Advancements in Gas Exchange Measurements: Introducing Dynamic Assimilation Technique for Non-Steady-State Assessments

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Introduction

- Traditionally, gas exchange measurements have relied on the steady-state method, assuming that both the instrument and biology reach a stable state and that assimilation by plants remains constant under specific environmental conditions.
- However, this method is problematic when studying transient responses or when high throughput is required.
- To address these limitations, LI-COR has developed the Dynamic Assimilation Technique (DAT), which allows gas exchange measurements under non-steady-state conditions.

Mass balance

$$A = \frac{u_e}{s}(1-W_r)\left(C_r - C_s + \frac{V\rho}{u_e}\frac{dC_s}{dt}\right) \quad (1)$$

$$A = \frac{u_e}{s}\left(C_r - Cs\,\frac{1-Wr}{1-Ws}\right) \quad (2)$$

A: CO_2 Assimilation (dry moles CO_2 m^{-2} s^{-1}); *s*: leaf area (m^2); u_e: flow of air entering chamber (mol s^{-1}); C_r and C_s: CO_2 reference and CO_2 sample (mol CO_2 (mol dry air)$^{-1}$); V: chamber volume (m^3); *ρ*: air density (mol m^{-3}); dC_s/dt: rate of change of CO_2 in the chamber (mol CO_2 (mol dry air)$^{-1}$ s^{-1}); W_r and W_s: water reference and water sample (mol H_2O (mol air)$^{-1}$)

- Equation (1): fundamental/dynamic CO_2 mass balance with the transient term dC_s/dt involved;
- Equation (2): becomes valid under steady-state conditions where $dC_s/dt = 0$.

Methods

- Survey measurements of CO_2 Assimilation, stomatal conductance to water vapor (g_{sw}), and H_2O Transpiration on greenhouse sunflower leaves (*Helianthus annuus*) were conducted, using both steady-state and non-steady-state methods of LI-6800 Photosynthesis System .
- We tested conditions where chamber controls matched the ambient environment and conditions where specific chamber controls (light, CO_2, relative humidity, and temperature) were not matched to ambient.
- Empty chamber tests were performed before and after each leaf measurement.
- IRGAs were matched over a range of CO_2 and H_2O concentrations using the LI-6800's range matching feature, after sufficient system warmup.
- Dynamic tuning was performed by running system equipped auto-programs, at constant flow rate that was used through all the experiments.

Figure 1: LI-6800 with sunflower leaf.

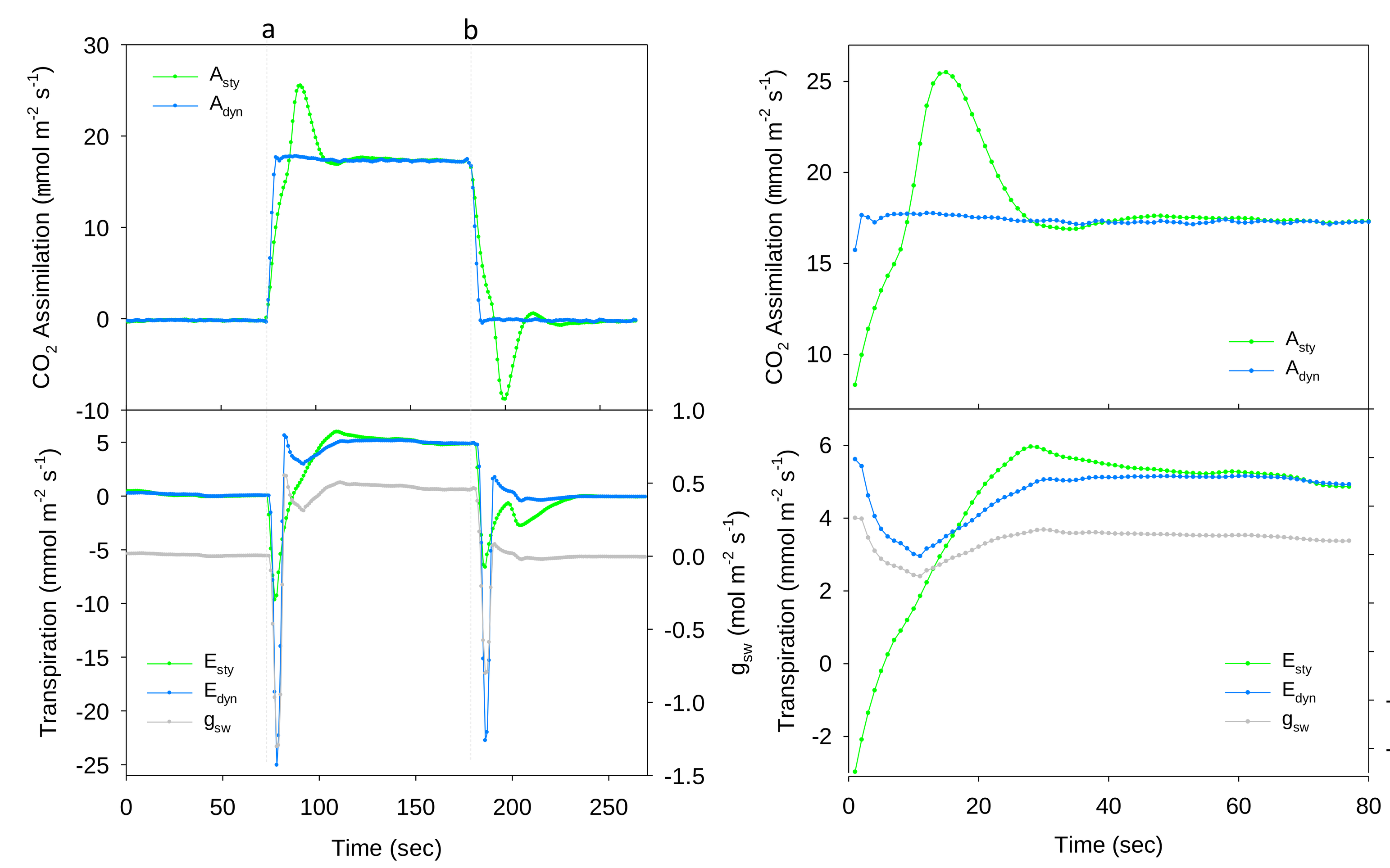
Results

In all graphs:

- A_{sty} (green): CO_2 assimilation computed with the steady state equation; A_{dyn} (blue): CO_2 assimilation computed with the dynamic equation;
- E_{sty} (green): H_2O transpiration computed with the steady state equation; E_{dyn} (blue): H_2O transpiration computed with the dynamic equation;
- g_{sw} (grey): Stomatal conductance to water vapor computed based on E_{dyn};
- At the **point labeled "a"**, empty chamber was opened and clamped on to a leaf.
- At the **point labeled "b"**, leaf was removed, and chamber was closed.
- **Inset graphs** indicate initial period when the leaf was clamped to the chamber, except that in Figure 4 Ⅰ.

Chamber controls were matched to the ambient environment

Figure 2: Survey measurement of sunflower leaf, with chamber controls (light, CO_2, temperature) matched to the ambient environment & chamber relative humidity level equals 65%.



Chamber controls were not matched to the ambient environment

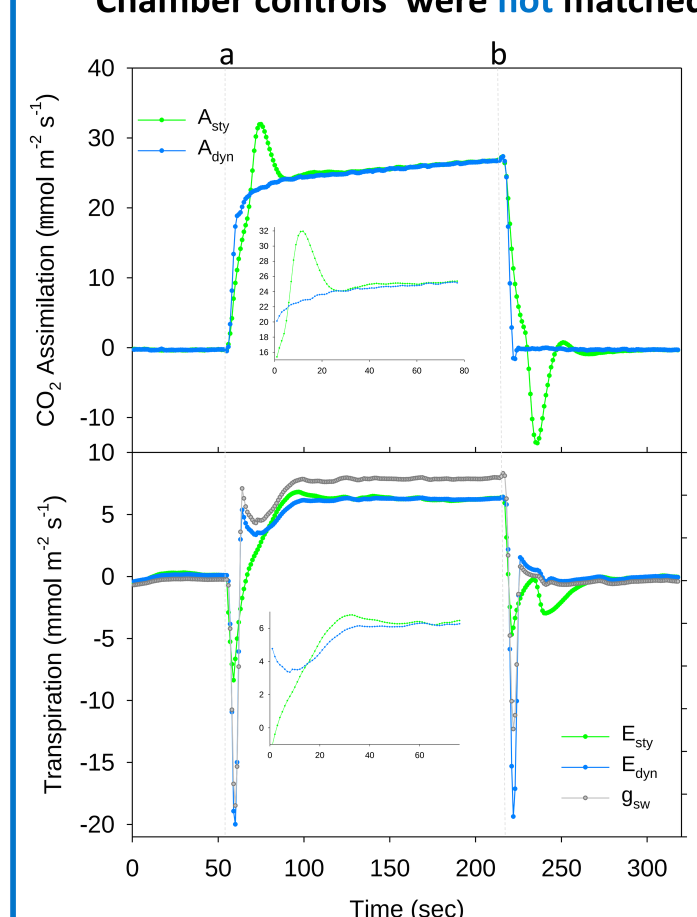


Figure 3: Chamber light control (2000 μmol m^{-2} s^{-1}) was not matched to ambient light intensity (~750 μmol m^{-2} s^{-1}). Other controls were matched & chamber relative humidity level equals 65%.

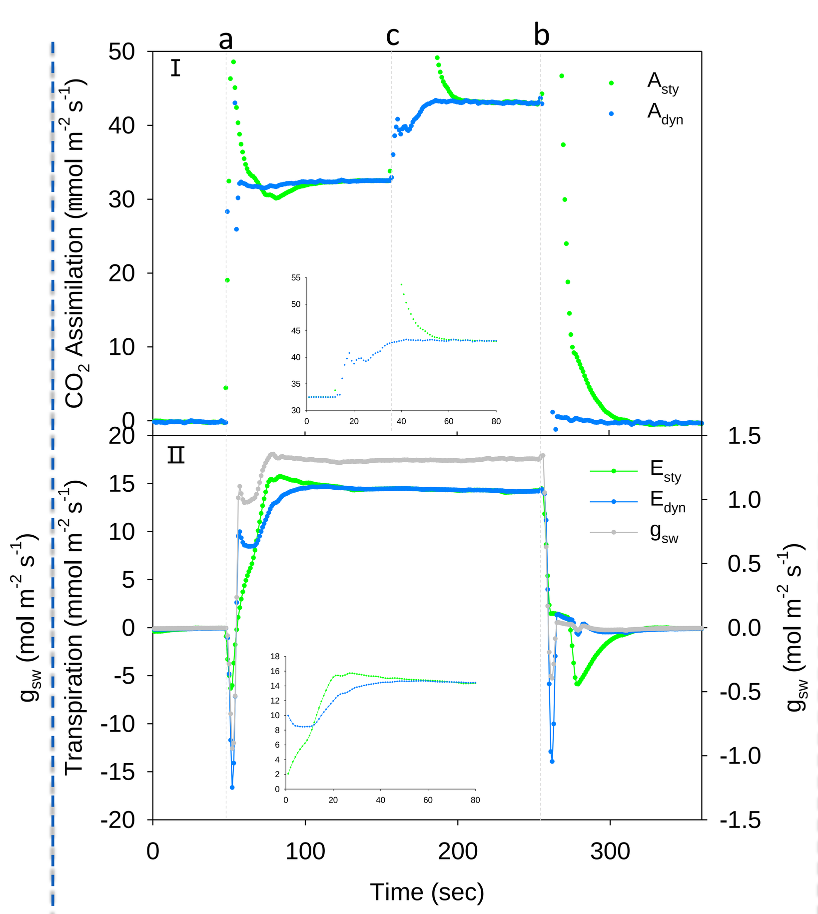


Figure 4: At the point labeled "c," there was a sudden shift in the CO_2 reference, rising from 460 to 1000 (μmol mol^{-1}). Initially, the chamber controls were set to match the ambient environment, with the chamber relative humidity set at 65%.

Inset graph in panel "Ⅰ" indicates initial period when CO_2 reference was elevated.

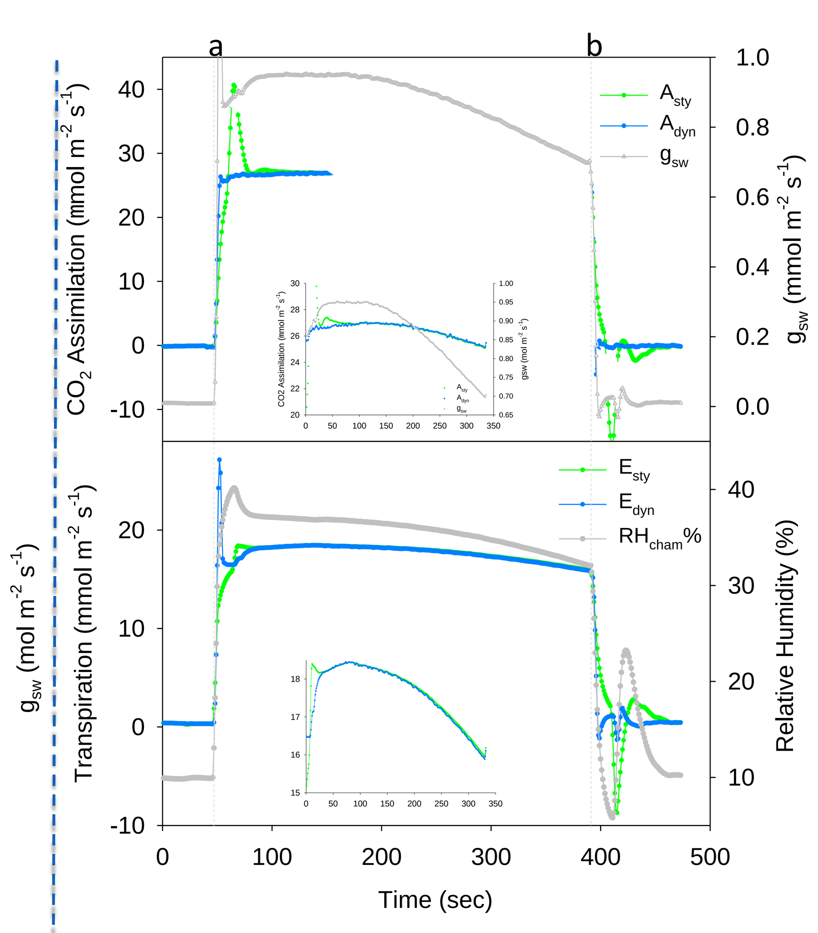
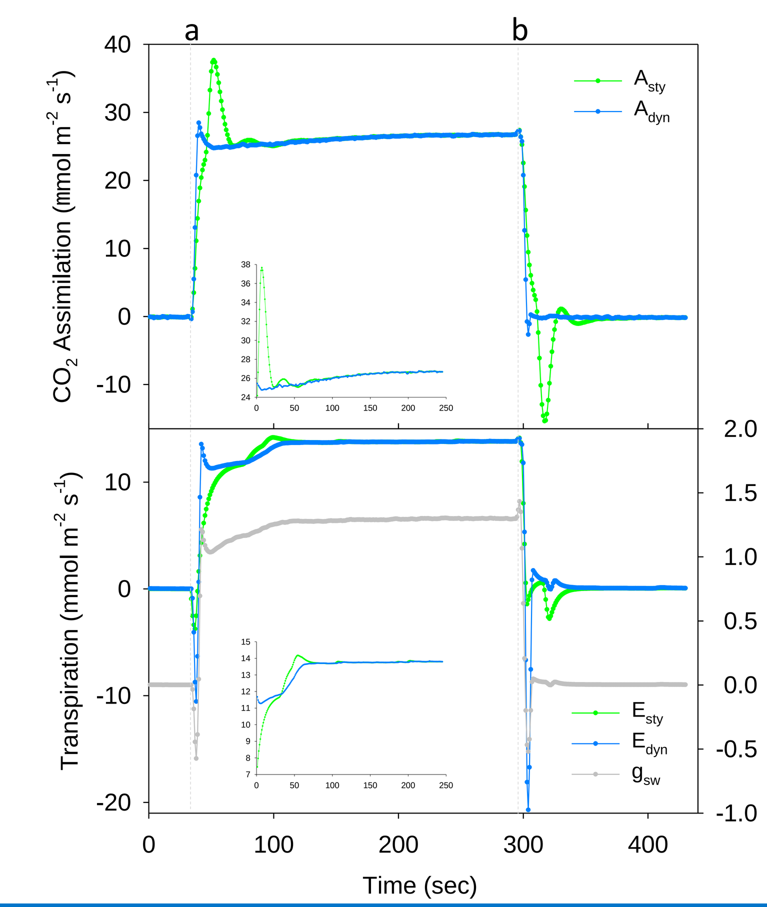


Figure 5: Chamber relative humidity level was below 40%, with other chamber controls matched to the ambient environment.

Results

Figure 6: Chamber air temperature (38 °C) was not matched to ambient temperature (~28 °C). Other controls were matched.



Conclusions

- When the chamber controls were adjusted to match the surrounding environment, biology reaches a stable state rapidly, requiring no adaptation to new conditions, and measurements mirror a snapshot of photosynthesis. However, when one of the chamber controls did not align with the plant's living environment or when the chamber lacks moisture, the biological response becomes evident, and the plant takes time to adapt to the new conditions.
- The LI-6800 gas exchange system, which implements both dynamic and steady-state mass balance, allows real-time observation of the differences between the two approaches.
- Dynamic assimilation equations eliminate the wait time for the chamber to flush, enabling rapid assessment of biological response to an environmental change. Differentiation between instrument and biological non-stability, facilitates the study of plant acclimation processes.
- Empty chamber measurements confirm that the dynamic assimilation value more quickly returns to the expected zero value for assimilation.
- When the instrument reaches a steady state, the transient term in the dynamic mass balance becomes zero, indicating convergence between dynamic and steady state calculations. This convergence serves as a useful indicator of stability, especially for new users, instilling confidence in the timing of data recording.
- Simultaneously utilizing both approaches allows users to differentiate instrument response from biological response, which is valuable for diagnostic purposes and for studying transient responses of biological processes.
- Additionally, our study emphasizes the importance of matching chamber controls to ambient conditions when conducting survey measurements.

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