Response Manipulation vs Diurnal Observation: A Direct Comparison of Two Methods for Quantifying Stomatal Behavior in Tropical Forests

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Abstract

A primary source of uncertainty in terrestrial biosphere model (TBM) projection of carbon uptake and water cycling from ecosystems is the relationship between CO_2 assimilation (A) and water loss via stomatal conductance (g_s). A common mathematical framework for modeling this relationship is the "Unified Stomatal model", which relates A to g_s over environmental conditions and is governed by two terms, the stomatal slope (g_1) and intercept (g_0). Given their importance in determining the relationship between forest productivity and climate, an accurate and mechanistic understanding of the g_1 and g_0 parameters is crucial, particularly in wet tropical broadleaf forests where changes in water cycling could impact global weather patterns. These stomatal parameters are estimated using leaf-level gas exchange by two alternative methods: (1) a response curve where the environmental conditions are modified for a single leaf, or (2) a survey approach, where repeated measurements are made on multiple leaves over a diurnal range of environmental conditions. We compare the curve and survey approaches by conducting a comprehensive measurement campaign in which we paired diurnal gas exchange surveys with leaf level response curves for the estimation of g_1 and g_0 on six tropical species across a full range of leaf phenological stages. We examine how these different estimates impact model projection of g_s, and how the consideration of a diurnal effect on g_1 and g_0 can improve predictions relative to a model using parameter estimates which are fixed over the photoperiod. Our results showed that age is an important factor to consider in estimates of g_0, however there was no effect of leaf age on estimates of g_1. The survey approach identified a diurnal trend associated with g_1 and g_0, which when accounted for improved model projections of diurnal trends in g_s. We found that while both approaches yield equally statistically valid estimates of g_1 and g_0 at a fixed point in time, they are not directly comparable across diurnal timescales, where shifting water supply and carbon demand lead to dynamic canopy scale water use efficiency (WUE). These results suggest that to improve the accuracy of modelled g_s in tropical forests, TBMs should recognize and implement diurnal variation in stomatal parameters which are associated with diurnal shifts in WUE.

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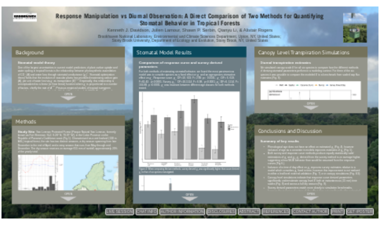
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BACKGROUND

Stomatal model theory

One of the largest uncertainties in current model predictions of plant carbon uptake and water cycling in tropical forests is the relationship between photosynthetic assimilation of CO_2 (A) and water loss through stomatal conductance (g_s)[1]. Stomatal optimization theory holds that the evolution of vascular plants has prioritized maximizing carbon gain (A), per unit of water lost via g_s as transpiration (E)[2-3]. Empirically, this relationship is conceptualized as a linear (or near linear) function where g_s is proportional to a number of factors, chiefly the rate of A[4-8]. Previous empirical studies of tropical evergreen forests have demonstrated that the "Unified Stomatal model" (USO) (Eqn. 1)[9] can be used to successfully predict g_s in this system[10].

Equation 1. $g_s = g_0 + 1.6 * (\frac{g_1}{\sqrt{D}})\frac{A}{Ca}$

In this model, a stomatal slope term (g_1) acts as the chief control on the ratio of g_s given A, a stomatal intercept term (g_0) serves as the model intercept and represents the minimum expected level of g_s for all photosynthetic photon flux density (PPFD) above the light compensation point[11], D represents the leaf to air vapor pressure deficit, and Ca represents the atmospheric level of CO_2. Conveniently, the USO parameter g_1 is inversely proportional to leaf water use efficiency (WUE)[9], allowing researchers to use g_1 to make informal assessments of ecosystem functioning.

Phenology has an unknown effect on stomata

Previous studies have shown a strong species level effect on g_1[10,12] as well as an overall correlation between g_1 and leaf mass per area (LMA)[10]. However, it remains to be seen what impact leaf phenology has on these parameter estimates. A strong phenological impact on g_1 would be an important component to include in terrestrial biosphere model simulation of forests and would be especially important to understand in evergreen forests, where leaves of all phenological stages may be co-occurring at any point in the year.

Method changes interpretation?

In addition, there are two methods available from which to fit stomatal parameters. In one method, termed the survey method, *in situ* measurements of instantaneous gas exchange are taken on intact leaves under ambient conditions, which are used to fit a regression from which g_1 and g_0 are obtained. In the second method, termed the response curve method, slow light response curves are used to elicit stomatal response on cut branch segments. The method chosen is often not closely considered, however it can have ramifications for the assumptions underlying the estimation, especially with regard to the impact of branch excision on stomatal behavior and the assumed constancy of g_1 through the diurnal period.

Objectives

Here we use a novel dataset from six evergreen tropical tree species, collected Jan-March 2020 in the San Lorenzo Protected Forest, Republic of Panama, to test the effect of both leaf phenology and stomatal measurement method on g_1 and g_0 estimates. Our objectives are:

1. Determine how g_0 and g_1 vary across leaf phenology, and whether the inclusion of phenology as a covariate in stomatal models is warranted.
2. Compare survey and response curve derived g_0 and g_1 to assess whether estimates are statistically comparable, and compare their pros and cons.
3. Using stomatal estimates generated from the two methods, make predictions about ecosystem scale rates of NPP and canopy E, comparing the latter to a known sapflux benchmark.

METHODS

Study Site: San Lorenzo Protected Forest (Parque Natural San Lorenzo, formerly known as Fort Sherman; SLZ; 9.28° N, 79.97° W), in the Colon Province on the Republic of Panama's Caribbean coast (Fig.1). Characterized as a wet lowland (130 m ASL) tropical forest, the site has two distinct seasons, a dry season spanning from late December to the end of April, and a rainy season that runs from May through mid-December. The dry season receives on average 655 mm of rainfall, approximately 20% of the yearly total.

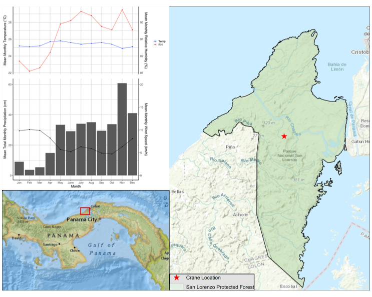


Figure 1. Location and monthly climate averages for the San Lorenzo Protected Forest.

Canopy Access: The Smithsonian Tropical Research Institute (STRI) maintains a canopy access crane at SLZ. The crane stands 52 m tall, with a boom length of 54 m and a total footprint of 0.9 hectares within a larger 6 hectare protected plot. There are over 240 species of trees and lianas within the crane footprint.

Study Species: Six tree species were selected for study, and were chosen to span early, mid, and late successional strategies. For each species, leaves were classified into one of three phenological stages, young, mature, or old (Fig. 2).

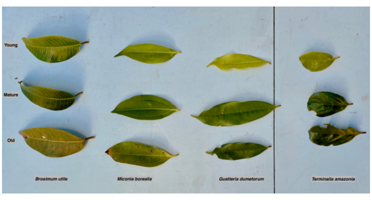


Figure 2. Graphical depiction of the three age classes for four of the six species in this study. Young leaves were characterized as lighter in color, softer more flexible texture, and a distal position on the branch. Mature leaves were the majority of the leaf material, characterized as fully expanded, having an intermediate color and texture as well as an intermediate position. Old leaves were very scarce for most species and tended to have some damage, were dark in color, and were in extreme proximal positions on branches.

Gas Exchange: All gas exchange was conducted using either one of five LI-6400XTs or an LI-6800 portable photosynthesis system (LI-COR Biosciences, Lincoln NE, USA). All survey gas exchange was collected using the LI-6800.

Response Curve Method: All response curves were conducted *ex situ* on cut branches. Branches were cut predawn and immediately recut under water to prevent embolism. For each curve, irradiance level was reduced from saturating down to 0 PAR in a stepwise fashion every 30-40 min, with increments chosen to produce an equal decrease in A (Fig. 3). Across all irradiance levels, leaf temperature, VPD, and Ca were held constant.

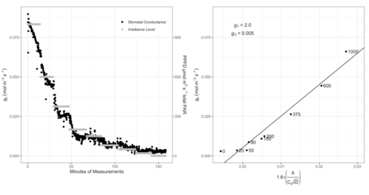


Figure 3. Illustration of the response curve method. Left panel shows raw data from a response curve, with each black point representing an observation of g_s. At each of the nine irradiance levels (horizontal grey bars) g_s drops then stabilizes, at which point irradiance is reduced to the next level. The right-hand panel shows a typical regression fit of the points extracted from the nine light levels, and the g_0 and g_1 estimates produced from this curve. For data analysis, many curves are combined to obtain one overall regression, with each curve acting as a random effect on the overall g_0 and g_1 estimates.

Survey Method: All survey measurements were conducted *in situ* on fully sunlit top of canopy leaves. Before each measurement, conditions (air temperature, C_a, PAR) inside the cuvette of the LI-6800 were matched to ambient conditions. Each survey measurement consisted of a 30-90 second acclimation period, followed by five measurements of A and g_s in quick succession. The five measurement points were averaged to produce a single survey point. Surveys were carried out from 06:30-18:30 on five different days, one day for each species examined.

Data Analysis: Both survey and response curve data were fit using non-linear mixed models of Equation 1. All models included a random individual level effect on g_1 and g_0. For response curves this random effect was the individual leaf (curve) while for the survey data the tree served as the random individual. Multiple fixed effects formulations on g_1 and g_0 were considered, and the most parsimonious model was chosen as the model which included all significant fixed effects and the lowest AIC score.

Canopy Level *E*: Canopy scale E simulations were done using the R package LeafGasExchange, which was designed to simulate leaf level gas exchange using a combined FvCB assimilation model, and Medlyn type stomatal conductance model. Model simulations were driven using meteorological data collected on site by STRI, and were parameterized using species level $V_{c,max}$, J_{max} and R_{dark} data collected in a previous study, and the mixed model derived parameters for g_0 and g_1. Leaf level data was scaled to the canopy using total canopy area and LAI estimates for each species, and a 15-80-5% breakdown for young, mature, and old leaves.

Sap Flux: Sap flux data were collected for three of the six study trees and serve as a model benchmark for canopy scale E. Sap velocity data were measured with Granier Model PS-TDP8 sensors (PlantSensors, Nakara, Australia), which use the heat ratio method to measure heat velocity in the sapwood of a tree. Raw velocity data were normalized and processed in the package AquaFlux to derive sap flow values, which were normalized by sapwood area to derive sap flux measurements at 15-minute intervals. These data were collected and processed as part of a larger NGEE-Tropics effort at SLZ (see "Disclosures" section).

STOMATAL MODEL RESULTS

Comparison of response curve and survey derived parameters

For both methods of estimating stomatal behavior, we found the most parsimonious model was to consider species as a fixed effect on g_1 and an age:species interactive effect on g_0. Response curve, g_0: DF=10, 619, F= 2.69, p= 0.0031, g_1: DF= 6, 619, F=61.42, p<0.0001. Survey, g_0: DF=10,1114, F= 6.98, p<0.0001, g_1: DF=6, 1114, F= 164.88, p <0.0001. g_1 was invariant between different age classes for both methods tested.

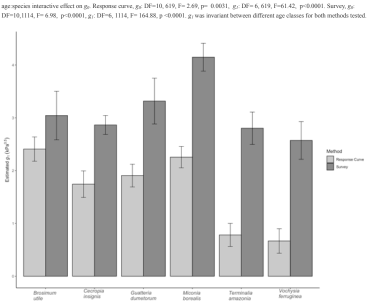


Figure 4. When comparing the two methods, survey derived g_1 was significantly higher than curve derived g_1 for five of six species investigated.

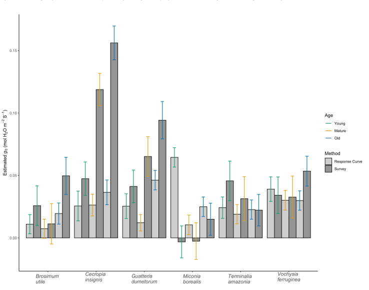


Figure 5. g_0 comparisons between different methods reveal a similar trend of elevated survey parameter estimates for four of the six species. There does not appear to be a consistent pattern between age and g_0 for either of the methods.

Investigation of a time of day effect

We also tested a third model which considered the interactive effect of time of day and species on g_1. It was only possible to fit this model to the survey dataset, as the response curves lack a temporal component. The effect was significant (DF=24, 623, F=41.9, p <0.0001).

When comparing the two models, the model including a time of day effect on g_1 has an improved calibration r^2 (0.80 vs 0.75) and RMSE (0.056 vs 0.063), lower AIC score (-1844 vs -1740) and a likelihood ratio tests reveals the models are significantly different (likelihood ratio= 139.8, p<0.0001).

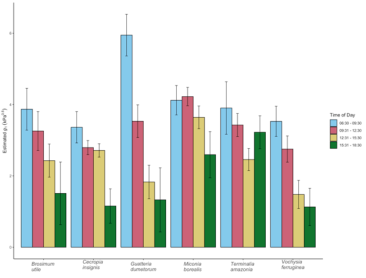


Figure 6. g_1 decreases progressively with increasing time of day for all six species investigated. The effect of time of day on g_0 was nonsignificant.

External model validation

To examine which of our three models predicts leaf level g_s most accurately, we performed a validation of the fixed effects components of the models using an additional survey derived dataset collected on four separate days.

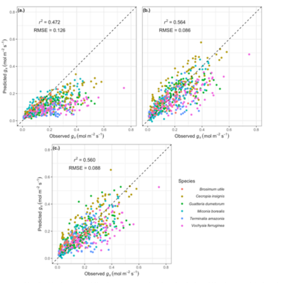


Figure 7. Observed values versus model predicted data for (a.) the model based on response curve derived g_0 and g_1, (b.) the model based on survey derived g_0 and g_1, and (c.) the survey model with an inclusion of a time of day effect on g_1. r2 and RMSE values in each panel show that the survey model improves predictions of g_s over the response curve model, however, the inclusion of the time of day effect slightly worsens model predictive accuracy.

CANOPY LEVEL TRANSPIRATION SIMULATIONS

Diurnal transpiration estimates

We simulated canopy scale E for all six species to compare how the different methods of deriving stomatal parameters perform in a modeling context. For three of the six species it was possible to compare the modeled E to a benchmark from scaled sap flux estimates (Fig. 8).

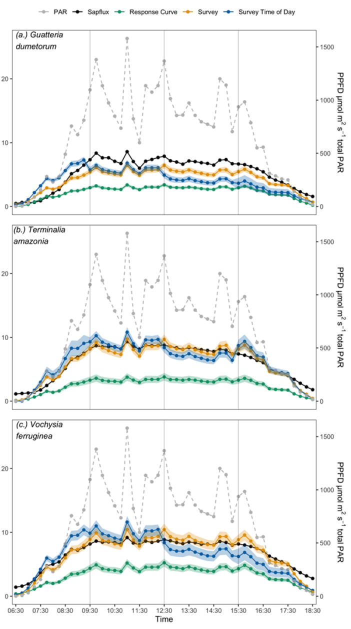


Figure 8. Simulated canopy scale E (g H_2O S^{-1}) at 15 min time points for (a) *Guatteria dumetorum*, (b) *Terminalia amazonia*, and (c) *Vochysia ferruginea* on 11 February 2020. Black points and line represent the sapflux benchmark, green points and line represent response curve derived simulation, 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. Colored shrouds around the simulated data represent error propagated into the simulation by using g_1 +/- SE of the estimated value. Grey dashed line represents the solar profile (PAR) and vertical grey lines show the transition between g_1 values for the survey time of day model.

Comparing simulations to the benchmark shows that the response curve method systematically underestimates diurnal flux as compared to the two survey approaches, a result which is consisted with the external model validation (Fig. 7).

Between the two survey methods, simulated E is very similar to the benchmark, with neither method systematically over or under estimating the benchmark values.

Finally, it is important to note that all three models underestimate the level of E at dawn and dusk, likely as a result of misfit g_0 values.

Mean daily transpiration estimates

We next compared estimates of mean daytime E for the six species, three methods and benchmark (when available, Fig .9). The calculation was done by assuming a constant rate of E for each 15 min interval.

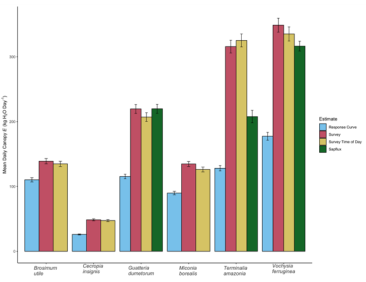


Figure 9. Comparison of estimated mean daily E (kg H_2O day^{-1}) for the six species in this study. Error bars represent the standard error of the mean.

To compare species level estimates we used a TukeyHSD test and found that for all six species the response curves produce significantly lower ($p<0.0001$) estimates of E than for the survey methods. In addition, we found no significant differences between the survey approaches for any of the six species (*Bu*: diff= -4.55, p= 0.729 ; *Ce*: diff= 1.32, p=0.818 ; *Gd*: diff= -13.95, p=0.473; *Mb*: diff= -9.47, p= 0.201; *Ta*: diff= 10.63, p=0.866 ; *Vf*: diff= -15.09, p=0.713).

In two of the three species for which we have a benchmark, the benchmark and survey estimates correspond well with no significant difference between either the survey method or the benchmark. However, in *Terminalia amazonia* benchmark E is significantly less than both survey estimates, and significantly greater than the response curve estimate ($p<0.0001$).

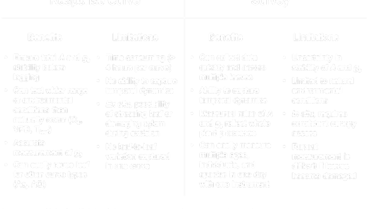
CONCLUSIONS AND DISCUSSION

Summary of key results

- Phenological age does not have an effect on estimated g_1 (Fig. 4), however inclusion of age as a covariate in models improves estimates of g_0 (Fig. 5).
- Both survey and response curve methods produce equally statistically valid estimations of g_1 and g_0; g_1 derived from the survey method is on average higher, suggesting a less WUE behavior than would be assumed from the response curves (Fig 6.).
- Inclusion of a time of day effect on g_1 improves survey estimates relative to a model which considers g_1 fixed in time, however this improvement is not realized in either a leaf level external validation (Fig. 7) or in canopy simulations (Fig. 8,9).
- Canopy level simulations indicate that response curve derived parameters significantly underestimate canopy level E both at instantaneous (15 min) time scales (Fig. 8) and across a full dry season (Fig. 9).
- Survey derived parameters match more closely to simulation benchmarks, however the inclusion of a time of day effect does not significantly alter estimated E (Fig. 8,9).

Conclusions

- While neither response curve estimation or survey estimation of g_1 and g_0 are without drawbacks (Fig. 10), here we observe that the survey derived parameters fit more closely to both leaf and canopy level flux benchmarks.
- The improvement in model prediction observed when g_1 is modeled as a diurnally dynamic parameter implies future model development should recognize and implement diurnal variation in stomatal parameters



Response Curve		Survey	
Benefits	Limitations	Benefits	Limitations
Ensure total A and g_s stability before logging Can test wider range of environmental conditions than naturally occur (C_a, VPD, T_{leaf}) Accurate measurement of g_0 Can easily reuse leaf for other curve types (A-c$_i$, AQ)	Time consuming (> 4 hours per curve) No ability to capture temporal dynamics Ex situ, possibility of stressing leaf or damaging xylem during excision No leaf-to-leaf variation captured in one curve	Can collect data quickly and across multiple leaves Ability to capture temporal dynamics Measured rates of A and g_s reflect whole plant processes Can easily measure multiple ages, individuals, and species in one day with one instrument	Uncertainty in stability of A and g_s Limited to natural environmental conditions In situ, requires consistent canopy access Repeat measurement is difficult if leaves become damaged

Figure 10. Summary of the benefits and limitations of using response curves or survey style measurements to estimate g_1 and g_0.

Lack of significant g_1 covariates

It is somewhat surprising to find a lack of significant fixed effects on g_1, especially as previous work in this forest[10] found a significant relationship between g_1 and LMA, as well as an effect of ψ_{leaf} on g_1. A possible explanation is that unlike that previous study, we chose to focus on six species which all had very similar LMA and diurnal ψ_{leaf} ranges, and as such those factors did not significantly vary between g_1 estimates.

The lack of a significant effect of age on g_1 is also surprising. Previous studies suggest that rates of A and g_s should be dynamic over the lifetime of a leaf, primarily driven by changes in hydraulics and nitrogen investment[13-23]. However, most of these studies focus on temperate deciduous forests, where a seasonal link to leaf aging occurs. In contrast, all six species in this study are evergreen, and have a near consistent annual leaf production. It is possible that the absence of a correlation between season and leaf age reduces nitrogen and hydraulic partitioning, which leads to no apparent change in g_1 over leaf phenology.

Difference in g_1 between methods

There are a number of possible explanations for the stark difference in g_1 estimates between survey and response curve estimation. It is possible that the predawn branch excision for the response curves is introducing air into the xylem of the branches, beginning the process of embolizing the tissue. This could lead to a shift toward the more water use efficient stomatal behavior we observe from curves. While previous work has shown that excision does not impair photosynthetic function[24], stomata should be affected more acutely by hydraulic damage[25].

There could also be error in the survey measurements. It is possible that if chamber conditions were improperly matched to the ambient conditions before a measurement was made, the stomatal behavior would appear more profligate than it really is. For example, if irradiance was underestimated at the time of the measurement, the measured rate of A would be lower than the ambient rate, while the g_s rate would be approximately the same, as stomata respond orders of magnitude slower than chloroplast to external stimuli[26]. This would make it appear as if g_1 was higher than it would be under steady state conditions.

Of the two possible explanations explored, the former seems more likely, as the latter would imply systematic underestimation of the radiation, temperature, and humidity environment during measurement. Due to the stochastic environmental conditions present in the upper canopy, we would expect equal over and underestimation, which would not dramatically shift estimates of g_1.

Inconsistent time of day effect

While the inclusion of time of day as a model covariate on g_1 resulted in a model fit with an improved r^2, RMSE, and AIC score, in both external validations of the model the time of day effect failed to produce a result significantly different from the model without this effect. The likely explanation for this is that the data which were used to generate the time of day model were all collected in a single day for each species, and thus were especially tuned to that day's meteorological dynamics. It is well accepted that stomatal behavior will shift diurnally in response to shifting carbon demand and water supply[2,3], however the magnitude of this diurnal modification should be somewhat mediated by the current and previous day's meteorology. With enough data it should be possible to tune expected diurnal fluctuations in g_1 and g_0 from their nominal average value, which would significantly reduce model uncertainty in E and NPP. We recommend future research explore diurnal shifts in g_1 in greater detail.

Choice in methods has an implication for canopy scale models

The method used to estimate stomatal parameters has a large impact on canopy scale model output, with the survey method producing species level values of E between 26% and 147% higher than model output using response curve derived parameters (110 vs 139 – 128 vs 316 kg H_2O day^{-1}; Fig. 9). Difference in species level predicted NPP ranged from 3% to 29% higher (1991 vs 2057 – 3817 vs 4933 g CO_2 day^{-1}). Clearly careful choices should be made with regard to the method used to estimate g_1, as choice in this parameter has the implication to radically alter predictions of tropical forest function.

For acknowledgments see “Disclosures” section.

DISCLOSURES

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ABSTRACT

A primary source of uncertainty in terrestrial biosphere model (TBM) projection of carbon uptake and water cycling from ecosystems is the relationship between CO_2 assimilation (A) and water loss via stomatal conductance (g_s). A common mathematical framework for modeling this relationship is the "Unified Stomatal model", which relates A to g_s over environmental conditions and is governed by two terms, the stomatal slope (g_1) and intercept (g_0). Given their importance in determining the relationship between forest productivity and climate, an accurate and mechanistic understanding of the g_1 and g_0 parameters is crucial, particularly in wet tropical broadleaf forests where changes in water cycling could impact global weather patterns. These stomatal parameters are estimated using leaf-level gas exchange by two alternative methods: (1) a response curve where the environmental conditions are modified for a single leaf, or (2) a survey approach, where repeated measurements are made on multiple leaves over a diurnal range of environmental conditions.

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Our results showed that age is an important factor to consider in estimates of g_0, however there was no effect of leaf age on estimates of g_1. The survey approach identified a diurnal trend associated with g_1, which when accounted for improved model projections of diurnal trends in g_s. We found that while both approaches yield equally statistically valid estimates of g_1 and g_0 at a fixed point in time, they are not directly comparable across diurnal timescales, where shifting water supply and carbon demand lead to dynamic canopy scale water use efficiency (WUE). These results suggest that to improve the accuracy of modelled g_s in tropical forests, TBMs should recognize and implement diurnal variation in stomatal parameters which are associated with diurnal shifts in WUE.

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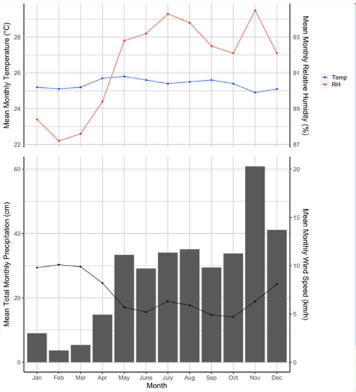

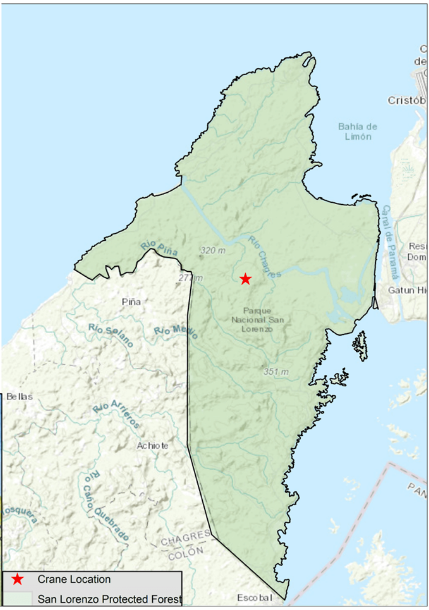Colón
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