**Table S1:** Trees sampled for A/Ci curves, with TPU-enabled SS parameter values presented. Thirteen trees representing twelve species were measured. The relative vertical position of each tree is given, with 1 being the tallest tree and 12 being the lowest tree. The lower the tree, the less ambient light. Two trees of the same species (*Abarema mataybifolia*) were located at the same position on the vertical gradient (position = 5). Mean and standard deviation parameter estimates are presented.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Family** | **Species** | **Relative Canopy Position** | **Overshoot?** | **TPU-enabled Mean Vcmax (μmol CO2 m−2·s−1)** | **TPU-enabled Mean Jmax (μmol CO2 m−2·s−1)** |
| Sapotaceae | *Manilkara elata* | 1 | YES | 63.3 (1.3) | 104.2 (6.3) |
| Fabaceae | *Tachigali chrysophylla* | 2 | YES | 73.7 (13.4) | 111.6 (12.5) |
| Rubiaceae | *Chimarrhis turbinata* | 3 | No | 25.7 (1.4) | 49.0 (1.2) |
| Lecythidaceae | *Eschweilera* sp. *trufle1* | 4 | YES | 33.6 (1.2) | 61.7 (8.9) |
| Fabaceae | *Abarema mataybifolia-1* | 5 | No | 22.3 (3.0) | 55.0 (4.8) |
| Fabaceae | *Abarema mataybifolia-2* | 5 | No | 14.4 (4.8) | 32.4 (11.8) |
| Burseraceae | *Protium apiculatum* | 6 | No | 21.8 (2.9) | 39.9 (4.7) |
| Melastomataceae | *Miconia egensis* | 7 | No | 35.7 (1.8) | 61.4 (0.9) |
| Rubiaceae | *Coussarea paniculata* | 8 | No | 25.3 (4.6) | 50.5 (6.4) |
| Euphorbiaceae | *Aparisthmium cordatum* | 9 | No | 43.4 (0.2) | 70.7 (1.7) |
| Hypericaceae | *Vismia cayennensis* | 10 | No | 26.3 (7.5) | 45.0 (7.0) |
| Lecythidaceae | *Couratari stellata* | 11 | Variable | 15.7 (NA) | 31.5 (NA) |
| Annonaceae | *Anaxagorea dolichocarpa* | 12 | YES | 22.8 (1.2) | 32.3 (4.5) |



Figure S1: Decline in Ci with increased Anet (“backward” trend) observed at the beginning of each DAT curve.

**Table S2.** Summary statistics for Dynamic Assimilation Technique (DAT) A/Ci curves and steady-state (SS) A/Ci curves.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Method** | **Mean**  (μmol CO2 m−2**·**s−1) | **Median**  (μmol CO2 m−2**·**s−1) | **SD**  (μmol CO2 m−2**·**s−1) | **Range**  (μmol CO2 m−2**·**s−1) |
| **TPU-Enabled (n = 27 pairs)** | | | | |
| Vcmax |  |  |  |  |
| DAT | 33.2 | 28.5 | 17.8 | 10.1, 80.9 |
| SS | 34.2 | 26.7 | 18.3 | 11.0, 83.2 |
| Jmax |  |  |  |  |
| DAT | 51.6 | 48.4 | 24.2 | 11.8, 112.4 |
| SS | 59.6 | 52.4 | 26.2 | 24.0, 120.5 |
| **No TPU (n = 27 pairs)** | | | | |
| Vcmax |  |  |  |  |
| DAT | 31.6 | 28.5 | 15.9 | 9.92, 69.9 |
| SS | 33.7 | 26.7 | 18.2 | 11.0, 83.2 |
| Jmax |  |  |  |  |
| DAT | 47.4 | 47.4 | 20.3 | 9.51, 100.4 |
| SS | 58.3 | 51.6 | 25.0 | 24.0, 114.5 |
| **TPU-enabled, *Only* Curves without Overshoot (n = 19 pairs)** | | | | |
| Vcmax |  |  |  |  |
| DAT | 29.7 | 24.7 | 14.0 | 10.1, 67.6 |
| SS | 30.6 | 25.9 | 14.2 | 11.0, 64.0 |
| Jmax |  |  |  |  |
| DAT | 51.3 | 47.8 | 19.1 | 22.7, 100.5 |
| SS | 55.6 | 51.6 | 19.9 | 24.0, 105.9 |
| **No TPU, *Only* Curves without Overshoot (n = 19 pairs)** | | | | |
| Vcmax |  |  |  |  |
| DAT | 28.9 | 24.7 | 13.5 | 9.9, 67.4 |
| SS | 30.6 | 25.9 | 14.2 | 11.0, 64.0 |
| Jmax |  |  |  |  |
| DAT | 50.2 | 47.4 | 17.5 | 23.6, 100.4 |
| SS | 55.6 | 51.6 | 19.9 | 24.0, 105.9 |



Figure S2: 1:1 comparison of TPU between DAT and SS curves. Error bars are smaller than the diameter of the points and are not readily distinguishable. Colors represent individual leaves. TPU was consistently underestimated in DAT curves as compared to SS, though we interpret this result with caution given the small sample size (n = 6).

Figure S3: Vcmax and Jmax parameter estimates between the full dataset and the overshoot-excluded subset. Red \* indicates a significant difference between DAT and SS. Vcmax was not significantly different when TPU was enabled (a) but was when TPU was omitted (b) in the full dataset. In the overshoot-excluded subset, both were found to have significantly greater Vcmax-SS. Jmax-SS was significantly greater regardless of the dataset used and if TPU was fit (c) or not (d).



Figure S4: Vcmax and Jmax differences (SS - DAT) on a species basis, ranked by tree height. Error bars represent the mean of the absolute standard deviations (SD) for DAT and SS parameter estimates. Species are organized by relative canopy height (top-down from tallest to shortest tree). Across species, greater differences between SS and DAT Jmax estimates were observed than between SS and DAT Vcmax. Among the Jmax differences, a roughly parabolic pattern can be observed with the greatest differences between methods occurring at the top and the bottom of the canopy.