearly identical methods (though only survey measurements were made for gas exchange) and focused on the same set of trees.

## References

Kyaw, T. Y., Alonzo, M., Baker, M. E., Eisenman, S. W., & Caplan, J. S. (2024). Predicting urban trees' functional trait responses to heat using reflectance spectroscopy. *Remote Sensing*, *16*(13), 2291. https://doi.org/10.3390/rs16132291

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