



## Research paper

# Leaf surface traits and water storage retention affect photosynthetic responses to leaf surface wetness among wet tropical forest and semiarid savanna plants

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While it is reasonable to predict that photosynthetic rates are inhibited while leaves are wet, leaf gas exchange measurements during wet conditions are challenging to obtain due to equipment limitations and the complexity of canopy–atmosphere interactions in forested environments. Thus, the objective of this study was to evaluate responses of seven tropical and three semiarid savanna plant species to simulated leaf wetness and test the hypotheses that (i) leaf wetness reduces photosynthetic rates ( $A_{\text{net}}$ ), (ii) leaf traits explain different responses among species and (iii) leaves from wet environments are better adapted for wet leaf conditions than those from drier environments. The two sites were a tropical rainforest in northern Costa Rica with ~4200 mm annual rainfall and a savanna in central Texas with ~1100 mm. Gas exchange measurements were collected under dry and wet conditions on five sun-exposed leaf replicates from each species. Additional measurements included leaf wetness duration and stomatal density. We found that  $A_{\text{net}}$  responses varied greatly among species, but all plants maintained a baseline of activity under wet leaf conditions, suggesting that abaxial leaf  $A_{\text{net}}$  was a significant percentage of total leaf  $A_{\text{net}}$  for amphistomatous species. Among tropical species,  $A_{\text{net}}$  responses immediately after wetting ranged from –31% (*Senna alata* (L.) Roxb.) to +21% (*Zamia skinneri* Warsz. Ex. A. Dietr.), while all savanna species declined (up to –48%). After 10 min of drying, most species recovered  $A_{\text{net}}$  towards the observed status prior to wetting or surpassed it, with the exception of *Quercus stellata* Wangerh., a savanna species, which remained 13% below  $A_{\text{net}}$  dry. The combination of leaf wetness duration and leaf traits, such as stomatal density, trichomes or wax, most likely influenced  $A_{\text{net}}$  responses positively or negatively. There was also overlap between leaf traits and  $A_{\text{net}}$  responses of savanna and tropical plants. It is possible that these species converge on a relatively conservative response to wetness, each for divergent purposes (cooling, avoiding stomatal occlusion, or by several unique means of rapid drying). A better understanding of leaf wetness inhibiting photosynthesis is vital for accurate modeling of growth in forested environments; however, species adapted for wet environments may possess compensatory traits that mitigate these effects.

**Keywords:** adaptation, occlusion, repellency, stomata, trichome.

## Introduction

Leaf wetness consists of water droplets or film that settles on the leaf surface and temporarily occludes stomata until it evaporates or drains (Ishibashi and Terashima 1995, Pandey and Nagar 2003). Most studies consider the susceptibility of wet surfaces to pathogen invasion (Hirano and Upper 2000, Jackson

et al. 2006) and the proliferation of algae, moss and other biofilms (Coley et al. 1993) or determine how irrigation affects crop yield (Cavero et al. 2009, Urrego-Pereira et al. 2013). Other studies focus on how foliar application of pesticides can affect photosynthesis (Kramer and Kozlowski 1960). Several of the above-mentioned studies are based on the assumption that

when water covers open stomata, photosynthesis is reduced, since carbon dioxide diffusion in water is extremely low when compared with diffusion in air (1:10,000) (Fogg 1947, Smith and McClean 1989). Although this diffusion limitation is known, some researchers argue that most plants species are hypostomatous, thus gas exchange would be less affected (Dietz et al. 2007, Letts et al. 2010), or that leaf wetness resultant from fog or dewfall are considered physiologically insignificant (Monteith 1963).

However, in wet environments, leaf wetness could have a measurable impact on photosynthesis, since water being intercepted by the forest canopy can range from 20% to 80% of precipitation in the tropics (Sollins and Drewry 1970, Loescher et al. 2005, Teale et al. 2014). In some tropical montane environments, other hydrological phenomena like dewfall and fog are also significant (Bruijnzeel and Veneklaas 1998, Gotsch et al. 2014). Given that tropical forest ecosystems can have mean annual precipitation from 2000 to 5000 mm, with peaks of up to 10,000 mm year<sup>-1</sup> (Juo and Franzluebbbers 2003, Holzman 2008), and that rainfall can occur daily and last throughout continuous days, the potential physiological impact of leaf wetness cannot be ignored. Although a decrease in the photosynthetic rate ( $A_{net}$ ) due to leaf wetness has been reported in some studies (Brewer and Smith 1995, Ishibashi and Terashima 1995, Letts and Mulligan 2005, Reinhardt and Smith 2008), an increase in photosynthetic activity has been reported in others (Smith and McClean 1989, Hanba et al. 2004, Johnson and Smith 2006, Urrego-Pereira et al. 2013). The optimum conditions created by wetness (cooler temperatures, high humidity) combined with favorable leaf anatomy traits are assumed to be the key elements for the species that increased photosynthesis.

Structurally and biologically diverse tropical rainforests, with a mixture of distinct and unique traits within plant functional groups, are likely to possess specialized adaptations for wet leaves. Adaptations to promote water shedding may include trichome coverage, surface roughness, and repellency features like cuticular wax (Kaul 1976, Rosado and Holder 2013). These adaptations help to mitigate or prevent the effects of water on stomata and enable photosynthesis during and after rain, fog or dewfall events (Holder 2007). Along with degree of leaf surface wettability, leaf arrangements within the canopy will dictate leaf wetness duration and its resulting photosynthetic suppression.

While tropical forests have the most frequent leaf wetness due to pronounced rainfall regimes, semiarid or water deficient environments may require fewer adaptations to cope with sporadic rain events interspersed by prolonged dry periods. On the other hand, semiarid plants experience frequent dew formations on plant surfaces (Monteith 1963), which can actually become an alternative water source through foliar uptake in some adapted species (Breshears et al. 2008, Limm et al. 2009), or can improve leaf and soil water content, consequently enhancing photosynthetic conditions (Zhuang and Ratcliffe 2012). Species

from the *Quercus* genus that occur in semiarid regions are characterized as possessing trichomes (diverse in types, patterns, and amount) on the bottom surface (Hardin 1979, Stein et al. 2003) to avoid damage caused by high radiation intensity and to help regulate leaf temperature and prevent dehydration on hot, dry days (Fernandez et al. 2014). However, they also feature a highly water-repellent abaxial surface, and some species even display foliar water uptake from the upper surface (Oliveira et al. 2005, Breshears et al. 2008, Fernandez et al. 2014, Yan et al. 2015).

Although these leaf traits are scientifically well-known and characterized extensively in the literature (e.g., Fernandez et al. 2014), their implications on physiological processes have not been well described, especially for *Quercus* species. Despite being relevant in various research fields (botany, atmospheric sciences, etc.) and across a global range of locations (Ishibashi and Terashima 1995), leaf wetness experiments are logistically challenging, especially since conducting measurements on rainy days or using high moisture levels can lead to equipment damage.

To study the effects of leaf wetness on rates of photosynthesis across a wide range of plant species, we performed leaf level gas exchange measurements under a controlled wetness gradient. Ten species were selected based on differences in leaf characteristics and growth habits. Species were further described in terms of their leaf water retention, stomatal features, temperature response and light response. Our study aimed to: (i) evaluate tropical and semiarid savanna species responses to simulated leaf wetness and test the hypothesis that leaf wetness reduces  $A_{net}$ ; (ii) identify leaf traits that affect leaf wetness duration such as surface features, repellency and stomatal arrangement and density; and (iii) address species traits generalization across biomes (wet tropical forests and semiarid savannas) and highlight the main physiological process drivers. Ultimately, these data can be used to improve models of photosynthesis under wet leaf conditions based on a mechanistic understanding of leaf-level processes and feedbacks in ecosystems spanning from wet to semiarid.

## Materials and methods

### Study sites and plant selection

**Tropical site** The study was conducted at Texas A&M University Soltis Center for Research and Education near San Isidro de Peñas Blancas in the Alajuela Province, Costa Rica (10°23'13"N, -84°37'33"W). The center is bordered by the Children's Eternal Rainforest, near the Monteverde-Arenal Mountain Cloud Forest Reserve. Measurements were performed between ~450 and ~600 m above sea level within a transitional tropical premontane moist forest and in a clearing at its edge (Holdridge 1967). This study site, denoted as 'tropical site', has an average annual temperature of ~24 °C, average relative humidity (RH) of 85% and mean annual rainfall of ~4200 mm, in which the

'dry season' can reach up to 200 mm month<sup>-1</sup> and the 'wet season' ~500 mm month<sup>-1</sup> (Teale et al. 2014).

In order to represent a variety of leaf traits (Table 1), we selected seven distinct tropical species (*Stachytarpheta jamaicensis* (L.) Vahl; *Tibouchina heteromalla* Cogn.; *Zamia skinneri* Warsz. Ex. A. Dietr.; *Calathea crotalifera* S. Watson; *Costus laevis* Ruiz & Pav.; *Carapa guianensis* Aublet.; *Senna alata* (L.) Roxb.), among which six were located in the open areas and one, was located inside the forest (Figure 1, Table 1). All plants are native to Costa Rica or the Caribbean region. Leaf traits were distinct for each species, but were mainly related to mesophyll surface coverage (bare or covered with trichomes), roughness, shape (including drip-tip, which was determined following Malhado et al. 2012) and size. Leaf surface water retention patterns, referred in this study as leaf surface ponding, were also observed immediately after rainfall events (Figure 2). This trait relates to how the water settles and drains from the leaf surface.

The species ranged from 1 to ~35 m in height (accessed through an adjacent 42-m micrometeorological tower) with leaf areas of individual leaves from 16.3 to 307.9 cm<sup>2</sup>. All measurements were made on sun-exposed leaves. To facilitate access, six species were sampled from open areas; however, all of them were commonly observed inside the forest understory, usually associated with dense epiphyll coverage and/or low light intensity.

**Savanna site** The second study site was at the Texas A&M University Ecology and Natural Resources Training Area (30°59'01"N, -96°35'65"W). The annual conditions for this study site (henceforth 'savanna site') include 1032 mm total annual precipitation, average annual temperature of 21 °C and average annual RH of 70% (NOAA 2015). We chose three *Quercus* species to examine potential contrasting plant adaptations between wet and dry biomes (*Quercus stellata* Wangenh. (Post oak—PO); *Quercus muehlenbergii* Engelm. (Chinkapin oak—CO); and *Quercus macrocarpa* Michx. (Bur oak—BO)). These species are known to resist harsh conditions (e.g., droughts and poor soil) and occur in the forests of the southeastern USA (Stein et al. 2003, Mickelbart and Jenks 2010). Each oak species had unique leaf surface texture, trichome presence and leaf area (58–105 cm<sup>2</sup>, Table 1).

### Leaf wetness duration categories

To determine leaf wetness duration, two fresh leaf replicates from each species were weighed over a precision scale and later artificially misted in the same manner as the leaves selected for wet gas exchange measurements (described below). After thorough wetting, leaves were weighed continuously until dry to obtain dry-down curves for each species. Leaves were considered to be dry when they reached their original fresh weight. Leaf surface water retention (g cm<sup>-2</sup>) was determined as the maximum water retained on the surface (g) divided by the leaf

Table 1. Leaf or leaflet traits of the 10 selected plant species.

Species code	Species	Microclimate condition	Height (m)	Leaf size (cm <sup>2</sup> ) <sup>1</sup>	Leaflet Count <sup>1</sup>	Texture	Trichomes	Drip-tip category <sup>2</sup>	Other morphological features
SJA	<i>Stachytarpheta jamaicensis</i> (L.) Vahl	Partially shaded	1–2	40.6 ± 10.5	N/A	Rough with multiple veins	Large and sparse on both sides	Acute	Thin and serrated margins
THE	<i>Tibouchina heteromalla</i> Cogn.	Full sun	~3	248.2 ± 59.7	N/A	Five midribs, multiple veins	Large and dense on both sides, mostly bottom	Acute	Intermediate thickness
ZSK	<i>Zamia skinneri</i> Warsz. Ex. A. Dietr.	Shaded	1–1.5	127.8 ± 43.0	20.8 ± 1.4	Parallel, dentated veins	None	Drip-tip	Thick and rigid
CCR	<i>Calathea crotalifera</i> S. Watson	Partially shaded	1–2	238.0 ± 11.8	N/A	Smooth, waxy	None	Small tip	Thin and flexible
CLA	<i>Costus laevis</i> Ruiz & Pav.	Shaded	2–3	149.3 ± 0.9	N/A	Smooth, waxy	Short (abaxial); none adaxial	Drip-tip	Thick and firm
CGU	<i>Carapa guianensis</i> Aublet.	Late successional/shade tolerant	15–35	79.6 ± 33.2	10.3 ± 0.6	Smooth	None	Rounded	Intermediate thickness and undulated margins
SAL	<i>Senna alata</i> (L.) Roxb.	Full sun	5–10	21.2 ± 4.9	26.0 ± 0.6	Smooth, water repellent	Short and numerous (abaxial); large and few (adaxial)	Rounded	Thin
PO	<i>Quercus stellata</i> Wangenh.	Full sun	~6	72.6 ± 10.3	N/A	Moderately coarse	Intermediate density on both sides	Absent	Thin and lobed margins
CO	<i>Quercus muehlenbergii</i> Engelm.	Full sun	~6	77.4 ± 14.0	N/A	Smooth	Dense (abaxial); none (adaxial)	Absent	Thin and sinuated margins
BO	<i>Quercus macrocarpa</i> Michx.	Full sun	~4	73.3 ± 11.1	N/A	Coarse	Intermediate density (abaxial); none adaxial	Absent	Thin and lobed margins

<sup>1</sup>Mean ± standard deviation.

<sup>2</sup>Classification according to Malhado et al. (2012).

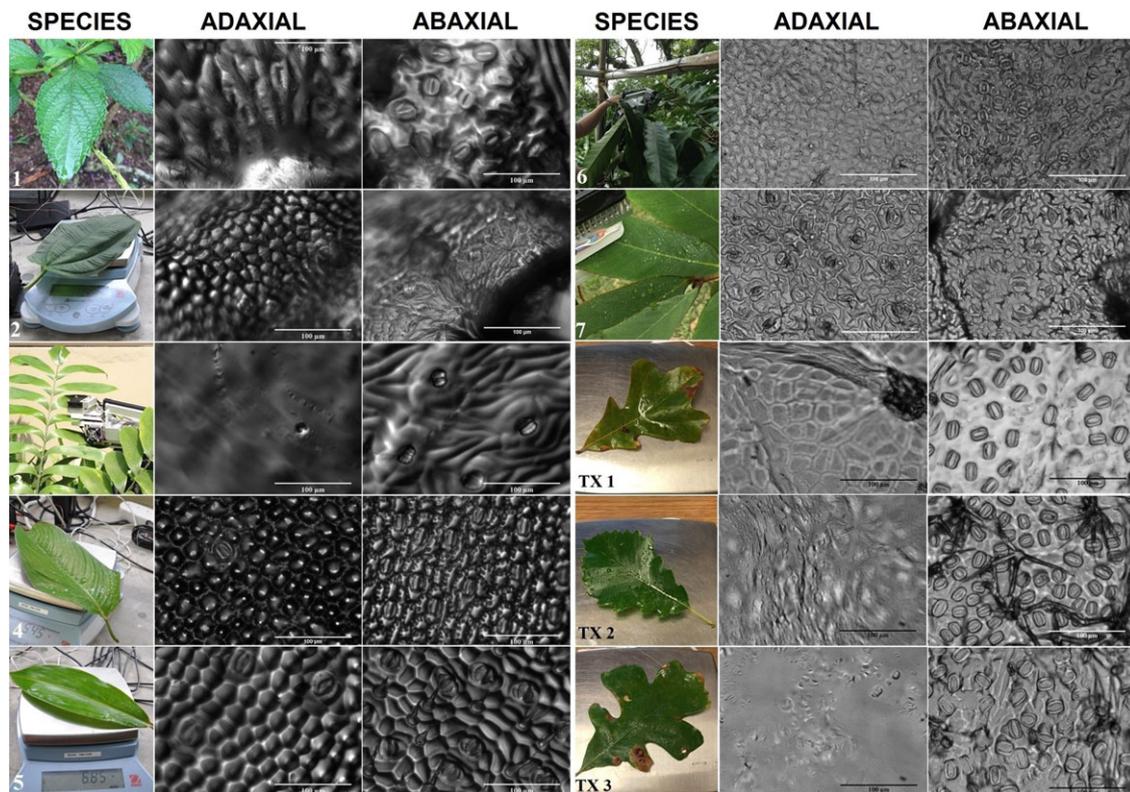


Figure 1. Selected tropical and savanna species subjected to wetness gas exchange experiment and followed by each adaxial and abaxial leaf surface photomicrograph at  $\times 20$  resolution, respectively. (1) *Stachytarpheta jamaicensis* (L.) Vahl (SJA); (2) *Tibouchina heteromalla* Cogn. (THE); (3) *Zamia skinneri* Warsz. Ex. A. Dietr. (ZSK); (4) *Calathea crotalifera* S. Watson (CCR); (5) *Costus laevis* Ruiz & Pav. (CLA); (6) *Carapa guianensis* Aublet. (CGU); (7) *Senna alata* (L.) Roxb. (SAL); (TX1) *Quercus stellata* Wangenh. (PO); (TX2) *Quercus muehlenbergii* Engelm. (CO); (TX3) *Quercus macrocarpa* Michx. (BO).

area ( $\text{cm}^2$ ). Plant species were grouped according to similarities in leaf surface water retention or dry-down rates to determine the significance of leaf traits in photosynthetic responses to leaf wetness. Simple linear regression models were applied to the dry-down curves for each replicate and, later, the resulting slopes and intercepts were averaged between the replicates. The categories were based on species with similar slopes and intercepts.

### Gas exchange measurements

'Tropical site' measurements were collected at midday under sunny and slightly cloudy conditions over 7 days between 9 and 15 June 2015. Similarly, 'savanna site' measurements were collected on 26 August 2015. Although greenhouse experiments have advantages in controlling environmental conditions, there is no guarantee that the plants would be developing (i.e., root-shoot growth, phenology) at the same rate as in the forest. Therefore, this experiment was conducted under natural conditions using established plants acclimated to the local light, temperature and soil environment. Midday gas exchange measurements were executed using a portable photosynthesis system (LI-6400XT, LICOR Inc., Lincoln, NE, USA) on five leaf replicates from each species. The physiological variable of

interest was photosynthetic rate ( $A_{\text{net}}$ ,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ); the chamber variables were leaf temperature ( $T_{\text{leaf}}$ ,  $^{\circ}\text{C}$ ), vapor pressure deficit ( $\delta e$ , kPa), and temperature difference between leaf and air ( $T_{\text{diff}}$ ,  $^{\circ}\text{C}$ ). Unfortunately, this method precluded the measurement of stomatal conductance and transpiration, since the equations used by the LI-6400XT to calculate these parameters were based on water concentrations inside the chamber. Due to the nature of the experiment design presented, these equations would not produce realistic values.

Measurements were conducted with fixed flow rate =  $500 \text{ mol s}^{-1}$ , photosynthetically active radiation (PAR) =  $1500 \mu\text{mol s}^{-1}$ , leaf area =  $6 \text{ cm}^2$  and constant  $\text{CO}_2$  concentrations  $\cong 400 \text{ mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ , stabilized by a buffer volume. All measurements were conducted when ambient conditions were dry. First, baseline dry leaf measurements were collected over a 3-min period. Later, leaves were thoroughly wetted, and then again placed inside the chamber for an additional 10 min of continuous measurements. At the time of wetting, leaves were wet completely through artificial misting using a spray bottle with fixed nozzle flow for a uniform application to simulate a recent rain event. Leaves were misted enough to cover the entire upper surface, but not forming large oversized droplets. We were careful to only mist the upper surface, since water droplets remain on the top leaf surface of

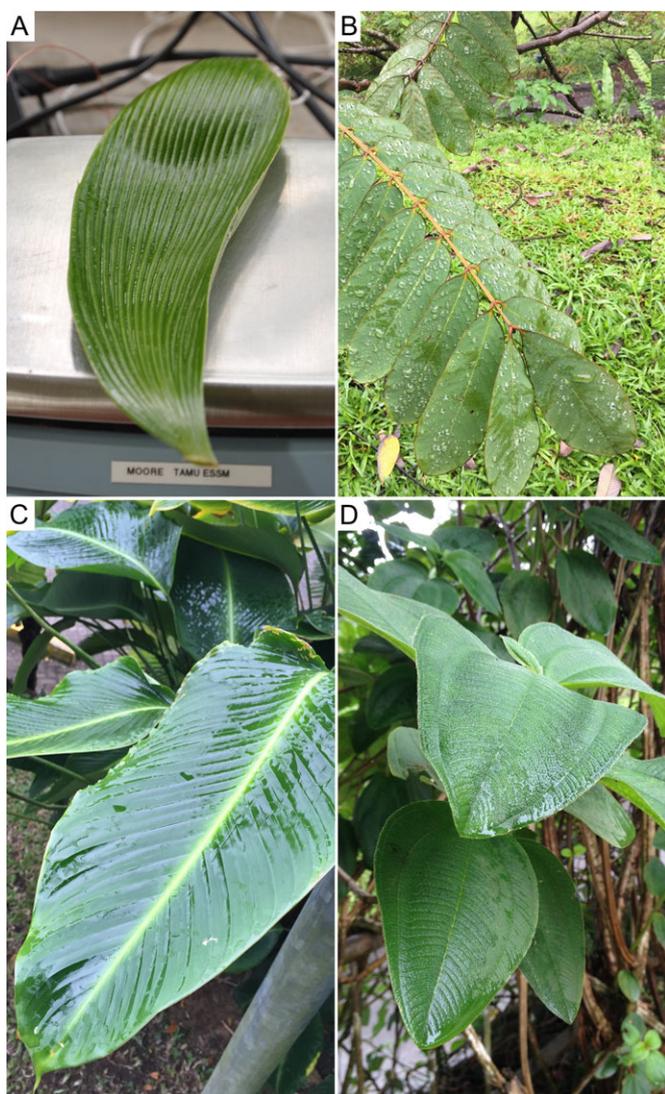


Figure 2. Examples of leaf water retention ('leaf ponding') in species with varying leaf traits. Only image A depicts artificial misting, while the others show intercepted rain. (A) *Zamia skinneri* Warsz. Ex. A. Dietr.: water channeled through leaflet indented venation; (B) *Senna alata* (L.) Roxb.: water beading on repellent leaf surface; (C) *Calathea crotalifera* S. Watson: thin, adhered water film on wettable surface; (D) *Tibouchina heteromalla* Cogn.: acute leaf tip water retention on wettable, trichome covered surface.

broadleaved plants under typical field conditions, unless leaf orientation is vertically upward or downward, which is rare (Bohman 2004, Dietz et al. 2007). Wet leaf measurements were logged at 1-min intervals up to 10 min, during which time leaves became increasingly drier inside the chamber.

Optimum PAR and leaf temperature was determined under sunny conditions through light and temperature curve measurements over a 10-day period between 26 January and 4 February 2016 for tropical species, while savanna species were measured on 25 May and 27 September. Temperature curves were generated under optimum PAR for each species, as determined by light curves. The LI-6400XT chamber conditions established

for light curves were the same as those adopted for the wet gas exchange measurements. Light curves followed a commonly applied PAR sequence (LI-COR 2012) of 800, 600, 400, 200, 500, 800, 1000, 1200, 1400, 1600, 1800, 2000, 2200, 2400, 2600, 2100, 1500, 800  $\mu\text{mol s}^{-1}$ . Most of the species had optimum PAR of 1500  $\mu\text{mol s}^{-1}$ , except for *S. alata* and *T. heteromalla*, which peaked around 1800 and 2000  $\mu\text{mol s}^{-1}$ , respectively. Adding these light intensity preferences into the chamber settings, we performed the temperature curves that followed the sequence: 27, 25, 23, 24, 27, 29, 31, 33, 35, 37, 32, 29, 27 °C (LI-COR 2012). It is important to highlight that the instrument uses water vapor to reduce the temperature from the ambient air until it reaches the desired leaf temperature; to increase, the opposite occurs. Therefore, we kept leaf temperature above 23 °C to prevent malfunctions due to excessive RH and condensation in its interior. Also, maximum leaf temperatures did not exceed 34 °C due to cooler ambient temperatures. Curves were fitted using Verhulst's (1838) proposed logistic model for growth or decay of population dynamics model (Zwanzig 1973, Archontoulis and Miguez 2015), except the temperature curve for species *S. jamaicensis*, which was better fitted with a quadratic equation. Light and temperature response curves were not recorded for *Z. skinneri* due to seasonal dormancy at the time these measurements were conducted. Since *Z. skinneri* is a well-known understory plant species in Costa Rica and Panama (Taylor et al. 2008, Acuña-Castillo and Marín-Méndez 2013), we assumed optimum temperatures were below 25 °C and light intensities not higher than 1500  $\mu\text{mol m}^{-2} \text{s}^{-1}$ .

### Stomatal density

Leaf surface impressions were used to determine stomatal density and to categorize each species as amphistomatous [stomata present on both abaxial (bottom) and adaxial (upper) surfaces] or hypostomatous (only on the bottom, Preninger 2013). Following a method similar to Long and Clements (1934) and Taft (1950), clear acrylic paint was applied on the adaxial and abaxial surfaces of three leaf replicates per species. After drying, the paint was carefully removed and stored in vials and microscope slides.

The samples were analyzed at Texas A&M Microscopy and Imaging Center using an upright, lighted microscope at 20 $\times$  objectives with phase contrast (Zeiss Axiophot, Carl Zeiss Microscopy, LLC, Thornwood, NY, USA) and coupled with a high resolution digital camera (DXM1200, Nikon Corporation, Tokyo, Japan). Image acquisition was controlled via MetaView software and resulting captured images processed using FIJI image processing software (Schindelin et al. 2012). Four 0.06  $\times$  0.06 mm<sup>2</sup> monochrome photomicrographs at 100  $\mu\text{m}$  scale were obtained from each side. These images were then used to collect stomata counts, which were later averaged to obtain stomatal density. Stomatal ratio (no. of stomata on adaxial/no. of

stomata on the abaxial) was also assessed; stomatal ratios close to one represent leaves with adaxial and abaxial surfaces with similar or equal number of stomata, whereas those equal to zero represent hypostomatous leaves.

Unfortunately, the dense layer of trichomes on both surfaces of the species *T. heteromalla* and the top surface of *S. jamaicensis* prevented the collection of reliable stomata impressions. Therefore, we relied on other studies, like Reis et al. (2005) and Iroka et al. (2015), to assume that its subfamily and species, respectively, are characterized as predominantly hypostomatous and amphistomatous, respectively. Likewise, stomata characterization was confirmed with prior studies in the literature (Meyer and Meola 1978, Reis et al. 2005, Camargo and Marengo 2012, Acuña-Castillo and Marín-Méndez 2013, Begum et al. 2014, Rozali et al. 2014, Singh et al. 2014, Iroka et al. 2015).

### Statistical analyses

Simple and multiple regressions (with additive and interaction terms  $A_{\text{net}} = a + bx$  and  $A_{\text{net}} = a + bx + cz + dxz$ , respectively; in which  $a$ ,  $b$ ,  $c$ ,  $d$  are regression coefficients and  $x$ ,  $z$  are independent variables) were used to compare photosynthesis response to leaf wetness within and among species and site. To determine the main photosynthesis drivers under wet conditions,  $A_{\text{net}}$  was the dependent variable, and chamber conditions ( $T_{\text{diff}}$ ,  $T_{\text{leaf}}$ ,  $\delta e$ ), species and site were the independent variables. Since  $T_{\text{leaf}}$  and  $T_{\text{diff}}$  were correlated, models included only one or the other variable, not both. Best model fit was determined through stepwise analyses and based on  $R^2_{\text{adj}}$ ,  $P$ -value ( $\alpha = 0.05$ , 0.01 and 0.001) and Mallow's  $C_p$ . We also compared dry and wet conditions through ANOVA and Tukey HSD multi-comparison post-hoc test per species and between conditions (no site distinction). All statistical analyses were performed with R version 2.6.2 software (R Development Core Team 2013).

## Results

### Leaf wetness duration and stomatal density

Dry-down curves were separated into three categories: (i) large leaf surface water retention, long drying period (LR/LD; slope =  $-7.4 \times 10^{-5} \pm 3.9 \times 10^{-6}$ ; intercept =  $0.005 \pm 0.004$ ), which included *T. heteromalla* and *Z. skinneri*; (ii) small leaf surface water retention, short drying period (SR/SD; slope =  $-1.3 \times 10^{-4} \pm 8.0 \times 10^{-6}$ ; intercept =  $0.004 \pm 0.0004$ ), which included *S. jamaicensis*, *S. alata* and all three savanna oak species (*Q. stellata*, *Quercus muehlenbergii*, *Q. macrocarpa*); and (iii) small leaf surface water retention, long drying period (SR/LD; slope =  $-4.7 \times 10^{-5} \pm 5.4 \times 10^{-6}$ ; intercept =  $0.003 \pm 0.0009$ ), which included *C. crotalifera*, *C. laevis* and *C. guianensis* (Figure 3, Table 2).

Stomatal density varied greatly among all species. All savanna oak species were hypostomatous and had a stomatal density that ranged from 676 to 1298  $\text{mm}^{-2}$ , higher than the density found

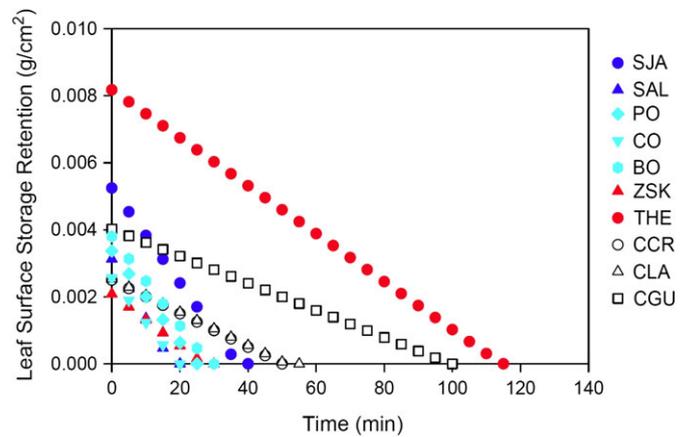


Figure 3. Dry-down curves and leaf categories. Gray: small leaf surface water retention, short drying period (SR/SD); black: large leaf surface water retention, long drying period (LR/LD); open: small leaf surface water retention, long drying period (SR/LD); light gray: savanna oak species—small water leaf surface water retention, short drying period (SR/SD).

on the abaxial surface of most of the tropical species (between 58 and 743  $\text{mm}^{-2}$ ) (Table 2). In contrast, among the tropical species, only two were hypostomatous (*T. heteromalla* and *C. guianensis*). The other five species were amphistomatous with stomatal ratios ranging from 0.03 to 0.75. Species with no trichomes on either surface were *T. heteromalla*, *C. laevis* and *C. guianensis*. Only *Z. skinneri* possessed stomatal crypts (Figure 1), which are pits in the mesophyll, covered by epidermis, that contain the stomata; they are known to protect the cells from excessive water loss during drought (Mauseth 2008) and to facilitate carbon dioxide diffusion through the tissue between the abaxial and adaxial surfaces of thick leaves (Hassiotou et al. 2009).

### Gas exchange measurements

**Measurement conditions (ambient and leaf)** At the tropical site, after applying artificial misting, leaves inside the chamber experienced 9% higher RH and 26% lower  $\delta e$  relative to dry conditions that were observed prior to artificial wetting. Leaf temperature ( $T_{\text{leaf}}$ ) was only 2% lower after wetting, but that amounted to nearly sixfold decrease in  $T_{\text{diff}}$  (Table 3). Similarly, at the savanna site, wetted leaves inside the chamber experienced 7% higher RH and 3% lower leaf temperatures associated, again, with a sixfold decrease in  $T_{\text{diff}}$ . However, those leaves at the savanna site experienced only a 4% decrease in  $\delta e$  and  $T_{\text{diff}}$  was closer to zero (before and after wetting), since the ambient conditions at this site were much warmer (+5 °C) and drier (nearly double) than the tropical site. The largest reductions of all three chamber variables after wetting was observed for *Z. skinneri* ( $\delta e = -82\%$ ;  $T_{\text{leaf}} = -36\%$ ;  $T_{\text{diff}} = -70\%$ ), while *Q. muehlenbergii* had the least variable chamber conditions ( $\delta e = -2\%$ ;  $T_{\text{leaf}} = -0.1\%$ ;  $T_{\text{diff}} = -40\%$ ), although *S. alata* had the smallest  $T_{\text{diff}}$  variation ( $-2\%$ ) within the chamber among all measured species.

Table 2. Summary attributes for each species (see Table 1) placed in one of three dry-down categories, including stomatal density (abaxial and adaxial,  $\text{mm}^{-2}$ ), stomatal ratio, initial photosynthetic response after 1 min equilibration time, expressed as a percentage relative to average dry state (initial response;  $A_{\text{net-dry}} - A_{\text{net-wet}}$ ), photosynthetic response after 10 min of dry-down (long-term response), expressed as a percentage relative to average dry state, and a general description of each plant's response while drying (dry-down  $A_{\text{net}}$  trend).

Species	Dry-down category	Adaxial stomatal density ( $\text{mm}^{-2}$ )	Abaxial stomatal density ( $\text{mm}^{-2}$ )	Stomatal ratio	Initial response (%)	Long-term response (%)	Dry-down $A_{\text{net}}$ trend
SJA	SR/SR	$200 \pm 49^1$	$267 \pm 14$	0.75	-20	-3	Fast recovery
SAL	SR/SR	$271 \pm 57$	$551 \pm 24$	0.57	-31	-1	Fast recovery
CCR	SR/LR	$8 \pm 10$	$255 \pm 46$	0.03	-15	-3	Recovery
CLA	SR/LR	$29 \pm 25$	$167 \pm 24$	0.18	-12	-31	Decrease/late recovery
ZSK	LR/LR	$13 \pm 16$	$58 \pm 22$	0.22	21	7	Increase
THE	LR/LR	0	Unknown <sup>1</sup>	0	-4	10	Increase
CGU	SR/LR	0	$689 \pm 86$	0	-9	-6	Recovery
PO	SR/SR	0	$676 \pm 49$	0	-11	-13	Decrease
CO	SR/SR	0	$1298 \pm 13$	0	-48	-31	Late recovery
BO	SR/SR	0	$731 \pm 51$	0	-11	14	Increase

<sup>1</sup>Highlights measurement uncertainties due to dense trichomes.

Table 3. Upper-half of the table: ambient and leaf/chamber conditions during measurements (overall, dry and wet average and standard deviation). Bottom-half: leaf/chamber conditions while dry and wet for each species and overall average per variable with respective standard errors. Note: ambient variables: air temperature ( $T_{\text{air}}$ ), relative humidity (RH) and photosynthetically active radiation (PAR); chamber variables: relative humidity (RH), vapor pressure deficit ( $\delta e$ ), leaf temperature ( $T_{\text{leaf}}$ ) and leaf-to-air temperature difference ( $T_{\text{diff}}$ ).

Ambient	Average	Leaf/chamber	Average	Dry	Wet	
<i>Costa Rica—tropical site</i>						
$T_{\text{air}}$ ( $^{\circ}\text{C}$ )	$32.8 \pm 2.4$	RH (%)	$67.1 \pm 4.1$	$62.2 \pm 4.3$	$68.6 \pm 4.2$	
RH (%)	$54.7 \pm 4.5$	$\delta e$ (kPa)	$1.5 \pm 0.3$	$1.9 \pm 0.3$	$1.4 \pm 0.3$	
PAR ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$427.0 \pm 250$	$T_{\text{leaf}}$ ( $^{\circ}\text{C}$ )	$32.5 \pm 1.9$	$32.9 \pm 1.2$	$32.3 \pm 1.0$	
		$T_{\text{diff}}$ ( $^{\circ}\text{C}$ )	$-0.35 \pm 0.4$	$0.07 \pm 0.3$	$-0.48 \pm 0.4$	
<i>Texas—savanna site</i>						
$T_{\text{air}}$ ( $^{\circ}\text{C}$ )	$38.4 \pm 0.8$	RH (%)	$35.5 \pm 2.0$	$33.4 \pm 1.1$	$36.1 \pm 2.3$	
RH (%)	$28.2 \pm 1.8$	$\delta e$ (kPa)	$4.4 \pm 0.2$	$4.5 \pm 0.2$	$4.3 \pm 0.3$	
PAR ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$564.4 \pm 240$	$T_{\text{leaf}}$ ( $^{\circ}\text{C}$ )	$38.3 \pm 0.9$	$38.4 \pm 0.5$	$38.3 \pm 0.6$	
		$T_{\text{diff}}$ ( $^{\circ}\text{C}$ )	$-0.1 \pm 0.13$	$-0.02 \pm 0.3$	$-0.12 \pm 0.1$	
Species	$\delta e$ —dry condition	$\delta e$ —wet condition	$T_{\text{leaf}}$ —dry condition	$T_{\text{leaf}}$ —wet condition	$T_{\text{diff}}$ —dry condition	$T_{\text{diff}}$ —wet condition
SJA	$1.97 \pm 0.4$	$1.23 \pm 0.3$	$35.93 \pm 1.4$	$34.21 \pm 1.4$	$-0.57 \pm 0.5$	$-1.91 \pm 1.3$
SAL	$1.79 \pm 0.4$	$1.66 \pm 0.4$	$32.41 \pm 1.4$	$32.05 \pm 1.4$	$1.11 \pm 0.3$	$1.13 \pm 0.3$
THE	$1.44 \pm 0.2$	$0.98 \pm 0.1$	$30.64 \pm 0.3$	$30.23 \pm 0.3$	$-0.23 \pm 0.3$	$-0.80 \pm 0.3$
ZSK	$2.22 \pm 0.4$	$1.39 \pm 0.4$	$34.02 \pm 1.4$	$33.38 \pm 1.1$	$0.25 \pm 0.2$	$-0.58 \pm 0.4$
CCR	$2.06 \pm 0.2$	$1.31 \pm 0.2$	$34.08 \pm 0.6$	$33.24 \pm 0.6$	$-0.24 \pm 0.2$	$-0.86 \pm 0.3$
CLA	$2.37 \pm 0.4$	$2.04 \pm 0.3$	$33.80 \pm 1.2$	$33.75 \pm 1.3$	$-0.14 \pm 0.2$	$-0.41 \pm 0.1$
CGU	$1.56 \pm 0.3$	$1.33 \pm 0.2$	$29.37 \pm 1.2$	$29.57 \pm 0.7$	$0.34 \pm 0.2$	$0.09 \pm 0.2$
PO	$4.17 \pm 0.3$	$4.07 \pm 0.4$	$37.35 \pm 0.8$	$37.37 \pm 1.1$	$-0.08 \pm 0.1$	$-0.22 \pm 0.1$
CO	$4.61 \pm 0.2$	$4.52 \pm 0.2$	$38.44 \pm 0.3$	$38.40 \pm 0.3$	$0.05 \pm 0.1$	$0.03 \pm 0.2$
BO	$4.66 \pm 0.1$	$4.35 \pm 0.1$	$39.28 \pm 0.5$	$39.04 \pm 0.4$	$-0.03 \pm 0.1$	$-0.18 \pm 0.2$

**Photosynthesis measurements** Light response curves confirmed our assumptions regarding the species' habitat preference (Figure 4A). Species classified as shaded (Table 1), in this case only *C. laevis*, had a sharp decline at  $1600 \mu\text{mol m}^{-2} \text{s}^{-1}$  after a constant optimum photosynthetic rate starting at low light intensities ( $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). This trend was not observed for any of the other sampled species. Another interesting trend was observed for *C. guianensis*, in which the light response curve

depicts the plasticity of a late successional species that are able to develop under shade and later adjust itself to higher light intensities (Fetcher et al. 1987). Although not showing an apparent light-saturation of photosynthetic rates, this species maintained the lowest photosynthetic rates among the tropical species. All other species (tropical and savanna) strongly increased their photosynthetic rates with increased light intensities, but light-saturation occurred at different intensity levels for each species.

Light-saturation over  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$  was observed for most of the species; while *T. heteromalla* became light-saturated at  $2200 \mu\text{mol m}^{-2} \text{s}^{-1}$ , followed by *S. jamaicensis* and *Q. stellata*, both with an inflection point at  $3000 \mu\text{mol m}^{-2} \text{s}^{-1}$ . The average optimum light intensity among all species was  $1671 \pm 734 \mu\text{mol m}^{-2} \text{s}^{-1}$ .

Overall, species that were intolerant of intense light had a higher tolerance for leaf wetness. Plants with small leaf surface water retention and long drying periods were also the ones that needed less light to photosynthesize ( $<1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ ); three of the species, however, had notably different behavior. *Quercus muehlenbergii* ( $\text{PAR}_{\text{opt}} = 1300 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and *Q. macrocarpa* ( $\text{PAR}_{\text{opt}} = 700 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), which had short drying times, had the lowest light intensity optimum among all species.

In contrast to the relationship observed between wetness duration and light tolerance, a species' photosynthetic response to temperature did not show a relationship with leaf wetness duration. However, there was a clear distinction between sites, in which savanna species were more tolerant to warmer temperatures (above  $40^\circ\text{C}$ ). Among both sites, temperature response curves indicated that photosynthesis in all but one species declined with increasing temperatures. Photosynthesis in *S. jamaicensis* appeared parabolic, with an optimum temperature of  $30^\circ\text{C}$  (Figure 4B). The main difference among the other species was at which temperature level photosynthetic rates declined and the relative sensitivity of  $A_{\text{net}}$  to temperature. Tropical species had the largest declines with increasing temperature when compared with the optimum conditions. Experimentally, when extrapolating temperature responses over the highest temperature measured ( $38^\circ\text{C}$ ) using the fitted models, five of the tropical species (Figure 4B) reached zero or near-zero rates of  $A_{\text{net}}$

( $<3 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) when leaf temperature was over  $40^\circ\text{C}$ . *Tibouchina heteromalla* did not reach zero but had a photosynthetic reduction of 81% between  $20$  and  $45^\circ\text{C}$ . In contrast, savanna species had a less steep decline of photosynthetic rates compared with tropical species, with none of the species'  $A_{\text{net}}$  below  $4 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ . Among the species, between  $20$  and  $45^\circ\text{C}$ , *Q. stellata* had the smallest reduction of photosynthetic rates (49%) and *Q. macrocarpa* the largest (71%). Even though species optimum temperature level varied, we believe that temperatures between  $24$  and  $32^\circ\text{C}$  would still maintain an optimum photosynthetic rate for all species ( $T_{\text{leaf-opt}} = 31.9 \pm 8.1^\circ\text{C}$ , while:  $T_{\text{tropical}} = 28.2 \pm 5.0^\circ\text{C}$  and  $T_{\text{savanna}} = 43.0 \pm 1.4^\circ\text{C}$ ).

Distinct leaf and successional traits influenced photosynthetic rates during dry and wet conditions. As a result, species and sites varied significantly ( $P < 0.05$ ) (Figure 5). Some species had higher  $A_{\text{net}}$  when dry or wet, while others were reduced. As a result, mean  $A_{\text{net-dry}}$  and  $A_{\text{net-wet}}$  did not differ ( $P = 0.32$ ). When assessing the trends in  $A_{\text{net-wet}}$  over the course of drying inside the chamber, most leaves experienced an initial reduction in  $A_{\text{net}}$  followed by a full or partial recovery to values similar to  $A_{\text{net-dry}}$ . Species photosynthetic response variability was also reflected on light and temperature responses for each species. Overall, leaves did not shut down photosynthesis when wet. However, tropical species generally maintained higher  $A_{\text{net-wet}}$  than savanna species. Wet *S. jamaicensis* and *T. heteromalla*, which both have trichomes in the surface, but different drying rates (fast and long period of drying, respectively), reached upwards of  $20\text{--}30 \mu\text{mol m}^{-2} \text{ s}^{-1}$  when wet. The other species ranged from a mean of  $2\text{--}10 \mu\text{mol m}^{-2} \text{ s}^{-1}$ . Interestingly, those with high  $A_{\text{net-dry}}$  did not necessarily have the highest  $A_{\text{net-wet}}$ . This was more pronounced in amphistomatous species. Although,  $A_{\text{net-dry}}$  of *C. laevis* was above the overall average of

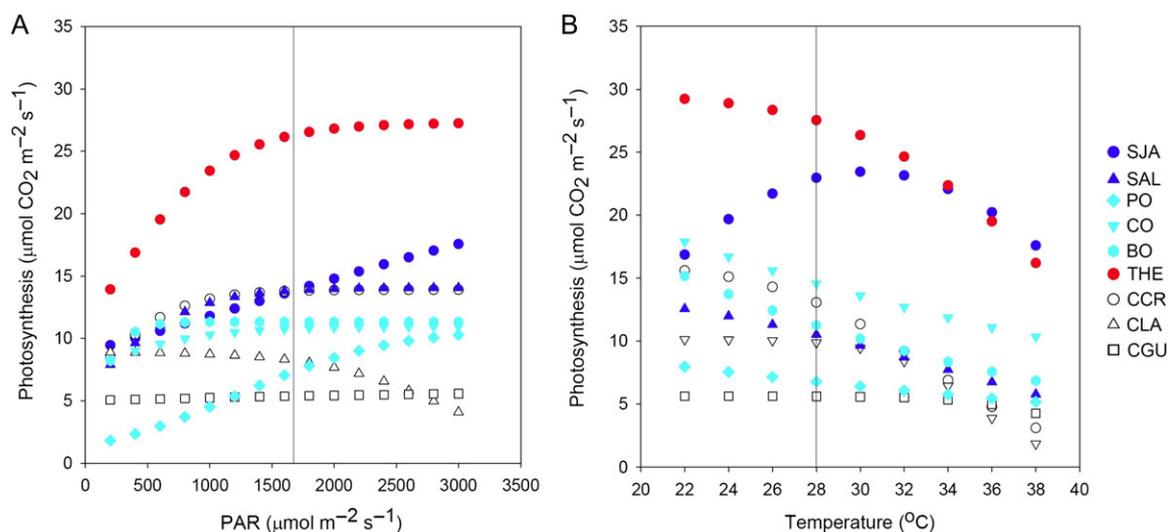


Figure 4. Fitted (A) light response curve ( $200\text{--}3000 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ) and (B) leaf temperature response curve ( $22\text{--}38^\circ\text{C}$ ) for each species. Gray vertical line represents average optimum condition among species ( $\text{PAR}_{\text{opt}} = 1700 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ;  $T_{\text{leaf-opt}} = 28^\circ\text{C}$  (tropical)). Symbol and line colors represent leaf wetness duration categories as in Figure 3.

$A_{\text{net-dry}}$ ,  $A_{\text{net-wet}}$  was reduced on average 25%. To standardize across taxa with greatly differing maximum  $A_{\text{net}}$  and to account for potential differences in leaf status at the time of measurements,  $A_{\text{net-wet}}$  was reported as the difference between wet and dry photosynthetic rates ( $\Delta A_{\text{net}} = A_{\text{net-wet}} - A_{\text{net-dry}}$ ; Figure 6) and as a percentage difference relative to  $A_{\text{net-dry}}$  at different time periods (initial and long-term response, and dry-down  $A_{\text{net}}$  status; Table 2). The large species variability (morphologically and physiologically) led us to further evaluate photosynthetic behavior based on whether a species was hypostomatous or amphistomatous.

**Hypostomatous species** Even though all savanna site species were hypostomatous with relatively small leaf surface water capacity and short drying period, all three displayed distinct photosynthetic patterns while drying (Figure 6A). All three species declined immediately, but after 10 min of wetting, *Q. stellata* continued responding negatively (to  $-13\%$  of  $A_{\text{net}}$  dry), *Q. muehlenbergii* recovered partially ( $-48\%$  to  $-31\%$  of  $A_{\text{net}}$  dry), and *Q. macrocarpa* recovered fully, ending with 14% higher  $A_{\text{net}}$  than recorded under dry conditions (Table 2).

At the tropical site, *T. heteromalla* and *C. guianensis* were also hypostomatous, yet differed markedly in wetness duration and photosynthetic patterns while drying (Figure 6A). Even though *T. heteromalla* had only a slight decrease during the first 2 min ( $-4\%$ ), afterwards there was a constant increase that reached 10% above the dry condition rates. Oppositely, *C. guianensis* had a late reaction to leaf wetness, as indicated by its largest decrease in photosynthetic rates after 4 min ( $-28\%$ ), but almost fully recovered by 10 min ( $-6\%$ ) (Table 2).

**Amphistomatous species** At the tropical site, *S. jamaicensis* and *S. alata* were categorized as amphistomatous with small leaf

surface water capacity and short drying period. Consequently, both species had the largest immediate decrease in  $A_{\text{net}}$ . These species were also the ones to possess the largest stomatal ratios within the tropical species (Table 2). But interestingly, both species were almost fully recovered after 10 min when the leaves were almost or fully dry (Figure 6B).

Amphistomatous species with small leaf surface water capacity and a long drying period (*C. crotalifera* and *C. laevis*) displayed different responses to wetness. *Calathea crotalifera* had an initial decrease of 15%, but like those that dried more quickly was almost fully recovered after 10 min ( $-3\%$ ). Although *C. laevis* had a similar initial photosynthetic rate decrease ( $-12\%$ ) to *C. crotalifera*, *C. laevis*  $A_{\text{net}}$  continued decreasing up to 9 min and started a late recovery afterwards (Table 2, Figure 6B).

*Zamia skinneri* was the only amphistomatous species with large leaf surface water capacity and long drying period. Similarly to *T. heteromalla*, this species had an immediate improvement (21%) of  $A_{\text{net}}$  dry, but subsequently started decreasing after 5 min and was almost equivalent (7%) to its dry condition rates at 10 min despite the fact that it was most likely still relatively wet at that time.

**Chamber variable relationships** The chamber variables ( $\delta e$ ,  $T_{\text{leaf}}$  and  $T_{\text{diff}}$ ) varied greatly between species, due to variable leaf traits (e.g., trichomes and surface roughness held water longer, hence affecting leaf temperature and  $\delta e$ ), but hypostomatous species had a smaller range of variation throughout the dry-down than amphistomatous species (Figure 6). While leaves were wet, the variables that had the largest variations were temperature difference and  $\delta e$  for most of the studied species and sites, consequently influencing the observed  $A_{\text{net}}$  trends. We

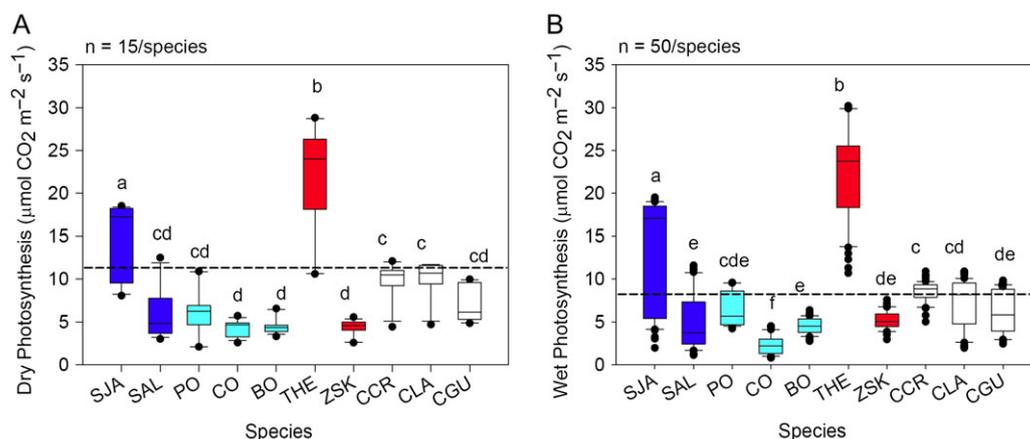


Figure 5. Range of photosynthesis measurements for dry (A) and wet (B) conditions for each species. Gray dashed line across each graph indicates the overall mean photosynthetic value for each condition (dry =  $8.8 \pm 6.0 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ; wet =  $8.2 \pm 6.5 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) and number of measurements per species is indicated above each graph. Order of species in the X-axis follow leaf wetness duration categories (from left to right: SR/SD (*Stachytarpheta jamaicensis*, *Senna alata*, *Quercus stellata*, *Quercus muehlenbergii* and *Quercus macrocarpa*), LR/LD (*Tibouchina heteromalla* and *Zamia skinneri*), SR/LD (*Calathea crotalifera*, *Costus laevis* and *Carapa guianensis*)). Box colors represent leaf wetness duration categories as in Figure 3. Box plots characterize this distribution, with the bottom and top part of the box indicating the 25th and 75th percentile, respectively; the two dashes the 10th and the 90th percentile, respectively; and the horizontal line within the box the median value. Tukey HSD denoted with letters indicate categories with significance differences, as indicated by ANOVA ( $P < 0.05$ ).

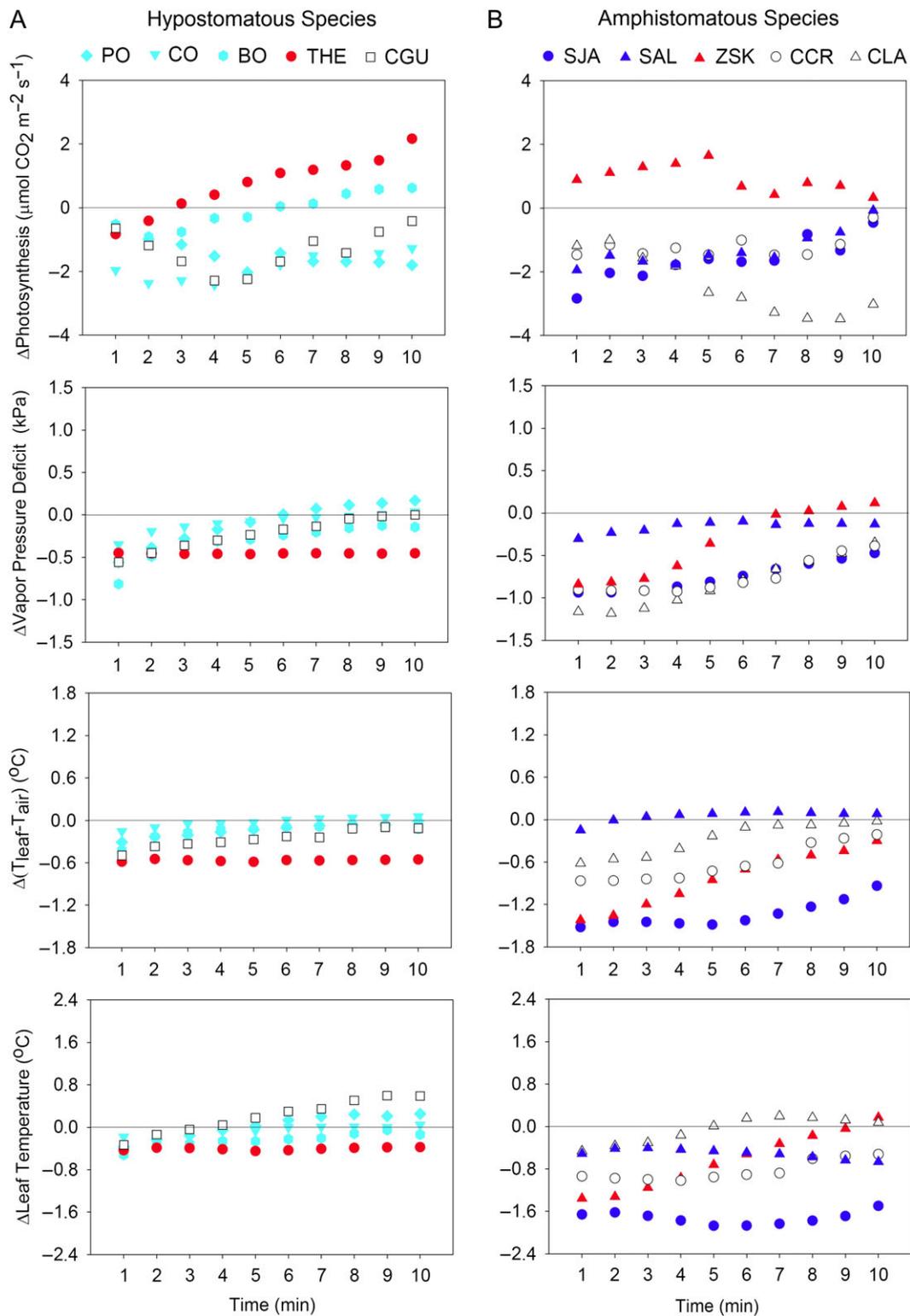


Figure 6. Normalized average photosynthetic response ( $\Delta A_{net} = A_{net-wet} - A_{net-dry}$ ) of the sampled species to simulated wetness and subsequent dry-out [ $x(t_0) = 0 \rightarrow \text{dry}$ ] and their respective micrometeorological drivers. (A) Hypostomatous species; (B) amphistomatous species. Notes: from top to bottom, respectively: photosynthesis ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), vapor pressure deficit (kPa), difference between leaf temperature and air temperature ( $^{\circ}\text{C}$ ) and leaf temperature ( $^{\circ}\text{C}$ ). Symbol and line colors represent leaf wetness duration categories as in Figure 3.

also found that effects of  $T_{diff}$  on  $A_{net}$  were slightly weaker or stronger, depending on species' leaf morphology (Tables 1 and 4). Morphological characteristics that may indicate a stronger  $T_{diff}$  response include higher water retention time or volume and/or presence of trichomes that may retain water (examples from Table 1), whereas weak responses to  $T_{diff}$  or greater tolerance for high leaf temperatures can be attributed to certain plant traits such as faster drying times, drip-tips and waxy leaf epidermal surfaces (Table 1). Vapor pressure deficit was highly correlated with photosynthetic response for most species or biome, although its influence is mainly dependent in the interaction with temperature difference (Table 4).

## Discussion

Rather than finding consistent reductions in  $A_{net}$  under wet leaf conditions, plant species were distinctly different from each other, as reflected in divergent photosynthetic responses that seemed to be related to leaf traits: leaf wetness duration, leaf surface features, stomata arrangement and successional stage (or growth habit). Hence, we cannot accept our hypothesis that leaf wetness reduced  $A_{net}$ , since results were highly inconsistent among species. Species with higher  $A_{net}$  when wet, relative to dry conditions, held more water on the surface, suggesting a preference for cooler and more humid conditions along with leaf traits that help cope with water retention (trichomes and grooved leaves, *T. heteromalla* and *Z. skinneri*, respectively) (Figure 2A and D). Others with longer drying periods and low water retention, immediately reduced their photosynthetic rates, but later recovered due to stomata placement and subsequent drier conditions resulted from leaf surface evaporation. Contrary to our expectations, having stomata on the adaxial surface of leaves did not consistently reduce  $A_{net}$  when wet. Apparently, other traits, such as faster drying times or lower water retention on the upper leaf surface, mitigated the impacts of wet upper stomata. This finding suggests that tropical species may be better adapted to wet leaf conditions than savanna species. It is notable that the species sampled were not consistent with the literature regarding stomata placement, in which xeric species are usually amphistomatous (Mott et al. 1982, Smith et al. 1998, Williams et al. 2004) and tropical species are hypostomatous (Smith et al. 1998, Camargo and Marengo 2011). Additionally, there are very few studies in the literature that measured wetness effects on photosynthesis in vivo (Doley et al. 1987, Letts and Mulligan 2005, Johnson and Smith 2006, Reinhardt and Smith 2008).

*Tibouchina heteromalla* possesses a dense layer of trichomes that could potentially help reduce transpiration, due to increase in leaf boundary layer thickness. Additionally, *T. heteromalla* had a long drying period, but did not experience photoinhibition, since the presence of trichomes may have provided protection under high light intensity (Ripley et al. 1999). Literature

suggests that some tropical species can perform foliar water uptake, as an alternative water source or to temporarily enhance cell turgidity (Eller et al. 2013, 2016, Goldsmith et al. 2013, 2016, Fu et al. 2016). *Tibouchina heteromalla* appears to be a good candidate for such behavior, especially since there has been recent evidence that trichome structures might play an important role in this process (Pina et al. 2016, Schwerbrock and Leuschner 2017), by increasing surface water storage. However, Goldsmith et al. (2013) hypothesizes that foliar water uptake occurs independent of phylogeny, morphology and growth habit; however, more information is required to actually identify plant anatomy traits that favor this process. Holder (2007) described *Tibouchina urvilleana* as having very similar leaf characteristics to *T. heteromalla* from our study, particularly its 'wettability' (i.e., water droplets spread and adhere to the leaf surface, rather than form a bead on the surface) (Figure 2B), while possessing an almost repellent abaxial surface (i.e., a 98° droplet contact angle just under the 110° threshold). Hence, the geographical occurrence of *T. heteromalla* (coastal or inland high altitude mountainous regions) and its preference for cooler temperatures (Luttge et al. 2015), also shown in its temperature curve (Figure 4B), leads to the assumption that leaf wetness actually creates a favorable boundary layer condition surrounding the leaves by retaining water droplets suspended from the leaf surface by the trichome tips. This type of retention can actually stimulate greater stomatal opening and higher water-use efficiency (Smith and McClean 1989, Brewer et al. 1991).

Favorable conditions can also be linked to the increase of  $A_{net}$  for *Z. skinneri*, as this species is mainly restricted to the tropical understory, which has low light intensity and high humidity. Although this species is amphistomatous, Acuña-Castillo and Marín-Méndez (2013) report several features that could allow for continued photosynthetic activity during wetness events: crypt stomata and adaxial stomata located in the elevated, ridged portions of the leaflet. In other words, stomata located in the elevated portions of the leaves are still dry and capable to continue photosynthetic activity, while stomata located inside the depressed portion are wet ('leaf ponding', Figure 2A). Although *Z. skinneri* retained a smaller surface water amount than SR/SD (*S. alata* and *S. jamaicensis* or any of the savanna species), this species' leaves took longer to dry than *S. alata*, which had the most similar water retention values (steeper slope). However, *Z. skinneri* also could not be considered as small water retention and long period of drying (SR/LD) because it did not take as long to dry as the species in those categories with similar water retention (*C. laevis* and *C. crotalifera*, shallower slopes). Thus, *Z. skinneri* had a larger storage capacity of water within deep leaf grooves that greatly enhanced leaf topography, in addition to a slope almost identical to *T. heteromalla* from the LR/LD dry-down category. Considering that tropical understory plants remain wet longer than more exposed overstory plants, these adaptations would enhance photosynthetic activity even under suboptimal

Table 4. Regression models for net photosynthesis ( $A_{\text{net}}$ ) as a function of conditions in the chamber, including leaf temperature ( $T_{\text{leaf}}$ ), leaf-to-air temperature difference ( $T_{\text{diff}}$ ) and vapor pressure deficit ( $\delta e$ ) for each species and biome (grouped and ungrouped by individual species). Best models were chosen by the largest adjusted coefficient of determination ( $R^2_{\text{adj}}$ ). All models include  $A_{\text{net}}$  as the dependent variable and one or more chamber conditions for the independent variable(s), separately or in combination, allowing for interactions between variables (full model:  $A_{\text{net}} = a + b \times T_{\text{leaf}} + c \times \delta e + d \times T_{\text{leaf}} \times \delta e$ , where  $a$ ,  $b$ ,  $c$  and  $d$  are model coefficients).

Species	Best model	$R^2_{\text{adj}}$
SJA	$\delta e$	0.77***
SAL	$T_{\text{leaf}} \times \delta e$	0.91**
THE	$T_{\text{leaf}} \times \delta e$	0.39 <sup>ns</sup>
ZSK	$\delta e$	0.49**
CCR	$\delta e$	0.35 <sup>ns</sup>
CLA	$T_{\text{diff}} \times \delta e$	0.98***
CGU	$T_{\text{leaf}} \times \delta e$	0.79**
PO	$\delta e$	0.72**
CO	$T_{\text{diff}} \times \delta e$	0.96***
BO	$T_{\text{diff}} \times \delta e$	0.98***
All tropical species <sup>ungrouped</sup>	$T_{\text{leaf}} \times \delta e$	0.57***
All tropical species <sup>grouped</sup>	Species $\times T_{\text{diff}}$	0.99***
All savanna species <sup>ungrouped</sup>	$T_{\text{diff}}$	0.53***
All savanna species <sup>grouped</sup>	Species $\times \delta e$	0.97***
All species (tropical and savanna) <sup>ungrouped</sup>	$T_{\text{leaf}} \times \delta e$	0.44***
All species (tropical and savanna) <sup>grouped</sup>	Species $\times T_{\text{diff}}$	0.99***

Significance value labels are denoted as \*\*\* $P < 0.001$ , \*\* $P < 0.01$  and  $ns$  as non-significant. Since  $T_{\text{leaf}}$  and  $T_{\text{diff}}$  were correlated, models included only one or the other variable, not both.

conditions. As seen in Figure 6, *Z. skinneri* photosynthetic rates started declining when vapor pressure deficit ( $R^2 = 0.49$ ,  $P < 0.01$ ) exceeded the initial dry state after 5 min.

Not only was leaf ponding important for highly complex anatomical species (Figure 2), it was also predominant in species with small water volume and long drying period. The species considered in this category had different volumes of water ponded on the surface depending upon leaf anatomy. One of the strategies adopted by tropical plant species to minimize water ponding is the presence of drip tips (Malhado et al. 2012, Goldsmith et al. 2016). *Calathea crotalifera* and *C. laevis* had a prominent and a small one, respectively; and *Z. skinneri* (from the other wetness category) also had leaflets with drip-tips (Table 1). Interestingly, all three sampled species with drip-tips are species commonly found in tropical understories. Malhado et al. (2012) found that Amazonian species that had this leaf feature were short plants with smaller trunk girths found in the understory. Additionally, Farji-Brener et al. (2002) and Meng et al. (2014) found that drip-tips were less common on canopy-exposed trees in Costa Rica and China, respectively, since leaf drying from solar radiation, wind and higher vapor pressure deficit is more dominant than at understory tree canopy level. Meng et al. (2014) also found that shaded species with drip-tips had smaller leaf inclination angles than sun-exposed species ( $\sim 10^\circ$

(flatter) and  $\sim 40^\circ$  (sloped), respectively) for optimized sun exposure to enhance carbon gain. Hence, drip-tip, leaf shape (mainly thick midrib with folded leaf margins) to facilitate water drainage, and flatter leaf angles can result in water ponding in portions of the leaves.

The surface area covered by the ponded water droplets along with leaf surface wettability can lead to longer drying period and, consequently, photosynthetic suppression in amphistomatous species or hypostomatous species with warmer leaf temperature preferences. Therefore, we theorize that the difference in  $A_{\text{net}}$  recovery from wetting events between *C. laevis* and *C. crotalifera* can be attributed to the due to low surface wettability (thinner and smoother waxy surface than the thicker, rougher waxy *C. laevis* leaves) and solar tracking (variable leaf angles) (Herbert and Larsen 1985). These features would lead to a better leaf drainage and result in a smaller area being affected by the drip-tip, while the major dry reminiscent area can continue gas exchange (Holder 2011) (Figure 2D). Additionally to *C. laevis*'s less favorable leaf features that lead to larger water retention on the surface, this species also had higher stomatal ratios than *C. crotalifera* (0.18 and 0.03, respectively).

*Carapa guianensis* leaflets, although hypostomatous, also had a surface design that favored leaf ponding (flat midrib and wavy mesophyll margins), but large pulvinus in the base of the leaflets and leaves can facilitate water drainage and leaf surface exposure to radiation to evaporate thin, retained water film (Figure 2C). Even though this species' photosynthetic rates varied little with leaf temperature variation (Table 3, Figure 4B), we assume that  $A_{\text{net}}$  reduction and later recovery was due to the combination of the vapor pressure deficit drop immediately after wetting along with the increase in leaf temperature ( $R^2 = 0.79$ ,  $P < 0.01$ ). Since conditions inside the chamber contrasted strongly with the large surface area of leaves outside the chamber (i.e., temperature and light intensities), this could have affected the leaf gas exchange characteristics of the leaf within the chamber (Long et al. 1996, Kaipainen and Pelkonen 2007), hence suggesting that leaf wetness' lower  $\delta e$  and cooling effect, even in species with stomata on the top, can cause a suppression effect without directly affecting stomatal  $\text{CO}_2$  diffusion rate. Urrego-Pereira et al. (2013) also found irrigated maize reduced  $A_{\text{net}}$  by 10–41% due to decrease in temperature below the optimum range. The same result was found by Hanba et al. (2004) on wettable soybean leaves.

Time of drying was a major indicator of rapid recovery from wetness. Water repellency has been documented as a major feature to cope with wetness (Rosado and Holder 2013), along with trichomes (Brewer et al. 1991), which were present on both species of this category (*S. alata* and *S. jamaicensis*, respectively). Unlike *T. heteromalla*, *S. jamaicensis* had a less dense layer of trichomes, which could have contributed to the drying process, but also with the initial photosynthetic decrease. Brewer and Smith (1994), while studying soybean leaves with

low trichome densities compared with native, non-agricultural species (Brewer et al. 1991), found that trichome layers retained water longer in patches and, consequently, reduced CO<sub>2</sub> assimilation by 15% compared with non-misted plants; however, leaf surface area beneath these patches of water film was small (when compared with bare leaf surfaces), promoting rapid evaporation. While water retention in the form of water film can reduce gas exchange, Brewer et al. (1991) states that more spherical water droplets on repellent leaf surfaces, as observed for *S. alata* (Figure 2B), will insure gas exchange on the dry remnant surfaces. Droplet roll-off also increases drying rate.

Leaf hypostomy provides extra protection against excessive transpiration and consequent dehydration of plant tissues (Brown and Wilson 1905). In drier environments, some savanna tree species (including the three sampled in this study) have thick leaves covered with trichomes. Additionally, a large volume and long period of water retention on the top surface can lead to pathogen invasions and fungi or lichen growth that can permanently disable stomata on an amphistomatous plant. Having stomata on the abaxial surface can primarily avoid surface wetness decreasing gas exchange during rain events, because the abaxial surface rarely gets wet (Dietz et al. 2007). Similar to *C. guianensis* hypostomy and its response to wetness, leaf anatomy traits and stomatal distribution required further chamber conditions assessment when analyzing the mixed responses among the savanna species. Although all three species had different  $A_{\text{net}}$  responses to wetness (Figure 6A), all were strongly influenced by the resulting combination of  $T_{\text{diff}}$  and  $\delta e$  variation (Table 4). Not only were all three species hypostomatous, but they also have a dense trichome layer on the abaxial surface, whereas the adaxial surface had variable surface roughness and trichome density (Table 1). Hence, we hypothesize  $A_{\text{net-wet}}$  responses were influenced by adaxial surface potential for retaining water and the subsequent cooling effect and/or leaf boundary layer thickening of each species.

Among the oak species, only *Q. stellata* is considered drought tolerant while the other two are considered intermediate (Dickson and Tomlinson 1996). Compared with *Q. macrocarpa*, *Q. muehlenbergii* may be more tolerant to high temperatures (Hamerlynck and Knapp 1994, Balok and Hilaire 2002) and its dominance in xeric environments (Abrams 1986). Our evidence supports this since *Q. macrocarpa* responded favorably to wetness and its associated cooler leaf temperatures (Table 3, Figure 6). Oppositely, even though *Q. muehlenbergii* had a similar reduction on those three chamber variables after wetting (Table 3), photosynthetic rates reduced immediately and only started improving after dry conditions were reached again after 7 min (Figure 6A). We also believe that the rough-textured (wax granules, Balok and Hilaire 2002) adaxial epidermis surface of *Q. macrocarpa*, even though trichome-free (Hardin 1979), leads to a more prolonged optimum microclimatic condition on the leaf by retaining water for a longer period. Also, Balok and Hilaire

(2002) found that *Q. macrocarpa* had less epicuticular wax content than *Q. muehlenbergii*, which not only indicates a vulnerability to drought due to high heat transmissivity through the mesophyll, but can possibly enhance the cooling effect on these species' leaves after rain events on hot, dry days.

*Quercus stellata*, as a drought-tolerant plant, has shown high tolerance to dry, hot conditions (Will et al. 2013). Contrary to our finding, we expected leaf wetness would have a beneficial effect of cooling (Table 3) the leaves of trees at the savanna site and even possibly allow for foliar water uptake, both of which may lead to greater stomatal conductance and stimulate  $A_{\text{net}}$ . Although there are some studies on dry environment or savanna tree's capacity in performing foliar water uptake (Breshears et al. 2008, Limm et al. 2009, Fernandez et al. 2014), there are few to no studies on the effect of leaf wetness on physiological processes for these types of trees.

Compared with tropical site species, we were surprised to find such similar responses to leaf wetness between biomes, given the large contrasts in plant adaptations to local environmental conditions. It is possible that these species converge on a relatively conservative response to wetness, each for divergent purposes (cooling, avoiding stomatal occlusion or by several unique means of rapid drying). To maximize their photosynthetic rates, tropical plants may have modified their leaf anatomy to cope with frequent leaf wetness. By contrast, leaf anatomy in savanna sites is more likely to have evolved traits to conserve water. Therefore, traits to reduce desiccation (e.g., trichomes or surface texture thickening leaf boundary layer) may not be comparable to traits that enhance leaf water shedding (e.g., hydrophobic cuticles or trichomes), while other traits may serve similar purposes (e.g., waxy cuticles or grooved leaf surface). Consequently, leaf wetness of savanna species will most likely suppress leaf gas exchange, while tropical species can steadily maintain these processes when wet, if vapor pressure deficit is sufficiently strong ( $\delta e > 0.5$ ).

## Conclusions

Our findings show strong evidence that leaf wetness duration was the main factor driving photosynthesis reduction or increase. However, this variable was only insightful if linked to one or more leaf traits, like stomatal distribution and trichome presence or leaf surface repellency/wettability. We surmise that tropical species have developed distinct adaptations to cope with wetness without drastically affecting photosynthetic rates, or have evolved strategies to enhance photosynthesis by using leaf wetness to their advantage. Unfortunately, there have been predictions that tropical regions will be affected by more frequent and extreme droughts in the near future, which could dramatically affect these species that depend on leaf wetness for their optimum physiological conditions. Additional studies to examine interactions between leaf traits and leaf wetness, and

potential functional adaptations in highly biodiverse ecosystems are crucial for a better understanding of canopy–atmosphere interactions and how that will affect forest yield and global climate modeling.

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## Conflict of interest

None declared.

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