

Variation in the resilience of cloud forest vascular epiphytes to severe drought

Sybil G. Gotsch¹, Todd E. Dawson² and Danel Draguljić³

¹Department of Biology, Franklin and Marshall College, PO Box 3003, Lancaster, PA 17603, USA; ²Department of Integrative Biology, University of California at Berkeley, 4006 Valley Life Sciences Building, Berkeley, CA 94720, USA; ³Department of Mathematics, Franklin and Marshall College, PO Box 3003, Lancaster, PA 17603, USA

Summary

Author for correspondence:

Sybil G. Gotsch

Tel: +1 717 358 4732

Email: sybil.gotsch@fandm.edu

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- Epiphytes are common in tropical montane cloud forests (TMCFs) and play many important ecological roles, but the degree to which these unique plants will be affected by changes in climate is unknown. We investigated the drought responses of three vascular epiphyte communities bracketing the cloud base during a severe, El Niño-impacted dry season.
- Epiphytes were instrumented with sap flow probes in each site. Leaf water potential and pressure–volume curve parameters were also measured before and during the drought. We monitored the canopy microclimate in each site to determine the drivers of sap velocity across the sites.
- All plants greatly reduced their water use during the drought, but recovery occurred more quickly for plants in the lower and drier sites. Plants in drier sites also exhibited the greatest shifts in the osmotic potential at full saturation and the turgor loss point.
- Although all individuals survived this intense drought, epiphytes in the cloud forest experienced the slowest recovery, suggesting that plants in the TMCF are particularly sensitive to severe drought. Although vapor pressure deficit was an important driver of sap velocity in the highest elevation site, other factors, such as the volumetric water content of the canopy soil, were more important at lower (and warmer) sites.

Introduction

In tropical mountains, ecosystems are compressed into narrow bands because of differences in climatic and geographic variables. The elevation of the tropical montane cloud forest (TMCF) varies depending on a number of factors, including exposure, the size of the mountain ('mass-elevation effect'), distance from the coastline and associated microclimatic variables, including temperature, cloud cover and precipitation (Flenley, 1995; Jarvis & Mulligan, 2010). In these regions, elfin forest, cloud forest, and upper and lower premontane rain forest exist within distances as short as a few kilometers (Bruijnzeel *et al.*, 2011). Such close proximity of different ecosystems provides opportunities to study how species in these distinct habitats respond to seasonal resource limitations and to understand how these habitats might be affected by projected changes in climate.

TMCFs exist on steep terrain; these forests provide critical local and regional hydrological services by promoting the infiltration of precipitation into the soil and subsurface, which, in turn, maintains stable stream flows (Zadroga, 1981; Brown *et al.*, 1996; Bruijnzeel *et al.*, 2010; Tognetti *et al.*, 2010). Because the microclimatic zones of TMCFs are narrow, this ecosystem is likely to be affected by projected changes in climate, including land surface warming and increases in cloud base heights (Lawton *et al.*, 2001). Changes in TMCF community structure may have

large impacts on landscape processes, although effects will depend on cloud water inputs and the intensity of land use practices (Muñoz-Villers, 2008; Tobón *et al.*, 2010; Bruijnzeel *et al.*, 2011).

Epiphytes reach their peak in biomass and diversity in the TMCF, where they supply up to 50% of the within-crown leaf area and provide substantial inputs of above-ground biomass to the entire ecosystem (Nadkarni, 1984; Nadkarni & Matelson, 1992; Hofstede *et al.*, 1993). In the TMCF of Monteverde, Costa Rica, large woody epiphytes, including epiphytic shrubs, hemi-epiphytes and single-stemmed woody species, are all abundant and comprise a substantial proportion of the living biomass in the canopy (40–60% of the biomass of vascular plants in all sites; A. Amici, unpublished data). These forest components intercept nutrient-rich cloud water, which is cycled locally in the canopy, and is then moved to the forest floor via litterfall and crownwash (Gotsch *et al.*, 2016). Furthermore, vascular epiphytes, bryophytes and associated dead organic matter have high water storage capacities, which can alter canopy microclimate and benefit the water relations of host trees (Stuntz *et al.*, 2002; Stanton *et al.*, 2014).

Vascular epiphytes exhibit a variety of traits that are likely adaptations to withstand dry periods by aiding in the maintenance of positive water balance and drought resistance. These characteristics include thick leaves and cuticles to resist water loss;

large tuber-like storage structures; and even in a few cases the induction of Crassulacean acid metabolism (CAM) photosynthesis when conditions are severe (Benzing, 1990; Zotz & Winter, 1994; Gotsch *et al.*, 2015). Despite having adaptations to cope with water limitation, these plants lack a direct connection to the ground, and so depend directly on atmospheric inputs of both water and nutrients. Whether vascular epiphytes can withstand projected changes in climate, such as long-term increases in temperature, cloud base heights and the number of days without rain, is largely unknown (Still *et al.*, 1999; Pounds *et al.*, 2006; Ray *et al.*, 2006; Karmalkar *et al.*, 2008; but see: Nadkarni & Solano, 2002). A loss of the epiphyte community or a change in epiphyte community composition could alter or diminish important services provided by the TMCF ecosystem, including water and nutrient cycling and storage, as well as food and habitat resources for a number of animals (Gotsch *et al.*, 2016 and references therein).

Deforestation in lowland regions in Costa Rica has caused an upward shift in the cloud base and this shift is likely to continue if land surface temperatures continue to rise (Lawton *et al.*, 2001). In addition, changes in precipitation patterns, including an increase in the number of dry days ($< 0.1 \text{ mm d}^{-1}$ recorded), have already been reported in Monteverde, a TMCF in Costa Rica, and are likely to continue (Still *et al.*, 1999; Pounds *et al.*, 2006). For example, the average number of dry days from 1973 to 1981 was 25, whereas from 2011 to 2016 this number rose to 104 (Pounds & Masters, 2017; A. Pounds, unpublished data). In 2016, the year of this study, precipitation patterns were also affected by the El Niño Southern Oscillation (ENSO). In this year, there were 129 dry days, which is the current record (Pounds & Masters, 2017; A. Pounds, unpublished data). Continued increases in the number of dry days are likely to result in widespread drought and shifts in community structure.

The evaluation of water relations traits, including sap flow and pressure–volume parameters, such as the turgor loss point (TLP), can be used to assess the resilience of epiphytes to periods of drought. Sap flow has been shown to respond to drought in this community, and pressure–volume parameters have been found to indicate drought tolerance in a number of ecosystems (Niinemets, 2001; Brodribb & Holbrook, 2003; Lenz *et al.*, 2006; Blackman *et al.*, 2010; Bartlett *et al.*, 2012; Darby *et al.*, 2016). In this study, we exploited an unprecedentedly intense dry season that occurred from February to April 2016 to determine the effect of drought on the water relations of common canopy epiphytes. Our study took place in three sites bracketing

the current cloud base: a TMCF site; a mid-elevation premontane rainforest site located near the current cloud base; and a site *c.* 300 m below the cloud base. We instrumented epiphytes at each site with sap flow sensors and measured water potential and pressure–volume curve parameters before and during the drought. We also followed plants at the beginning of the rainy season to document variation in drought recovery across sites and species. We investigated the following questions. Is there variation in drought responses between or within epiphyte communities that reside in different microclimatic zones? Are TMCF epiphytes more vulnerable to drought than epiphytes in canopies at lower elevations? How do the patterns of water use vary in canopy epiphyte communities that reside in different microclimates? Are there differences in the microclimatic drivers of sap flow (water use) across the sites?

Materials and Methods

Site description

This research took place in three sites that bracket the cloud base in the tropical montane region of central Costa Rica (Table 1). The highest elevation site is located in TMCF in the Monteverde Reserve (CCT, 1554 m above sea level (asl)). The average annual rainfall is *c.* 4000 mm (Pounds *et al.*, 1999; A. Pounds, unpublished data). The dry season generally occurs from February to April (Nadkarni, 1984, 1994), which include periods of rain and mist, but less than that at other times of the year. The middle elevation site is located near the current cloud base in an upper pre-montane rain forest (Curi Cancha Reserve or LCC, 1474 m asl). The lowest elevation site is located in a premontane rain forest and has an average annual precipitation of 2300 mm (<http://weather.uga.edu/index.php?content=gp&site=SANLUIS>; University of Georgia-Costa Rica, or UGA, 1100 m asl). The seasonality of the weather patterns is more severe in the lower elevation sites (data, this study; see Fig. 2).

At each site, two dominant canopy trees (but not emergent) were chosen within 50 m of one another (Fig. 1). Specific host species were not targeted, because, unlike lowland forests, montane forest trees do not appear to have striking host specificity (Nadkarni, 1984). The host tree genera included *Ficus* (Moraceae), *Ocotea* (Lauraceae), *Pouteria* (Sapotaceae) and *Sapium* (Sapindaceae), which are among the most common in all sites (Haber, 2000). We selected trees that had a large and healthy crown and trunk and what appeared to be a

Site	Elevation	Location	Forest type	Latitude	Longitude
CCT	1554	Monteverde Reserve	Primary	10°18'18.84"N	084°47'39.30"W
LCC	1474	Curi Cancha Reserve, Monteverde	Secondary with remnant trees	10°18'22.51"N	084°48'16.45"W
UGA	1100	University of Georgia, San Luis	Secondary	10°16'58.83"N	084°47'51.06"W

Table 1 Elevation, forest type, reserve name and GPS coordinates for each of the study sites

CCT, a cloud forest site; LCC, an upper premontane rainforest near the cloud base; UGA, a lower premontane rainforest.



Fig. 1 Each image was taken from a study crown in each of the three sites. In the cloud forest (upper site and panel), the epiphyte community is most lush and diverse. Near to the cloud base (middle site and panel), the biomass of the canopy community is high, but the diversity is lower than that in the cloud forest site. In the premontane site (lowest site and panel), the diversity and abundance are lowest.

representative epiphyte community for the site (Fig. 1). We sampled individuals in two trees to maximize our sample sizes within a species and to ensure that sampled individuals (which often grow in clonal patches) were genetically distinct.

Epiphyte study species

The most abundant canopy epiphyte species (based on biomass measurements; A. Amici, unpublished data) were measured in two host trees in each of the sites (Table 2). Because sap flow measurements were central to this research, we limited our species selection to those with long and straight internodes. When possible, we included individuals from both host trees at each site. Our study species represented different functional groups (shrubs, single-stemmed woody species, herbaceous, hemi-epiphytes) at the LCC and CCT sites. In CCT, the

epiphyte community is well mixed, whereas, in LCC, shrubs were the most abundant functional group and, in UGA, shrubs and bromeliads were most abundant (S. Gotsch, unpublished data). At UGA, only one species yielded usable sap flow data.

Microclimate

Microclimate stations were installed near the center of one tree crown in each site between June and July of 2014. Each station contained one data logger that recorded air temperature and relative humidity (HOBO U23 Pro v2 External U23-002, Onset Corporation, Cape Cod, MA, USA), and an Em50 data logger (Decagon Devices, Pullman, WA, USA) which collected data from a Davis cup anemometer, a soil moisture probe, a soil temperature probe and a leaf wetness (LWS) sensor (Decagon Devices). Soil probes were placed at a 45° angle into the epiphyte mat to a depth of 10–15 cm. Inner canopy epiphyte mats (where weather stations were placed) ranged in depth from 10 to 15 cm in the lowest site to ≥ 30 cm in the upper two sites. All mats consisted of dead organic matter and had a high fine and coarse root biomass relative to terrestrial soil samples at a similar depth (S. G. Gotsch, pers. obs.) It was not possible to analyze the *absolute* volumetric water content (VWC) of canopy soils, as calibration is needed in each location to ensure that the baseline values across the sites are comparable. Because these sensors are deployed until 2019, this calibration was not possible. To compare across sites, we calculated the percentage of the maximum VWC (% max soil) in each site for the study period, which represented the dry season and 3 wk of wet-up when the rainy season began (after Starr & Paltineanu, 2002). As canopy epiphyte mats occupy a small area relative to terrestrial soil, we assumed that the wet-up period in this study was sufficient to saturate the mats. We limited the microclimate data used to the time at which we also had sap flow data, as one of our goals was to determine the microclimatic drivers of sap flow across the sites.

Sap flow

Sap flow sensors were installed on 16 individuals in the two focal trees in each of the three study sites (four to five species per tree, two to four replicates per species per tree). Sap flow stations were installed throughout the months of January and February of 2016. All stations were functioning by 1 March 2016. We maintained these stations throughout the driest months of the dry season (March and April) and for 2 wk after the onset of the rainy season, in early May.

The sap flow probes used measure sap velocity with the heat ratio method, which allows the measurement of low and reverse flows (Burgess *et al.*, 2001). To minimize intrusive damage to the small epiphyte stems, we used external sensors constructed following the methods of Clearwater *et al.* (2009) and Darby *et al.* (2016). The sensor head is held firm to the stem with Parafilm, which also keeps the sensor head dry. The sensor array was powered by a 12 V battery. The data were logged every 10 m using a CR-1000 datalogger connected to an AM 16/32 multiplexer (Campbell Scientific, Logan, UT, USA). Sap velocity and

Table 2 Taxonomic and growth form information, and summary sap flow statistics for the focal study species

Site	Species	Family	Epiphytic growth form	Drought	SE	Recovery	SE	<i>n</i>
CCT	<i>Cavendishia capitulata</i>	Ericaceae	Shrub	14.2	6.0	46.9	6.0	3
	<i>Chorigyne ensiformis</i>	Cyclanthaceae	Herbaceous	43.9	10.8	65.0	3.2	2
	<i>Neomirandea croatii</i>	Asteraceae	Single-stemmed woody	24.5	na	45.7	na	1
	<i>Notopleura pithecobia</i> *	Rubiaceae	Single-stemmed woody	35.2	17.8	40.2	9.8	3
	<i>Peperomia</i> sp.*	Piperaceae	Herbaceous	61.2	na	81.6	na	1
LCC	<i>Schefflera rodrigueziana</i>	Araliaceae	Hemi-epiphyte	32.9	6.8	64.9	16.3	3
	<i>Cavendishia quercina</i>	Ericaceae	Shrub	27.0	na	56.9	na	1
	<i>Gonocalyx costaricensis</i>	Ericaceae	Shrub	43.0	10.7	90.5	5.6	5
	<i>Macleania insignis</i>	Ericaceae	Shrub	31.0	5.4	100.0	1.7	5
	<i>Neomirandea croatii</i>	Asteraceae	Single-stemmed woody	68.6	27.7	70.8	4.1	3
UGA	<i>Hillia loranthoides</i> *	Rubiaceae	Shrub	na	na	na	na	0
	<i>Macleania insignis</i>	Ericaceae	Shrub	59.2	6.8	100.0	9.7	7

These species were evaluated using sap flow probes and pressure–volume curves, and their water potential was also measured under field conditions, throughout the experiment. *n* refers to the sample size for sap flow. Sensor failure prohibited sap flow data from being collected from *Hillia laurenthoides*, but this species was used for other measurements in the study. ‘Drought’ refers to the percentage of maximum sap flow (l h^{-1}) for the study species during the height of the drought (week 8) and ‘Recovery’ refers to the percentage of maximum sap flow following a week of rain (week 10). Uneven sample sizes were caused by sensor failure. All Ericaceous shrubs contained lignotubers. Species marked with an asterisk were those with succulent leaves. Not applicable (na) is indicated for species for which only one individual was measured as the standard error (SE) could not be measured.

volumetric sap flow calculations were performed on the 35 sensors that remained functional throughout the study period. The heat pulse velocity, V_h (cm h^{-1}), was calculated using the equation

$$V_h = \frac{k}{x} \ln \left(\frac{\delta T_1}{\delta T_2} \right) \quad \text{Eqn 1}$$

where x represents the distance between the heater and the thermocouple (0.5 cm), δT_1 and δT_2 are the downstream and upstream temperature changes ($^{\circ}\text{C}$) and k represents the thermal diffusivity constant estimated from

$$k = \frac{x^2}{4t_m} \quad \text{Eqn 2}$$

where t_m is the time between the heat pulse and the maximum temperature measured x distance above and below the heater (Clearwater *et al.*, 2009). This allowed for the determination of the sap flow velocity, V_s , from

$$V_s = \frac{V_h \rho_b (c_w + m_c c_s)}{\rho_s c_s} \quad \text{Eqn 3}$$

where ρ_b is the stem density, c_w is the specific heat capacity of the wood matrix ($1200 \text{ J kg}^{-1} \text{ }^{\circ}\text{C}^{-1}$ at 20°C), c_s is the specific heat capacity of water ($4182 \text{ J kg}^{-1} \text{ }^{\circ}\text{C}^{-1}$ at 20°C), m_c is the water content of the xylem and ρ_s is the density of water (Burgess *et al.*, 2001).

Variation in sensor construction can be corrected by severing the xylem above and below the installation to obtain true zero values, but this method was not appropriate because the plants underwent continued observation after this study. We therefore obtained zero reference values when environmental conditions suggested a period with minimal transpiration but no foliar water

uptake (i.e. at night-time when the vapor pressure deficit (VPD) was low and the leaf surfaces were dry; Ambrose *et al.*, 2009, 2010; Gotsch *et al.*, 2014). The data for each sensor were then adjusted by adding or subtracting this value from the rest of the dataset. These corrected values were then scaled up to the volumetric flow by multiplying the velocity data by the cross-sectional area of the stem on which the sensor was placed. These estimates are probably an overestimate of the total volumetric flow as there are non-conductive tissues included in the total cross-sectional area. For woody species, this error is probably reduced as nearly all of the entire cross-section of the stem appeared to be functional xylem (S. G. Gotsch, pers. obs.). For herbaceous species, this error is probably greater as there are more non-conductive components in these stems. Both sap velocity and estimates of volumetric flow are reported in the results for comparison. The species and sample sizes for the sap flow sensors that remained functional throughout the experiment can be seen in Table 2.

Water potential and pressure–volume curves

Leaf water potential was measured with a Scholander-style pressure chamber (Model 1505, PMS Instrument Co., Corvallis, OR, USA) on 5–10 individuals of each of the study species in the three sites. Measurements occurred before the drought (January), in the middle of the dry season (March) and at the end of the dry season (April), and were taken at both predawn (04:30–05:30 h) and midday (11:30–12:30 h) during these time periods. Leaves were sealed in plastic bags and measured within 45 min of collection.

Pressure–volume curves were conducted on 10–12 individuals per species per site to determine the TLP and the osmotic potential at full saturation. Different individuals were used from those instrumented with sap flow sensors. Small shoots were harvested, sealed into plastic bags and brought to a laboratory within 3 h. In the laboratory, stems were recut underwater and placed into a black plastic bag, whilst maintaining the cut end in water. This

humidity chamber served to rehydrate the samples. To conduct a pressure–volume curve, water potential and fresh weight were measured 12–15 times as each sample was bench-dried in ambient air until *c.* 40% of the mass was lost. This process occurs very slowly in epiphytes and, in many cases, pressure–volume curves took 2–3 d to complete, depending on the degree of succulence of the species (Gotsch *et al.*, 2015). During this time, the thickness of succulent leaves decreased and veins became clearly visible. Since the laboratory conditions were similar to those of the cloud forest (> 80% relative humidity and 16–22°C), samples were placed in a light box to accelerate desiccation. Once a curve had been completed, samples were dried to a constant weight to determine the leaf dry mass. The relationship between the inverse water potential and relative water content was plotted and analyzed following the methods described by Sack *et al.* (2011).

Data analyses

To analyze differences in the weekly meteorological variables across the sites, we performed an analysis of covariance (ANCOVA) for each of the following variables: LWS, relative humidity, air temperature, wind speed, percentage of the maximum volumetric soil water content and VPD. The week of measurement and the site were treated as fixed variables.

We also performed ANCOVAs on sap velocity, volumetric sap flow and percentage of the maximum sap flow (% max sap flow) within and across sites, as the evaluation of these parameters sheds insights into the different processes driving water movement in epiphytes. For example, sap velocity will be driven more by variation in microclimate, whereas volumetric sap flow will be affected to a large degree by the size of individuals, and the % max sap flow is also influenced by the capacity for sap flow of different species (Supporting Information Figs S1, S2). The week of measurement and the site were also treated as fixed variables in these analyses. To determine sap flow recovery following the drought, we calculated the average sap flow in each site for an entire week (week 11) following a week with high rainfall (week 10). We then divided that value by the weekly average sap flow during the first week of the study. The first week of the study was considered to be the maximum sap flow as conditions were sunny but had been wet in all sites for a month before the study. The recovery values were then evaluated using an analysis of variance (ANOVA) to determine whether the site rates were significantly different from one another.

Canopy water potential data were analyzed using a two-way ANOVA in which the effects tested were month, site and month \times site, where the months corresponded to the wet season, the middle of the dry season and the end of the dry season. We conducted two of these two-way ANOVAs, one for predawn and another for midday leaf water potential. We also used two-way ANOVAs to determine the effect of site, season and the interaction of these effects on the TLP and the osmotic potential at full saturation. The effect of species identity could not be included in this analysis as only one species was studied in UGA. The data exhibited either a normal or log-normal distribution. These analyses were conducted in JMP v.10 (SAS Institute Inc., Cary, NC, USA).

To compare the microclimatic drivers of sap velocity at different elevations, we performed multiple mixed-effect regressions with backward selection for the three sites using R v.3.1.1. To examine the differences in the microclimatic drivers for *Macleania insignis*, we performed the same backward selection on LCC for *M. insignis* only and compared it with the analysis for UGA, as only *M. insignis* was measured in UGA. Finally, we performed a multiple mixed-effect regression for shrubs and for single-stemmed species within CCT to compare drivers of sap velocity of these two functional groups. In each backward selection procedure, the full model contained the random effects for species and plant nested within species if different species were present. The fixed effects for VPD, wind, gusts, direction, VWC, soil temperature, LWS, temperature and relative humidity were considered for inclusion in the full model. All variables were first evaluated to assess multi-collinearity, as variables with high correlation must be excluded from regression analyses. After examination of individual variables and possible multi-collinearity issues, the analyses in CCT excluded wind, gusts, direction, temperature and relative humidity. In LCC and UGA, only relative humidity was excluded. The fixed effects included main effects, quadratic effects and all two-way interactions. All parameters were estimated via maximum likelihood. These analyses were performed following the methods detailed in Darby *et al.* (2016). The detailed summaries of the final models are shown in the tables in Supporting Information.

Results

Microclimate

Microclimatic parameters varied significantly across the sites. There was a significant effect of site (but not week) on VPD ($F=579.6$, $P<0.0001$); VPD was generally highest in the lowest elevation site and lowest in the highest site (Fig. 2a). There was a significant effect of site and week on LWS (week: $F=1028.7$, $P<0.0001$; site: $F=1113.5$, $P<0.0001$), which appeared to be driven largely by the cloud forest site, which had high LWS at the beginning of the study because of steady mist during this period (Fig. 2b; S. G. Gotsch, pers. obs.). Air temperature exhibited a slight increase from the beginning to the end of the time period at all sites and the highest temperatures were consistently at the lowest site (Fig. 2c; week: $F=260.5$, $P<0.0001$; site: $F=995.1$, $P<0.0001$). The middle and lowest elevation sites were the most closely related in temperature despite the middle and upper sites being closer in elevation (Fig. 2c). Wind speed was variable in the lower two sites, whereas, in the highest site, the wind speed was close to zero throughout the study (Fig. 2d; week: $F=888.6$, $P<0.0001$; site: $F=1323$, $P<0.0001$). The relative humidity was generally highest in the highest site and lowest in the lowest site, and the effect of site (but not week) was significant (Fig. 2e; site: $F=543.3$, $P<0.0001$). The middle elevation site often exhibited intermediate relative humidity, but there were weeks when the relative humidity was nearly identical to the upper or lower sites (Fig. 2e). The percentage of the maximum soil water content (% max soil) exhibited significant variation across the

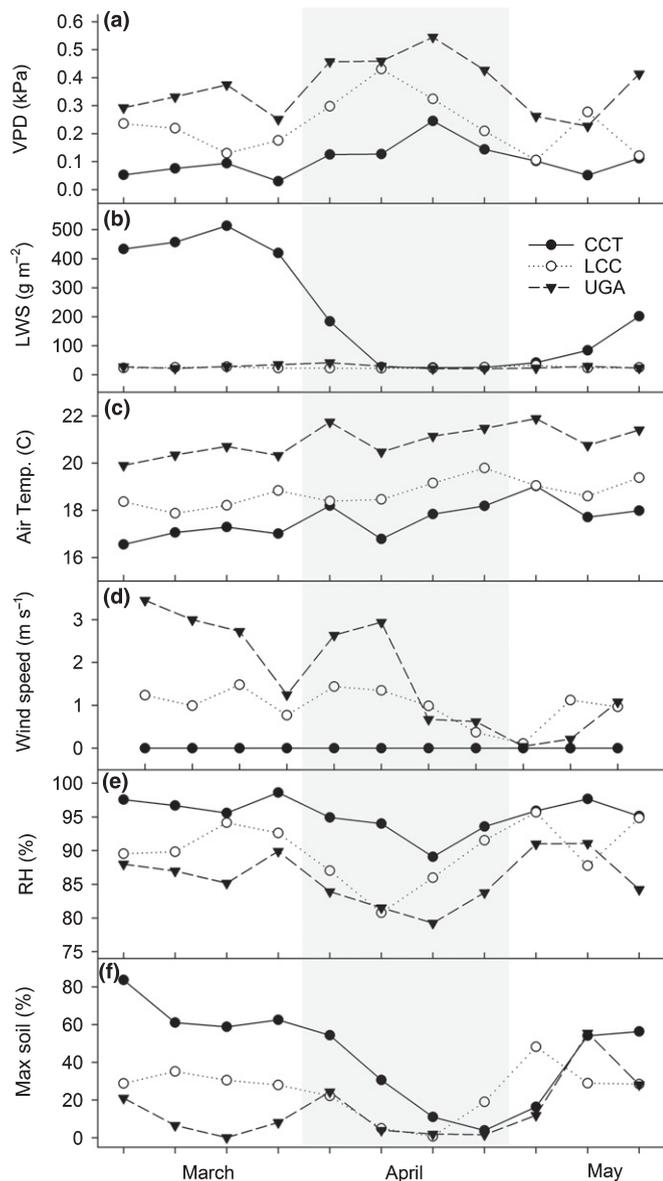


Fig. 2 Weekly mean canopy microclimate during the study period. The cloud forest (CCT) is the uppermost site, the upper premontane site lies just below the cloud base (LCC) and is the middle elevation site, and the lower premontane cloud forest (UGA) is the lowest site. (a) Vapor pressure deficit (VPD), (b) leaf wetness (LWS), (c) air temperature, (d) wind speed, (e) relative humidity (RH) and (f) percentage of the maximum volumetric soil water content in each of the sites. The shaded bar represents four consecutive weeks with little to no rainfall.

sites throughout the study period (Fig. 2f; week: $F=432.6$, $P<0.0001$; site: $F=1434.5$, $P<0.0001$). At the beginning of the study, % max soil was consistently greater in the upper site, but, during the drought, % max soil approached zero in all sites. The uppermost site maintained higher water content throughout the majority of the study than the lower two sites (Fig. 2f).

Volumetric sap flow

Throughout the measurement period, the sap flow averaged across all individuals per site was greatest at the middle elevation

site (Fig. 3; week: $F=5.7$, $P=0.02$; site: $F=5.8$, $P=0.01$). At the beginning of the time series, the volumetric sap flow was similar in the upper and lower sites, although these trajectories differed during the drought and recovery periods (Fig. 3). There was substantial variation in the range of sap flow values within and across species in the upper and mid-elevation sites (see Supporting Information). From March to April, decreases in volumetric sap flow occurred in all sites (Fig. 3). Dry season depression of sap flow rates was greatest in the highest elevation site; sap flow remained close to zero for the last 2 wk of April. By contrast, in the middle and lower elevation sites, sap flow continued at low rates throughout the drought (Fig. 3). When the rainy season began, there was some degree of recovery of sap flow in all sites and the differences across sites were statistically significant (ANOVA: $F=4.64$, $P=0.01$). After a week of substantial precipitation, plants at the lowest site returned to an average sap flow rate of 78% of the first week of the time series, whereas plants in the middle elevation site experienced a 49% recovery and plants from the upper elevation cloud forest site experienced a 44% recovery of sap flow.

Percentage of maximum sap flow

The weekly percentage of the maximum volumetric sap flow (% max sap flow) followed similar trajectories in all sites (Fig. 4). Sap flow was high at the beginning of the period, rates dropped during the drought and then exhibited a partial recovery with the return of the rains. The % max soil often exhibited similar patterns to the % max sap flow, although the patterns of both variables differed significantly across sites (Fig. 4; week: $F=7.4$, $P=0.01$; site: $F=4.18$, $P=0.03$). In the highest elevation site, the percentages of both soil and sap flow overlapped for the majority of the study ($r^2=0.81$, Fig. 4a). At the middle elevation site, there was least agreement between % max soil and % max sap flow ($r^2=0.25$, Fig. 4b). In this site, the % max soil was low at the beginning of the study, and remained low until the rainy season returned. In the lowest elevation site, the correlation was highest between % max sap flow and % max soil, but the values were substantially lower for % max soil water ($r^2=0.95$, Fig. 4c). In the upper two sites, there was evidence of a time lag between these two variables, which was seen during the recovery period when the rains returned (Fig. 4a,b). In the upper elevation site, the % max sap flow increased more than % max soil water in late April, whereas % max soil showed improvement before the sap flow signal in the mid-elevation site. We also found that the difference in the % max sap flow for *Macleania insignis* in these two sites was not significant (Fig. S1). The % max sap flow for all study species in the height of the drought (week 8) and following a week of rain (week 10) can be seen in Table 2.

Sap velocity: site-specific patterns

The average weekly sap velocity was significantly different across sites (Fig. 5a; week; $F=16.8$, $P=0.0003$, site: $F=3.9$, $P=0.03$). Although the overall volumetric flow rates were highest at the beginning of the study in the mid-elevation site (Fig. 3), the

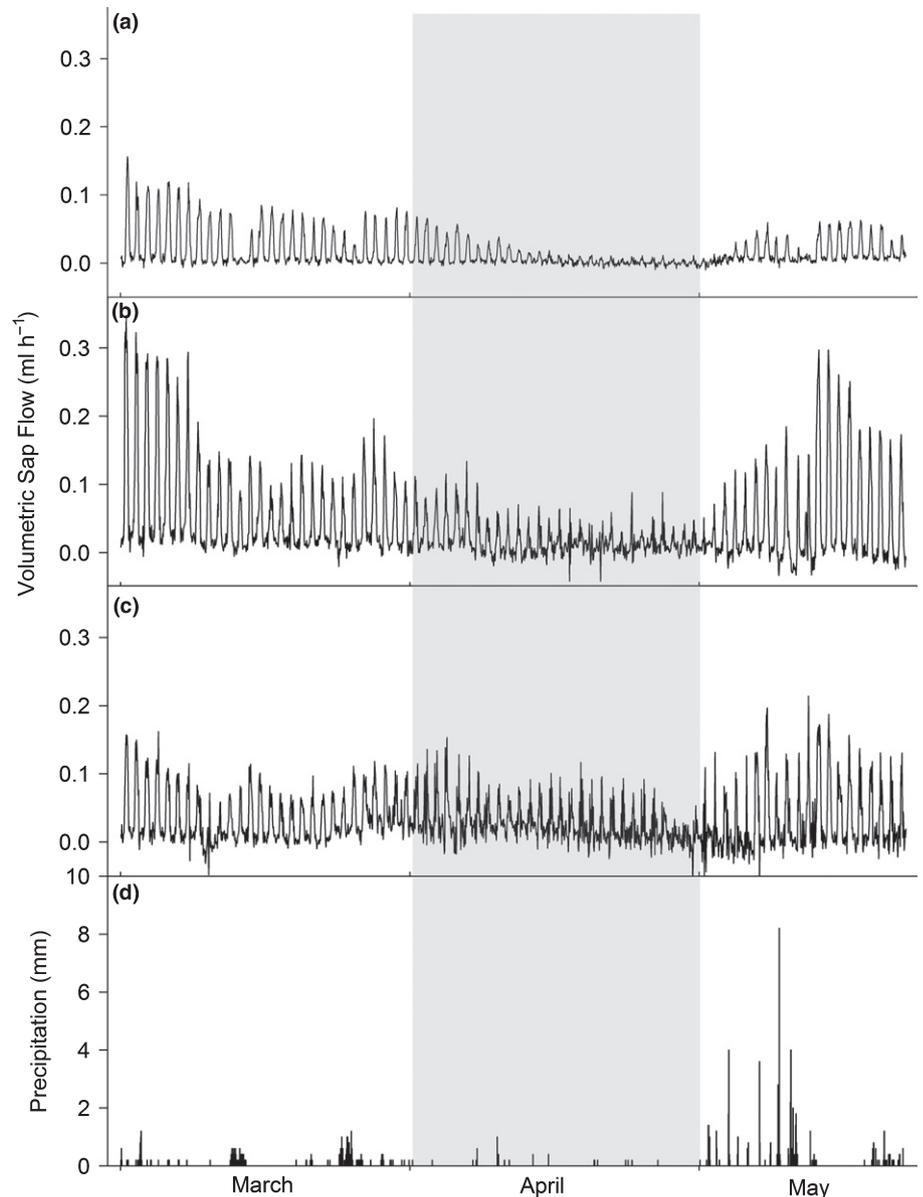


Fig. 3 Average hourly volumetric sap flow throughout the dry season (March and April 2016) and during a month of recovery (May). (a) The average sap flow of all canopy plants in the cloud forest site (CCT), (b) the average sap flow in a middle elevation site (just below the cloud base, LCC) and (c) plants at the lowest elevation site (UGA). Daily rainfall totals are depicted for CCT in (d). Although rainfall events are probably larger at CCT than at the other sites, the patterns of rainfall will be similar. The shaded bar represents four consecutive weeks with little to no rainfall.

velocity was 33% higher in the highest elevation site in week 1 (Fig. 5a). There was a delay in the drought-related reduction in sap flow (Fig. 5a) for the lowest and highest sites.

We also analyzed patterns of sap velocity within *M. insignis*, a wide-ranging species that was common in both the mid-elevation and lower elevation sites (Table 2). In the low-elevation site, *M. insignis* expressed values of sap velocity more than two-fold greater than those in the mid-elevation site, and these values remained higher through the majority of the study (Fig. 5b). ANCOVA results indicate a significant effect of site and week in the analysis (week: $F=4.96$, $P=0.03$, site: $F=19.41$, $P=0.0003$).

In the high-elevation site, we documented the sap velocity of different growth forms. We found clear differences in sap velocity between shrub and non-shrub (woody and herbaceous) epiphytes ($F=6.79$, $P=0.01$). Single-stemmed woody and herbaceous species were grouped together as their patterns of sap flow were

not statistically significantly different from one another (data not shown). *Schefflera rodrigueziana*, a hemi-epiphyte, exhibited a statistically indistinguishable pattern of sap flow from the shrub, *Cavendishia capitulata*. Individuals of this hemi-epiphyte were less than a meter tall, and it was likely that these individuals had not yet established contact with terrestrial soil. Given the similarity of sap flow patterns and size of the individuals, these two shrub-like growth forms were grouped together. We found that these shrubs exhibited a more profligate water use strategy than single-stemmed plants. At the beginning of the study, rates of sap velocity were more than two-fold greater than the rates of the single-stemmed species (Fig. 5c). These rates remained higher than those of the single-stemmed species for all weeks in the study, with the exception of the two driest weeks (Fig. 5c). In comparison, the single-stemmed species maintained low and consistent sap velocity throughout the study (Fig. 5c).

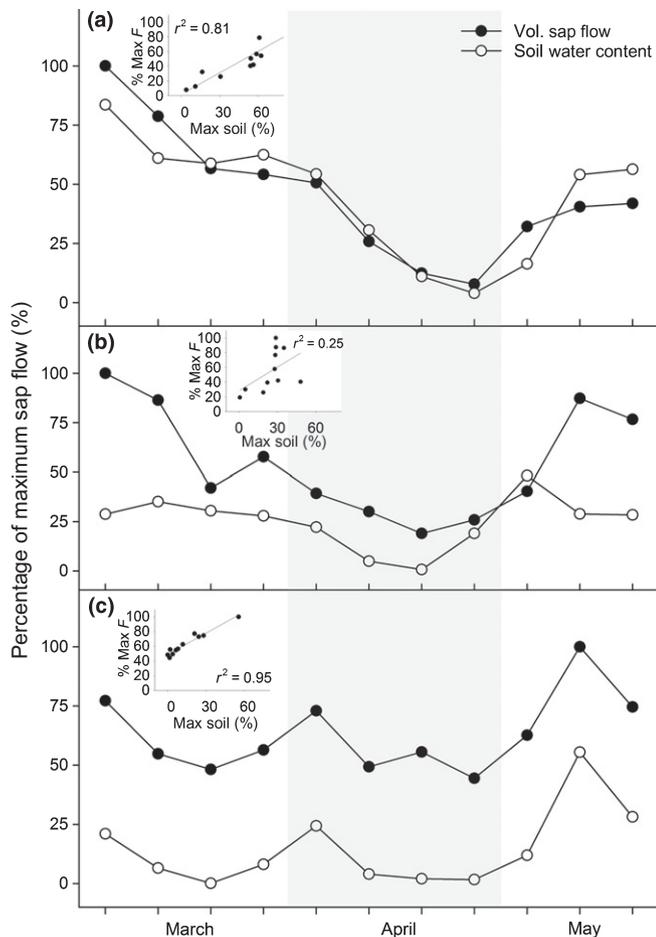


Fig. 4 Percentage of maximum volumetric weekly sap flow for all study species and soil water content in (a) the cloud forest site (CCT, upper site), (b) the mid-elevation site just below the cloud base (LCC, middle site) and (c) the lower premontane rain forest site (UGA, lowest site). The shaded bar represents four consecutive weeks with little to no rainfall. The inset panels are linear regressions of the relationship between the percentage maximum soil water content and the percentage maximum of sap flow in each of the sites. The shaded bar represents four consecutive weeks with little to no rainfall.

Microclimatic drivers of sap flow

The two most important microclimatic drivers of sap velocity for each site are shown in Table 3 (the detailed analysis is shown in Table S1). Tables S2–S7 show the output for the models obtained by backward selection for each site. These models were difficult to interpret in terms of the relative influence of each variable, because the relative importance of a particular environmental driver (i.e. VPD) is the combination of the main effect plus all other effects that included that main effect (i.e. interactions, quadratic terms, etc.). In order to compare the main effects directly, we conducted an additional series of calculations (Table S1). We calculated the instantaneous rate of change in \log_{10} sap velocity (the partial derivative) with respect to each microclimatic driver. Then, we evaluated each derivative at the average observed value for each microclimatic driver. This quantity gave us the instantaneous rate of change of \log_{10} sap velocity

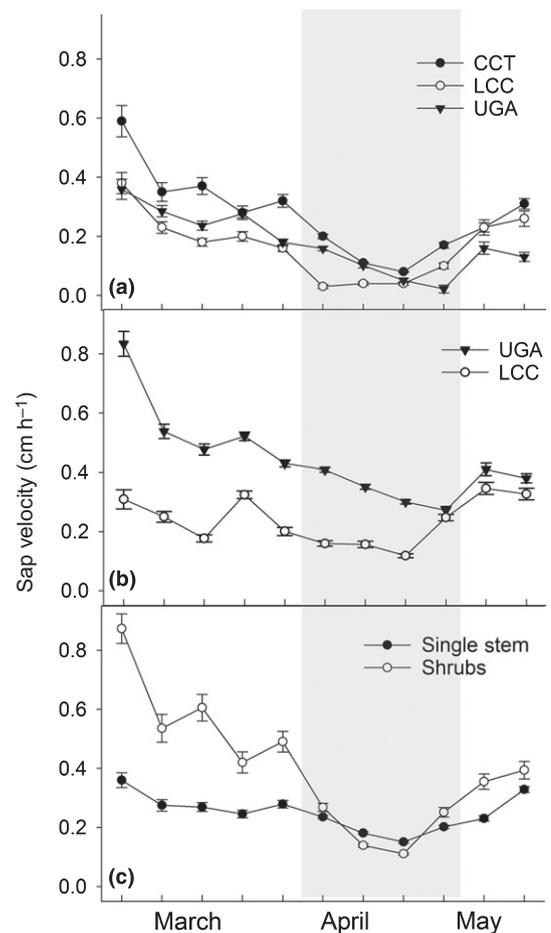


Fig. 5 Weekly sap velocity for (a) all study species across all sites, (b) *Macleania insignis*, a common epiphytic shrub, in a middle elevation site (LCC) and (c) the lowest elevation site (UGA) and between different functional groups in the cloud forest. The shaded bar represents four consecutive weeks with little to no rainfall. Error bars represent \pm SEM.

as a function of each microclimatic driver, when all microclimatic drivers were at their observed average values. Table 3 (and Table S1) indicates that, at the highest elevation site (CCT), VPD and LWS exhibit the largest effects on sap velocity. In the mid-elevation site (LCC), soil temperature and VWC show the largest impacts on sap velocity (see Tables 3, S1, S3). In the lowest elevation site (UGA), average wind speed and VWC show the largest influence on sap velocity (see Tables 3, S1, S4). In all sites, there was a positive effect between the drivers and sap velocity.

The relative role of microclimatic drivers on sap velocity in *M. insignis* at mid- and low-elevation sites differed. In the mid-elevation site, air temperature and VWC had the greatest effect on sap velocity (see Tables 3, S1, S5). In the lowest elevation site, sap flow in *M. insignis* was primarily influenced by the average wind speed and VWC. All effects for these analyses had a positive influence on sap velocity (see Tables 3, S1, S4).

In the high-elevation cloud forest site, the sap velocity of both shrubs and single-stemmed plants (see definition of groups above) were influenced primarily by VPD and VWC. All parameters had a positive effect on sap velocity (see Tables 3, S1, S4, S6, S7).

Table 3 Primary and secondary drivers of sap velocity as determined by multiple mixed-effect regression analyses with backward selection and additional analyses to account for the influence of main effects in interactions and quadratic terms (Supporting Information Table S1)

Focal dataset for multiple mixed-effect regression analyses	Primary driver	Secondary driver
CCT	VPD (+)	LWS (+)
LCC	Soil temp (+)	VWC (+)
UGA (<i>Macleania</i>)	Wind (+)	VWC (+)
CCT–shrubs	VPD (+)	VWC (+)
CCT–single stemmed	VPD (+)	VWC (+)
LCC– <i>Macleania</i>	Air temp. (+)	VWC (+)

The focal datasets were as follows: cloud forest (CCT), upper premontane rainforest (LCC), lower premontane rainforest (UGA), cloud forest–shrubs only (CCT–shrubs), cloud forest–single stemmed species only (CCT–single stemmed) and upper premontane rainforest–*Macleania insignis* only (LCC–*Macleania*). As only *Macleania insignis* individuals were measured for sap flow at UGA, this analysis is used in the site-level comparisons as well as to examine differences in *Macleania* at different sites. The ‘primary drivers’ were the microclimatic variables that were associated with the highest instantaneous rate of change in \log_{10} transformed sap flow velocity values when all drivers were held at their average observed values, whereas the ‘secondary drivers’ had the second largest rates of change. The output tables containing the scaled values for all significant effects in each of the analyses can be found in Supporting Information. The symbol listed after the driver indicates whether the associated rate of change of sap velocity is positive or negative. VPD, vapor pressure deficit; VWC, volumetric soil water content; LWS, leaf wetness; Air temp., air temperature; Wind, average wind speed; Soil temp., soil temperature.

Canopy water potential

Predawn and midday leaf water potentials were generally high throughout the study period (Fig. 6a,b). Results of the two-way ANOVAs (one for predawn and one for midday) indicated that there were no significant effects of the month of measurement in either analysis. By contrast, for midday values, there was a significant effect of the site and the interaction between the month of the measurement and the site (ANOVA for site: $F=6.6$, $P=0.0038$; site \times month: $F=4.5$, $P=0.0047$), whereas, for predawn values, there was only a significant effect of the interaction term (ANOVA: $F=9.5$, $P<0.0001$). We also calculated the distance from TLP, which was the difference between TLP and the average minimum leaf water potential for all species measured in each time period. The distance from TLP decreased in all sites between January (a relatively wet month) and March (middle of the dry season) (Fig. 6). In CCT, the distance from TLP ranged from -0.2 to -1.15 MPa, whereas the distance in LCC and UGA was substantially larger (-1.1 to -3.1 MPa, Fig. 6c).

Pressure–volume curves

There was significant variation in TLP (Ψ_{TLP}) and the osmotic potential at full saturation (Ψ_o) across sites (Ψ_{TLP} : $F=55.01$,

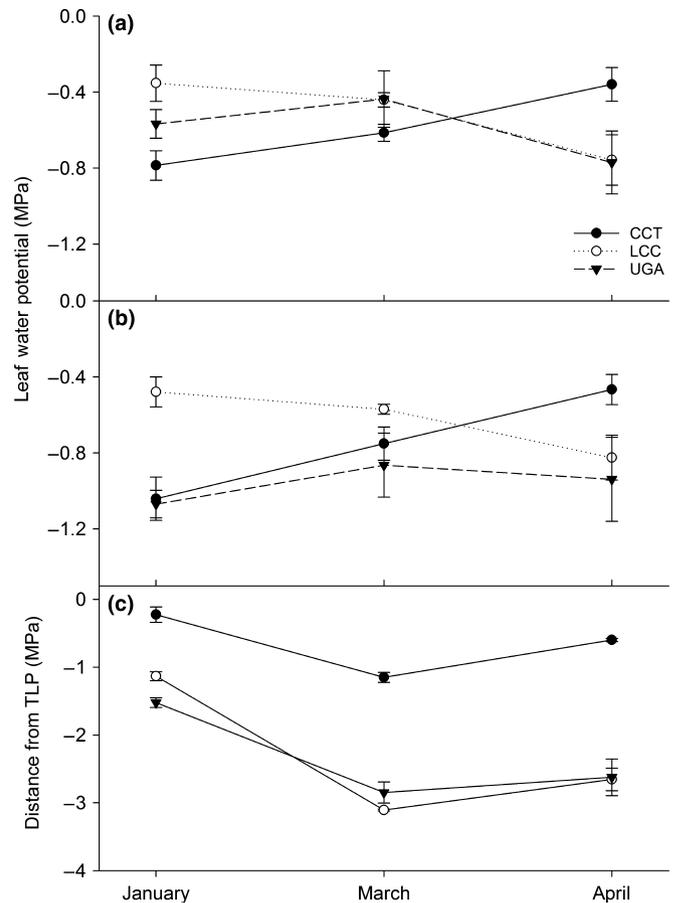


Fig. 6 (a) Predawn and (b) midday leaf water potential for the epiphyte species included in this study. The first measurement in January was before the dry season started, March was in the middle of the dry season and April was at the end of the dry season. (c) The difference between the minimum leaf water potential and the turgor loss point (TLP) for each of these times across the sites. Error bars represent \pm SEM.

$P<0.0001$; Ψ_o : $F=45.6$, $P<0.0001$). Average values decreased from -1.67 MPa (Ψ_{TLP}) and -1.59 MPa (Ψ_o) in the highest elevation site to -2.71 (Ψ_{TLP}) and -2.57 (Ψ_o) in the lowest elevation site. In addition, there were significant decreases in these parameters from the wet to the dry season (Ψ_{TLP} : $F=167.8$, $P<0.0001$; Ψ_o : $F=205.1$, $P<0.0001$; Fig. 7). The interaction term between the season and the site was also significant for both parameters (Ψ_{TLP} : $F=23.1$, $P<0.0001$; Ψ_o : $F=205.1$, $P<0.0001$). The greatest decrease in Ψ_{TLP} and Ψ_o occurred in the lowest elevation site followed by the middle elevation site (Fig. 7). Although the highest elevation site also experienced a significant shift in these parameters, the variation within this site was greater and the average decrease was the lowest of the three sites (Fig. 7). We did not find significant differences in Ψ_{TLP} and Ψ_o of *Macleania* from the lower to mid-elevation sites (ANOVA effect of site, $P=0.35$ for Ψ_{TLP} and $P=0.55$ for Ψ_o). However, there was a significant downward shift in these parameters from the wet to dry season in both sites (ANOVA effect of season, $F=216.8$ and $P<0.0001$ for Ψ_{TLP} and $F=235.4$ and $P<0.0001$ for Ψ_o).

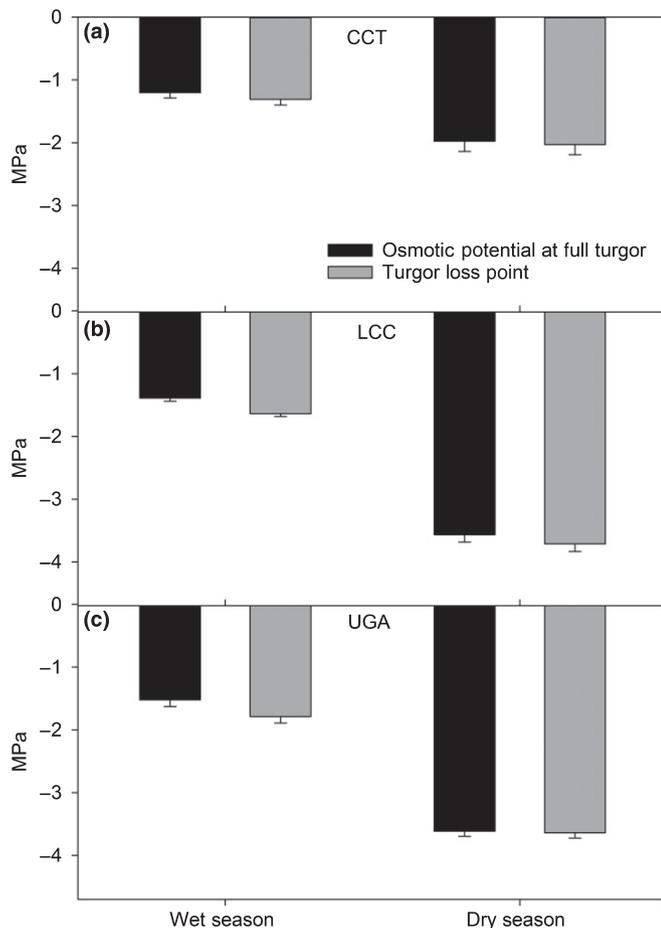


Fig. 7 The turgor loss point (gray bars) and the osmotic potential at full turgor (black bars) across sites and seasons. The upper panel represents plants from CCT (highest, cloud forest site), the middle panel represents plants from LCC (middle elevation site) and the lowest panel represents plants from UGA (lowest site). The error bars represent \pm SEM.

Discussion

In this study, we took advantage of an unprecedented dry season influenced by an ENSO event to document variation in the resilience of epiphyte communities in three ecotones in a tropical montane region. This is the first study that we are aware of to document variation in the water relations of epiphytes that live in different microclimates in a TCMF region that also documents the responses of these communities to extreme drought.

Microclimatic variation across sites

We found significant variation in all microclimatic variables across the sites. This variation was undoubtedly caused by the effect of elevation on cloud formation and other associated changes in microclimate. The cloud forest site (highest elevation in this study) was generally cooler, more humid and had a higher soil water content and LWS than the lower sites (Fig. 2). The air temperature and % max soil in the middle and lowest elevation sites were closer to one another than between the upper two sites, even though the middle and upper sites were in closer proximity.

The middle elevation site is nearest to the current cloud base; there may be a greater difference in microclimate on either side of this boundary than variation caused by elevation alone (Lawton *et al.*, 2001). Furthermore, there was greater variability in relative humidity in the middle elevation site than in the other two sites (Fig. 2d). Such variability may be caused by shifts in microclimate when this site is within vs below the cloud layer. If low-lying clouds are present, the upper site will consistently be inundated, whereas the low site will be dry. The middle elevation site therefore experiences a microclimate that is overall more similar to that of the lower elevation site, but occasionally receives cloud moisture that is routine to the upper site.

Drought effects on water relations across sites

Sap flow In all three sites, reduced rates of sap velocity and sap flow occurred during the drought, although the extent of the reduction, as well as the recovery, varied significantly across sites (Figs 3, 4). Variation across sites is probably the result of variation in species-specific differences in the degree of osmotic adjustment of the plants (see section below, ‘Tissue water relations’) across the sites, as well as differences in morphology. Volumetric sap flow was lowest during the drought in the upper elevation site, whereas the middle and lower elevation sites continued to move sap at low levels throughout the drought (Fig. 3). When the rainy season returned, plants in all sites experienced a recovery of sap flow and sap velocity, although the recovery was greater in the lower elevation sites. Our sap flow measurements were performed on stems; we acknowledge that hydraulic segmentation at the stem–leaf boundary may confer unique stem–leaf water use behaviors in the same plants that could additionally influence the relationship between stem hydraulic properties and environmental relations beyond that which was imposed by the drought.

In the upper and lower sites, we found significant relationships between soil water and sap flow, whereas there was greater discord between these variables in the middle elevation site (Fig. 4). In this mid-elevation site, the canopy community is dominated by large shrubs, which contain thick lignotubers containing stored water which probably serve to buffer these plants from drought (Evans & Vander Kloet, 2010). Although the lower site contains shrubs as well, they tend to be smaller in stature and their lignotubers are also smaller (S. G. Gotsch, pers. obs.).

Tissue water relations Osmotic adjustment occurred for plants in all sites during the dry season, and many of these shifts were more substantial than those reported previously for epiphytes (Griffiths *et al.*, 1989; Smith, 1989; Martin, 1994; Stiles & Martin, 1996; Nowak & Martin, 1997). The greatest shifts in Ψ_o and Ψ_{TLP} were found in the plants inhabiting the lowest sites, where both sap velocity and sap flow continued to be positive throughout most of the drought. The dry season osmotic potentials for the epiphytic shrubs in the lowest site were among the lowest ever measured in epiphytes (Zotz & Hietz, 2001; Martin *et al.*, 2004). The lower osmotic potentials detected in this study may be a result of the different ecological niches occupied by our study species. Most of the research to date on epiphytes has

focused on bromeliads and smaller functional groups, such as ferns and orchids, which have minimal, if any, rooting potential in the mat. By contrast, many of the individuals in this study were larger woody species with greater rooting potential and perhaps greater ability for osmotic adjustment. The species studied here are all common taxa in the study sites, and the epiphytic shrubs, in particular, comprise a substantial proportion of the total biomass of the lower two sites and are also common in the cloud forest site. These epiphytic shrubs and other large woody species, although not as common in other rainforest ecosystems, are common and abundant in TMCFs, and play important roles in this ecosystem (A. Amici, unpublished data; Gotsch *et al.*, 2016). Ψ_{TLP} has been found to be an indicator of drought tolerance across a wide range of plants and ecosystems (Bartlett *et al.*, 2012). Such seasonal shifts in Ψ_o and Ψ_{TLP} conferred a greater distance from Ψ_{TLP} in these sites and probably facilitated the continued exchange of gases despite water limitation (Mulkey *et al.*, 1991; Ishida *et al.*, 2010; Figs 6, 7). Even though plants in the lowest site experienced the most severe drought conditions, substantial osmotic adjustment allowed water uptake from dry soils to continue (Fig. 4). Osmotic regulation was less substantial in the mid-elevation site, which may have limited the ability of plants to maintain levels of uptake during the drought.

Osmotic regulation may be an important adaptation for epiphytes living in drier sites to enable continued water uptake from canopy soils. The shift in these parameters enabled plants to function further from Ψ_{TLP} in the lower sites, providing protection from hydraulic failure (Brodribb & Holbrook, 2003; Blackman *et al.*, 2010; Fig. 7). In the cloud forest site, plants function much closer to Ψ_{TLP} in both the wet and dry seasons, despite the prolonged duration of the drought (Fig. 6c). This may indicate limited plasticity in these traits across sites. Species turnover across microclimatic gradients is high in epiphyte communities; rarely is a species found over a wide range of elevations (Cardelús *et al.*, 2006; Ding *et al.*, 2016; E. Hollenbeck, unpublished data). Such a high degree of specialization may make communities in cooler and moister regions particularly vulnerable to projected changes in climate.

Combining traits Although epiphyte communities are characterized by a high degree of species turnover across elevation gradients, there are some species that occupy wider ranges. *Macleania insignis*, a common epiphytic shrub, for example, is one of a few species that resides in both the lower and mid-elevation sites. *Macleania insignis* had a higher sap velocity in the lowest site, where the evaporative demand was greatest; however, these effects diminished when plant size was taken into account. Furthermore, we found significant seasonal shifts in Ψ_{TLP} or Ψ_o in both sites. These results indicate that a shift in allocation patterns (e.g. altered leaf area to sapwood area ratio) in *M. insignis* may allow for a positive water balance to be maintained in a wide variety of microclimatic conditions, and thereby confer colonization by this species of a wider range of elevations than found in other species.

Lastly, we examined differences in sap velocity within the cloud forest site, where we had the best representation of different

functional groups. We found statistically significantly different sap velocity rates between epiphytic shrubs and single-stemmed epiphytes throughout the study (Fig. 5c). In general, shrubs exhibited greater water use throughout most of the study and, on average, exhibited lower rates of recovery when compared with single-stemmed species. However, variation in rates between individuals was high and the sample size was low, resulting in the differences in recovery rates not being statistically significant. In another study, we found that epiphytic shrubs exhibited higher rates of photosynthesis than herbaceous species (S. G. Gotsch, unpublished data). The extended drought may have depleted these stores as many of these individuals did not return to pre-drought rates of sap flow after a week of substantial rainfall. We suggest more focused work on this important functional group in the future to better understand the effects of projected changes in climate on the survival and performance of epiphytic shrubs.

Microclimatic drivers of sap flow

Some epiphytes are referred to as 'atmospheric plants' because they are physically isolated from terrestrial soils, and ambient atmospheric conditions have been shown to drive sap flow (Eller *et al.*, 2015; Berry *et al.*, 2016; Darby *et al.*, 2016). We also found that atmospheric conditions, specifically VPD, were important drivers of the physiological responses observed in this study. However, to our knowledge, we are the first to demonstrate the importance of canopy soil in also driving the dynamics of sap velocity in this important community. In the cloud forest, extensive canopy mats can form of decomposing organic material and deposited dust, which are held together by a tight lattice of canopy roots (Nadkarni, 1984). Such canopy mats can be 50 cm in depth, extend the length of the branch and provide a substantial water supply (Nadkarni, 1984). This water supply was important (together with VPD) in influencing (driving) patterns of dry season sap velocity in the cloud forest when single-stemmed and shrubby species were evaluated separately (Table 3). VWC is also an important driver in the mid-elevation site and even in the lowest elevation site where canopy mats are smaller and discontinuous. The epiphytes in this site are shrubs (*M. insignis*) that are overall smaller and have smaller storage organs (lignotubers) than their counterparts in the mid-elevation site (S. G. Gotsch, pers. obs.). As a result, the small supply of water in the mats becomes an important predictor of sap velocity.

In the lowest elevation site, average wind speed was the primary driver of sap velocity. The average within-canopy wind speed in this site was often over 2 m s^{-1} and gusts were often twice that (S. G. Gotsch, unpublished data); wind speed is known to have an important influence on rates of evapotranspiration (ET; Campbell & Normal, 2000) and probably influenced ET at this site. In the mid-elevation site, soil temperature and VWC are the primary drivers of sap velocity. This site has the highest overall epiphytic biomass of the three sites and shrubs comprise a substantial component of the epiphytic biomass (A. Amici, unpublished data). These large individuals may be particularly dependent on canopy soil conditions (Fig. 4b). We acknowledge that microclimatic niches may vary greatly within

the canopies in which this study took place. We limited our measurements to the inner crown to try to minimize differences in microclimate experienced by our study individuals, but this could also play a role in the variation in water use detected within and across sites.

Implications in an increasingly variable climate

Changes in rainfall patterns are likely to affect the ability of epiphytes to refill water stores and withstand drought. In the Monteverde region, precipitation has been measured continuously over the last 45 years, allowing for the analysis of relatively long-term patterns of rainfall. On average, annual rainfall has been increasing, but the variability from year to year has been increasing as well (Pounds *et al.*, 1999; A. Pounds, unpublished data). Simultaneously, together with an increase in average rainfall, the number of dry periods has been increasing and is projected to increase in the future (Pounds *et al.*, 1999; Still *et al.*, 1999; Lawton *et al.*, 2001; A. Pounds, unpublished data). Rain is likely to fall in large storms more often now than in the recent past; these shifts will probably have a substantial effect on many epiphytes. As epiphyte mats occupy a small area relative to terrestrial soil water stores, these mats will probably reach saturation quickly during a storm, but stored water will also be lost quickly during dry periods. Consistent inputs of water from light rain, mist and cloud immersion would maintain high soil water content of canopy epiphyte mats. Although there are periods with such favorable conditions, they are projected to decrease as rainfall patterns change and cloud base heights increase (Pounds *et al.*, 1999; Still *et al.*, 1999; Ray *et al.*, 2006; A. Pounds, pers. comm.). Epiphytic shrubs in the cloud forest utilize foliar water uptake to offset water loss (Gotsch *et al.*, 2015). This functional group may be particularly vulnerable to changes in climate, given the projected increases in drought, temperature and cloud base heights (Still *et al.*, 1999; Pounds *et al.*, 2006; Ray *et al.*, 2006; Karmalkar *et al.*, 2008; A. Pounds, unpublished data). Herbaceous epiphytes, such as those studied here, as well as epiphytes with more specialized water storage mechanisms (i.e. a greater degree of leaf/stem succulence, CAM photosynthesis, water tanks, etc.) are likely to be less affected than epiphytic shrubs by projected changes in climate, because of a greater ability to regulate gas exchange and store water. A shift in the cloud forest epiphyte community as a result of the loss of shrubs is likely to have a significant effect on many processes in the TMCF because of the prevalence of this functional group in the canopy and the many other species that rely on epiphytic shrubs for food resources and habitat (Gotsch *et al.*, 2016).

Conclusions

Vascular epiphytes in TMCFs provide important ecosystem services, including substantial leaf area, and food and habitat resources for many species of invertebrates, birds and mammals (Gotsch *et al.*, 2016). Therefore, marked changes and, particularly, the loss of this component of the plant community are likely to have important impacts not only on the overall ecology,

but also on water and nutrient cycling in these already vulnerable ecosystems. Epiphytes in our cloud forest site may be vulnerable to increases in temperature and drought. These plants experienced a slower recovery of sap flow following a severe drought than plants from lower elevations. Within the cloud forest site, shrubs exhibited a more profligate water use strategy than smaller single-stemmed woody species and herbaceous plants, even though all plants were exposed to nearly a month without precipitation. This functional group may be more sensitive to hydraulic failure if exposed to increasingly severe dry seasons. Although epiphytes have been assumed to be vulnerable to projected changes in climate, this is the first study that we are aware of to provide evidence for a mechanism that can explain the sensitivity of this community to drought. Future research is needed to determine feedbacks between changes in epiphyte community composition and the physiology of their host trees, as well as stand-level water and nutrient cycling.

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Author contributions

S.G.G. and T.E.D. developed the ideas that resulted in this work. S.G.G. participated in and oversaw the collection of all data, conducted data analyses on sap flow, canopy water potential and pressure–volume curves, and wrote the manuscript. D.D. conducted the multiple mixed-effect regression analyses and contributed to text relating to these analyses. All authors edited the manuscript and gave feedback on the presentation of data.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

Fig. S1 The percentage of the maximum rate of volumetric sap flow across all sites, of *Macleania insignis*, a common epiphytic shrub, in a middle elevation site (LCC) and the lowest elevation site (UGA) and between different functional groups in the cloud forest.

Fig. S2 Box plots of volumetric sap flow throughout the study period.

Table S1 A summary of the estimated rates of change in sap velocity in all sites with respect to each of the microclimatic variables

Table S2 A summary of the effects of microclimate on sap velocity in CCT using mixed-model regressions and backward selection

Table S3 A summary of the effects of microclimate on sap velocity in LCC using mixed-model regressions and backward selection

Table S4 A summary of the effects of microclimate on sap velocity in UGA (*Macleania insignis* only) using mixed-model regressions and backward selection

Table S5 A summary of the effects of microclimate on sap velocity for *Macleania insignis*, an epiphytic shrub, in LCC using mixed-model regressions and backward selection

Table S6 A summary of the effects of microclimate on sap velocity for shrub species in CCT using mixed-model regressions and backward selection

Table S7 A summary of the effects of microclimate on sap velocity for single-stemmed species in CCT using mixed-model regressions and backward selection

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