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Protocol for leaf-level gas exchange measurement for photosynthesis model calibration V.1

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Abstract

Reliable leaf-level measurements of photosynthesis are critical for calibrating biochemical and empirical models of carbon assimilation, which support crop simulation, land surface modeling, phenomics, and ecophysiology. In turn, these models help to inform predictions across scales including seasonal crop yields and decadal atmospheric CO_2 trends. However, available data and published model parameters for analysis and forward prediction remain sparse and inconsistent (Rogers, 2014), and a detailed, standardized protocol focused on efficient gas exchange data collection for photosynthetic model calibration is absent. Establishing such a protocol could accelerate progress in both climate modeling (Rogers et al., 2017) and crop improvement (Bailey-Serres et al., 2019) by providing a consistent foundation for model calibration and comparison.

The most widely used model of C_3 photosynthesis is the Farquhar, von Caemmerer, Berry (FvCB) biochemical model (Farquhar et al., 1980), often extended with a triose-phosphate utilization (TPU) rate limiting state (Harley and Sharkey, 1991), peaked temperature responses (Bernacchi et al., 2003; Medlyn et al., 2002), and a more flexible light response function (Farquhar and Wong, 1984). The model predicts the net carbon assimilation rate of photosynthesis (A) as a function of internal CO_2 concentration (C_i), absorbed photosynthetically active radiation (Q), and leaf temperature (T_ℓ), parameterized with dozens of genotype- and environment-dependent parameters. This model and its numerous variants have been widely applied across the plant sciences, from molecular biology to agricultural decision support (e.g., Feyissa et al., 2019; Muller and Martre, 2019), and integrated into large Earth systems models (Rogers et al., 2017) used to investigate future climate scenarios. A major bottleneck in model utilization is obtaining data of sufficient quality and quantity for robust model calibration for a given set of plants in question.

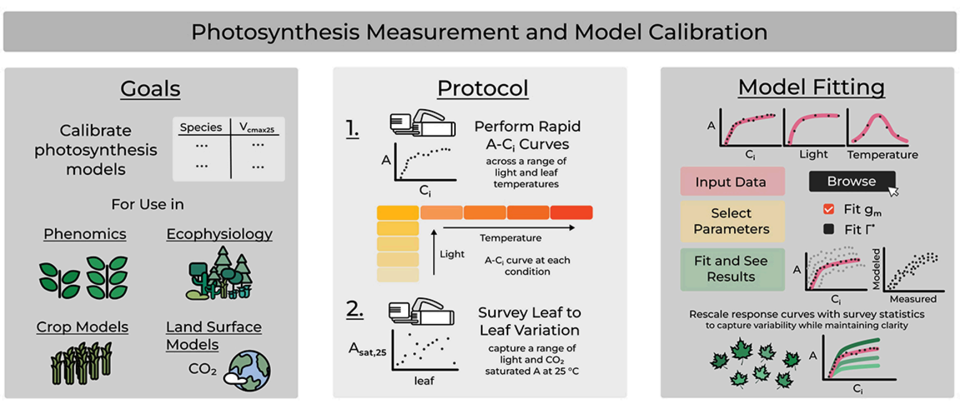
For decades, portable gas exchange systems equipped with infrared gas analyzers (IRGAs) have enabled precise quantification of CO2 and H2O fluxes at the leaf level (Busch et al., 2024). These gas exchange measurements paired with those of environmental conditions provide the means for deriving key photosynthetic parameters. However, many experimental designs used throughout the literature to obtain environmental response curve data vary in approach and often fall short of supporting full photosynthetic model parameterization (Rogers et al., 2017). Furthermore, calibrated parameters are highly sensitive to fitting techniques (Long and Bernacchi, 2003; Gu et al., 2010) and model formulations (Lochocki and McGrath, 2025). These challenges result in published parameter sets that can be incomplete, inconsistent, and incomparable across studies.

Traditional approaches for estimating photosynthetic parameters often perform steady-state responses ofAA to C_i (known colloquially as “A-C_i” curves), as well as to light ($A-Q$) and temperature ($A-T$). Measurements are time consuming, and each environmental response curve is typically generated independently and fit independently instead of together, as one unified set of measurements for model parameter extraction. Recent advances in the speed of A-C_i curve measurement (Saathoff and Welles, 2021; Tejera-Nieves et al., 2024) and in robust FvCB model fitting (Lei et al., 2025), have increased the throughput of reliable model calibration.

Yet another challenge in obtaining data for calibrating photosynthetic models is the trade-off between measurement resolution and canopy representation. Photosynthetic parameters can vary substantially from leaf to leaf, often driven by differences in age and nutrient content – particularly nitrogen and phosphorous – linked to

position in the canopy (Field, 1983; Wilson et al., 2000; Schultz, 2003; Walker et al., 2014; Bloomfield et al., 2018). However, conducting the full suite of photosynthetic response curves across CO_2, light, and temperature, at a sufficient resolution to extract key traits, such as the temperature optimum of photosynthesis, is time intensive, rendering comprehensive canopy coverage impractical. An alternative approach is to conduct point-wise survey measurements across many leaves, leveraging ambient environmental variation to generate canopy-scale “response curves”. While more sample efficient, this approach introduces substantial noise that obscures the nonlinear shape of physiological responses, complicating parameter estimation and interpretation within models based on mechanistic, leaf-level processes.

To address these challenges, we present a semi-automated protocol for measuring leaf-level gas exchange for photosynthetic model calibration that attempts to balance quality and efficiency. The protocol utilizes rapid A-C_i response curves based on the Dynamic Assimilation™ Technique of the LI-6800 Portable Photosynthesis System (LI-COR Biosciences, Lincoln, NE, USA) across a range of light and temperature values, as well as survey measurements to capture leaf-to-leaf variation in the magnitude of Aunder saturating conditions. FvCB model calibration is demonstrated using a drag-and-drop user interface that provides simple parameter extraction and quick result visualization. Approximately 60% of the protocol can be automated with scripts that set instrument cuvette conditions, run the response curves, and log the data. The end-to-end solution extracts 24 possible FvCB parameters with a single portable gas exchange system in 2.5 hours. By standardizing and automating response curve measurements and streamlining model calibration for users of all technical ability, this protocol provides a means for reliable, reproducible, and efficient extraction of photosynthetic parameter values to support the growing demand in the photosynthesis modeling community.



Schematic depiction of a protocol for calibrating leaf-level models of photosynthetic assimilation (A) with respect to internal CO_2 concentration (C_i), absorbed light flux, and leaf temperature. Rapid A-C_i curves are performed on one or two leaves across different temperatures and light levels. Survey measurements at saturating light and CO_2 and at a common reference temperature are then taken to capture the natural leaf-to-leaf variability. The survey measurements are used to rescale the A-C_i curves performed on a single consistent leaf to better reflect the average of the canopy. Model fitting is performed using PhoTorch Studio, a graphical user interface for the PhoTorch fitting package (Lei et al., 2025).

Attachments

PhotosynthesisProtoc...

1.2MB

Materials

Specific instrument settings described in these protocols are for the LI-COR instruments listed below. Adaptations may be needed in order to use instruments from other manufacturers.

- LI-6800 Portable Photosynthesis System (LI-COR Biosciences, Lincoln, NE, USA)

List of optional software:

- LI-6800 Background Programs for automated measurements of light and temperature sweeps (**https://github.com/PlantSimulationLab/LI6800-automated-scripts)**
- PhoTorch (**https://github.com/GEMINI-Breeding/photorch)**, for advanced users looking to automate workflows of fitting large datasets with Python
- PhoTorch Studio (**https://github.com/ktrizzo/photorch-studio)**, for simple drag-and-drop fitting with a graphical user interface

Materials and conditions

1 **Ambient Conditions Checklist**

These protocols are intended to be applied on plants in the field, and certain ambient weather conditions will limit their feasibility. Model calibration will be most effective with ambient light reaching 2000 µmol m^{-2} s^{-1} (or the typical maximum diurnal light intensity of a specific location) and leaf vapor pressure difference ranges of at least 10-30 mmol mol^{-1}. This can most consistently be achieved on a day with:

1. Full sun and no or minimal cloud coverage
2. Minimal wind
3. A daily high air temperature of at least 18°C (65°F)

A rule of thumb to keep in mind is the LI-6800 chamber conditions can achieve roughly ±10°C from the console temperature, and console temperature can reach 5-15°C above ambient air temperature when heated in full sun.

Preferred conditions for performing this protocol typically occur between mid-morning and solar noon, when gas exchange rates are naturally near a peak, water potentials are not too low, and a range of cuvette temperatures can be reached.

This protocol could be adapted to perform measurements in the laboratory, but this will likely limit the range of possible environmental conditions. In this case, it would be desirable to measure plants growing in the brightest light and warmest air temperature possible.

2 **Plant Material**

This protocol was developed to be used on intact, fully mature, healthy leaves of broadleaf plant species. The underlying photosynthetic parameters extracted from the data are expected to exhibit seasonal, phenological, and interannual change, so a modeler should be aware of when calibration data was collected and for what period in time model parameters will be used in simulation or for comparison.

3 **Software**

The protocol can be semi-automated with Background Programs written for the LI-6800. Three programs are provided, one that carries out the light sweep, one that carries out the temperature sweep, and one that carries out light sweep followed by the temperature sweep. The protocol can also be applied without the automation script by manually varying each environmental setpoint and running the CO_2 response program already included in the instrument firmware.

In addition to the data collection, we also discuss parameter calibration based on PhoTorch (Lei et al. 2025).

List of optional software:
- LI-6800 Background Programs for automated measurements of light and temperature sweeps (**https://github.com/PlantSimulationLab/LI6800-automated-scripts)**
- PhoTorch (**https://github.com/GEMINI-Breeding/photorch)**, for advanced users looking to automate workflows of fitting large datasets with Python
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Initial Instrument Set-up

30m

4 **LI-6800 initial setup**

Prior to use in respective protocols, the chosen instruments will need to be set up, typically once per day or per measurement session. The set-up procedure described is not meant to be exhaustive and represents only typical set-up tasks performed before each measurement session. Users should consult manufacturer documentation for a complete guide on instrument set-up.

4.1 Connect head (hand-held unit) via cable and tube.

4.2 Check the head has correct aperture size installed. The largest aperture should be used that can be consistently covered entirely by sampled leaves.

4.3 Connect H_2O add column, and ensure there is plenty of water in the column.

4.4 Install new CO_2 cartridge.

4.5 Check the desiccant level. If there is less than 1/2 orange Sorbead® (or blue Drierite®) left, replace it. Note that it may be possible to replace only the half of the column that has lost its color.

4.6 Remove red cover from light sensor.

4.7 Power on instrument.

4.8 Aperture size: On the *'Start up'* →*'Chamber Setup'* tab, click the button for the appropriate aperture size that was installed in the previous step above.

4.9 Dynamic Equations: On the *'Start up'* → *'Chamber Setup'* tab, ensure *'Add Dynamic Equations'* is checked.

4.10 Clock Synchronization: On the *'Start up'* →*'System Setting'* →*'Date Time & Clocks'*, press *'Sync Head Clock'*.

4.11 Then, on the *'Constants'* →*'Gas Exchange'* tab, check *'UseDynamic'*. (If the A-Ci curves look very off, not having this box checked could be the issue; this can be fixed post-facto by changing *'UseDynamic'* from **FALSE** to **TRUE** in the exported .xlsx file.)

5 **LI-6800 Calibration**

5.1 Table 1. Close the chamber and leave it empty. Go to the 'Environment' tab and set the environmental
parameters in the table below.

	Group	Parameter	Value
	Flow	Flow	Auto - 500 µmol/mol
		Overpressure	Auto - 0.1 kPa
		Pump (speed)	Auto
	H2O	RH_air	50%
	CO2	CO2_r	400 µmol/mol
		Soda Lime - Scrub	Auto
	Fan	Fan Speed	10,000 rpm
	Temperature	Temperature	Off

	Group	Parameter	Value
	Light	Head Light Source Setpoint	2,000 µmol/m²/s

Table 1. Instrument settings for LI-6800 (LI-COR Biosciences, Lincoln, NE, USA) to be used for initial setup and daily calibrations.

5.2 Perform CO_2 and H_2O point matching: on the '*Measurements*' tab, click '*Match IRGAs*'. In the upper right, click '*Auto*' under '*Matching at a single reference point*'. When both the CO_2 and H_2O lights turn green simultaneously, matching is complete. Double-check the '*Time Since Last Match*' field to verify success.

5.3 Perform CO_2 and H_2O range matching: '*Constants*' →'*Range Match*'. Click '*CO2 Range Match*' → '*Start*'. Select the Normal (5 min) match and set Flow s/Flow r = 1.13. Repeat for H_2O, selecting the Quick (3 min) match.

Note

Range matches are temperature sensitive and should be repeated between subsequent completions of the protocol in a field setting, or any time the ambient environment changes substantially.

5.4 Perform CO_2 and H_2O dynamic tuning: '*Constants*' →'*Dynamic*'. Ensure CO_2 and H_2O control are on. '*Utilities/Tests*' tab →choose '*CO2 Test Current*' →'*Start*'. After 2 min, select '*Yes*' to store. Repeat for '*H2O Test Current*'.

A-Ci Curve Light/Temperature Response

1h 30m

6 For each sample temperature and light combination in Table 3, collect an A-C_i curve measurement. Instructions are first given for manual setting of chamber conditions for temperature and light sweeps, followed by the procedure when using automated scripts to sweep through environmental conditions.

Note

Highly recommended: In the '*Measurements*' tab, graph A versus C_i to observe each A-C_i curve in real time. Curves should appear as increasing hyperbolas that saturate at high CO_2 (e.g., Fig. 2); deviations may indicate instrument or leaf issues.

Light (μmol m^{-2} s^{-1})	Air temperature (°C) 25	30	35	38	40	43
2000	1/7/S	8	9	10	11	12
1600	2					
1200	3					
600	4					
200	5					
0	6					

Table 3. A-C_i response curve light and temperature combinations. Each numbered entry represents an A-C_i curve measurement, with its position on the grid denoting the light-temperature setting. Measurements 1-6 (colored in red) are performed on the same leaf, and 7-12 (colored in blue) performed on a different second leaf. Maximum achievable temperatures will depend on ambient conditions. '**S**' denotes the survey measurements, which should each be performed on a different leaf.

7 Procedure for Manual Control of Light/Temperature Sweeps

This procedure option outlines how to collect measurements listed in Table 3 by manually setting each light/temperature combination. This option is slower than the automated option (Section 8) because you must manually monitor the instrument(s) and adjust the light and temperature values in Table 3 for each A-C_i curve.

7.1 **(Manual Option)** Choose a fully-expanded, outer-canopy sunlit leaf that is large and appears healthy. Clamp the chamber onto the leaf, ensuring it covers the entire aperture.

7.2 **(Manual Option)** Create a folder with the crop name and any desired identifiers.

7.3 **(Manual Option)** Open a log file: *'Log Setup'* → *'Open a Log File'*. Use the naming convention: **{YYYY-MM-DD}_{time}_Q{light}_T{temperature}**.

7.4 **(Manual Option)** Set the appropriate air temperature: *'Environment'* → *'Temperature'*. Use Tair = 25, 30, 35°C, etc. If ambient air temperature is less than 30°C (86°F), cuvette temperatures greater than 35°C may be difficult to achieve; use the highest achievable temperature.

7.5 **(Manual Option)** Set the light flux set-point: *'Environment'* → *'Light'* → *'Head Light Source'*. Use 2000, 1600, 1200 μmol m^{-2} s^{-1}, etc.

7.6 **(Manual Option)** Wait until all environmental set-points have been reached (green **7/7** badge on the '*Environment*' tab).

7.7 **(Manual Option)** Run a rapid A-Ci curve: '*Programs*' → '*BP Builder*'. Browse to **dynamic/DAT CO2 continuous.py**, select it, and click '*Start BP*'. Use the parameters in Table 4.

	Parameter	Value
	Starting CO2	200 µmol/mol
	Pre-ramp Wait	1 min
	Ending CO2	2,000 µmol/mol
	Ramp rate	300 µmol/mol
	Logging interval	3 sec

Table 4. BP Builder parameters for rapid A-C_i program

7.8 Select '*Continue*' to run the program.
The status '*1 BP Active*' appears in the upper right. After a 1-min pre-ramp wait, the CO_2 ramp begins;
monitor on the '*BP Monitor*' tab.

When the BP is complete, close (saving) the log file, change light and/or temperature to the next
combination, wait for the environment to reach all **7/7** set-points, open a new log file, and repeat.
If using the automated scripts instead of the above manual option, perform the following after selecting
and clamping onto a leaf as described above.

8 **Procedure for Automated Control of Light/Temperature Sweeps**

This procedure option outlines how to collect measurements listed in Table 3 by automatically sweeping through each light/temperature combination using a Python script. This option is faster than the manual option (Section 7) because the script automatically adjusts the light and temperature values in Table 3 for each A-C_i curve.

Note

To use the automated Background Program scripts, first transfer them into the LI-6800's BP Directory.

- Download the **.py** script files (e.g., **ACi_Light_Sweep.py**) from the **automated-scripts repository** above, and transfer to a USB thumb-drive.
- Connect the LI-6800 head to the console (file access will not be available otherwise) and turn the machine on. Plug the USB thumb-drive into the LI-6800.
- Navigate to the LI-6800 file browser under '*Tools*' → '*Manage Files*'. Select '*Copy files to LI-6800*', and under the '*Copy to*' drop-down, select '*BP Directory*'. Find the protocol **.py** files, select them and press '*Copy*' to transfer the files to the device. Safely eject the USB with '*Eject*'.
- (**Important**) Add user variables under the '*Constants*' → '*User*' tab. Press '*Add*' followed by '*Edit*' and rename the variable **CurveID**. Make another variable and rename it **Response**. This step is necessary to log curve ID's throughout the protocol.

8.1 **(Automated Option)** Choose a fully-expanded, outer-canopy sunlit leaf that is large and appears healthy. Clamp the chamber onto the leaf, ensuring it covers the entire aperture.

8.2 **(Automated Option)** Open a log file: *'Log Setup'* → *'Open a Log File'*. Use the naming convention: **{YYYY-MM-DD}_{time}_light** followed by a unique leaf identifier if measuring more than one leaf in this folder.

8.3 **(Automated Option)** Run the light-sweep Background Program in *'Programs'* → *'BP Builder'*. Browse to the location of the downloaded program **ACi_Light_Sweep.py**, select it, and press *'Start BP'*. Use the default settings. Allow the program to run for 40 minutes. Close the log file.

8.4 **(Automated Option)** Open a log file: *'Log Setup'* → *'Open a Log File'*. Use the naming convention: **{YYYY-MM-DD}_{time}_temperature** followed by a unique leaf identifier if measuring more than one leaf in this folder.

8.5 **(Automated Option)** Run the temperature-sweep Background Program in *'Programs'* → *'BP Builder'*. Browse to the location of the downloaded program **ACi_Temperature_Sweep.py**, select it, and press *'Start BP'*. Use the default settings. Allow the program to run for 50 minutes. Close the log file.

Survey Measurement

30m

9 This section outlines collection of "survey" measurements to capture leaf-to-leaf variability in photosynthetic capacity.

Before starting survey measurements, complete the following steps.

9.1 Perform CO2 and H2O point matching as described above in Sect. 5.2.

9.2 Open a log file: '*Log Setup*' → '*Open a Log File*'. Name it **{YYYY-MM-DD}_{time}_A_survey** and
save it in the same folder as the A-C_i curves.

10 Randomly select 15–25 healthy, sunlit, fully-expanded leaves (as time allows) and collect one measurement per leaf following the steps below:

10.1 Set air temperature to Tair = 25°C.

10.2 Set light flux set-point to 2000 µmol m^{-2} s^{-1} .

10.3 Ensure other environment values match Table 5.

10.4 Wait until all environmental set-points are met (green **7/7**).

10.5 Choose a healthy, mature, sunlit leaf and clamp the chamber.

10.6 Wait until the A reading stabilizes (less than 3% deviation from a mean value across 10 seconds; typically after 30 seconds - 2 minutes); record the measurement by pressing the analog '*Log*' button on the center of the head handle, or by pressing the digital '*Log*' button in the upper right corner of the console display. Confirmation of a successfully recorded log will be indicated with a chime from the instrument.

10.7 Move to the next leaf and repeat Steps 10.1-10.6

Data Analysis

11 **Re-scaling of Response Curves with Survey Statistics**

A complete sampling of A-Ci curves across several cuvette light and temperature conditions on a single leaf produces the most internally consistent and least error-prone dataset for photosynthetic model parameter extraction. However, leaves in the same plant canopy exhibit variability in photosynthetic parameters primarily due to different nitrogen content as a result of age or developmental plasticity (Niinemets et al., 2004; Xu et al., 2019). To address this issue, we use survey measurements at saturating light and CO_2 and at a reference temperature to re-scale the A-Ci curves using the median saturated A from the survey measurements (Fig. 2). Each set of response curves performed on a single leaf are re-scaled by a factor according to

$$A_{i,scaled} = A_i \cdot \frac{\text{median}(\mathbb{A}_{\text{sat},T_{\text{ref}},\text{survey}})}{A_{\text{sat},T_{\text{ref}},\text{response}}} ,$$

for each i^{th} data point of the same leaf, where $\mathbb{A}_{\text{sat},T_{\text{ref}},\text{survey}}$ is the set of all surveyed light- and CO_2-saturated A at a constant reference temperature, T_{ref} , of a sample of leaves, and $A_{\text{sat},T_{\text{ref}},\text{response}}$ is the saturated (maximum) value of A obtained in the A-C_i response curve at the same conditions as the survey measurements (Q_{in} = 2000 µmol m^{-2} s^{-1}, C_a = 2000 µmol mol^{-1}, $T_{air} = T_{\text{ref}}$). Put simply, each group of same-leaf A-C_i curves is shifted up or down so that the end of the $T_{\text{ref}} - Q_{2000}$ curve is aligned with the center of the survey data. T_{ref} used in the example data shown (Figs. 2, 4, and 5) is 25°C.

Note

This approach inherently assumes that leaf-to-leaf variation of photosynthetic responses within a species, cultivar, or genotype exists in the scale of the response, while the shape is conserved. Evidence demonstrates that shape is not entirely conserved (Ogren, 1993), but analysis of Koyama and Kikuzawa (2010) demonstrates that shape conservation is a good working approximation.

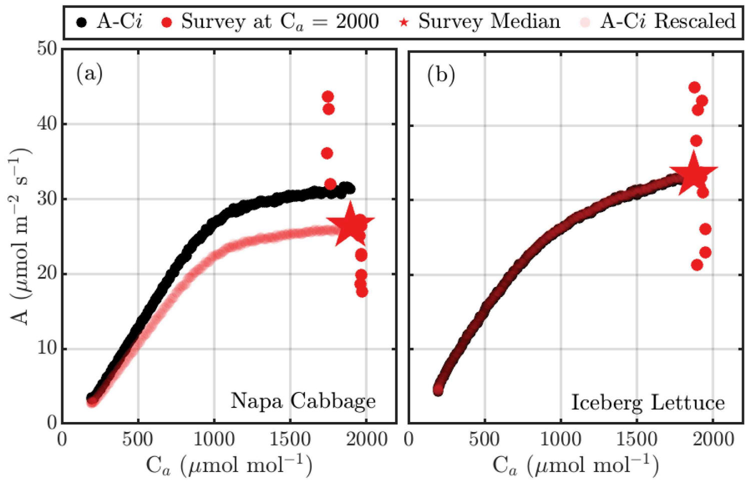

Figure 2. A-C_i curves (black points) of napa cabbage (*Brassica rapa* subsp. *pekinensis*) in (a) and iceberg lettuce (*Lactuca sativa* var. *capitata* 'Calmar') in (b) re-scaled using the approach described above with survey measurements (red points) of ~15 randomly sampled healthy, mature leaves at matching cuvette conditions to the A-C_i curves -- T_{air} set to 25°C, Q_{in} set to 2000 µmol m^{-2} s^{-1} -- and at CO2_r set to 2000 µmol mol^{-1}. The median of the survey measurements (red star) were used in the rescaling factor. The leaf chosen for the A-C_i curves in napa cabbage happened to be higher than the median, while the leaf chosen in iceberg lettuce happened to be nearly exactly at the median. Rescaling based on survey measurements helps alleviate the risk that a single leaf chosen for multiple A-C_i is not representative of the canopy median. All subsequent A-C_i curves performed on the same leaf are divided by the same factor.

12 **Model Fitting**

Fitting photosynthetic response curves to the FvCB model, or models of similar complexity, may be conceptually straightforward, but the task has proven unexpectedly challenging in practice (Long and Bernacchi, 2003; Gu et al., 2010; Lochocki and McGrath, 2025), leading to several independently developed fitting solutions. In the R programming language, there are several packages (Liu et al., 2021), including plantecophys (Duursma, 2015), msuRACiFit (Gregory et al., 2021), photosynthesis (Stinziano et al., 2021), PhotoGEA (Lochocki et al., 2025). In Python, there is PhoTorch (Lei et al., 2025). For this protocol, we demonstrate PhoTorch Studio (https://github.com/ktrizzo/photorch-studio), a browser-based user interface that packages the robust capabilities of PhoTorch into a simple point-and-click solution. The interface allows for quick drag-and-drop LI-6800 file uploading, seamless rescaling from survey measurements, simple toggling of fitting options including which parameters to keep constant, and plotting of the resulting fit with error metrics. Example results are shown in Figs. 4-6 and Table 6. See Lei et al. (2025) for the full FvCB model description and list of all parameters considered. Detailed instructions for PhoTorch and PhoTorch Studio can be found in their code repositories.

A key feature of this protocol is its ability to resolve the temperature optima of photosynthetic rates. Component rates of carboxylation, electron transport, and triose-phosphate utilization, V_{cmax}, J_{max}, and TPU, respectively, can exhibit peaked temperature responses of the form

$$k(T) = k_{\mathrm{ref}} \exp\left[\frac{\Delta H_a}{R}\left(\frac{1}{T_{\mathrm{ref}}} - \frac{1}{T}\right)\right] \frac{f(T_{\mathrm{ref}})}{f(T)},$$

$$f(T) = 1 + \exp\left[\frac{\Delta H_d}{R}\left(\frac{1}{T_{\mathrm{opt}}} - \frac{1}{T}\right) - \ln\left(\frac{\Delta H_d}{\Delta H_a} - 1\right)\right],$$

resulting in an emergent temperature optimum of photosynthesis (Fig. 5 that varies with both light and CO_2 (Fig. 7). This formulation has four parameters, the rate at a reference temperature k_{ref}, the rate's temperature optimum T_{opt}, the activation enthalpy ΔH_a, and the deactivation enthalpy ΔH_d; R is the ideal gas constant (0.008314 kJ mol^{-1} K^{-1}), and T_{ref} is typically taken to be 298.15 K (25°C). The six temperatures listed in the protocol were chosen to balance measurement time and confident extraction of these parameters, which facilitates modeling the effective photosynthetic temperature optimum (Fig. 7).

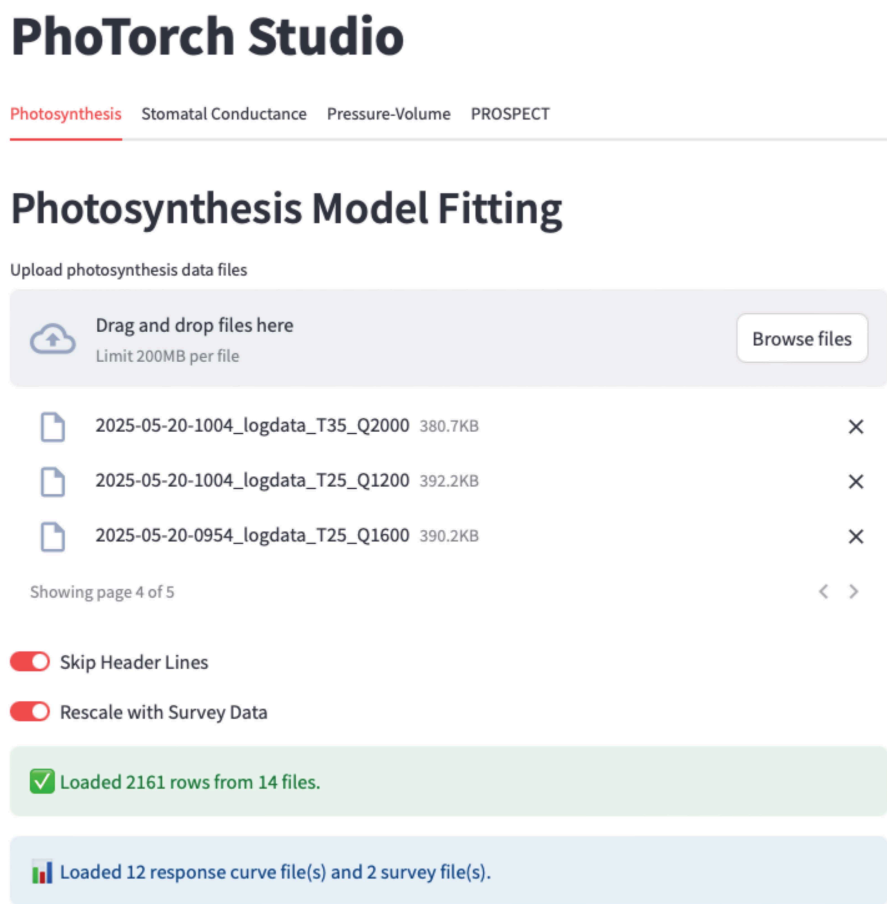

Figure 3. PhoTorch Studio, a graphical user interface for the Python-based PhoTorch plant model fitting package. Files directly from the LI-6800, or minimally pre-processed .xlsx or .csv files containing photosynthetic response curve data may be simply dragged and dropped into the app, and fit within seconds. Survey data files may also be included, and optionally used to re-scale response curves. After fitting, results are graphed similarly to Figs. 4-6.

Example Results

13

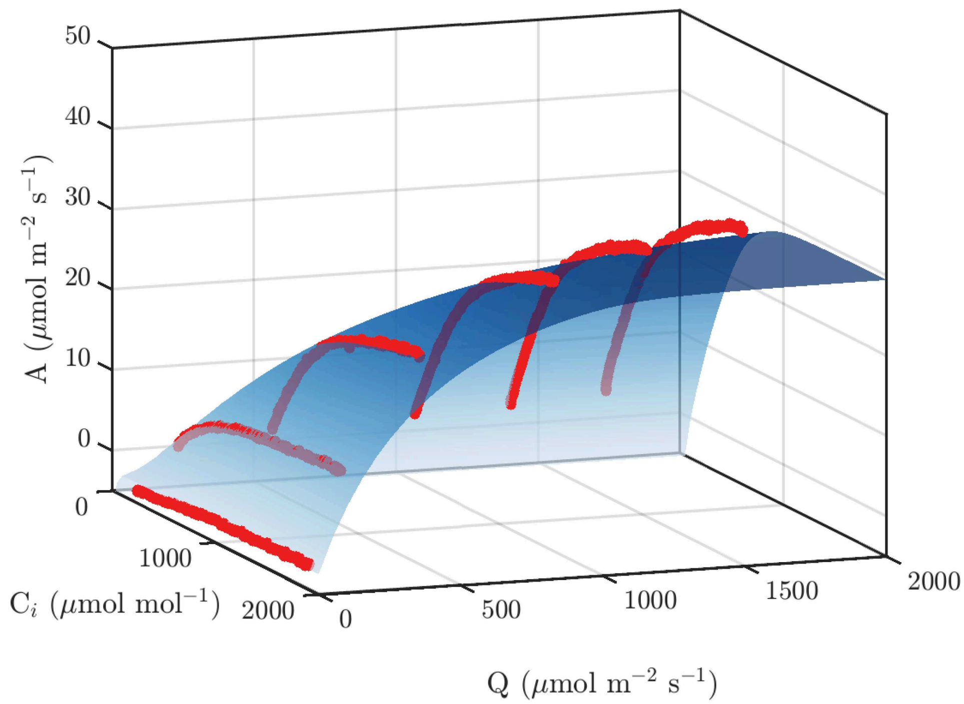

Figure 4. Measured A-C_i curves (red) performed with the Dynamic Assimilation^{TM} technique of the LI-6800 (LI-COR Biosciences, NE, USA) at different light intensities (Q), shown alongside the best fit Farquhar, von Caemmerer, Berry (1980) model surface (blue). The surface is characterized by one consistent set of parameters fit to a number of A-C_i curves at different light intensities and temperatures, and was fit to using the Python package PhoTorch (Lei et al., 2025). The coefficient of determination, R^2, of the model fit was 0.974.

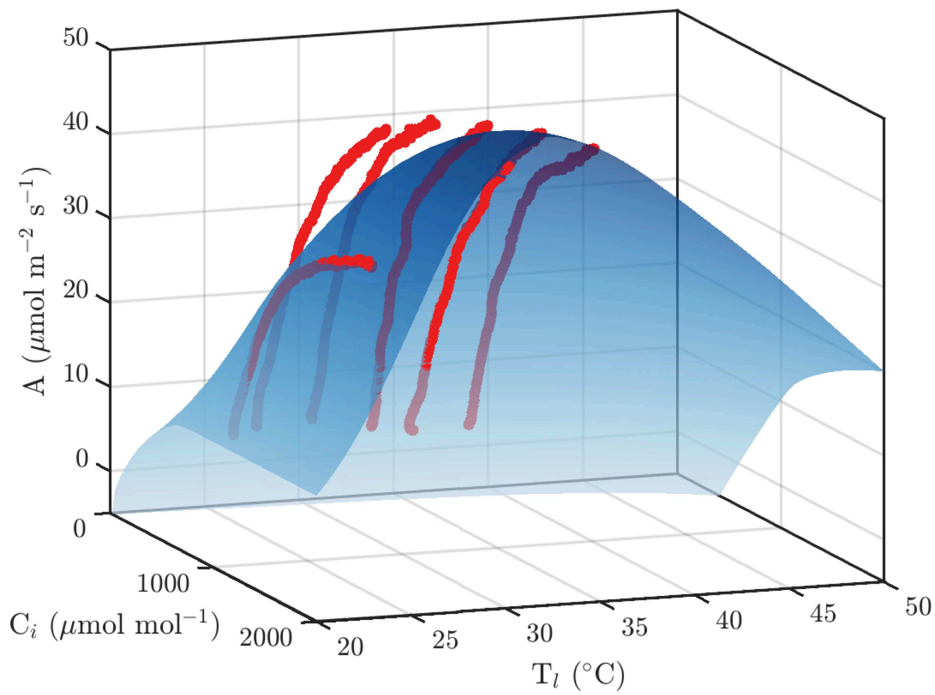

Figure 5: Measured A-C_i curves (red) performed with the Dynamic Assimilation™ technique of the LI-6800 (LI-COR Biosciences, NE, USA) at different leaf temperatures (T_ℓ), shown alongside the best fit Farquhar, von Caemmerer, Berry (1980) model surface (blue). The surface is characterized by one consistent set of parameters fit to a number of A-C_i curves at different light intensities and temperatures, and was fit to using the Python package PhoTorch (Lei et al., 2025). The coefficient of determination, R^2, of the model fit was 0.974.

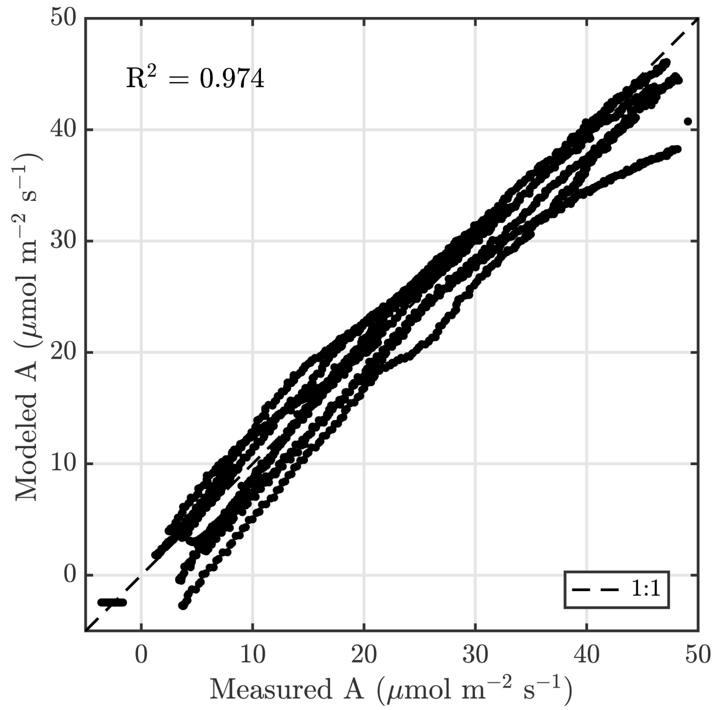

Figure 6. Measured versus modeled net assimilation (A). The measured data consists of A-C_i response curves performed with the Dynamic Assimilation^{TM} technique of the LI-6800 (LI-COR Biosciences, NE, USA) at different light intensities (Q) and leaf temperatures (T_ℓ). Modeled A was obtained by fitting all of the curves together to the Farquhar, von Caemmerer, Berry (1980) model for one set of parameters using the Python package PhoTorch (Lei et al., 2025), and then predicting A given the inputs of C_i, Q, T_ℓ, and the best fit parameters.

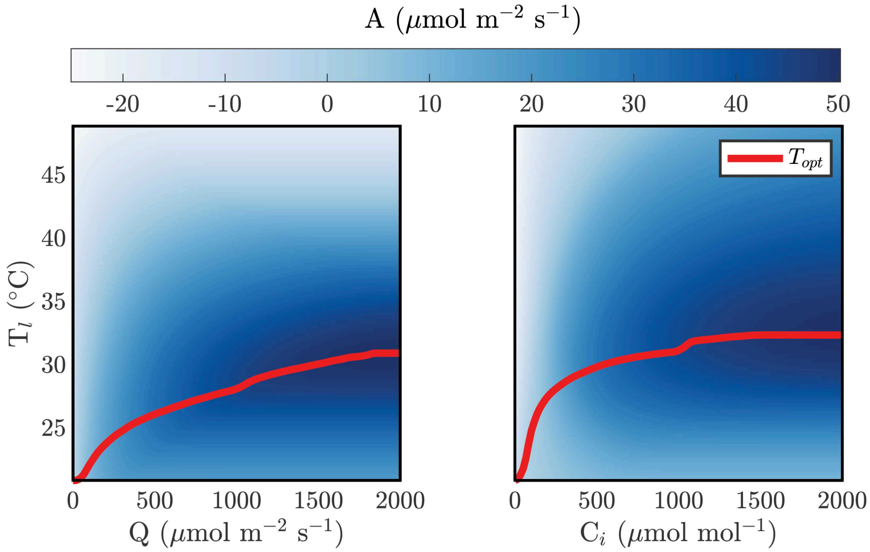

Figure 7. A demonstration of the photosynthetic temperature optimum (T_{opt}; red) as it varies across leaf temperature (T_ℓ), absorbed photosynthetically active radiation (Q), and internal CO_2 concentration (C_i). Modeled assimilation (A) shown with a colormap was produced using the best-fit Farquhar, von Caemmerer, Berry (1980) model parameters in Table 6, fit from the data in Figs 4 and 5.

Parameter	Value	Fit or Fixed	Description	Units
V_{cmax25}	77.07	fit	max carboxylation rate (Rubisco)	μmol m^{-2} s^{-1}
J_{max25}	191.7	fit	max electron transport rate (RuBP regeneration)	μmol m^{-2} s^{-1}
TPU_{25}	9.607	fit	triose-phosphate utilization rate	μmol m^{-2} s^{-1}
R_{d25}	2.442	fit	mitochondrial respiration rate	μmol m^{-2} s^{-1}
α	0.4365	fit	quantum efficiency (light response initial slope)	unitless
θ	0.1216	fit	light response curvature	–
$\Gamma^*{}_{25}$	72.86	fit	photorespiratory CO_2 compensation point	μmol mol^{-1}
K_{c25}	435.3	fit	Michaelis-Menten constant of Rubisco for CO_2	μmol mol^{-1}
K_{o25}	238.7	fit	Michaelis-Menten constant of Rubisco for O_2	mmol mol^{-1}
O	213.5	fixed	O_2 concentration	mmol mol^{-1}
g_{m}	0.4232	fit	mesophyll conductance	mol m^{-2} s^{-1}
α_g	0.000	fit	fraction of photorespiratory glycolate recycling	–
$T_{\text{opt},\text{V}_{\text{cmax}}}$	310.8	fit	optimum temperature of V_{cmax}	K
$T_{\text{opt},\text{J}_{\text{max}}}$	307.4	fit	optimum temperature of J_{max}	K
$T_{\text{opt,TPU}}$	305.5	fit	optimum temperature of TPU	K
$\Delta\text{H}_{\text{a},\text{V}_{\text{cmax}}}$	186.7	fit	activation enthalpy of V_{cmax}	kJ mol^{-1}
$\Delta\text{H}_{\text{a},\text{J}_{\text{max}}}$	167.0	fit	activation enthalpy of J_{max}	kJ mol^{-1}
$\Delta\text{H}_{\text{a,TPU}}$	202.5	fit	activation enthalpy of TPU	kJ mol^{-1}
$\Delta\text{H}_{\text{a},\text{R}_{\text{d}}}$	46.39	fit	activation enthalpy of R_{d}	kJ mol^{-1}
$\Delta\text{H}_{\text{a},\Gamma^*}$	37.83	fixed	activation enthalpy of Γ^*	kJ mol^{-1}
$\Delta\text{H}_{\text{a},\text{K}_{\text{c}}}$	79.43	fixed	activation enthalpy of K_{c}	kJ mol^{-1}
$\Delta\text{H}_{\text{a},\text{K}_{\text{o}}}$	36.38	fixed	activation enthalpy of K_{o}	kJ mol^{-1}
$\Delta\text{H}_{\text{d},\text{V}_{\text{cmax}}}$	223.9	fit	deactivation enthalpy of V_{cmax}	kJ mol^{-1}
$\Delta\text{H}_{\text{d},\text{J}_{\text{max}}}$	210.1	fit	deactivation enthalpy of J_{max}	kJ mol^{-1}
$\Delta\text{H}_{\text{d,TPU}}$	242.5	fit	deactivation enthalpy of TPU	kJ mol^{-1}

Table 6. Extracted Farquhar, von Caemmerer, Berry model parameters of data shown in Figs. 4 and 5, fit with PhoTorch, resulting in a model coefficient of determination, R^2, of 0.974 (Fig. 6). Input data consisted of 12 Dynamic Assimilation^{TM} Technique A-C_i (LI-6800, LI-COR Biosciences, NE, USA) response curves across a range of cuvette light setpoints from 0-2000 µmol m^{-2} s^{-1} and cuvette air temperatures from 25-40°C, comprised of 3232 total data points. Response curves varying light were performed on one leaf of an iceberg lettuce (*Lactuca sativa* var. *capitata* 'Calmar'), and curves varying temperature on another, and scaled using the median of survey measurements (Fig. 2).

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