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K. J. Davidson

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Short term variation in leaf level water use efficiency in a tropical forest

Kenneth J. Davidson^{1,2}, Julien Lamour¹, Alistair Rogers¹, Kim S. Ely¹, Qianyu Li¹, Nate G. McDowell^{3,4}, Alexandria L. Pivovarov⁵, Brett T. Wolfe^{6,7}, S. Joseph Wright⁷, Alfonso Zambrano⁷, and Shawn P. Serbin¹

1. *Department of Environmental and Climate Sciences, Brookhaven National Laboratory, Upton, NY, USA*
2. *Department of Ecology and Evolution, Stony Brook University, Stony Brook, NY, USA*
3. *Atmospheric Sciences and Global Change Division, Pacific Northwest National Lab, PO Box 999, Richland, WA, 99352*
4. *School of Biological Sciences, Washington State University, PO Box 644236, Pullman, WA 99164-4236*
5. *Biology Division, Glendale Community College, Glendale, CA, USA*
6. *School of Renewable Natural Resources, Louisiana State University, Baton Rouge, LA, USA*
7. *Smithsonian Tropical Research Institute, Apartado, 0843-03092, Balboa, Panama*

Name	ORCID
Kenneth J. Davidson	0000-0001-5745-9689
Julien Lamour	0000-0002-4410-507X
Alistair Rogers	0000-0001-9262-7430
Kim S. Ely	0000-0002-3915-001X
Qianyu Li	0000-0002-0627-039X
Nate G. McDowell	0000-0002-2178-2254
Alexandria L. Pivovarov	0000-0002-3104-1900
Brett T. Wolfe	0000-0001-7535-045X
S. Joseph Wright	0000-0003-4260-5676
Alfonso Zambrano	0000-0001-5442-5897
Shawn P. Serbin	0000-0003-4136-8971

	Summary	Introduction	Materials and Methods	Results	Discussion	Total
Words	199	1284	1995	1236	2703	7218

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Supporting information: 16 figures, 4 tables, 3 notes

Keywords: stomatal optimization, stomatal conductance, diurnal response, water use efficiency, climate model, leaf phenology, sap flux, transpiration

Summary:

- The representation of stomatal regulation of transpiration and CO₂ assimilation is key to forecasting terrestrial ecosystem responses to global change. Given its importance in determining the relationship between forest productivity and climate, accurate and mechanistic model representation of the relationship between stomatal conductance (g_s) and assimilation is crucial.
- We assess possible physiological and mechanistic controls on the estimation of the g_1 (stomatal slope, inversely proportional to water use efficiency) and g_0 (stomatal intercept) parameters, using diurnal gas exchange surveys and leaf level response curves of six tropical broadleaf evergreen tree species.
- g_1 estimated from *ex-situ* response curves averaged 50% less than g_1 estimated from survey data. While g_0 and g_1 varied between leaves of different phenological stages, the trend was not consistent among species. We identified a diurnal trend associated with g_1 and g_0 that significantly improved model projections of diurnal trends in transpiration.
- The accuracy of modelled g_s can be improved by accounting for variation in stomatal behavior across diurnal periods, and between measurement approaches, rather than focusing on phenological variation in stomatal behavior. Additional investigation into the primary mechanisms responsible for diurnal variation in g_1 will be required to account for this phenomena in land surface models.

Introduction:

Properly parameterizing Earth system models (ESMs) is of vital importance to forecasting terrestrial ecosystems' responses to novel future climates (Rogers *et al.*, 2017). In particular, the ESM representation of the terrestrial biosphere in land-surface models (LSMs) used to predict the dynamics, responses, and feedbacks of vegetation to the climate system is a critical requirement for climate modeling (Bonan, 2008). This includes the representation of photosynthetic carbon assimilation (A), which directly determines ecosystem net carbon uptake (Ainsworth & Rogers, 2007). Transpiration (E), which is a dominant flux in the hydrologic cycle (Schlesinger & Jasechko, 2014), is fundamentally governed by stomatal conductance (g_s), which regulates both A and E (Meidner & Mansfield, 1968; Hetherington & Woodward, 2003; Lawson & Vialet-Chabrand, 2019). Despite the large influence of g_s on global carbon and water cycles, parametrization of g_s in models remains one of the largest uncertainties in current predictions of net ecosystem productivity (Dietze *et al.*, 2014; Bauerle *et al.*, 2014; Jefferson *et al.*, 2017; Franks *et al.*, 2018; Ricciuto *et al.*, 2018).

The LSMs that are commonly used in Earth system models tend to represent g_s using an assumed linear relationship between g_s and A where the slope parameter governs the ratio of $g_s:A$ (Damour *et al.*, 2010). The majority of these formulations are phenomenological (e.g., Wong *et al.*, 1979; Farquhar & Wong, 1984; Ball *et al.*, 1987; Leuning, 1995; Damour *et al.*, 2010), however, Medlyn *et al.*, (2011) argued that the relationship could be modeled in terms of stomatal optimality theory. The principal of stomatal optimization is that the evolution of vascular plants has prioritized maximizing A , per unit of water lost via E (Cowan & Fraquhar, 1977; Cowan, 1978). Stated in terms of water, stomatal regulation should minimize g_s so that E is minimized for a given amount of A , at least during periods of water limitation. This is mathematically represented by $\partial E/\partial A = \lambda$, where $1/\lambda$ represents the whole plant water use efficiency (WUE). This relationship is conceptualized in the “Unified Stomatal Optimization model” (USO; Medlyn *et al.*, 2011; Eqn. 1).

Equation 1.

$$g_s = g_0 + 1.6 \left(1 + \frac{g_1}{\sqrt{D}} \right) \frac{A}{C_s}$$

Unlike phenomenological models, the g_1 term in the USO model relates directly to optimality theory (Cowan & Fraquhar, 1977; Cowan, 1978). The stomatal slope term (g_1) is approximately

inversely proportional to WUE. The stomatal intercept (g_0) is the expected g_s when net assimilation (A_{net}) = 0 and occurs at an irradiance level equivalent to the light compensation point. The g_0 parameter was an empirical addition, intended to help constrain the model and ensure predicted g_s is always positive, rather than a feature predicted by optimality (Duursma *et al.*, 2019). The other terms in the USO model relate to environmental conditions, including the leaf-to-air vapor pressure deficit (D) and the CO₂ concentration at the leaf surface (C_s). Previously, Wu *et al.*, (2019b) tested four different stomatal model formulations in the wet tropics and found that the USO model was best suited to represent g_s in this system.

Data used to fit the USO model can be collected either by taking survey measurements at ambient conditions (Bernacchi *et al.*, 2006; Lin *et al.*, 2015; Wu *et al.*, 2019b) or by the use of a response curve method (Ball *et al.*, 1987; Leuning, 1995; Leakey *et al.*, 2006; Domingues *et al.*, 2014; Wolz *et al.*, 2017; Davidson *et al.*, 2022; Lamour *et al.*, 2022). In the survey method, *in-situ* measurements of instantaneous gas exchange from many individual leaves are collectively used to fit a regression from which g_1 and g_0 are obtained. In the curve method, slow irradiance, vapor pressure deficit (VPD), or CO₂ response curves are used to illicit stomatal response, usually on a cut branch segment. For this approach it is typical to calculate g_0 and g_1 for an individual leaf.

Much of the work to date on assessing variation of stomatal parameters has focused on specific biotic or abiotic drivers. Research into the effects of drought, soil moisture (H  roult *et al.*, 2013), and temperature (Lin *et al.*, 2015; Wolz *et al.*, 2017) have shown that, in some systems, the abiotic control on g_1 may be stronger than any biotic controls (Zhou *et al.*, 2014, 2016). Other work has linked leaf phenology and photosynthetic properties in tropical evergreen forests, where the rate of leaf production and leaf longevity both influence leaf function (van Schaik *et al.*, 1993). Leaves with longer average lifespans have reduced maximum carboxylation rate of the enzyme Rubisco (V_{cmax}) as compared with shorter lived leaves (Kitajima *et al.*, 1997, 2002). Similarly, Wu *et al.*, (2016) found that V_{cmax} changed over the lifetime of tropical leaves, and this leaf-age variation is an important factor to consider when modeling tropical forest carbon assimilation (Wu *et al.*, 2017). Yet, it remains to be seen what impact leaf phenology has on stomatal parameters in tropical species. A strong phenological partitioning of leaf level stomatal response, if it occurs, would most likely be an important development for ESMs given the large impact phenology has on terrestrial carbon dynamics (De Weirdt *et al.*, 2012). This is especially true for evergreen tropical forests,

where leaves are found across a particularly wide range of phenological stages and where phenological changes drive a large variation in seasonal carbon uptake (Wu *et al.*, 2016, 2017).

Other research has focused on the temporal consistency of stomatal parameters (Resco de Dios *et al.*, 2020), or the conditions in which the g_s response should be optimized (Katul *et al.*, 2010). Plants may optimize their stomatal operation across a variety of time scales, from near instantaneous response to stimuli such as sunflecks (Way & Pearcy, 2012; Campany *et al.*, 2016), diurnal responses such as those to drying soil (Knapp, 1993; Zhang *et al.*, 2018), and long-term responses such as those which optimize water and nutrient uptake by roots (Franklin, 2007). Thus, whole plant WUE is the integration of all these factors, with leaf level WUE lying at the end of a hierarchy of responses (Katul *et al.*, 2010). Wu *et al.*, 2019b addressed the seasonal consistency of tropical WUE by analyzing dynamics of g_1 during a drought event and found that species specific g_1 did not significantly vary by month, suggesting that at the whole canopy scale WUE is stable through time. However, the diurnal consistency of leaf level WUE in this system remains untested. If they occur, diurnal adjustments in WUE may be in response to rapidly changing external stimuli such as changes in VPD during the day (Katul *et al.*, 2009), or may be the consequence of shifting water supply and carbon demand dynamics within the leaf (Bonan *et al.*, 2014; Dewar *et al.*, 2018). Understanding the degree to which WUE varies diurnally, and the main mechanisms which regulate these diurnal changes, may be helpful in developing more representative and robust process-based models of g_s and E in tropical broadleaf evergreen forests.

In this study, we measured leaf gas exchange on six evergreen tropical tree species to identify key biotic and abiotic controls on stomatal parameters and WUE. We had four main objectives: 1) compare survey and *ex-situ* response curve derived g_0 and g_1 to assess whether these two commonly used approaches provide comparable estimates of the key parameters; 2) determine if g_0 and g_1 vary with leaf phenological stage; 3) assess if g_0 and g_1 vary over a diurnal period; and 4) conduct simulations within a simplified canopy-scale model to understand the impacts of parameterization on E , comparing our estimated flux to a model benchmark derived from sap flux measurements. By addressing these four objectives, our goal was to develop a more detailed process-based understanding of stomatal function in wet tropical forests and identify several key axes of biotic and abiotic variation which could be targeted for further investigation.

Materials and Methods:

Site and species description

Data collection occurred from January 6 to March 23, 2020 in the San Lorenzo Protected Forest (9.281°N, 79.974°W), on the Colón Province on the Republic of Panama's Caribbean coast (Fig. 1). The site is characterized as a wet lowland (130 m ASL) tropical forest, with a mean annual temperature of 23.4°C and an average of 3330 mm of rainfall (Paton, 2020). We used a crane operated by the Smithsonian Tropical Research Institute (STRI) for canopy access. Six tree species were selected for study, chosen to span early, mid, and late successional growth strategies (Table 1). For each species, we sampled from two trees that were exposed to full sun throughout the day. Four of the trees had previously been instrumented for measurement of sap flux.

161 **Table 1. Values for species specific photosynthetic and conductance parameters and canopy scaling factors used in simulations**
162 **of leaf level A and g_s .** For details regarding parameter estimates and tree functional traits see section “Canopy E simulations.” Data on
163 leaf lifespan were previously reported in Osnas *et al.*, (2018).

Species	$V_{cmax,25}$ ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	$J_{max,25}$ ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	$R_{dark,25}$ ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	g_1 ($\text{kPa}^{0.5}$)	g_0 ($\text{mol m}^{-2} \text{s}^{-1}$)	DBH (cm)	LAI ($\text{m}^2 \text{m}^{-2}$)	Crown Area (m^2)	Sapwood Area (m^2)	Successional Status	Leaf Lifespan (days)
<i>Brosimum</i> <i>utile</i> (Kunth)	Young: 68.84 Mature: 51.28	84.61 60.44	1.36 1.17	Response Curve: 2.40 Survey: 3.04	0.013 0.029	-	-	-	-	Late	324
Oken	Old: 35.22	49.17	0.89								
<i>Cecropia</i> <i>insignis</i> Liebm.	Young: 77.48 Mature: 78.70 Old: 60.50	109.48 108.48 89.33	1.50 1.81 1.68	Response Curve: 1.75 Survey: 2.87	0.031 0.107	-	-	-	-	Early	231
<i>Guatteria</i> <i>dumetorum</i> R. E. Fr.	Young: 38.66 Mature: 48.18 Old: 35.79	65.71 67.53 64.71	1.24 0.82 0.97								
<i>Miconia</i> <i>borealis</i> (Bonpl.) DC.	Young: 64.59 Mature: 71.60 Old: 54.06	106.29 108.38 94.55	1.81 0.92 0.79	Response Curve: 2.20 Survey: 4.15	0.034 0.003	34.0	1.15	74.42	0.080	Mid	189
<i>Terminalia</i> <i>amazonia</i> (J.F. Gmel.) Exell	Young: 70.28 Mature: 77.85 Old: 51.34	106.07 116.16 87.52	1.42 1.14 1.24								
<i>Vochysia</i> <i>ferruginea</i> Mart.	Young: 46.57 Mature: 57.46 Old: 43.66	76.14 78.47 78.62	1.67 1.18 0.78	Response Curve: 0.67 Survey: 2.57	0.036 0.040	58.0	2.0	153.24	0.204	Mid	215

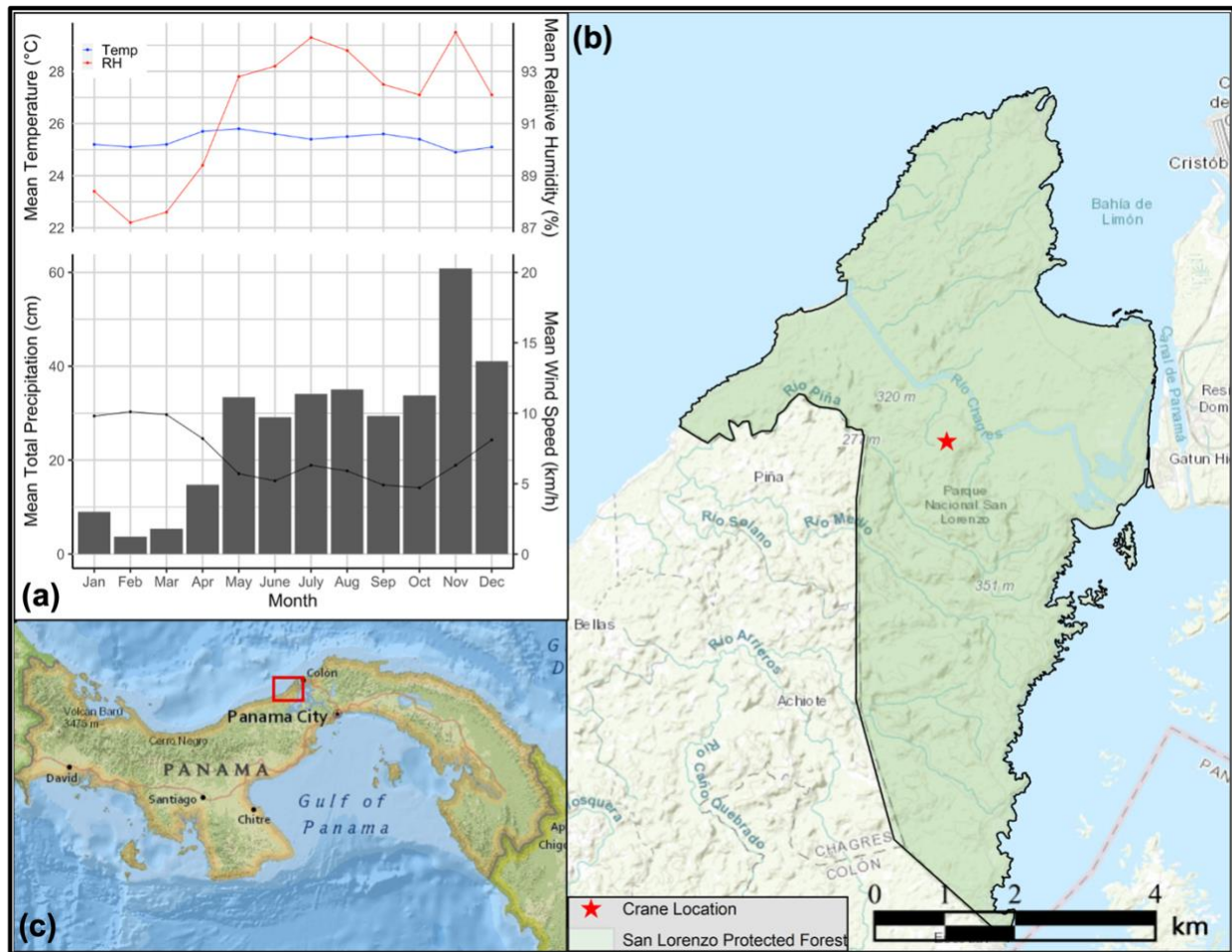


Figure 1. Geographic location of the crane site within the San Lorenzo Protected Forest and monthly climate averages collected at the site between 1997 and 2019. The period between January and April is considered the dry season, with less than 20% of annual rainfall (black bars). During the period of study, mean temperature was 25.5°C and mean daily precipitation was 1.3 mm. Basemap sources: National Geographic and Esri.

Gas exchange and leaf trait data collection

Gas exchange measurements were carried out using five LI-6400XT portable photosynthesis systems using a 6400-02(B) LED light source and an LI-6800 portable photosynthesis system using a Multiphase Flash Fluorometer leaf chamber (6800-01A) (LI-COR Biosciences, Lincoln NE, USA). For all measurements the color spectrum of the irradiance was set to a fixed ratio of 90% red:10% blue.

We used two different methods of gas exchange: an *ex-situ* response curve approach and an *in-situ* survey approach. Stomatal response curves on cut branch segments were carried out as described previously (Leakey *et al.*, 2006; Davidson *et al.*, 2022; Lamour *et al.*, 2022). Upper canopy, fully sun exposed branches were cut predawn and immediately recut under water to ease xylem tension and prevent embolism (Sperry, 2013). We recut a total of at least 50 cm back from the first cut to remove open vessels (Wolf *et al.*, 2016) with final branch lengths of approximately 1.5 m. Cut branches were transferred in buckets to a shaded ($\sim 150 \mu\text{mol PAR}$) location where all response curves were carried out. Each species was measured on 10-15 different days, spread across the duration of the field campaign. For each curve, irradiance level was reduced from saturating down to $0 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD in a stepwise fashion every 20-40 min (Fig. 2a; Lamour *et al.*, 2022). The irradiance value at saturation was derived for each species using photosynthetic response curves collected at multiple locations from across the top of each canopy (Lamour *et al.*, 2021b; Rogers *et al.*, 2021).

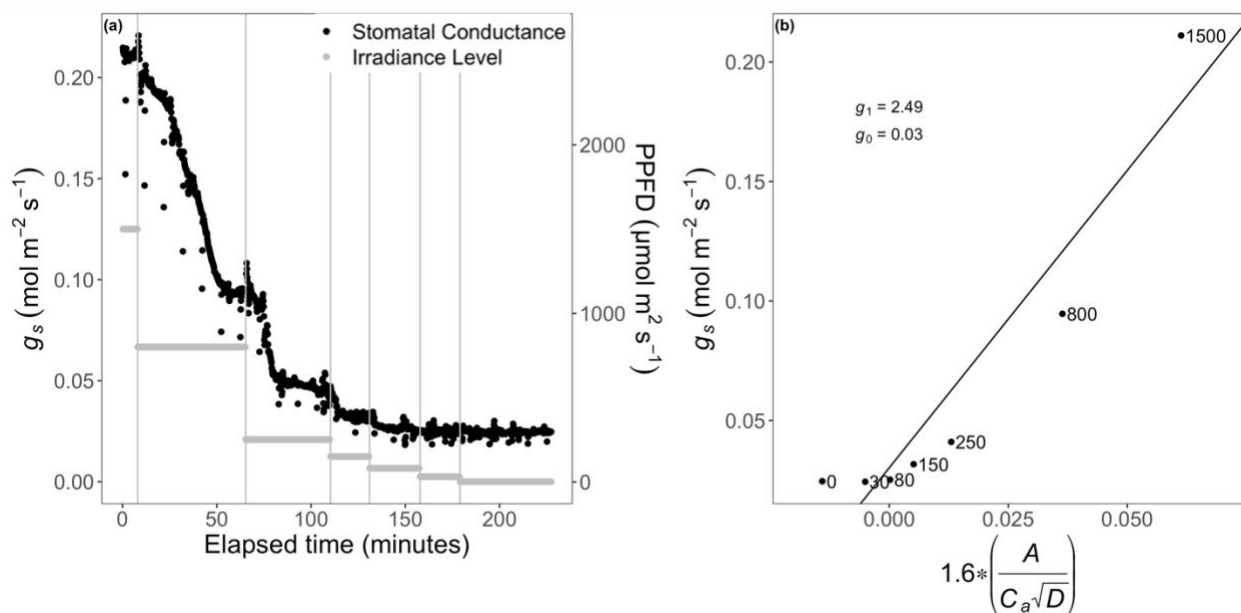


Figure 2. Schematic representation of the response curve method. (a.) raw data from a response curve, with each black point representing an observation of g_s . At each irradiance level (horizontal grey bars) g_s drops then stabilizes, at which point irradiance is reduced to the next level (vertical grey bars). Across all irradiance levels, leaf temperature and VPD_{leaf} , were held constant and close to ambient conditions. The CO_2 inside the leaf chamber was maintained at $400 \mu mol \text{ mol}^{-1}$. (b.) a typical regression fit of the points extracted from the seven irradiance levels, and the g_1 and g_0 estimates produced from this curve. In this example, the non-linearity of the response curve data, explored in detail by Lamour *et al.*, (2022), is readily apparent. For data analysis, many curves are combined to obtain one overall regression, with each curve acting as a random effect on the overall g_1 and g_0 estimates.

Survey measurements followed previous approaches (Rogers *et al.*, 2004; Wu *et al.*, 2019b). Fully sunlight top-of-canopy leaves were measured with the LI-6800. Before each measurement, the conditions within the leaf chamber were set to match ambient air temperature ($22.8\text{-}32.6^\circ\text{C}$), CO_2 concentration ($\sim 400 \text{ ppm}$), and irradiance ($3 - 2400 \mu mol \text{ PAR}$). The relative humidity in the leaf chamber was maintained at 70% for all measurements, a level which allowed for VPD_{air} to be close to mean daytime conditions (Paton, 2020, Fig. S1), while minimizing condensation risk in the instrument. Each survey measurement consisted of a 30-90 second stabilization period, followed by five measurements of A and g_s at five second intervals, which were then averaged to produce a single data point.

Surveys were carried out from 0630h (approximate sunrise) to 1830h (approximate sunset) across eight different days. Two survey schemes were used, one in which we measured as many leaves as possible at three positions on a single tree crown for 40 minutes every hour for four hours, and a second where each of the 12 tree crowns (two per species, three positions per tree) was measured as many times as possible for 12 minutes, replicating this pattern five times in a 12-hour period. The aim of the first scheme was to assess the diurnal dynamics of a single species on a given day, while the aim of the second scheme was to broadly survey the forest. We used the data from the first scheme to parameterize our models (see Note S1), and data from the second scheme, along with Wu *et al.*, (2019b), to validate our models.

Immediately following the single species gas exchange survey measurements the leaf was cut at the base of the petiole, and stored in a humid, cool, dark box for further processing (Leach *et al.*, 1982; Rodriguez-Dominguez *et al.*, 2022). Processing included using a Scholander-type pressure chamber to measure leaf water potential (Ψ_{leaf} ; Scholander *et al.*, 1964). Following measurement of Ψ_{leaf} , samples were dried to constant mass and weighed to determine leaf mass

area (LMA; dry leaf mass per unit leaf area). Measured leaves were classified into one of three phenological stages, young, mature, or old (Table 1, Fig. 3).

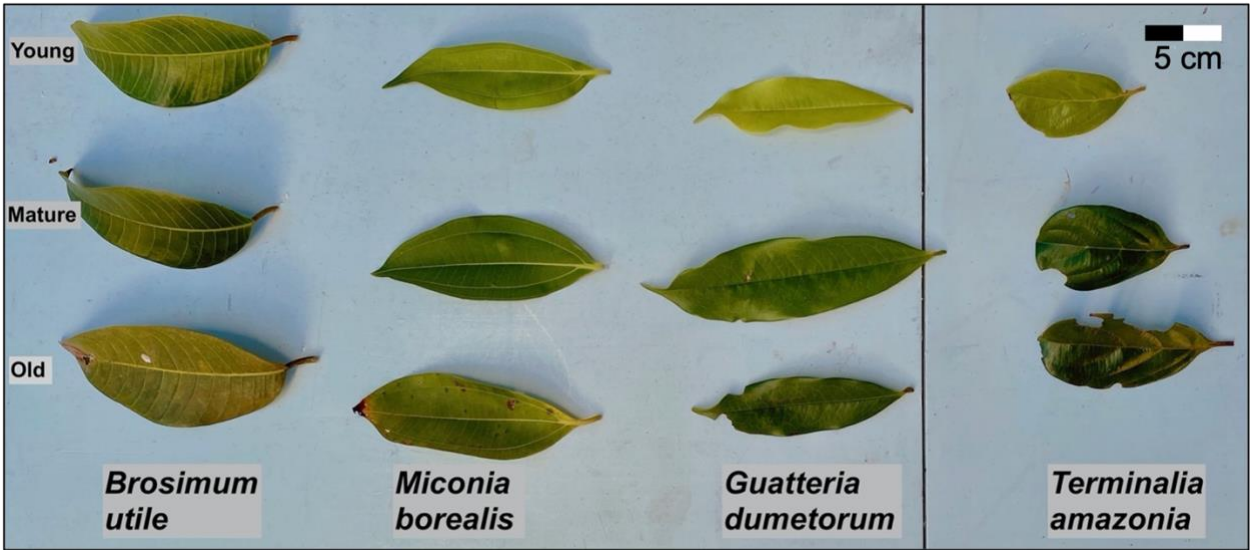


Figure 3. Examples of the three phenological stages for four of the six species in this study. Young leaves were characterized as lighter in color, with minimal damage, softer more flexible texture, and a distal position on the branch. Mature leaves comprised the majority of the leaf material, characterized as fully expanded, having an intermediate color and texture as well as an intermediate position. Old leaves were very scarce for most species and tended to have minor surface damage, were dark in color, and were in extreme proximal positions on branches.

Reflectance spectroscopy

We used a full range spectroradiometer (PSR 3500+, Spectral Evolution, Inc., Lawrence, MA, USA) together with an LC-RP-Pro leaf clip foreoptic (Spectra Vista Corporation, Poughkeepsie, NY, USA) containing an internal, full-spectrum calibrated light source to measure leaf reflectance on all leaves following completion of stomatal response curves. The reflectance data were used to estimate $V_{cmax,25}$ and $J_{max,25}$ using the spectral methods and model presented in Lamour *et al.*, (2021).

Sap flux measurement

Sap flux velocity (cm s^{-1}) data were collected for four of the 12 study trees. Sap flux velocity was measured with Granier-type sensors (Model PS-TDP8, PlantSensors, Nakara, Australia), which use heat dissipation to empirically infer sap flux velocity (Granier, 1987). Each tree was instrumented with two sensors, located at breast height, and positioned perpendicular to each other at a depth of 2 cm into the sapwood of the trees. Raw temperature data were processed in the R

package ‘AquaFlux’ (v 1.0.0 Speckman, 2019) to derive sap flux velocity. VPD data obtained on site from the STRI meteorological station was used to normalize minimum flux. Sap flux velocity data were multiplied by sapwood area (Meinzer et al., 2001) to derive sap flow (g s^{-1}) at 15-minute intervals, with the assumption that velocity was consistent across the sapwood radial profile. In total there were 42 diurnal (05:30-18:30) periods of complete sap flux coverage for each of the four study trees during our period of study (Pivovarovoff *et al.*, 2021).

Estimation of conductance parameters

Stomatal response was evaluated using the USO model (Medlyn *et al.*, 2011, Fig. 2b, Equation 1, Note S1). g_1 represents the slope in the relationship shown in Equation 2, a reconfiguration of Equation 1 where g_1 directly acts as the slope between g_s and A , atmospheric CO_2 concentration (C_a), and VPD. The parameters g_1 and g_0 are mathematically identical in Equations 1 and 2 (Lamour *et al.*, 2022), however Equation 2 provides for simpler graphical depiction of the data. Equation 2 also uses C_a in place of C_s , as when using gas exchange systems, the terms are very similar (Lamour *et al.*, 2021c).

Equation 2.
$$g_s - \frac{1.6 \cdot A}{C_a} = g_0 + g_1 * \frac{1.6 \cdot A}{(\sqrt{D} \cdot C_a)}$$

As with any linear model, a limited range of measured g_s can affect the model fit by introducing uncertainty in the regression (g_1). The wider the range of the data, the lower the uncertainty in the fit of the model slope, assuming that the variation around the mean slope is consistent (e.g., no heteroscedasticity). For response curve data, we captured a full range of conductance values, as we are theoretically measuring g_s from its maximum (light saturated) value to its minimum (dark adapted) value. A limited range of g_s can also lead to uncertainty in estimating a value for g_0 , especially if most measurements occurred far above the light compensation point. To keep analysis consistent between datasets, we chose to fit g_0 for the survey dataset rather than prescribe a value (Duursma *et al.*, 2019).

R_{dark} was calculated from dark adapted measurements of A_{net} (the final response curve point) and was scaled to 25°C ($R_{\text{dark},25}$) using an inverse Arrhenius equation (Equation 3; Bernacchi *et al.*, 2001; Von Caemmerer, 2013):

Equation 3.
$$R_{\text{dark},25} = \frac{R_{\text{dark}}}{\exp\left(\frac{H_a}{(R \cdot T_{25})} - \frac{H_a}{(R \cdot T_{\text{leaf}})}\right)}$$

where H_a is the activation energy of mitochondrial respiration ($H_a = 46390 \text{ J mol}^{-1}$), and R is the ideal gas constant ($R = 8.314 \text{ J mol}^{-1} \text{ K}^{-1}$).

Canopy E simulations

As a method of evaluating the performance of different stomatal parameterizations against a benchmark data set, we modeled canopy scale E response to ambient environmental conditions. We chose not to evaluate the model performance in simulating canopy scale A , as we have no benchmark which to compare model estimates. We used the R package ‘LeafGasExchange’ (v 1.0.1, Lamour & Serbin, 2021), which includes a coupled leaf scale steady state assimilation, conductance, energy balance, and radiation interception model. Details of model parameters and equations used can be found in Supplemental Note 2.

The model simulates environmental conditions around the leaf using local meteorological data (Paton, 2020). To simulate the radiation environment at the leaf surface the model uses the radiation interception model developed by Norman, (1979), which partitions incoming radiation into direct and diffuse streams. In each simulation we assumed six canopy layers with a uniform distribution of leaf area index (LAI) between layers (Béland & Baldocchi, 2021). To simulate wind speed within the canopy we assumed an exponential decrease with LAI within the canopy following the model presented in Equation 14 in Buckley *et al.*, (2014). Values for $V_{cmax,25}$, $J_{max,25}$, $R_{dark,25}$, g_0 , and g_1 , estimated as described above, were averaged by species and by phenological stage (Table 1). In each model run, uncertainty was simulated by rerunning the model using g_1 and g_0 values corresponding to ± 1 SE of the fitted value.

Leaf level estimates of E were scaled to the canopy by first summing across canopy layers, then multiplying by total canopy area as estimated using the allometric function presented in Martínez Cano *et al.*, (2019). LAI estimates were made for each species (Condit *et al.*, 1996; Sirri *et al.*, 2019), which allow for an appropriate accounting of leaf area per ground area.

We then conducted a model simulation sensitivity analysis to explore the potential impacts of biotic variation on emergent, canopy scale E . To do this, we simulated different canopies with different combinations of leaf phenological classes, with leaf physiological properties based on leaf phenological stage. For this analysis we assumed “natural” conditions were a 15-80-5% partitioning between young, mature, and old leaves, an estimate that is in line with Wu *et al.*, (2017b). We compared our simulation results for canopy scale E with measured sap flow as a

benchmark. Here we expected that there was a delay between flux measured at the base of the tree and E in the canopy, so we averaged flux across a 15 minute interval to minimize this discrepancy (Meinzer *et al.*, 2003)

Statistical analysis

To compare g_1 and g_0 estimates between tree species and phenological stages within tree species we used mixed effects models constructed and analyzed in the R package ‘nlme’ (v 3.1, Pinheiro *et al.*, 2020). For response curve data, the leaf served as the random effect, while for survey data, the location within the canopy of the tree served as the random effect. For all data we considered the species and phenological stage as fixed effects. The equations and coefficients for the mixed models can be found in Supplemental Note 1 and Table S1-3.

To examine which of our three statistical models (response curve, survey, survey + time-of-day; Note S1) predicts leaf level g_s most accurately, we performed a validation of the fixed effects components of the models using two additional survey derived datasets, one which was collected on four separate days during our 2020 field campaign (described above as scheme 2), and a second data set collected by Wu *et al.*, (2019b) at the same site in 2016 (Rogers *et al.*, 2021). We then used validation statistics (r^2 , RMSE, likelihood ratio test) to determine which model predicted g_s most accurately.

We assessed the effect of species and species:leaf phenological stage on $V_{cmax.25}$, $R_{dark.25}$, and the effect of LMA, Ψ_{leaf} , and successional stage on g_1 and g_0 using ANOVA and post-hoc Tukey tests. Finally, we compared canopy level transpiration simulations using ANOVA at the species level, and a post-hoc Tukey’s honest significant difference test to distinguish significance between factor levels. All analysis was performed using the R open source software environment (v 3.6.2, R Core Team, 2013).

Results:

Gas exchange dynamics of diurnal surveys and response curves

Here we present the results from an analysis of 80 stomatal response curves (Fig. S2a) and 665 survey data points (Fig. 4) (Lamour *et al.*, 2021b). Both A and g_s respond strongly to the diurnal cycle of irradiance during the survey measurements (Fig. 4). VPD_{leaf} also exhibits a diurnal trend, peaking around solar noon, due to higher leaf temperatures, and a small increase in VPD_{air} (Fig.

S1). Stomatal response curves can be used to examine potential maximum photosynthetic and stomatal fluxes. Here we see that among species, there is significant variation in maximum g_s , with *Brosimum utile*, *Cecropia insignis*, and *Miconia borealis* having maximum g_s values up to three times that of the other three species (Fig. S2b).

We can compare the relationship between g_s and environmental conditions for both datasets (Fig. S2). We see that not only do the survey data have a larger overall range of conductance, there is also more within-species variation in conductance for a given set of environmental conditions. We can also see the non-linearity of the response curve data, explored in detail by Lamour *et al.*, (2022), which is much less pronounced for the survey data set.

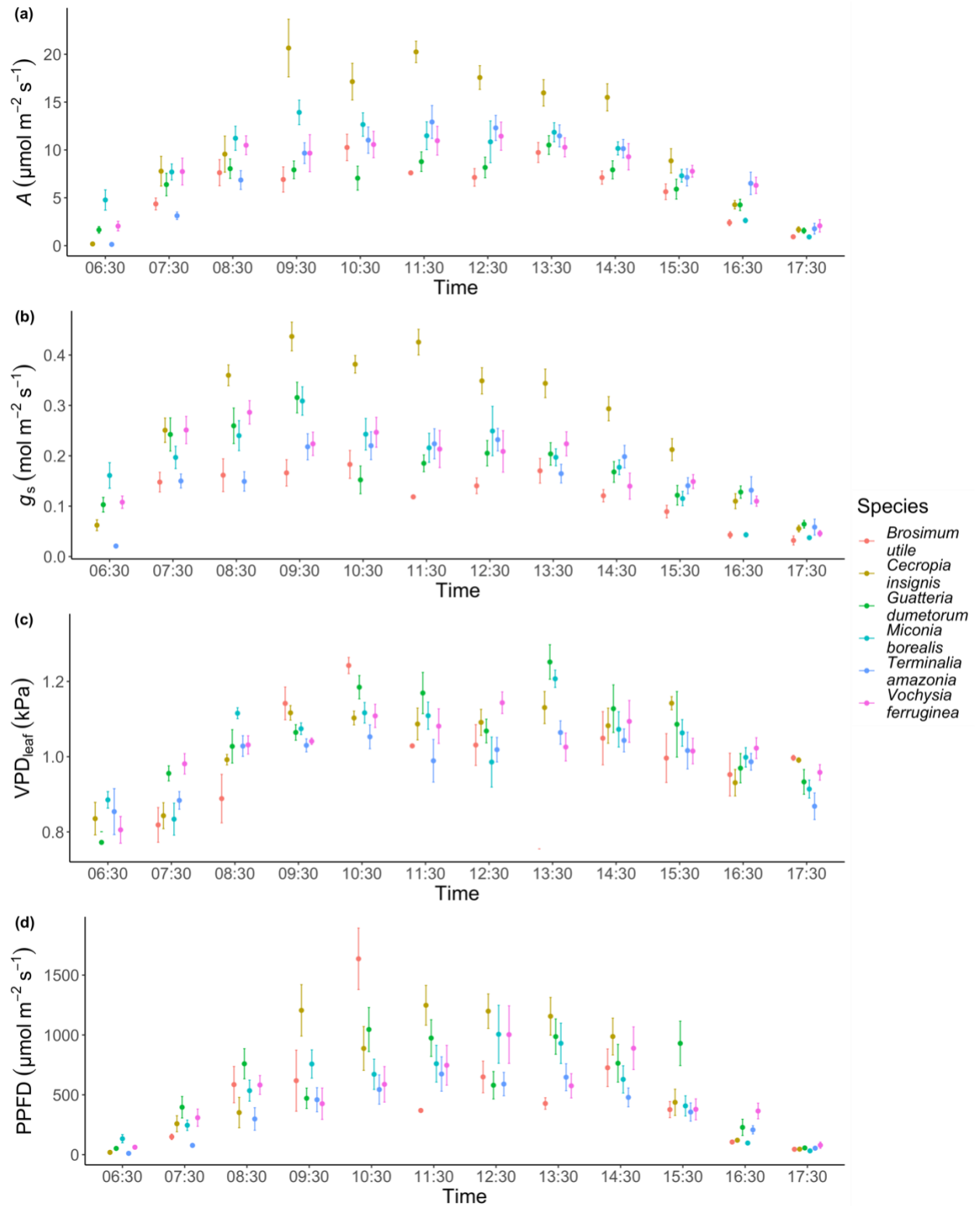


Figure 4. Species specific averages of hourly (a) photosynthesis, (b) stomatal conductance, (c) vapor pressure deficit at the leaf surface, and (d) irradiance collected during diurnal survey measurements. Error bars represent ± 2 SE of the mean.

Model comparison and validation

We evaluated the accuracy of the models built on the different datasets (response curve, survey, survey + time-of-day), first assessing their calibration scores, and then by predicting g_s measured by the survey method at the same site in 2016 (Rogers *et al.*, 2021) and using our 2020 validation dataset. In the calibration phase, the survey and survey + time-of-day models can be compared directly as they are derived using the same data set. The model including a time-of-day effect on g_1 and g_0 has an improved calibration r^2 (0.80 vs 0.75) a lower RMSE (0.0564 vs 0.0627 mol m⁻² s⁻¹), a lower AIC score (-1844 vs -1741) and a likelihood ratio test reveals the models were significantly different ($p < 0.001$). However, during the validation phase, the model calibrated on the survey dataset without a time-of-day effect performed the best, with the lowest RMSE, and the highest validation r^2 (Fig. 5).

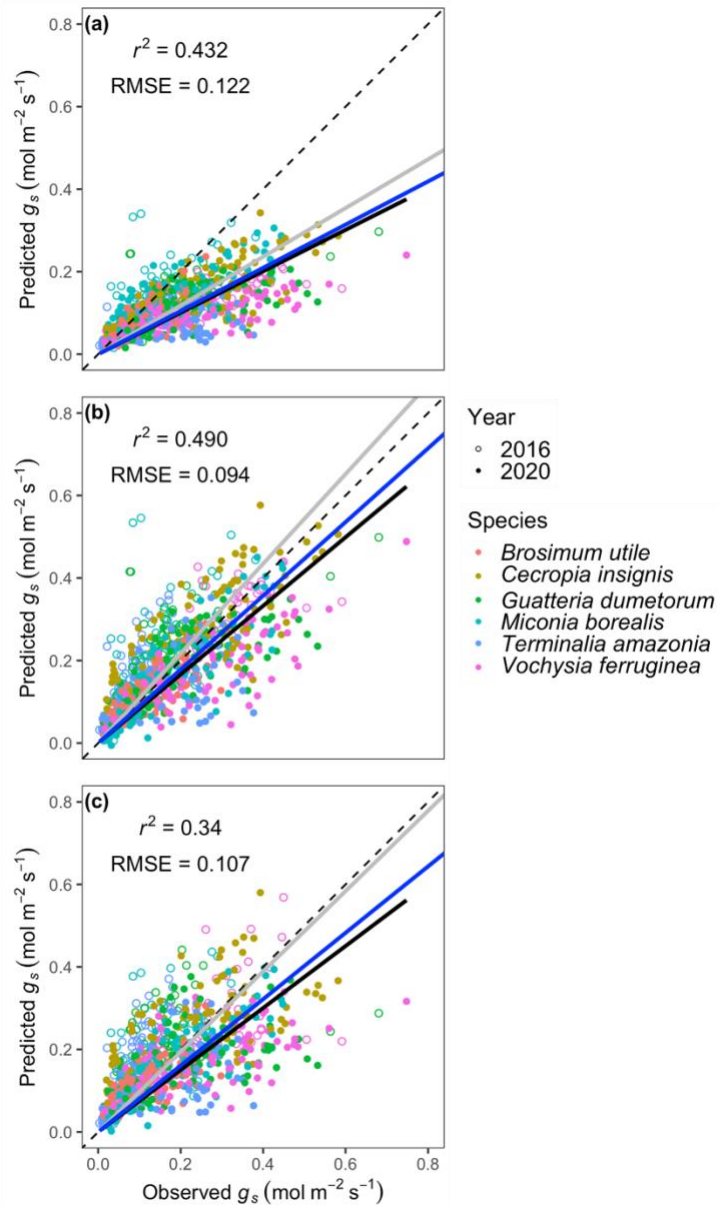


Figure 5. Validation plots of observed values of leaf level g_s versus model predicted data for (a.) the model based on response curve derived g_0 and g_1 , (b.) the model based on survey derived g_0 and g_1 , and (c.) the survey model with an inclusion of a time-of-day effect on g_1 . r^2 and RMSE values in each panel show that the survey model improves predictions of g_s over the response curve model, however, the inclusion of the time-of-day effect slightly worsens model predictive accuracy. Open circles were from the 2016 dataset collected by Wu et al. (2019), while filled circles correspond to the 2020 validation data. Solid black, grey, and blue trend lines correspond to the lines of best fit for the 2016 data, 2020 data, and all the data respectively. Dashed black line is a 1:1 reference line.

g_1 values estimated using the response curve dataset were significantly ($p < 0.001$) lower than when they were estimated using the survey data set (Fig. 6). On average, the response curves

produced estimates of g_1 which were 50% lower than estimates obtained from survey data. Only in the species *Brosimum utile* was there no significant difference in estimated g_1 (Fig. 6).

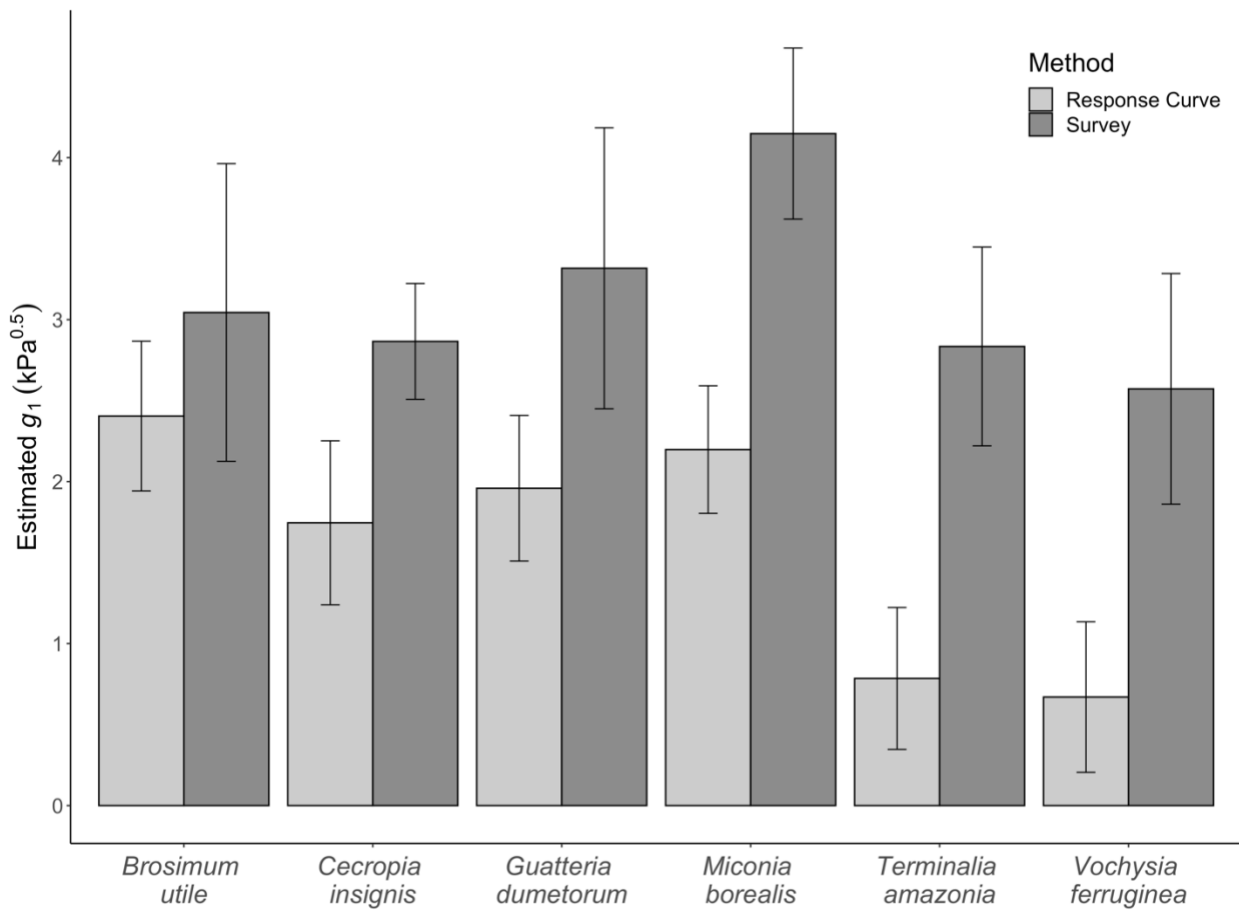
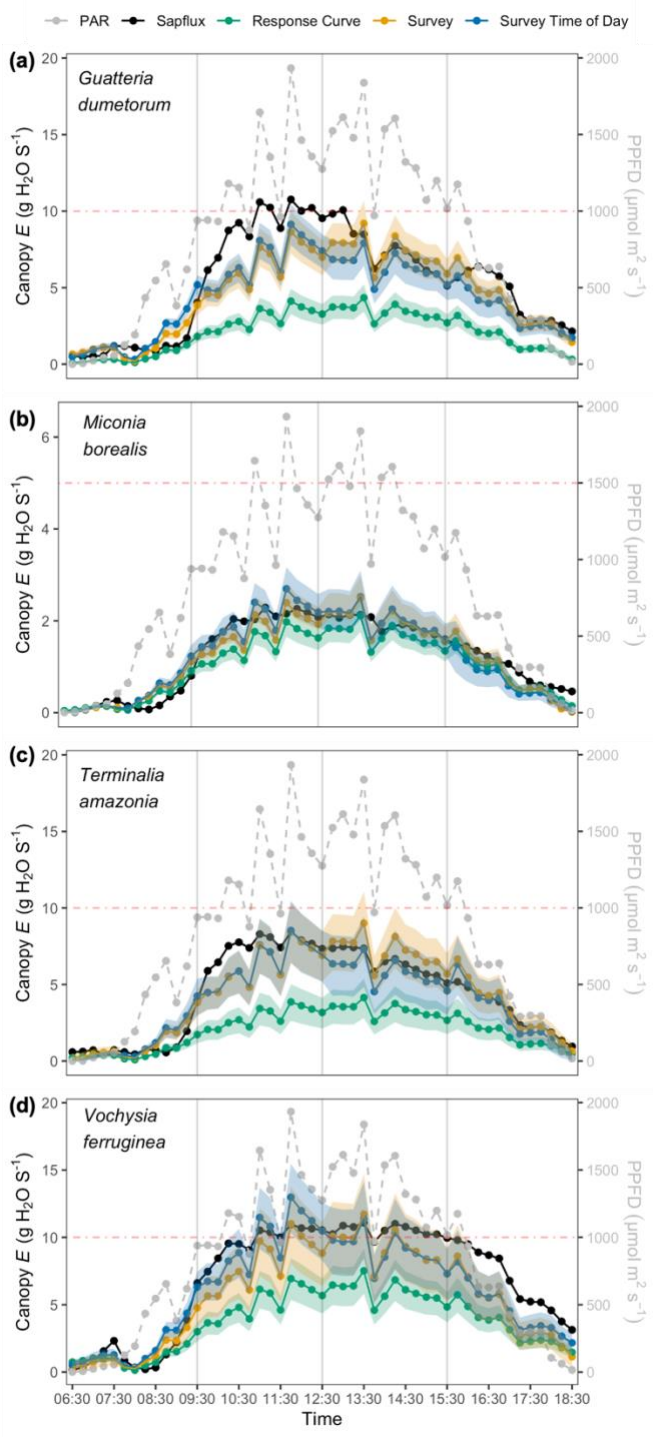


Figure 6. Fitted values of g_1 by species from the response and survey methods. For all species other than *Brosimum utile* g_1 from response curves was significantly lower than g_1 estimated from the survey data ($p < 0.001$). Error bars represent ± 2 SE of the mean.

Finally, we simulated canopy scale E for all six species to compare how the different methods of deriving stomatal parameters perform in a modeling context at the larger whole-tree scale. Four of the six species also had scaled sap flux velocity measurements (Pivovarov *et al.*, 2021) to compare the modeled E allowing us to benchmark results (Fig. 7). We found that when comparing estimated and observed E , the survey + time-of-day parameterization had the lowest RMSE (0.013 g H₂O m⁻² s⁻¹) followed by survey (0.014 g H₂O m⁻² s⁻¹), and the response curve method (0.022 g H₂O m⁻² s⁻¹) (Fig. S4). All three methods closely tracked changes in irradiance. However, the two

396 survey methods captured the magnitude of the change in canopy E more closely than the response
 397 curve method (Fig. 7).



398
 399 Figure 7. Simulated canopy scale E (g H₂O s⁻¹) at 15 min time points for (a.) *Guatteria dumetorum*,
 400 (b.) *Miconia borealis*, (c.) *Terminalia amazonia*, and (d.) *Vochysia ferruginea* on the 20th of
 401 February 2020. Black points and line represent the sapflux benchmark, green points and line
 402 represent response curve derived simulation, gold points and line represent survey derived

simulation, and blue points and line represent survey derived simulation with g_1 changing based on time-of-day. Shading around the simulated data represent error propagated into the simulation by using $g_1 \pm 1$ SE of the estimated value. Grey dashed line represents the solar profile (PAR), vertical grey lines show the transition between g_1 values for the survey time-of-day model and horizontal red line shows the level of saturating irradiance for each species.

Effect of leaf phenological stage on estimated stomatal parameters

For the response curve data set, both g_1 and g_0 varied by tree species ($p < 0.001$) and phenological stage ($p = 0.009$) with a significant stage:species interaction ($p < 0.001$, Figs. 8a, S5, Table 2). For the survey data set, g_0 varied by tree species ($p < 0.001$), phenological stage ($p < 0.001$) with a significant stage:species interaction ($p < 0.001$, Fig. S5, Table 2) but g_1 only varied among tree species ($p < 0.001$; Fig. 8b, Table 2).

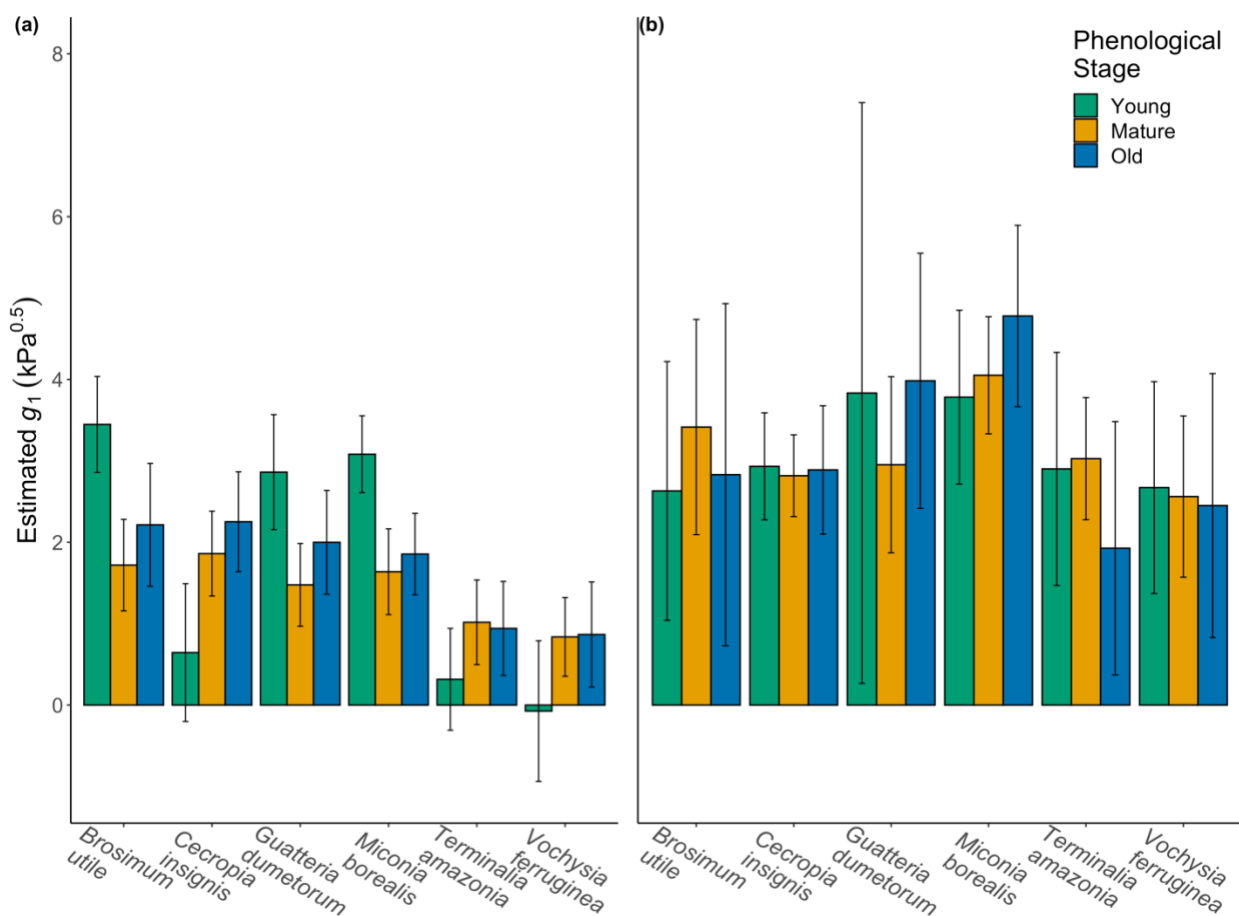


Figure 8. Fitted values of g_1 for (a.) response curve data, and (b.) data from survey measurements. Data in panel (a.) are significant to the species and phenological stage levels, while data in panel (b.) were only significant at the species level. Error bars represent ± 2 SE of the mean.

421 **Table 2. Fixed effects components from the mixed effects models tested.**

Model ¹	Effect	Numerator df	Denominator df	F-value	p-value
Response Curve	$g_0 \sim \text{species}$	6	607	94.30	< 0.0001
	$g_0 \sim \text{stage}$	2	607	10.30	< 0.001
	$g_0 \sim \text{stage:species}$	10	607	3.13	< 0.001
	$g_1 \sim \text{species}$	6	607	110.67	< 0.001
	$g_1 \sim \text{stage}$	2	607	4.73	0.009
	$g_1 \sim \text{stage:species}$	10	607	5.31	< 0.001
Survey	$g_0 \sim \text{species}$	6	629	630.08	< 0.001
	$g_0 \sim \text{stage}$	2	629	25.61	< 0.001
	$g_0 \sim \text{stage:species}$	10	629	9.10	< 0.001
	$g_1 \sim \text{species}$	6	629	118.21	< 0.001
	$g_1 \sim \text{stage}$	2	629	0.08	0.922
	$g_1 \sim \text{stage:species}$	10	629	0.50	0.889
Survey + time-of-day	$g_0 \sim \text{species}$	6	623	764.05	< 0.001
	$g_0 \sim \text{stage}$	2	623	31.06	< 0.001
	$g_0 \sim \text{stage:species}$	10	623	11.04	< 0.001
	$g_1 \sim \text{species}$	6	623	143.35	< 0.001
	$g_1 \sim \text{time}$	3	623	124.29	< 0.001
	$g_1 \sim \text{time:species}$	18	623	8.11	< 0.001

422 ¹A full description of each model, and the random effects components are provided in Note S1.

423
424 When assessing the differences between the interactive effects of phenological stage and
425 species on g_1 for response curves we only found four significant effects, *Brosimum utile* young
426 and mature ($p = 0.003$, 66.9% decrease), *Miconia borealis* young and mature ($p = 0.006$, 61.2%
427 decrease), *Miconia borealis* young and old ($p = 0.041$, 49.7% decrease), and *Cecropia insignis*
428 young and old ($p = 0.007$, 111% increase). We also used response curve data to investigate
429 continuous relationships between g_0 and g_1 with other related leaf physiological traits, but did not
430 observe a significant effect of LMA, Ψ_{leaf} , or successional stage on g_1 or g_0 (Fig. S6, S7).

Finally, we analyzed the interactive effects of phenological stage and species on $V_{cmax.25}$ and $R_{dark.25}$, derived from leaf spectroscopy. The relationships between stage and $R_{dark.25}$ ($p < 0.001$) and stage and $V_{cmax.25}$ ($p < 0.001$) were significant, ~40% decrease in values between the mature and old phenological stages (Fig. S8, Table 1).

Investigation of a time-of-day effect

Using the survey dataset (Fig. 4), we tested if the time-of-day influenced g_0 and g_1 . We found a significant decline in g_1 from morning to evening for the six species ($p < 0.05$, Table 2). g_1 was on average 2.5 times higher in the early morning than in the late afternoon, however, the magnitude of the decline differed between species (Fig. 9). Unlike g_1 , g_0 did not differ by the time-of-day for the six species ($p = 0.432$).

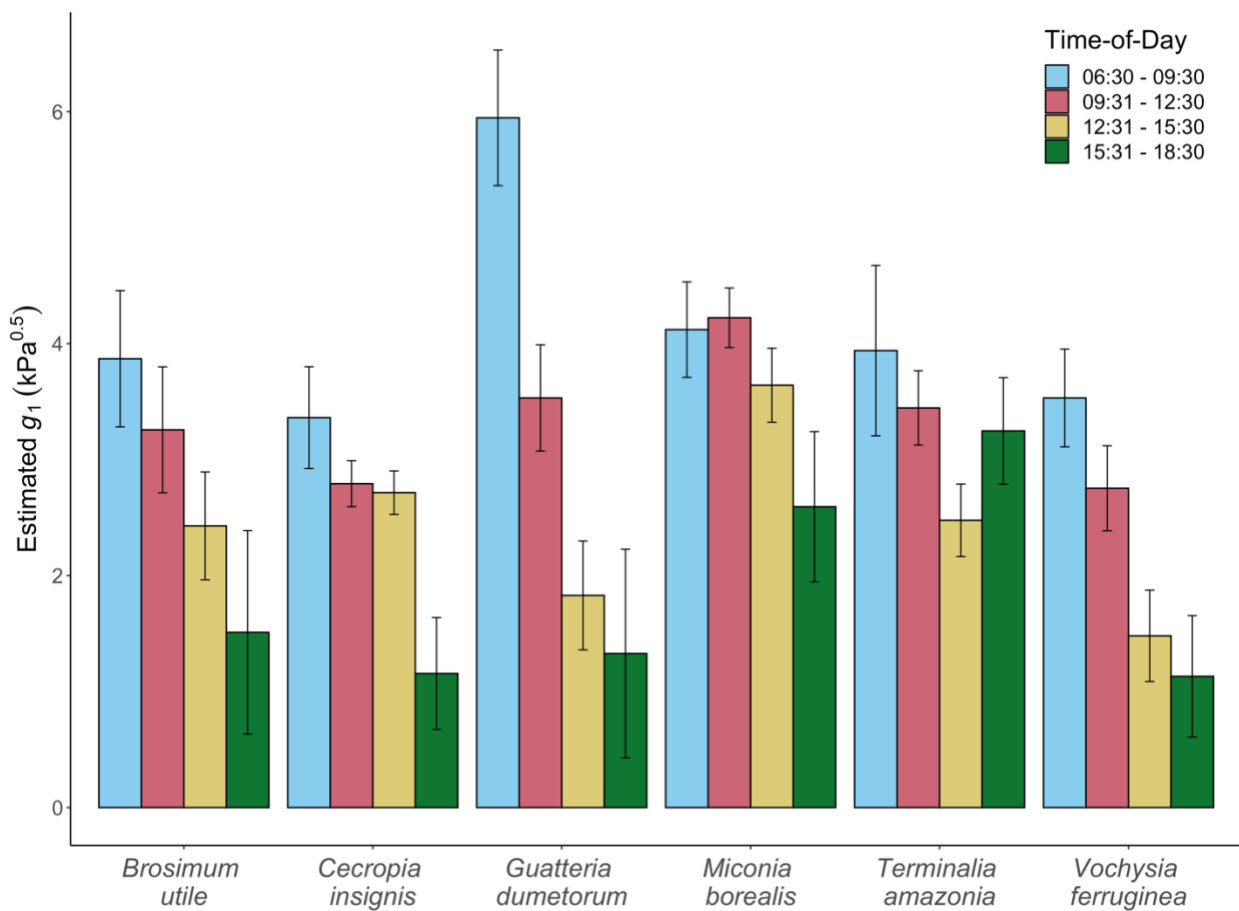


Figure 9. Trends in fitted values of g_1 by time-of-day and species. g_1 decreases significantly with increasing time-of-day for all six species investigated. *Brosimum utile*, *Cecropia insignis*, *Guatteria dumetorum*, and *Vochysia ferruginea*, all exhibit a very strong and consistent decline in

g_1 , whereas *Miconia borealis* and *Terminalia amazonia* exhibit a more moderate decline. Error bars represent ± 2 SE of the mean.

Canopy level transpiration simulations

To assess the impact of data collection methodology on model performance, we compared estimates of mean daytime E for the six species, three methods and sap flux benchmark (when available, Fig. 10). We assumed a constant flux of E for each 15 min interval and averaged E for each 12-hour diurnal period. For five of the six species the survey data produce significantly higher ($p < 0.001$) estimates of E than for the response curve data (*Brosimum utile* =26.0%, *Cecropia insignis*= 87.1%, *Guatteria dumetorum* = 124.9%, *Terminalia amazonia* =107.4%, and *Vochysia ferruginea* = 44.3% increase). In *Miconia borealis* there was no significant difference between E as estimated from curve derived values, and E as estimated from survey derived values ($p = 0.152$), even though in *Miconia borealis* the g_1 value estimated from response curves was nearly half that of the g_1 value estimated from survey measurements (Fig. 6). However, g_0 values for *Miconia borealis* were especially low with no significant difference between curve and survey-based estimates (Fig. S5), and *Miconia borealis* has a saturating irradiance which was 50% higher than the other five species in this study Rogers *et al.*, (2021).

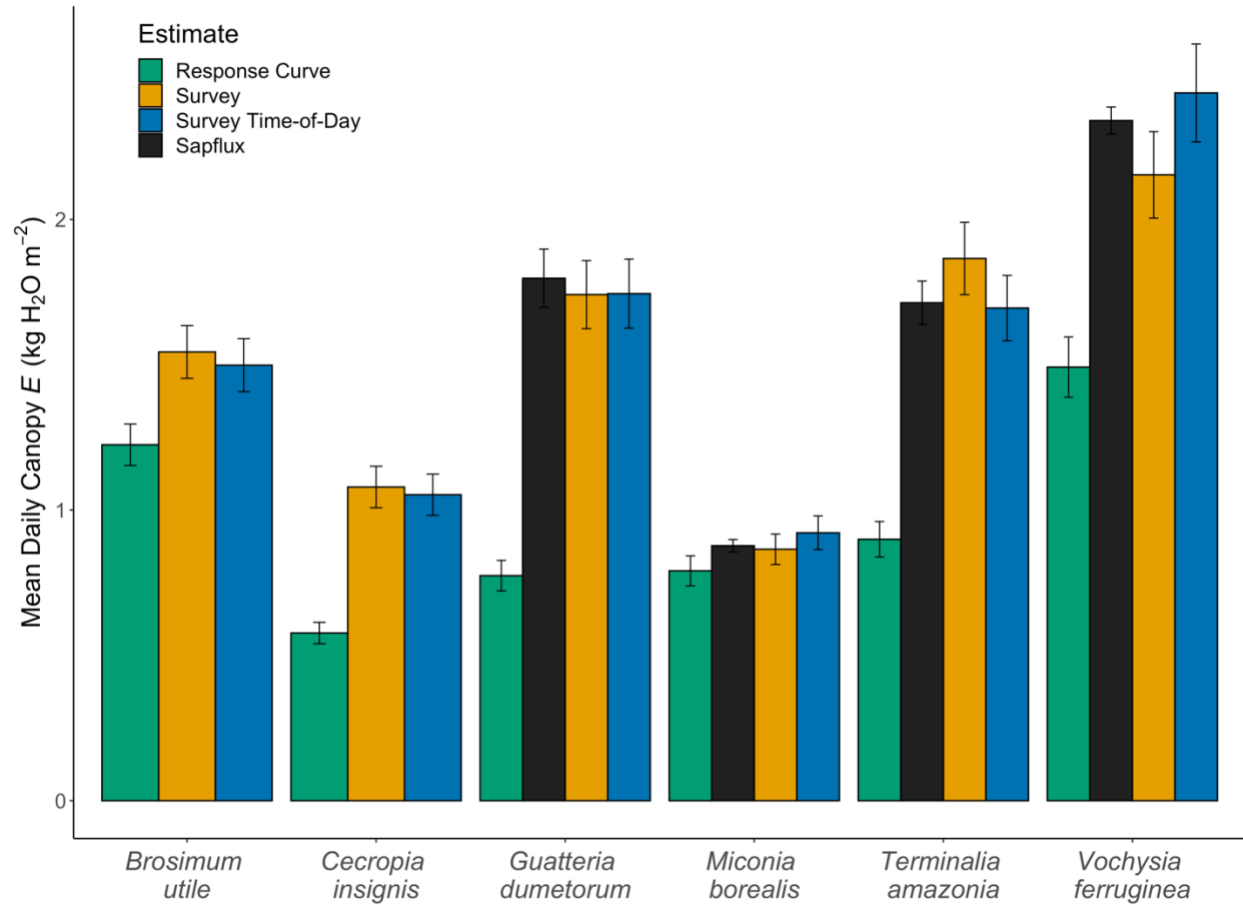


Figure 10. Comparison of estimated mean total daily E ($\text{kg H}_2\text{O m}^{-2}$ ground area) using the three models (Note S1). Sapflux benchmark values are presented for four of the six species in this study. Mean values are the average integrated total of E over the 42 diurnal (05:30-18:30) periods of complete sapflux coverage. Error bars represent ± 2 SE of the mean.

We also found no significant differences between the survey approaches for any of the six species. In all four species for which we have a benchmark, the benchmark and survey estimates showed strong correspondence with estimates being statistically indistinguishable ($p > 0.05$).

Finally, we examined the impact of leaf phenological stage distribution assumptions on the modeling of canopy E (Fig. S9). For this analysis we reran the model prescribing different distributions of leaf phenology, including even (33.3% young, 33.3% mature, 33.3% old), all-young, all-mature, and all-old. Interestingly, we found no significant differences in predicted daily canopy transpiration between the even phenology distribution, the “natural” (15% young, 80% mature, 5% old) phenology distribution, or the all-mature phenology distribution for any species when using any of the three models of stomatal parameters. However, when modeled using a single

phenological stage, we see strong variation in estimates of daily E (Fig. S9) with daily estimates of E varying by up to 150% in some species.

Discussion:

A key step toward reducing ESM uncertainty is to account for the abiotic and biotic effects on the stomatal parameters g_0 and g_1 . In this study we demonstrate that *ex-situ* stomatal response curves and *in-situ* survey style measurements produce statistically different estimations of stomatal parameters in tropical broadleaf evergreen forests (Fig. 6). These differences in parameterization resulted in large (between 26% and 125%) differences in simulated fluxes of water (Fig. 10). Further, we found that leaf phenology plays a role in regulating stomatal traits, however this role was not consistent across species (Fig. 8, S5) was not observed consistently using the different measurement approaches (Fig 6), and, for a given methodology, did not drive variation in modelled E (Fig. S9). We also observed a consistent decline in g_1 during the photoperiod (Fig. 9), with g_1 declining by an average of 250%. When these results are taken together and used to model forest function, we found that models using stomatal parameters derived from *ex-situ* response curves significantly underestimated canopy level E both at instantaneous time scales (Fig. S4) and across a full dry season (Fig. 7), and that while leaf traits do vary among leaf phenological stage, models which only include mature vegetation perform similarly to those that explicitly simulate three leaf stages (Fig. S9).

Derivation of stomatal model parameters is highly sensitive to the method of data collection

One of the clearest observations from this study is the significant difference in fitted values of g_1 between response curves and survey style measurements. In five of the six species studied, g_1 was significantly lower (50% reduction) for response curves than for survey measurements (Fig. 6). We explore several possible mechanistic explanations for the observed difference in g_1 estimates.

All response curves were measured on excised branch segments. While previous work on tropical tree species has shown that excision does not impair photosynthetic function (Verryck et al., 2020) stomata will be affected more acutely by hydraulic damage than chloroplasts if branches are not allowed to sufficiently rehydrate before measurement (Wolf et al., 2016; Lawson & Viallet-Chabrand, 2019). This decline in g_s , without a commensurate decline in A (Davidson et al., 2022)

may result in a decrease in estimated g_1 . An excision effect was previously observed in plants collected from the same site as this study, where levels of A and g_s diminished from pre-excision values within 60 minutes of branch removal (Santiago & Mulkey, 2003). Here, the researchers attributed excision induced embolism as the likely mechanism underlying their result. Missik *et al.*, (2021) found that immediately following excision, levels of g_s were reduced by up to 62% in woody deciduous trees, again attributing this decline to embolism. However, in both studies branches were cut during the daytime when transpiration fluxes are higher, and the risk of embolism is much greater (Sperry, 2013). Further, Santiago & Mulkey, (2003) limited their experiment to just 60 minutes, which may not be sufficient time for branches to rehydrate and acclimate to a new leaf level environment. Other studies (e.g., Meng & Arp, 1993; Miyazawa *et al.*, 2011) have found mixed, and sometimes contrasting effects of excision on gas exchange measurements.

A more relevant comparison may be drawn between this study and Davidson *et al.*, (2022) who, in a comparison of cut and uncut branch segments, demonstrated that in a woody deciduous species when excision is carried out predawn, stomatal behavior is not significantly altered in leaves measured within 8h of excision. We observed no significant difference in g_0 or g_1 estimated from curves conducted in the morning (07:00-12:00) and the afternoon (12:01-18:00) local time (Fig. S11) suggesting that excision stress did not alter stomatal function over the course of measurements. In addition, longer duration response curves were correlated with higher estimates of g_1 (Fig. S12), again suggesting that a stress response is not accumulating during measurement.

It is also possible that the direction of the response curve (increasing irradiance versus decreasing irradiance), combined with an asymmetrical response of stomata to long term changes in irradiance (Lawson & Blatt, 2014) may have led to the negative bias observed for g_1 . To test this, we collected response curves in both directions on a similar set of tropical species (Fig. S10) however we did not observe any evidence for a negative bias associated with curves in which irradiance is reduced.

There could also be error associated with the survey measurements. Accurate survey assessment of the $A:g_s$ relationship relies upon the assumption that levels of A and g_s are at a steady state (Hölttä *et al.*, 2017), and are acclimated to the environment surrounding the leaf, however errors when selecting an appropriate level of PPFD, VPD_{leaf} , or C_a will cause alteration of the leaf level environment. Taking irradiance as an example; if an incorrect level of irradiance is chosen,

the level of assimilation will change rapidly in response to the new irradiance, however the stomatal response will lag, which will alter the estimate of g_1 . Stomata are known to react more slowly to increases in irradiance than decreases in irradiance (Lawson & Vialet-Chabrand, 2019). This means that if, on average, we misestimated irradiance in the same proportion (equal under and over-estimation), we would expect a slightly lower WUE (higher g_1) for survey data, which is consistent with our findings (Fig. 6). This issue of improperly estimated irradiance is not insignificant, as irradiance can fluctuate dramatically, particularly under cloudy conditions (Fig. 7), which may lead to errors matching the natural environment to the leaf chamber conditions.

A final factor to consider is the leaf-to-leaf variability implicit in each measurement type, with survey measurements consisting of roughly 10 times the number of individual leaves as response curves (Fig. S2). Survey measurements showed a slightly poorer performance, and a significantly higher variance, in an internal model calibration than curve derived measurements (Fig. S13, Tables S1 and S2). This may be due to the larger leaf-to-leaf variability captured using the survey method, or it could be due to unstable ambient conditions at the time of measurement which can create noise in survey data (Fig. S2b and S3c-d). When the two models were validated using an external dataset comprised of ~120 new individual leaf estimates per species, both models had similar residual variation, while the response curve model exhibited a clear bias, underestimating levels of g_s (Fig. 5).

Clearly, both methods have benefits and drawbacks, and neither is free from error. Survey style measurements can integrate the diurnal dynamics of stomatal response, which may be an appealing feature for those who model stomatal response to a range of multivariate environmental conditions. Response curves capture stomatal response at truly stabilized conditions, which while stability of environment rarely occurs in nature, is useful for developing generalizable parameter estimates which can be used in ESMs.

The effect of leaf phenology and traits on g_0 and g_1

Previous work documenting phenological changes in leaf properties in wet evergreen tropical forest found a strong effect of leaf phenology on V_{cmax} (Kitajima *et al.*, 1997; Wu *et al.*, 2017). This change in V_{cmax} leads to a change in potential A , and an assumed commensurate change in the potential g_s , driven by changes in leaf level metabolism (Tobin & Rogers, 1992; Wingler *et al.*, 2004). However, if the relationship between A and g_s is not coordinated across leaf development,

g_1 will vary between leaf phenological stages. Theoretically, a phenology related increase in g_1 , as observed in *Cecropia insignis* (Fig. 8a) may be adaptive as a means of maximizing the photosynthetic efficiency of older vegetation. If V_{cmax} declines in older vegetation, as we observe in this study (Fig. S8), we may also expect an increase in g_1 as a higher level of intercellular carbon (C_i) is required for the same rate of A . However, in this study we did not find evidence across all species for a consistent effect of leaf phenological stage or relative leaf age on g_1 or g_0 (Fig. 8). Here we explore two possible explanations for the lack of an effect observed in our study.

In the USO model, the relationship between A and g_s is the chief control on the g_1 parameter. Coordination between A and g_s means that for a given level of A , there is a given level of g_s and thus a given level of C_i . Conversely, if A and g_s lack coordination, at a given level of A , g_s , and thus C_i , can vary. Therefore, tight coordination between A and g_s will result in leaves maintaining a fixed ratio of $C_i:C_a$ across changes in photosynthetic capacity (Wong *et al.*, 1979, 1985; McDowell *et al.*, 2006). We did not find significant differences in $C_i:C_a$ across any of the phenological stages across species (Fig. S14), suggesting that although V_{cmax} may shift with leaf phenology (Fig. S8a), g_s will change in proportion to supply and maintain a stable level of intercellular CO_2 (Long & Hällgren, 1993). This coordination in V_{cmax} and g_{smax} (g_s at saturating irradiance) is indicative of stable WUE or g_1 across phenological stages (Fig. 8). However, this does not suggest a lack of optimization of carbon capture through ontogeny. Even though the ratio of A and g_s does not change with leaf phenology, V_{cmax} changes with leaf phenology (Fig. S8a).

Time-of-day effect on g_1

While g_1 is often thought to be fixed over daily to seasonal timescales (Medlyn *et al.*, 2011), others suggest that at the leaf level g_1 may vary over shorter, diurnal time scales (Katul *et al.*, 2010). Daytime adjustments in g_1 may be driven by rapidly changing external stimuli such as irradiance (Way & Pearcy, 2012; Company *et al.*, 2016), or VPD and C_a (Katul *et al.*, 2009), to which individual leaves may alter their stomatal response in order to maximize assimilation, or minimize water loss. Supply and demand dynamics may also effect stomatal behavior, with a reduction in the water supply to the substomatal cavity (Bonan *et al.*, 2014), leading to a more conservative response. Here we observed declining g_1 over the diurnal periods for all six species (Fig. 9).

A decreased supply of water to the leaf late in the day (Venturas *et al.*, 2017), may be the most likely explanation for the observed decrease in g_1 . In this case, lower g_s at the same level of stomatal index, (a term representing A at a combination of leaf level environmental conditions) would result in a decrease in the fitted value of g_1 (Fig. S15). These findings are consistent with other studies of stomatal response (Manzoni *et al.*, 2011) which suggest that WUE responds to short-term changes in the supply of water. Longer term adjustments in WUE in response to seasonal or yearly drought, do not appear to be occurring at this site (Wu *et al.*, 2019b).

The finding that g_1 declines throughout the day is also consistent with models of stomatal optimality which apply a penalty on stomatal opening, either in the form of vascular damage (Wolf *et al.*, 2016; Sperry *et al.*, 2017; Anderegg *et al.*, 2018), or in non-stomatal limitations on A (Hölttä *et al.*, 2017; Dewar *et al.*, 2018; Kennedy *et al.*, 2019). Many of these models apply a function where stomata reduce their aperture in response to Ψ_{leaf} (Klein, 2014). To investigate if Ψ_{leaf} can help explain the diurnal dynamics of g_1 , we implemented the optimality model described in Anderegg *et al.*, (2018). This model applies a reduction in estimated g_s which is proportional to the assumed decline in stem xylem conductivity with increasing Ψ_{leaf} (see also Note S3). However, we found that the Ψ_{leaf} penalty from Anderegg *et al.*, (2018) poorly explained observed patterns of g_s (Fig. S16). Examination of stomatal model residuals versus measured Ψ_{leaf} (Fig. S7) demonstrates that Ψ_{leaf} , as an explanatory factor alone, is not capable of explaining the variation in g_1 observed, consistent with previous observations (Wu *et al.*, 2019b). As an alternative to Ψ_{leaf} based models, many ESMs use a β factor, dictated by soil moisture, to downregulate V_{cmax} and g_s . Recently, Li *et al.*, (2022) tested the applicability of a β factor at the same site in this study and found that, like Anderegg *et al.*, (2018), it over penalized drought-related loss of productivity. Several other hydraulic parameters such as hydraulic capacitance, and leaf hydraulic conductance may be more pertinent, and may play a larger role in stomatal regulation, however we lack those data in this current study.

The low morning WUE observed across all species may be the result of circadian resonance (Green *et al.*, 2002) whereby sunrise anticipation linked to a plant's endogenous rhythm may prime stomata to achieve their largest opening early in the day. This behavior, in systems with a fixed photoperiod, is thought to be adaptive as a means of maximizing carbon assimilation (Resco de Dios *et al.*, 2016). Higher than optimal morning g_s would lead to elevated estimates of g_1 , which

is consistent with previous findings that suggest circadian regulation fails to help to optimize diurnal variations in stomatal conductance (Resco de Dios *et al.*, 2020).

The failure of a time-of-day effect to significantly improve predictions of g_s (Fig. 5) suggests that while an overall pattern of declining g_1 is expected, the magnitude of the decline each day is the byproduct of abiotic processes that we may not have fully captured in this study, such as dynamics in the soil-plant-atmosphere continuum (Zhang *et al.*, 2018), or an effect of ABA on regulating stomata (McAdam & Brodribb, 2015). Without these data, and a more detailed understanding of the response of stomata to small changes in VPD (Buckley, 2016) it may not be possible to model diurnal fluctuations in WUE. Thus, we recommend future research explore diurnal shifts in g_1 in greater detail, as an understanding of the mechanisms behind these dynamics has the potential to significantly advance our model representation of canopy E .

Impact of measurement method on models of canopy function

Our simulations of canopy E revealed that using parameters derived from response curves leads to a systematically underestimated (26-125%) diurnal flux (Figs. 7, 10), consistent with our external model validation (Figs. 5, S4). This underestimation is in part attributable to lower g_1 values from response curves (Fig. 6) which limit daytime E , due to the assumption that at an irradiance level greater than saturating, $g_s = g_{smax}$.

All three models underestimate the level of E at low irradiance as the simulations assume that any time irradiance is below the light compensation point, $g_s = g_0$. However, this assumption may be flawed due to the combination of cuticular conductance and stomatal leakage (Machado *et al.*, 2021), measurement artifacts (Duursma *et al.*, 2019) or unaccounted for transpiration from stored crown water supplies (Meinzer *et al.*, 2003). The integrated diurnal flux of water (Fig. 10) also reveals a large impact of parameterization, with the survey method producing species level values of E between 9% and 77% higher than model output using response curve derived parameters, underscoring the impact that g_1 can have on model estimation of ecosystem function (Dietze *et al.*, 2014) and reinforcing the need for careful choices with regard to the method used to estimate g_1 .

Finally, in our assessment of the impact of selected phenological distribution on simulations of canopy E (Fig. S9) we observed no significant difference in daily E between models run using a natural leaf distribution an even phenological distribution or an all-mature distribution. Previous work in the Brazilian Amazon has suggested that appropriate estimation of leaf phenological

distribution is essential for accurate estimation of gross primary productivity (GPP), and canopy A_{net} (Wu *et al.*, 2016, 2017). The fact that our models of E are not sensitive to leaf age distribution may be due in part to the limited role leaf phenology plays in stomatal traits (Fig. 8), with the majority of between phenological stage variation being modeled as variation in V_{cmax} (Fig. S8, Wu *et al.*, 2019a). While V_{cmax} plays a role in determining g_s and therefore E , variation in the g_1 and g_0 parameters would be expected to manifest in larger variation in E (Rogers *et al.*, 2017). Our findings underscore the need to closely consider the method used, and assumptions implicit to that method, when parameterizing generic PFTs, or when applying trait based approaches to ESMs (Van Bodegom *et al.*, 2012).

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Author Contributions:

KJD, JL, AR, SJW and SPS conceived of the study. KJD, JL, AR, KSE, and SPS contributed to the leaf level data collection and quality checking. NM, ALP, BTW, and AZ contributed to the sap flux data. KJD, JL, AR, ALP, BTW, and SPS analyzed the data. KJD drafted the manuscript, and all authors contributed to the final version.

Data Availability:

Gas exchange data that support the findings of this study are publicly available in: Lamour J; Davidson K; Ely K; Anderson J; Serbin S; Rogers A (2021): Leaf gas exchange and fitted parameters, San Lorenzo, Panama, 2020. 1.0. NGEE Tropics Data Collection. (dataset). <http://dx.doi.org/10.15486/ngt/1781004>. and Rogers A; Serbin S; Ely K; Wu J; Wolfe B; Dickman T; Collins A; Detto M; Grossiord C; McDowell N; Michaletz S (2022): Diurnal leaf gas exchange

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Leaf trait data that support the findings of this study are publicly available in: Lamour J; Davidson K; Ely K; Anderson J; Rogers A; Serbin S (2022): Leaf structural and chemical traits, and BNL field campaign sample details, San Lorenzo, Panama, 2020. 1.0. NGEE Tropics Data Collection. (dataset). <http://dx.doi.org/10.15486/ngt/1783737>.

Spectroscopic data that support the findings of this study are publicly available in: Lamour J; Davidson K; Ely K; Anderson J; Rogers A; Serbin S (2021): Leaf spectral reflectance and transmittance, San Lorenzo, Panama, 2020. 1.0. NGEE Tropics Data Collection. (dataset). <http://dx.doi.org/10.15486/ngt/1781003>.

Sap flux data that support the findings of this study are publicly available in: Pivovarovoff A; McDowell N; Davies S; Detto M; Wolfe B; Wright J; Zambrano A (2020): Sap flow data from San Lorenzo, Panama (PA-SLZ) from January - June 2020. 1.0. NGEE Tropics Data Collection. (dataset). <https://doi.org/10.15486/ngt/1678733>

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Supporting Information:

For full supporting information see the document “Supporting_Information.docx” Single sentence summaries of all SI items are provided below.

Figure S1. Histograms of VPD_{air} recorded by the STRI metrological station positioned on top of the canopy crane from 06:00-18:30 for the duration of the study and recorded inside the cuvette of the LI-6800 during survey measurements.

Figure S2. Stomatal response regression fit following the form of Equation 2 of individual observations of from response curve data, and survey derived data.

Figure S3. Histograms showing the distribution of individual observations of photosynthesis, stomatal conductance, vapor pressure deficit at the leaf surface, and irradiance, collected during diurnal survey measurements, divided out by time of day and by species.

Figure S4. Observed versus predicted fluxes of canopy scale E for *Guatteria dumetorum*, *Miconia borealis*, *Terminalia amazonia*, and *Vochysia ferruginea* using three different methods to derive stomatal parameters.

Figure S5. g_0 comparisons between different methods of data collection the six species studied broken out by phenological stage.

Figure S6. The relationship between response curve derived g_1 and g_0 and LMA, Ψ_{leaf} , and successional stage.

Figure S7. The relationship between leaf water potential and model residual (difference between observed and predicted g_s) for the survey model including a time-of-day effect.

Figure S8. Relationship between leaf phenological stage and (a.) $V_{\text{cmax},25}$, and (b.) $R_{\text{dark},25}$, for the six species in this study.

Figure S9. Simulations of daily canopy E by species when using different leaf phenology distributions.

Figure S10. g_1 as estimated from response curves collected with decreasing (saturating to 0 $\mu\text{mol PAR}$) and increasing (0 $\mu\text{mol PAR}$ to saturating) levels of irradiance.

Figure S11. Comparison of fitted values for g_1 and g_0 by species for curves conducted in the morning (07:00-12:00) and afternoon (12:01-18:00).

Figure S12. Estimated g_1 versus length of response curve measurement.

Figure S13. Calibration plots for the curve derived data and the survey derived data used to build the models of g_1 and g_0 .

1038 Figure S14. Mean estimated intercellular carbon mole fraction (C_i) by species and leaf age.

1039

1040 Figure S15. Conceptual illustration of the link between stomatal slope, and water use efficiency.

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1042 Figure S16. Observed vs predicted g_s when a Ψ_{leaf} penalty is applied (see Note S3), and when no
1043 penalty is applied.

1044

1045 Note S1: Description of mixed effects models and their parameter estimates.

1046

1047 Table S1: Parameter estimates and their standard error for the response curve model

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1049 Table S2: Parameter estimates and their standard error for the survey model

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1051 Table S3: Parameter estimates and their standard error for the survey + time-of-day model

1052

1053 Note S2: Description of the leaf scale photosynthesis, conductance, and energy balance model
1054 from the R package LeafGasExchange (Lamour & Serbin, 2021)

1055

1056 Note S3: Description of the Ψ_{leaf} penalty function introduced by Wolf *et al.*, (2016) and tested by
1057 Anderegg *et al.*, (2017).

New Phytologist Supporting Information

Article title: Short term variation in leaf level water use efficiency in a tropical forest

Authors: Kenneth J. Davidson, Julien Lamour, Alistair Rogers, Kim S. Ely, Qianyu Li, Nate G. McDowell, Alexandria L. Pivovarov, Brett T. Wolfe, S. Joseph Wright, Alfonso Zambrano, and Shawn P. Serbin

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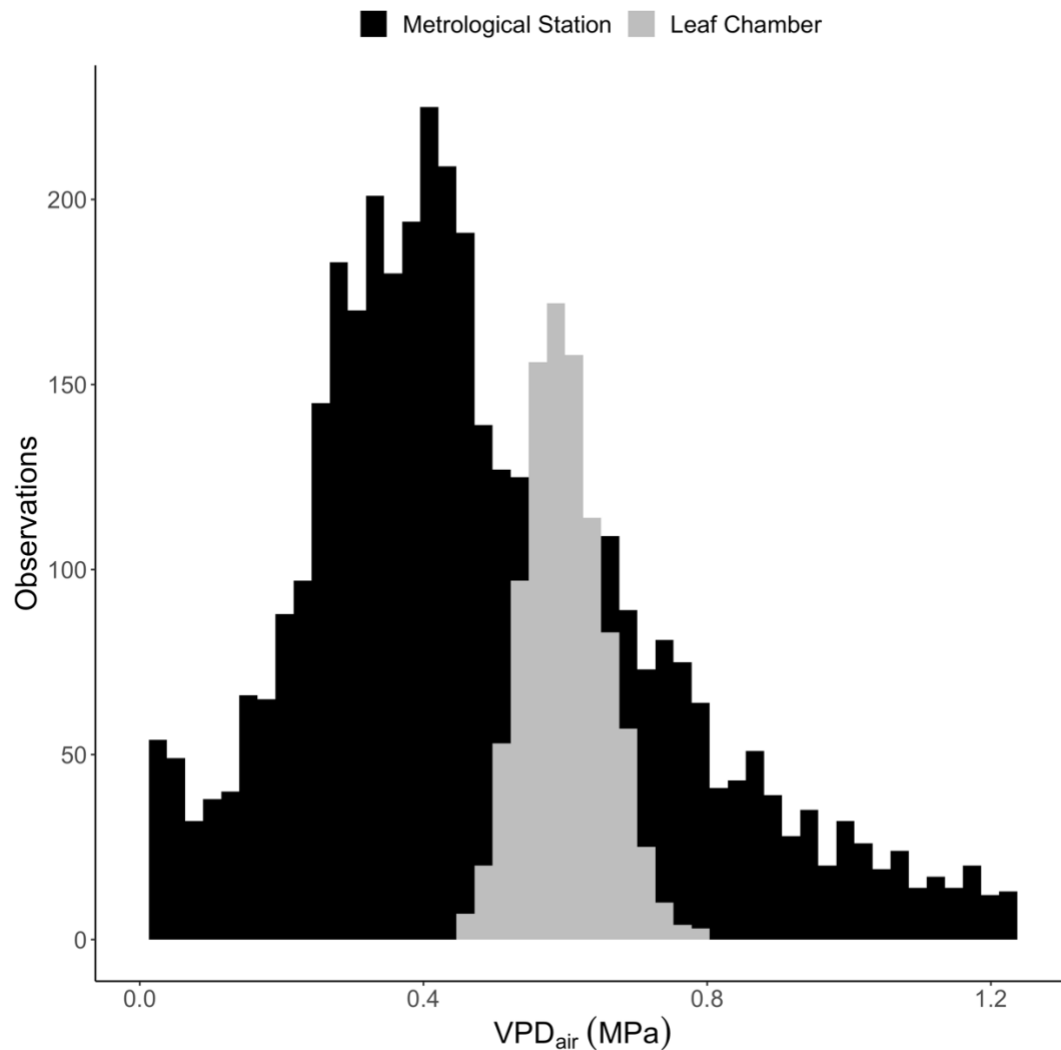


Figure S1. Histograms of VPD_{air} recorded by the STRI metrological station positioned on top of the canopy crane from 06:00-18:30 for the duration of the study (black) and recorded inside the cuvette of the LI-6800 during survey measurements (grey).

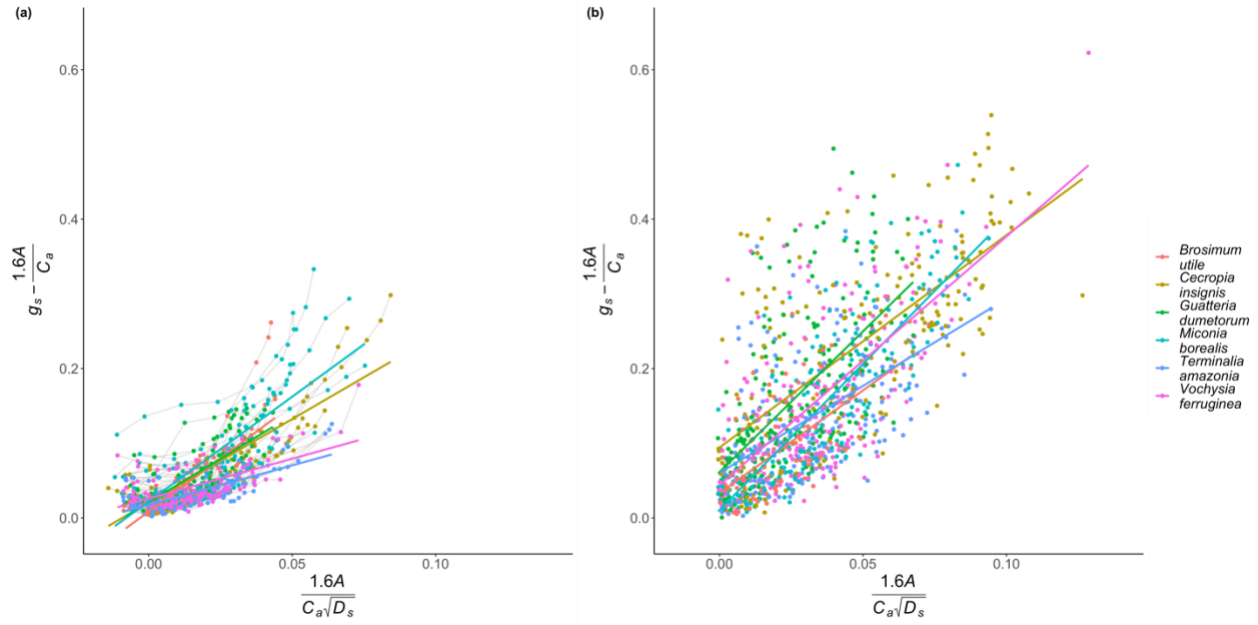


Figure S2. Stomatal response regression fit following the form of Equation 2 of individual observations of from (a) response curve data, and (b) survey derived data. For the response curve data, points which were collected on the same leaf are connected by a grey line.

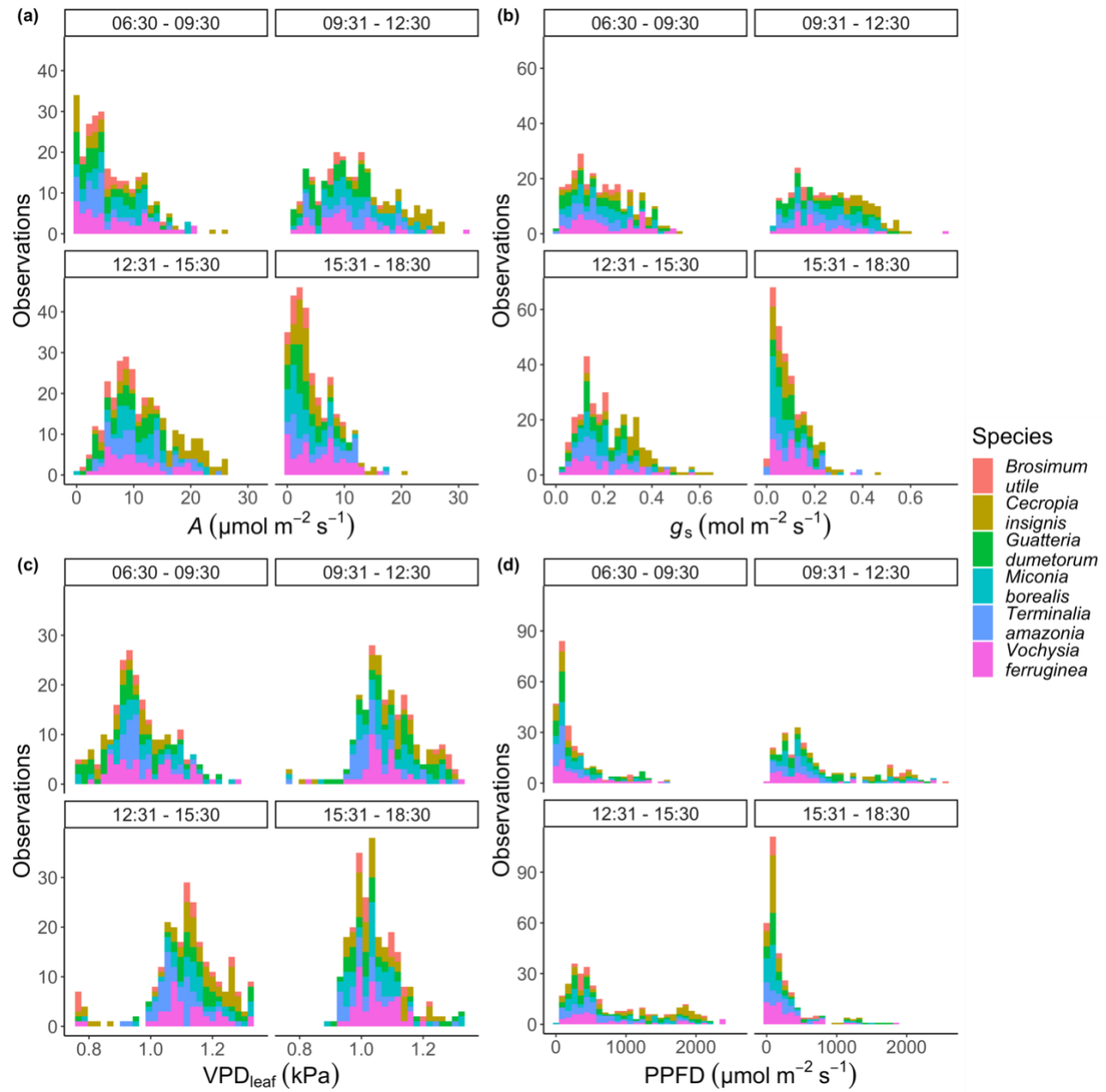


Figure S3. Histograms showing the distribution of individual observations of (a) photosynthesis, (b) stomatal conductance, (c) vapor pressure deficit at the leaf surface, and (d) irradiance, collected during diurnal survey measurements, divided out by time of day and by species.

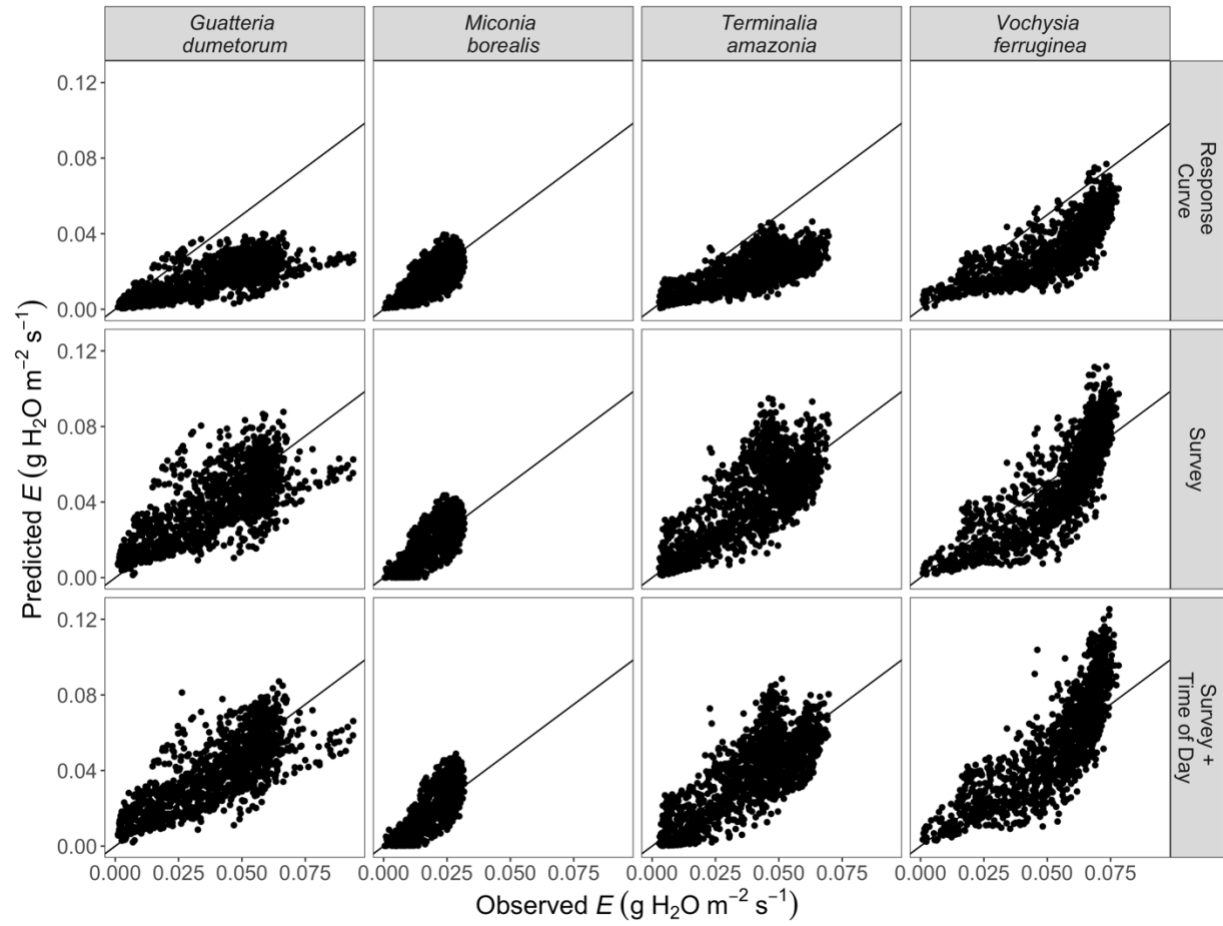


Figure S4. Observed versus predicted fluxes of canopy scale E for *Guatteria dumetorum*, *Miconia borealis*, *Terminalia amazonia*, and *Vochysia ferruginea* using three different methods to derive stomatal parameters. Observed data are scaled from sapflux measurements.

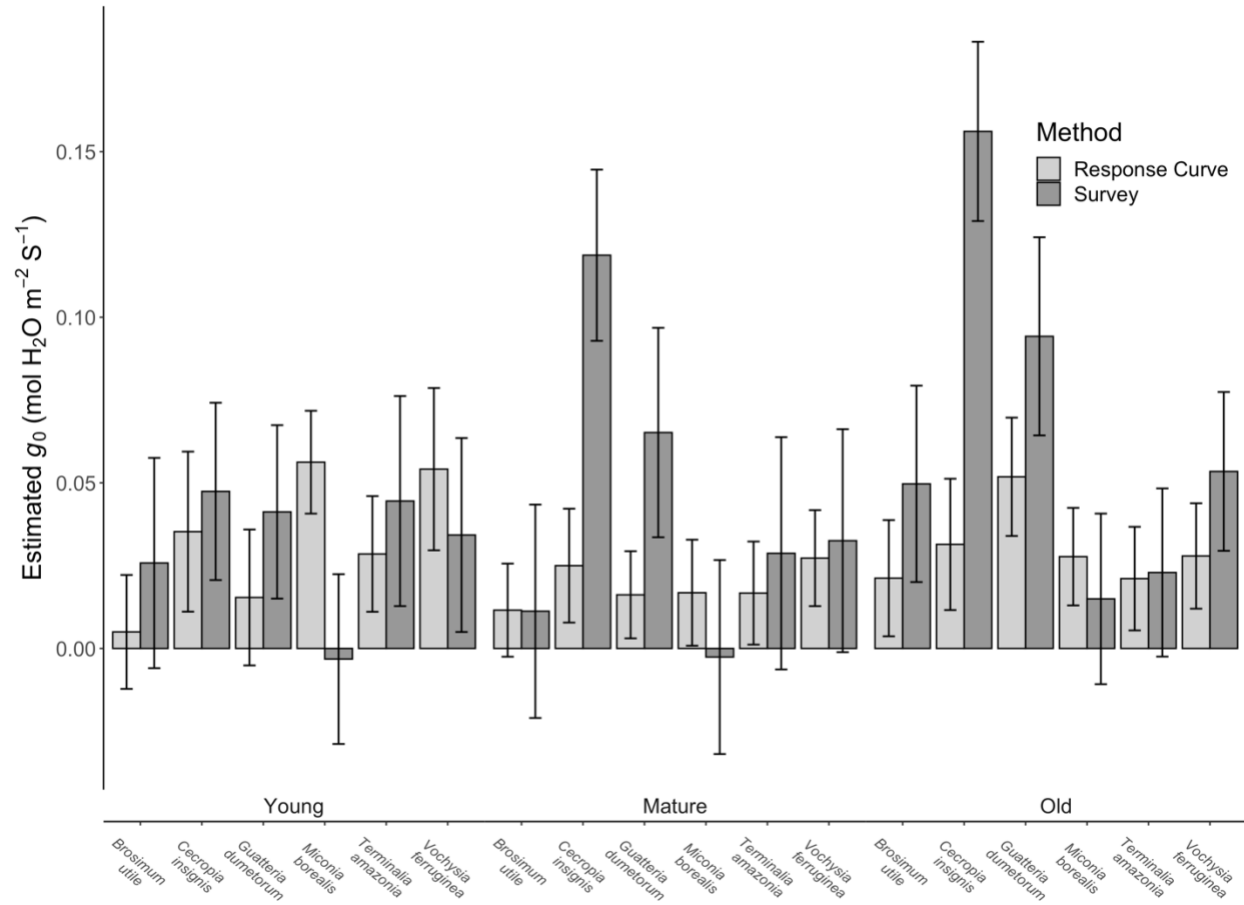


Figure S5. g_0 comparisons between different methods reveal a similar trend of elevated survey parameter estimates for four of the six species. There does not appear to be a consistent pattern between phenological stage and g_0 for either of the methods. Error bars represent ± 2 SE of the mean.

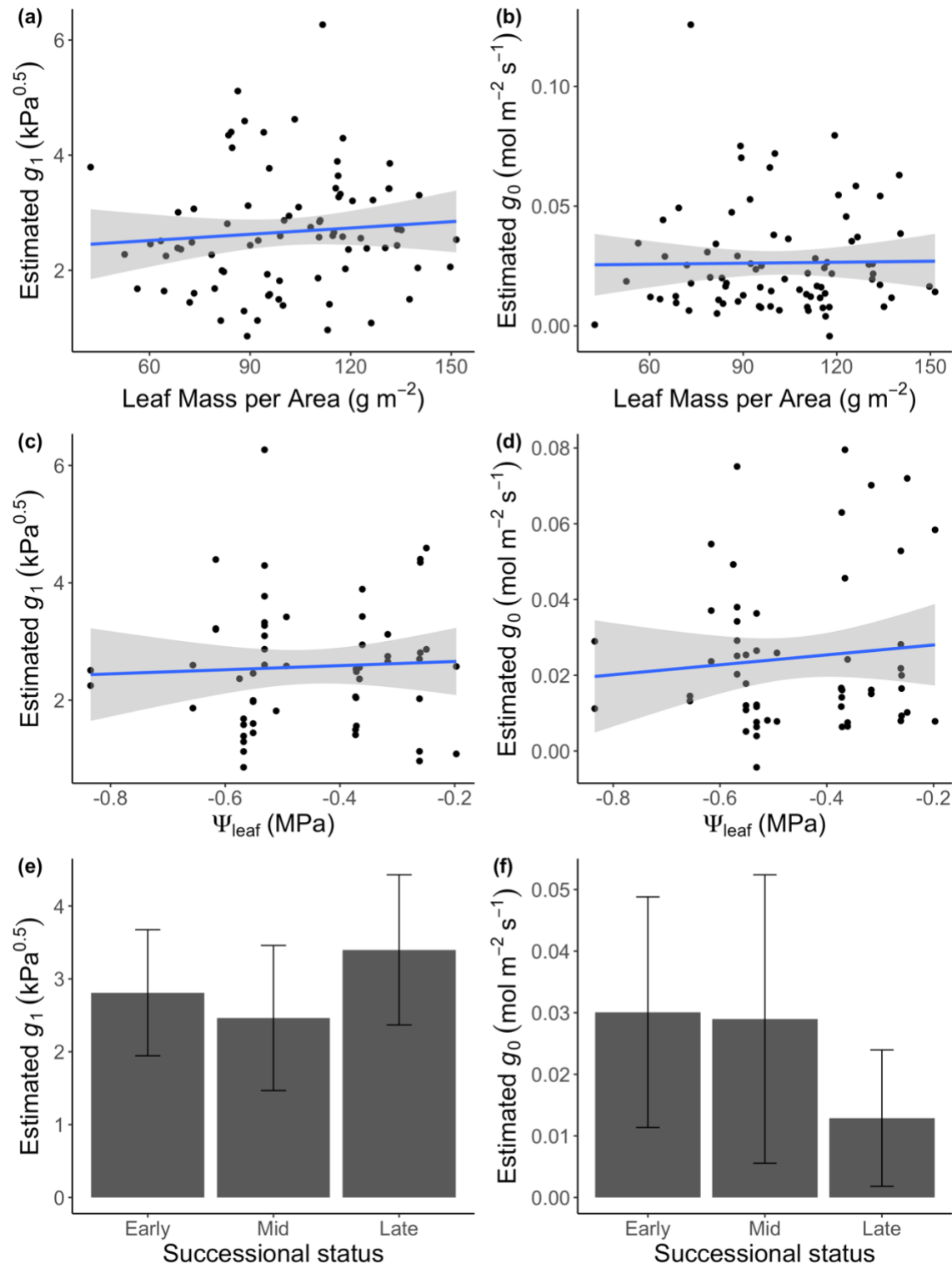


Figure S6. The relationship between response curve derived g_1 and g_0 and LMA (a-b), Ψ_{leaf} (c-d), and successional stage (e-f). None of the relationships shown are significant. Error bars represent ± 1 SD of the mean.

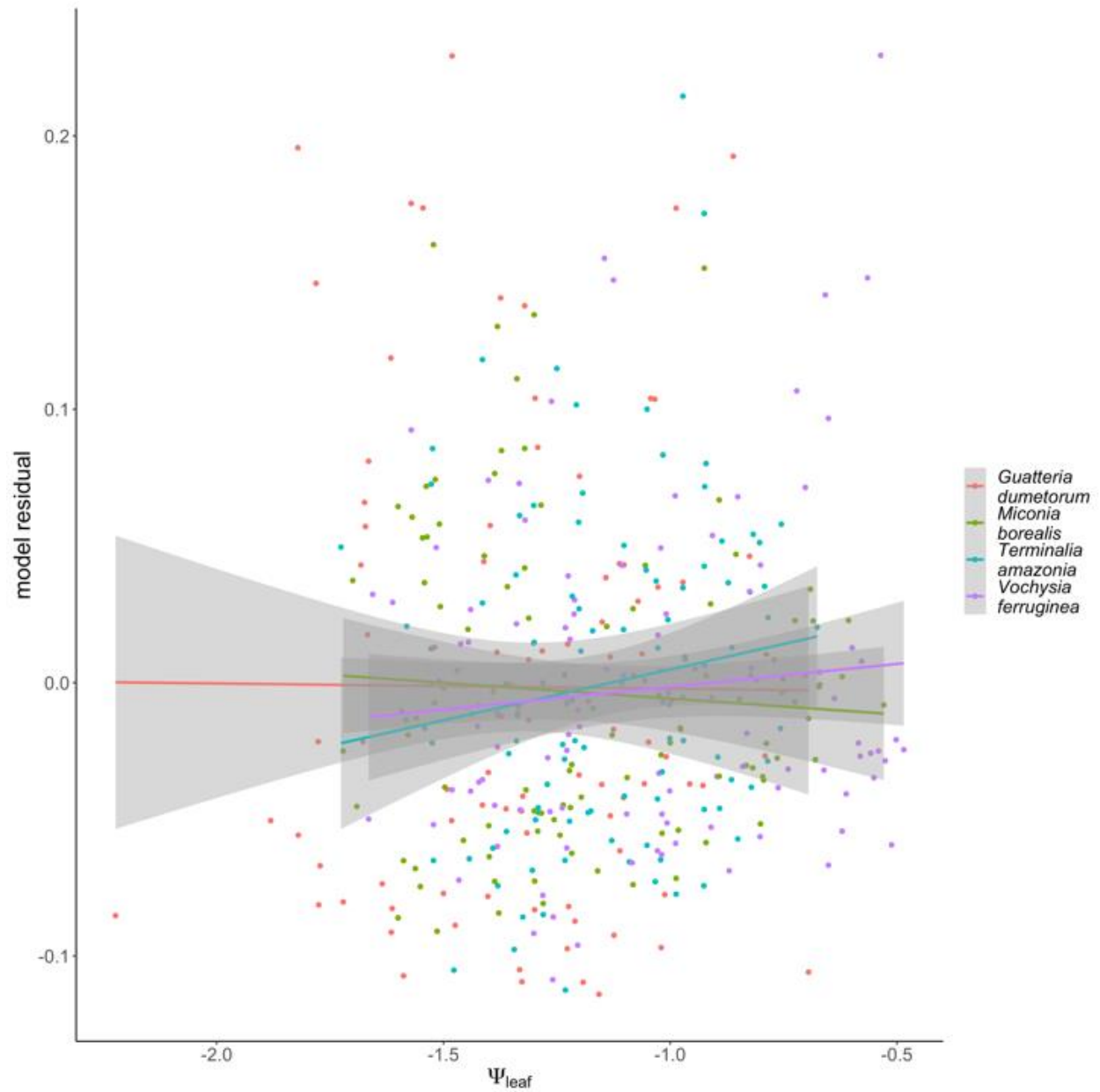


Figure S7. The relationship between leaf water potential and model residual (difference between observed and predicted g_s) for the survey model including a time-of-day effect. Grey shroud around the lines of best fit indicates the interval of prediction, the expected range for a new value given the data.

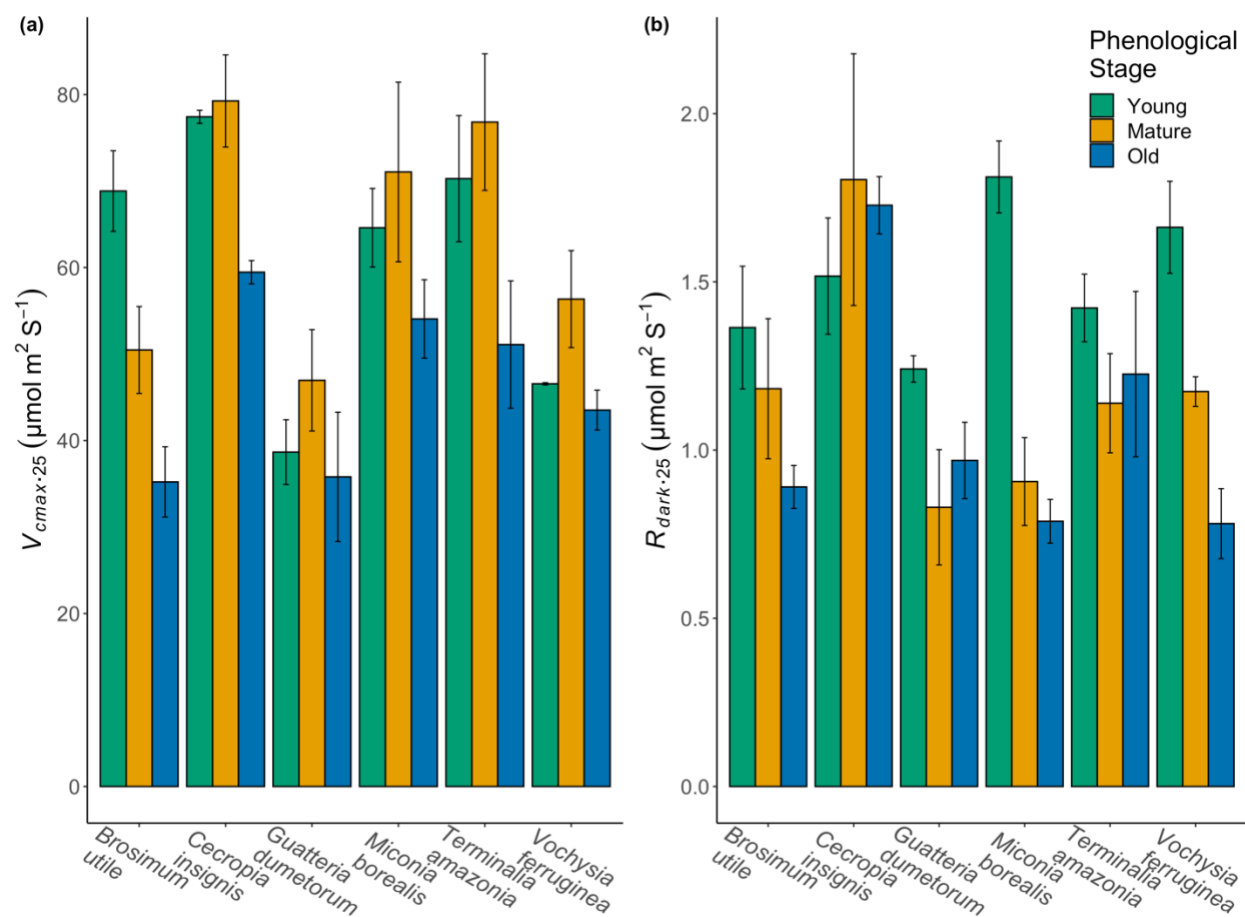


Figure S8. Relationship between leaf phenological stage and (a.) $V_{c,max,25}$, and (b.) $R_{dark,25}$, for the six species in this study. Variation in both $V_{c,max,25}$, and $R_{dark,25}$ is significant to the species and phenological stage levels. Error bars represent ± 2 SE of the mean.

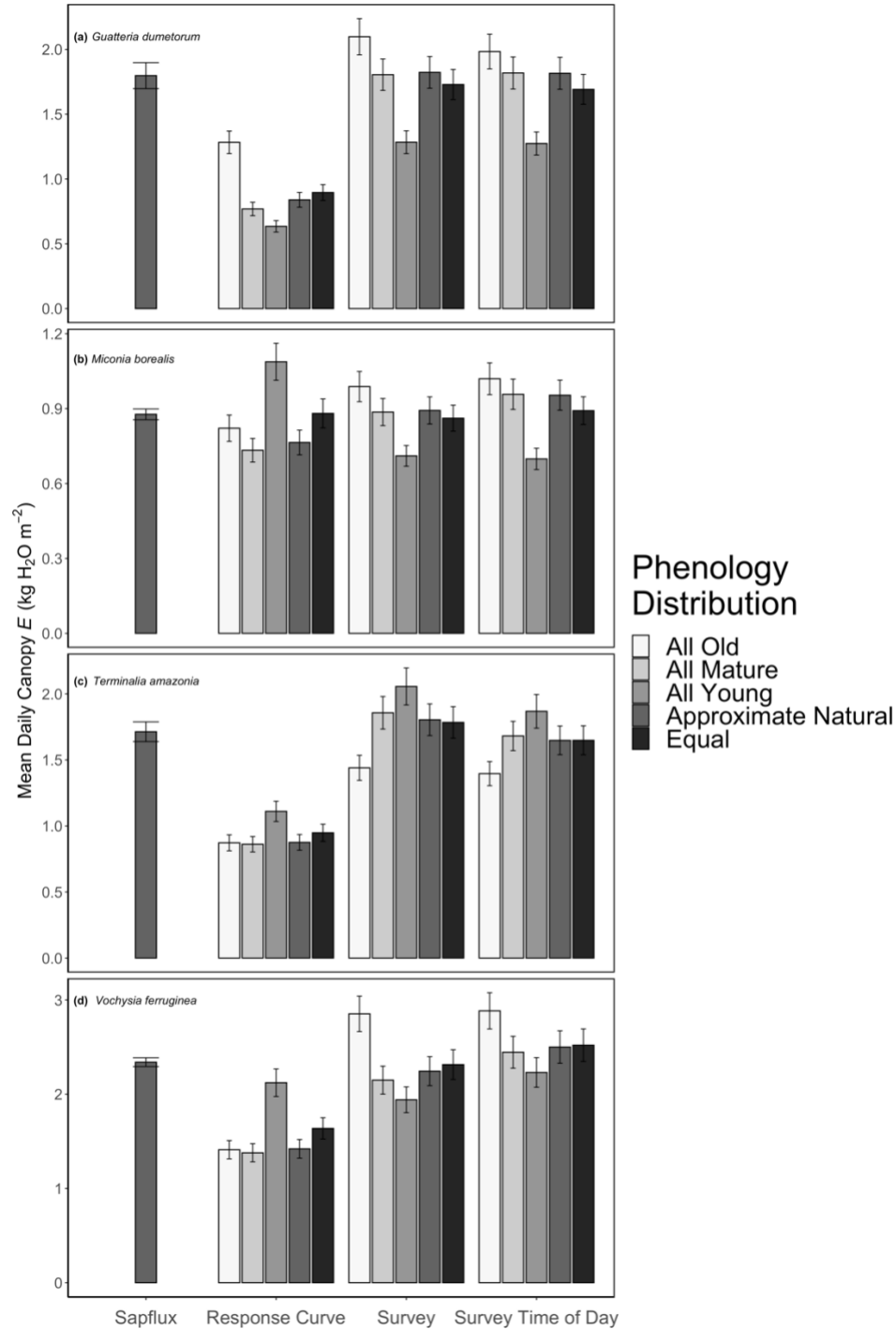


Figure S9. Simulations of daily canopy E by species when using different leaf phenology distributions. Distributions are as follows; All Old (0% young, 0% mature, 100% old), All Mature (0% young, 100% mature, 0% old), All Young (100% young, 0% mature, 0% old), Approximate Natural (15% young, 80% mature, 5% old), Equal (33.3% young, 33.3% mature, 33.3% old). X axis labels indicate which model was used to derive the conductance parameters for each simulation. Error bars represent ± 2 SE of the mean.

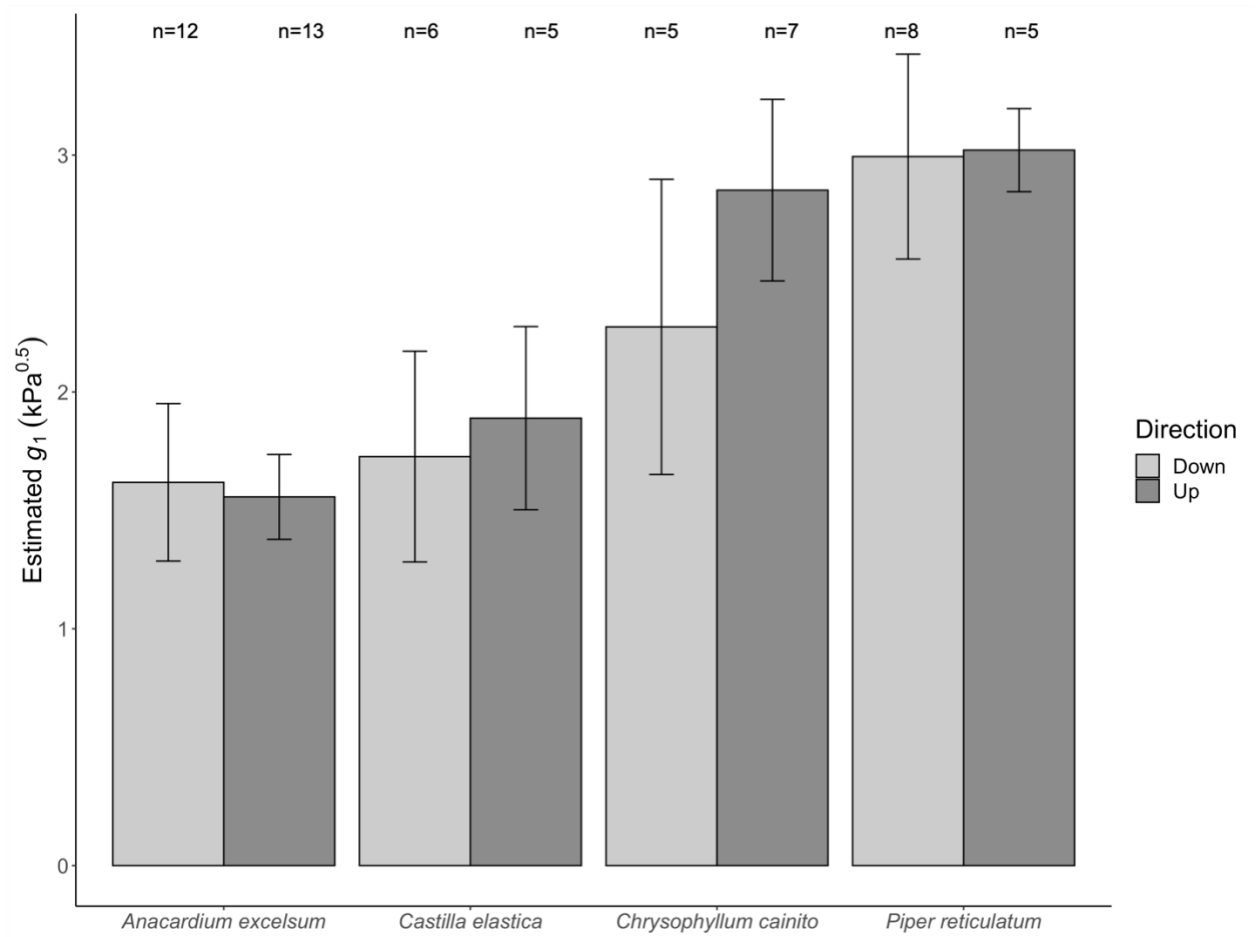


Figure S10. g_1 as estimated from response curves collected with decreasing (saturating to 0 μmol PAR) and increasing (0 μmol PAR to saturating) levels of irradiance. None of the within species g_1 estimates were significantly different. Error bars represent ± 2 SE of the mean.

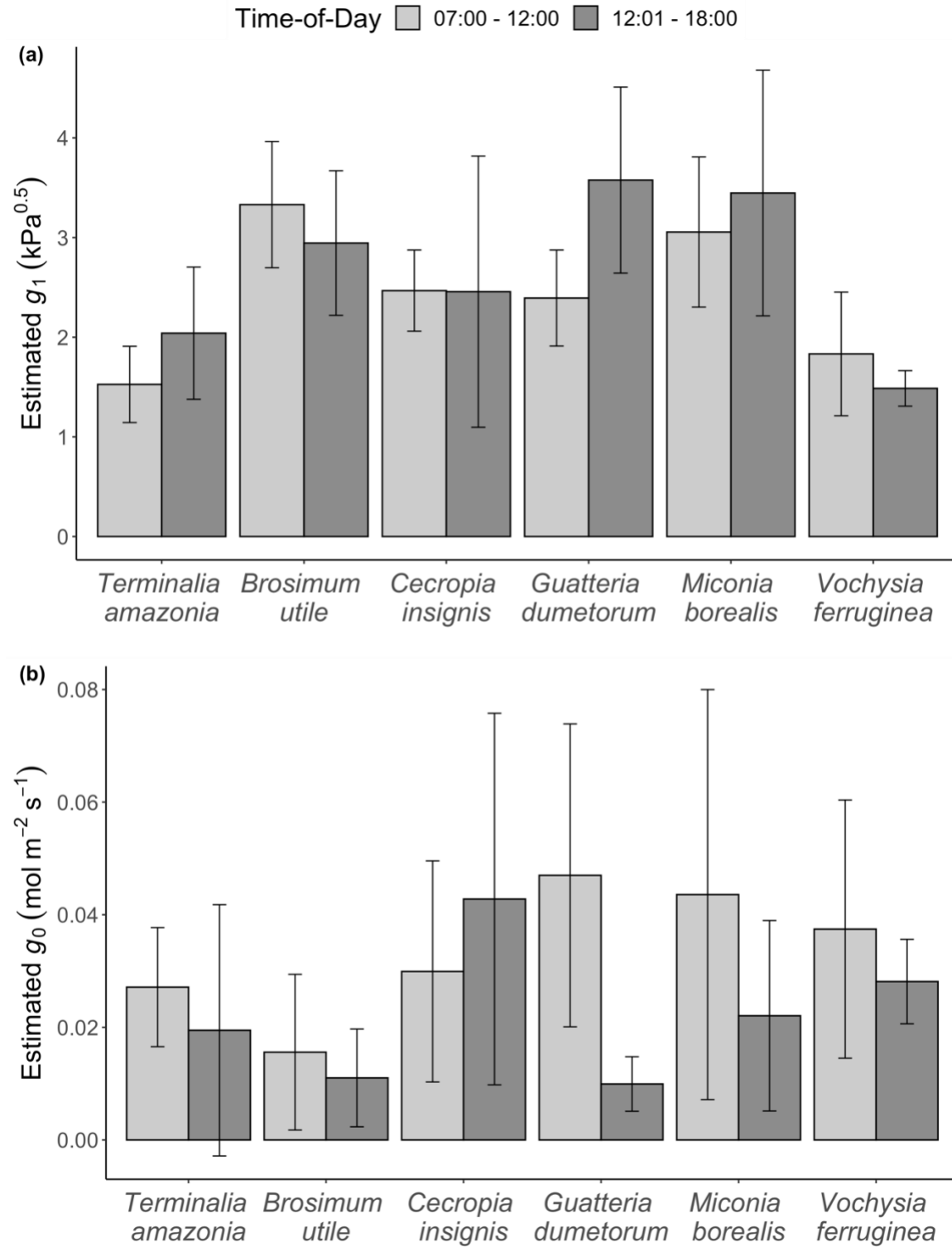


Figure S11. Comparison of fitted values for g_1 and g_0 by species for curves conducted in the morning (07:00-12:00) and afternoon (12:01-18:00). None of the within species comparisons shown are significant. Error bars represent ± 1 SD of the mean.

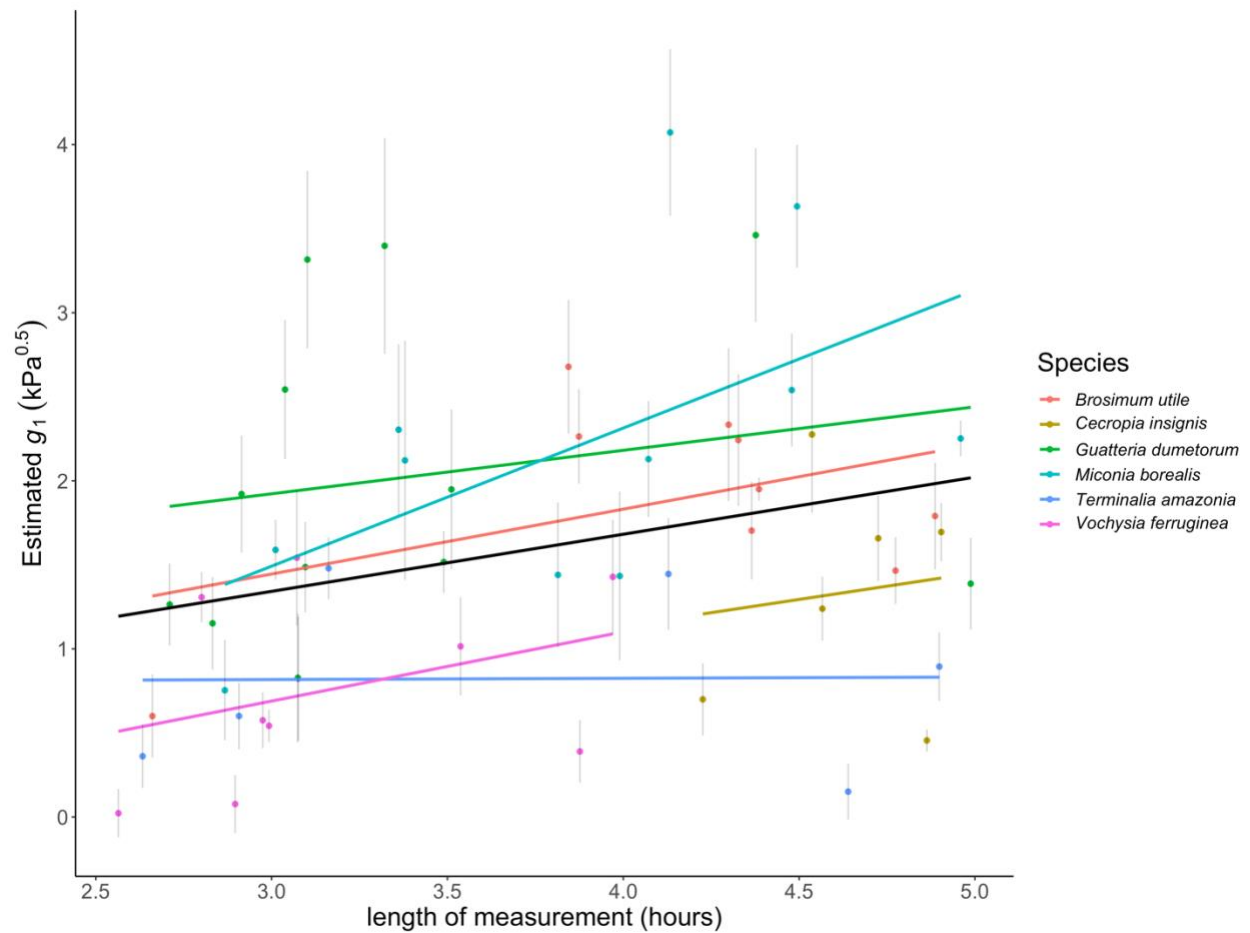


Figure S12. Estimated g_1 versus length of response curve measurement. Colored lines corresponded to species specific slopes, while black line is the line of best fit for all the data. Error bars on each point represent ± 2 SE of the estimate. Across all species estimated g_1 increased with measurement time.

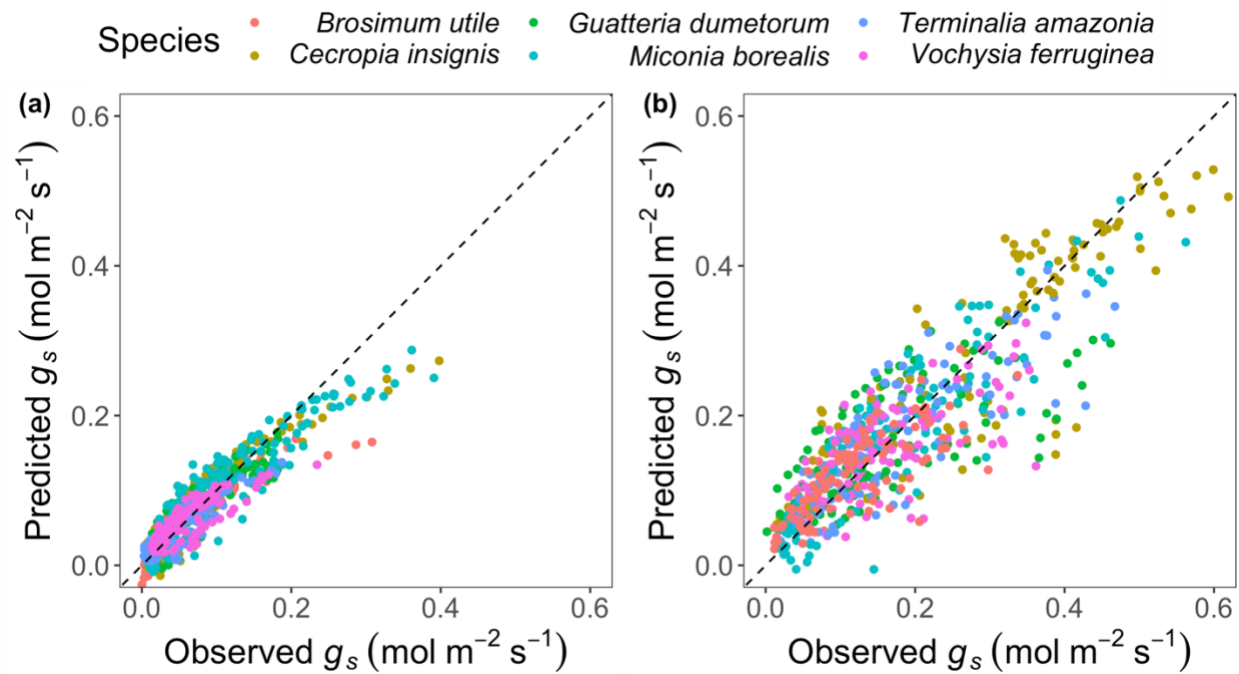


Figure S13. Calibration plots for (a.) the curve derived data, and (b.) the survey derived data used to build the models of g_1 and g_0 . From these calibration plots it is apparent that the curve model has a bias at high g_s levels, while the survey model has considerably more error at all levels of g_s .

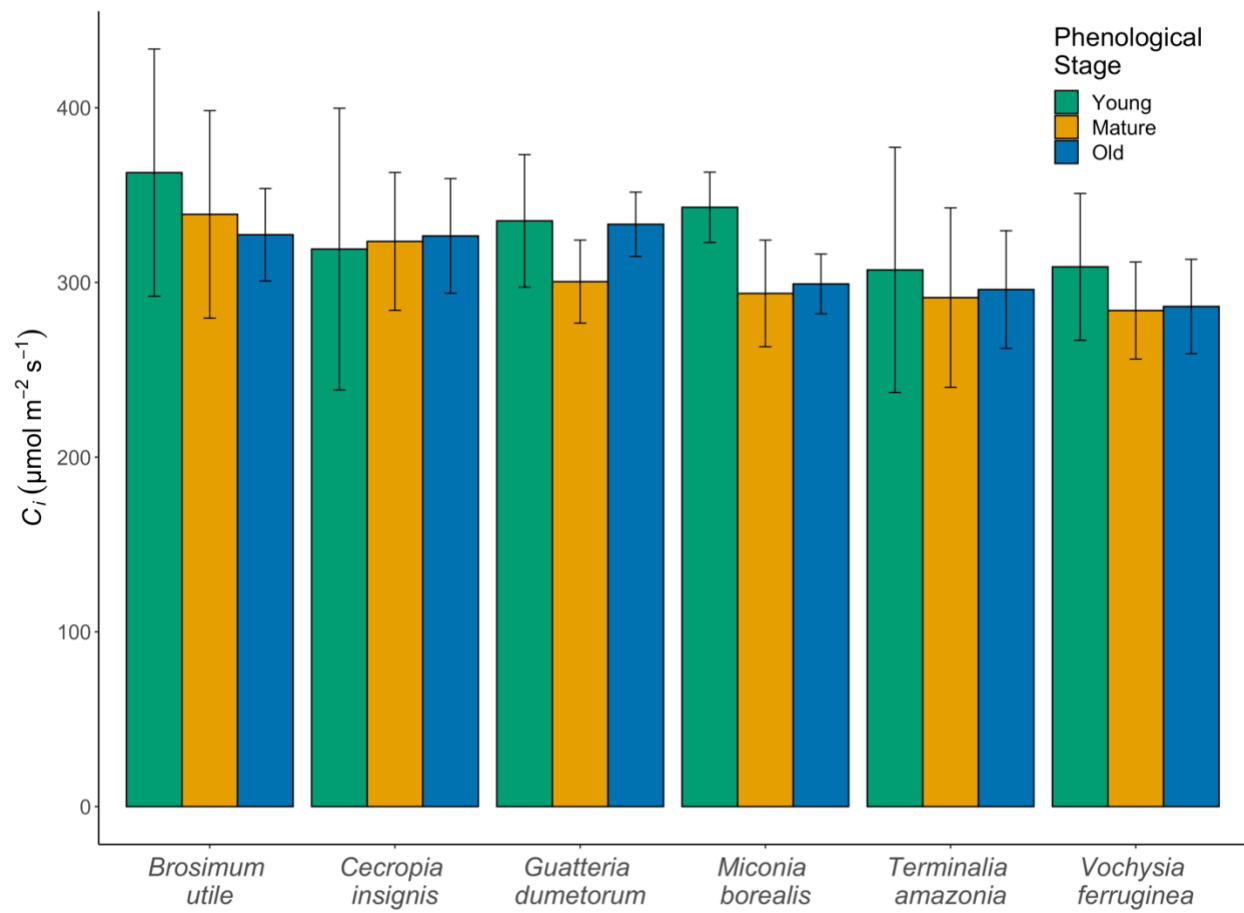


Figure S14. Mean estimated intercellular carbon mole fraction (C_i) by species and leaf age. Data are from response curve measurements and are an average across all irradiance levels. Error bars represent ± 2 SE of the mean.

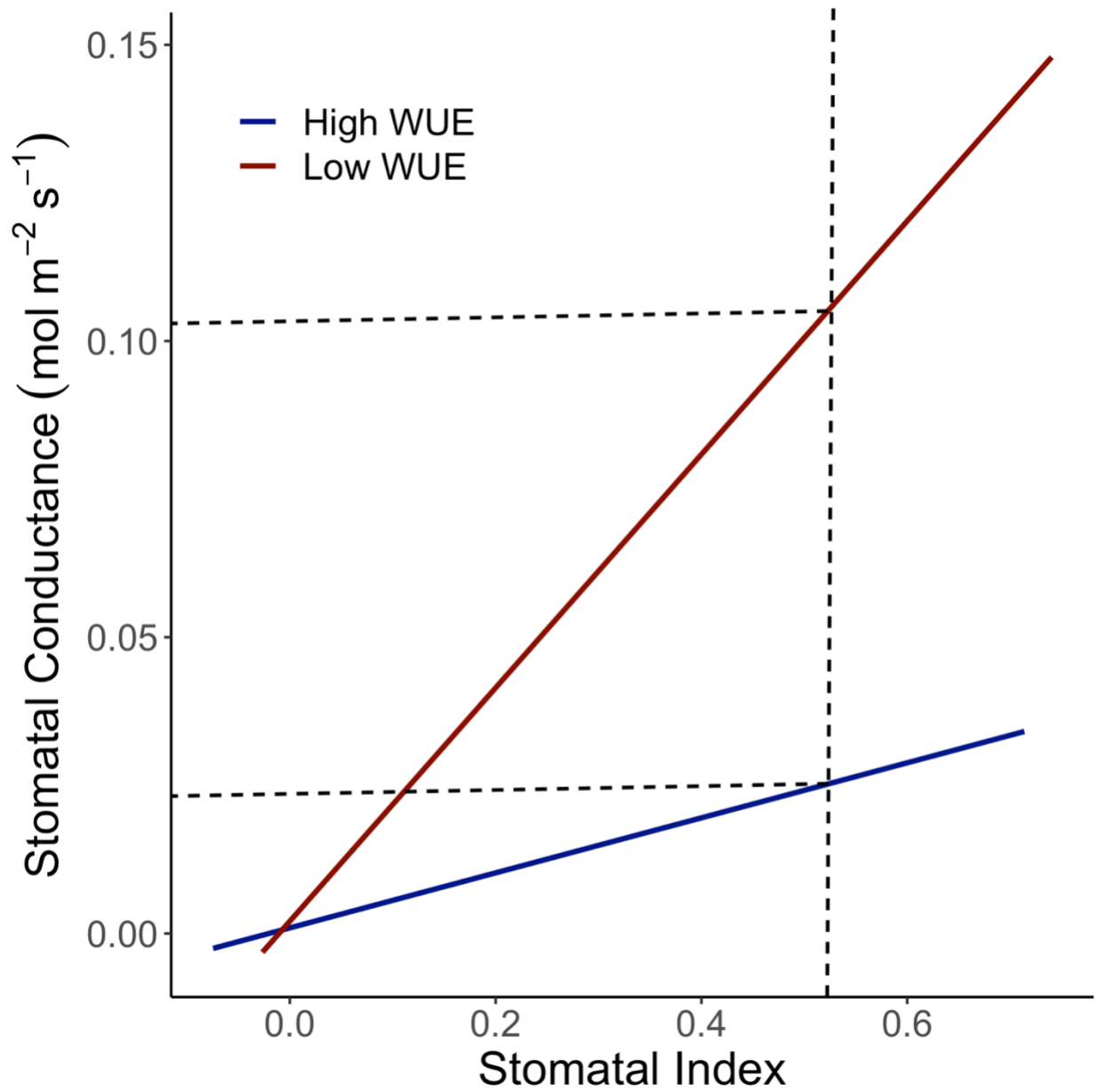


Figure S15. Conceptual illustration of the link between stomatal slope, and water use efficiency. At the same value of stomatal index (a term representing A at a combination of leaf level environmental conditions) on the x axis (vertical dashed line) the two data series have different levels of g_s (horizontal dashed lines).

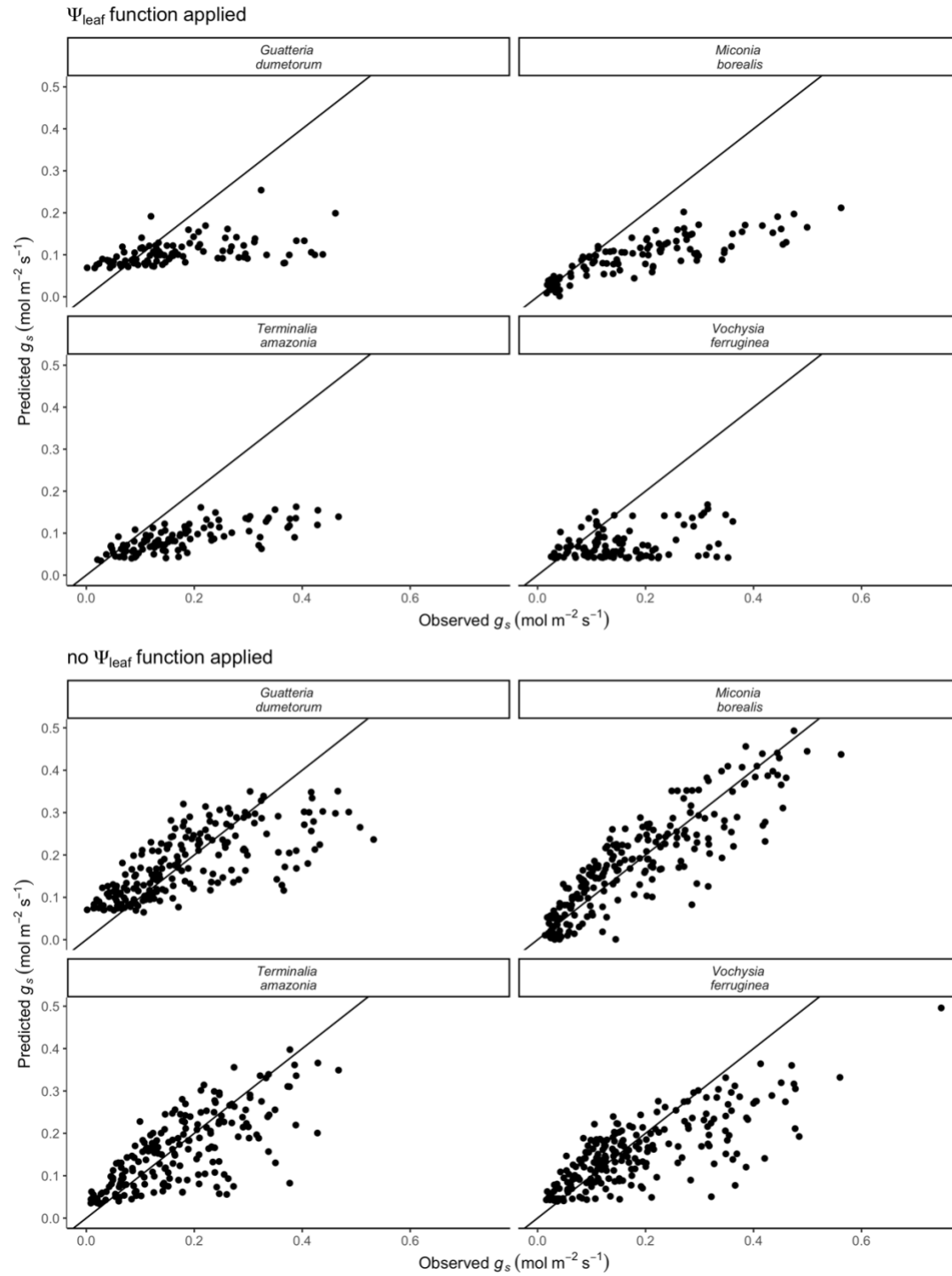


Figure S16. Observed vs predicted g_s when a Ψ_{leaf} penalty is applied (upper panels, Note S3), and when no penalty is applied (lower panels). All plots use survey collected data and the survey model described in Note S1.

Note S1: Description of mixed effects models and their parameter estimates.

USO Model:
$$g_s = g_0 + 1.6 \left(1 + \frac{g_1}{\sqrt{D}} \right) \frac{A}{c_a}$$

Response curve model:
$$g_{s,l,i} = (g_0 + \mu_{0,species_l:stage_l} + a_l) + 1.6 \left(1 + \frac{(g_1 + \mu_{1,species_l:stage_l} + b_l)}{\sqrt{D}} \right) \frac{A}{c_a} + \varepsilon_{l,i}$$

Survey model:
$$g_{s,t,i} = (g_0 + \mu_{0,species_t:stage_t} + a_t) + 1.6 \left(1 + \frac{(g_1 + \mu_{1,species_t} + b_t)}{\sqrt{D}} \right) \frac{A}{c_a} + \varepsilon_{t,i}$$

Survey + time-of-day model:
$$g_{s,t,i} = (g_0 + \mu_{0,species_t:stage_t} + a_t) + 1.6 \left(1 + \frac{(g_1 + \mu_{1,species_t:quarter_i} + b_t)}{\sqrt{D}} \right) \frac{A}{c_a} + \varepsilon_{t,i}$$

Where $g_{s,l,i}$ and $g_{s,t,i}$ are the i^{th} stomatal conductance to water vapor measured on leaf l and on canopy position t , respectively. g_0 represents the population reference intercept of the model. $\mu_{0,species:stage}$ represents the fixed interactive effect of species and phenological stage on g_0 . g_1 represents the population reference “slope” for the model. $\mu_{1,species}$ and $\mu_{1,species:quarter}$ represents the fixed effect of species and the interactive fixed effect of species and time-of-day (e.g., 6:30-09:30, 09:30-12:30, 12:31-15:30, and 15:31-18:30) respectively on g_1 such that each combination of species and of the four measurement periods have a fixed mean g_1 .

a_l and a_t are the random effect of the leaf or canopy position, respectively, on g_0 . The random effects represent the fact that g_0 for each leaf or canopy position might deviate from the reference g_0 corresponding to other individuals of the same age and the same species. Similarly, b_l and b_t are the random effects of the leaf or canopy position on g_1 , respectively. $\varepsilon_{l,i}$ and $\varepsilon_{t,i}$ are the residual of the models. It is assumed that the variables a and b are independent and identically distributed and that $a \sim N(0, \sigma_a^2)$, $b \sim N(0, \sigma_b^2)$ and $\varepsilon_i \sim N(0, \sigma_i^2)$.

Table S1: Parameter estimates and their standard error for the response curve model

Parameter	Value	SE
$\mu_{0,BROSUT:YOUNG}$	0.005	0.009
$\mu_{0,CECRIN:YOUNG}$	0.035	0.012
$\mu_{0,GUATDU:YOUNG}$	0.015	0.010
$\mu_{0,MICOBO:YOUNG}$	0.056	0.008
$\mu_{0,TERMAM:YOUNG}$	0.029	0.009
$\mu_{0,VOCHF:YOUNG}$	0.054	0.012
$\mu_{0,BROSUT:MATURE}$	0.012	0.007
$\mu_{0,CECRIN:MATURE}$	0.025	0.009
$\mu_{0,GUATDU:MATURE}$	0.016	0.007
$\mu_{0,MICOBO:MATURE}$	0.017	0.008
$\mu_{0,TERMAM:MATURE}$	0.017	0.008
$\mu_{0,VOCHF:MATURE}$	0.027	0.007

$\mu_{0,BROSUT:OLD}$	0.021	0.009
$\mu_{0,CECRIN:OLD}$	0.031	0.010
$\mu_{0,GUATDU:OLD}$	0.052	0.009
$\mu_{0,MICOBO:OLD}$	0.028	0.007
$\mu_{0,TERMAM:OLD}$	0.021	0.008
$\mu_{0,VOCHF:OLD}$	0.028	0.008
$\mu_{1,BROSUT:YOUNG}$	3.447	0.295
$\mu_{1,CECRIN:YOUNG}$	0.645	0.423
$\mu_{1,GUATDU:YOUNG}$	2.861	0.353
$\mu_{1,MICOBO:YOUNG}$	3.081	0.236
$\mu_{1,TERMAM:YOUNG}$	0.317	0.313
$\mu_{1,VOCHF:YOUNG}$	-0.074	0.432
$\mu_{1,BROSUT:MATURE}$	1.719	0.281
$\mu_{1,CECRIN:MATURE}$	1.860	0.261
$\mu_{1,GUATDU:MATURE}$	1.476	0.254
$\mu_{1,MICOBO:MATURE}$	1.638	0.263
$\mu_{1,TERMAM:MATURE}$	1.017	0.260
$\mu_{1,VOCHF:MATURE}$	0.837	0.242
$\mu_{1,BROSUT:OLD}$	2.214	0.378
$\mu_{1,CECRIN:OLD}$	2.252	0.307
$\mu_{1,GUATDU:OLD}$	1.999	0.319
$\mu_{1,MICOBO:OLD}$	1.855	0.250
$\mu_{1,TERMAM:OLD}$	0.941	0.289
$\mu_{1,VOCHF:OLD}$	0.867	0.323
σ_a^2	0.016	-
σ_b^2	0.482	-
σ_{li}^2	0.015	-

Table S2: Parameter estimates and their standard error for the survey model

Parameter	Value	SE
$\mu_{0,BROSUT:YOUNG}$	0.026	0.016
$\mu_{0,CECRIN:YOUNG}$	0.047	0.013
$\mu_{0,GUATDU:YOUNG}$	0.041	0.013
$\mu_{0,MICOBO:YOUNG}$	-0.003	0.013
$\mu_{0,TERMAM:YOUNG}$	0.045	0.016
$\mu_{0,VOCHF:YOUNG}$	0.034	0.015
$\mu_{0,BROSUT:MATURE}$	0.011	0.016
$\mu_{0,CECRIN:MATURE}$	0.119	0.013
$\mu_{0,GUATDU:MATURE}$	0.065	0.016
$\mu_{0,MICOBO:MATURE}$	-0.003	0.015
$\mu_{0,TERMAM:MATURE}$	0.029	0.018
$\mu_{0,VOCHF:MATURE}$	0.033	0.017
$\mu_{0,BROSUT:OLD}$	0.050	0.015
$\mu_{0,CECRIN:OLD}$	0.156	0.014
$\mu_{0,GUATDU:OLD}$	0.094	0.015

$\mu_{0,MICOBO:OLD}$	0.015	0.013
$\mu_{0,TERMAM:OLD}$	0.023	0.013
$\mu_{0,VOCHFEE:OLD}$	0.053	0.012
$\mu_{1,BROSUT}$	3.044	0.459
$\mu_{1,CECRIN}$	2.866	0.179
$\mu_{1,GUATDU}$	3.317	0.434
$\mu_{1,MICOBO}$	4.149	0.264
$\mu_{1,TERMAN}$	2.834	0.307
$\mu_{1,VOCHFEE}$	2.573	0.356
σ_a^2	0.026	-
σ_b^2	0.36	-
$\sigma_{t,i}^2$	0.068	-

Table S3: Parameter estimates and their standard error for the survey + time-of-day model

Parameter	Value	SE
$\mu_{0,BROSUT:YOUNG}$	0.029	0.015
$\mu_{0,CECRIN:YOUNG}$	0.060	0.013
$\mu_{0,GUATDU:YOUNG}$	0.044	0.012
$\mu_{0,MICOBO:YOUNG}$	0.004	0.012
$\mu_{0,TERMAM:YOUNG}$	0.033	0.015
$\mu_{0,VOCHFEE:YOUNG}$	0.046	0.014
$\mu_{0,BROSUT:MATURE}$	0.019	0.015
$\mu_{0,CECRIN:MATURE}$	0.132	0.012
$\mu_{0,GUATDU:MATURE}$	0.071	0.015
$\mu_{0,MICOBO:MATURE}$	0.011	0.014
$\mu_{0,TERMAM:MATURE}$	0.017	0.017
$\mu_{0,VOCHFEE:MATURE}$	0.046	0.016
$\mu_{0,BROSUT:OLD}$	0.056	0.014
$\mu_{0,CECRIN:OLD}$	0.169	0.013
$\mu_{0,GUATDU:OLD}$	0.090	0.014
$\mu_{0,MICOBO:OLD}$	0.022	0.012
$\mu_{0,TERMAM:OLD}$	0.018	0.012
$\mu_{0,VOCHFEE:OLD}$	0.059	0.011
$\mu_{1,BROSUT:Morning}$	3.869	0.587
$\mu_{1,CECRIN:Morning}$	3.362	0.439
$\mu_{1,GUATDU:Morning}$	5.946	0.585
$\mu_{1,MICOBO:Morning}$	4.121	0.411
$\mu_{1,TERMAN:Morning}$	3.940	0.734
$\mu_{1,VOCHFEE:Morning}$	3.532	0.422
$\mu_{1,BROSUT:PreNoon}$	3.257	0.543
$\mu_{1,CECRIN:PreNoon}$	2.792	0.198
$\mu_{1,GUATDU:PreNoon}$	3.532	0.458
$\mu_{1,MICOBO:PreNoon}$	4.223	0.257
$\mu_{1,TERMAN:PreNoon}$	3.446	0.320

$\mu_{1,VOCHF\bar{E}:PreNoon}$	2.753	0.366
$\mu_{1,BROSUT:PostNoon}$	2.429	0.465
$\mu_{1,CECRIN:PostNoon}$	2.715	0.188
$\mu_{1,GUATDU:PostNoon}$	1.829	0.469
$\mu_{1,MICOBO:PostNoon}$	3.641	0.319
$\mu_{1,TERMAN:PostNoon}$	2.477	0.313
$\mu_{1,VOCHF\bar{E}:PostNoon}$	1.481	0.394
$\mu_{1,BROSUT:Evening}$	1.511	0.877
$\mu_{1,CECRIN:Evening}$	1.156	0.482
$\mu_{1,GUATDU:Evening}$	1.328	0.899
$\mu_{1,MICOBO:Evening}$	2.594	0.648
$\mu_{1,TERMAN:Evening}$	3.248	0.459
$\mu_{1,VOCHF\bar{E}:Evening}$	1.131	0.524
σ_a^2	0.052	-
σ_b^2	0.485	-
$\sigma_{t,i}^2$	0.054	-

Note S2: Description of the leaf scale photosynthesis, conductance, and energy balance model from the R package LeafGasExchange (Lamour & Serbin, 2021)

To simulate leaf surface temperature (T_{leaf}), CO_2 concentration (C_s), and leaf surface vapor pressure deficit (VPD_{leaf}) the model uses a leaf energy budget model published by Muir, (2019, 2020) which iteratively estimates T_{leaf} , intercellular CO_2 concentration (C_i), and boundary layer conductance (g_{bw}). This is accomplished by first assuming $T_{\text{leaf}} = T_{\text{air}} + 1\text{ }^\circ\text{C}$, $C_s = \text{atmospheric } \text{CO}_2 \text{ concentration } (C_a)$, then solving for A , g_s , C_i , T_{leaf} , and g_{bw} accounting for the temperature dependencies of the photosynthetic parameters (Bernacchi et al., 2001, 2003; Leuning, 2002). The model then compares the resulting estimation of T_{leaf} to the prior value, and if the values differ by less than $0.1\text{ }^\circ\text{C}$, assumes the model has converged. If the values differ by more than $0.1\text{ }^\circ\text{C}$, the model is rerun using the updated values of T_{leaf} , C_s , and VPD_{leaf} .

Stomatal conductance is simulated using the USO model (Medlyn et al., 2011) presented in Equation S1. Photosynthesis is simulated using the FvCB assimilation model (Farquhar et al., 1980) which assumes that net photosynthesis (A) is the minimum of carboxylation limited assimilation (A_c) and RuBp regeneration limited assimilation (A_j), less the respiration rate that is not attributable to the photorespiratory pathway (R_{dark}). The model uses parameters for the maximum rate of electron transport at 25°C ($J_{\text{max},25}$), maximum rate of carboxylation at 25°C ($V_{c,\text{max},25}$), and the leaf dark respiration rate at 25°C ($R_{\text{dark},25}$) which are scaled to T_{leaf} using an Arrhenius function or a modified Arrhenius function (Bernacchi et al., 2001, 2003; Leuning, 2002). The model also scales $V_{c,\text{max},25}$ between canopy layers with cumulatively increasing LAI using the exponential decline function presented in Lloyd et al., (2010). While traditionally models consider a third limitation on photosynthesis, the export limited assimilation rate, also known as the rate of triose phosphate utilization (A_p), there is a growing body of evidence suggesting that this limitation is unlikely to limit photosynthesis under current growth conditions (Rogers et al., 2021). Therefore, we chose not to implement this limitation in our model.

Equation S1.

$$g_s = g_0 + 1.6 \left(1 + \frac{g_1}{\sqrt{D}} \right) \frac{A}{C_s}$$

Note S3: Description of the Ψ_{leaf} penalty function introduced by Wolf et al., (2016) and tested by Anderegg et al., (2017).

To evaluate an additional constraint on the USO model, we tested if the inclusion of a function linking Ψ_{leaf} imposed hydraulic limitation on potential g_s improved modeled rates. Here we applied the function in Equation S2 to Equation S1 to arrive at Equation S3 and a new predicted rate of g_s .

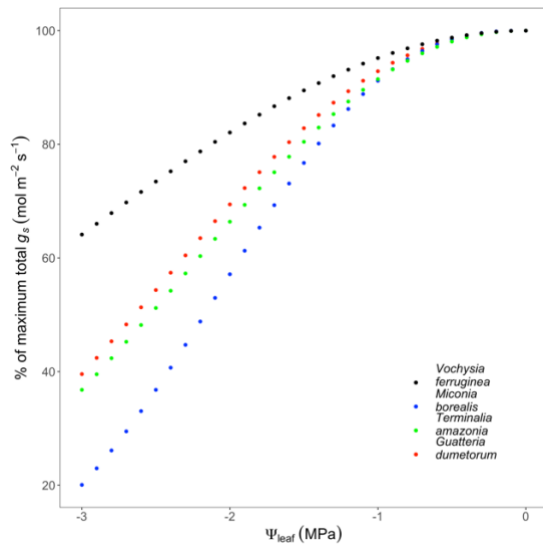
Equation S2.
$$f\Psi_{\text{leaf}} = e^{-\left(\frac{\Psi_{\text{leaf}}}{c}\right)^b}$$

Equation S3.
$$g_s = g_0 + 1.6 \left(1 + \frac{g_1}{\sqrt{D}}\right) \frac{A}{C_s} * f\Psi_{\text{leaf}}$$

Here Ψ_{leaf} is the observed Ψ_{leaf} (MPa) after each measurement of g_s , and b and c are species specific values, derived from Weibull functions of xylem conductivity Ψ_{stem} obtained from Wu et al., (2019), where Ψ_{leaf} is assumed to correlate with Ψ_{stem} such that at $\Psi_{\text{leaf}} = 0$ MPa, the rate of hydraulic conductance is maximized (Sperry et al., 2017). Values for b and c along with a diagnostic plot showing the functional decline in potential g_s as a function of Ψ_{leaf} are reported below.

Table S4: Species specific Weibull function values corresponding to xylem conductivity versus Ψ_{stem} . Data are reproduced from Wu et al., (2019).

Species	b parameter	c parameter
<i>Guatteria dumetorum</i>	1.3	2.1
<i>Miconia borealis</i>	1.6	1.5
<i>Terminalia amazonia</i>	1.2	2.0
<i>Vochysia ferruginea</i>	1.0	3.5



Reduction in potential g_s between Equation 1(100% at any Ψ_{leaf}) and Equation 3 as a function of Ψ_{leaf} for four species.

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