Foggy days and dry nights determine crown-level water balance in a seasonal tropical montane cloud forest

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ABSTRACT

The ecophysiology of tropical montane cloud forest (TMCF) trees is influenced by crown-level microclimate factors including regular mist/fog water inputs, and large variations in evaporative demand, which in turn can significantly impact water balance. We investigated the effect of such microclimatic factors on canopy ecophysiology and branch-level water balance in the dry season of a seasonal TMCF in Veracruz, Mexico, by quantifying both water inputs (via foliar uptake, FU) and outputs (day- and night-time transpiration, NT). Measurements of sap flow, stomatal conductance, leaf water potential and pressure–volume relations were obtained in *Quercus lanceifolia*, a canopy-dominant tree species. Our results indicate that FU occurred 34% of the time and led to the recovery of 9% (24 ± 9.1 L) of all the dry-season water transpired from individual branches. Capacity for FU was independently verified for seven additional common tree species. NT accounted for approximately 17% (46 L) of dry-season water loss. There was a strong correlation between FU and the duration of leaf wetness events (fog and/or rain), as well as between NT and the night-time vapour pressure deficit. Our results show the clear importance of fog and NT for the canopy water relations of *Q. lanceifolia*.

Key-words: *Quercus lanceifolia*; canopy ecophysiology; canopy microclimate; environmental drivers of transpiration; evaporative demand; fog; heat pulse technique; Mexico; sap flow; seasonality.

INTRODUCTION

The presence of fog, mist and clouds plays an important role in the water balance and ecosystem services of fog-affected ecosystems (Dawson 1998; Bruijnzeel, Mulligan & Scatena 2011; Giambelluca *et al.* 2011). While fog is an ubiquitous feature of tropical montane cloud forests (TMCFs), many TMCFs experience marked seasonality in water availability and fog frequency (Ritter, Regalado & Aschan 2009; Holwerda *et al.* 2010). Cloud water interception (CWI), defined as additional water inputs from impaction of fog droplets onto canopy surfaces and subsequent drip from the

canopy, has been studied in TMCFs worldwide (Bruijnzeel 2005; Holwerda *et al.* 2006; Delay & Giambelluca 2010). The proportional contribution of CWI to total water input is often very high in foggy areas with low rainfall, while in wetter TMCFs, fog inputs vary greatly, but generally contribute less to the total water balance (reviewed in Bruijnzeel *et al.* 2011). For example, in dry ecosystems such as fog-affected forests in Dhofar and Chile, CWI can account for as much as twice the amount of the total precipitation and thus maintain a vegetation type that would not be possible in the absence of fog (de-Val *et al.* 2006; Hildebrandt *et al.* 2007). In the coastal redwood forests of California, CWI accounts for 34% of the annual water input and plays a significant role in the ability of the large *Sequoia sempervirens* trees to maintain positive water status in the dry summer months (Dawson 1998; Burgess & Dawson 2004). In contrast, in the seasonal TMCF at our current study location in Veracruz, Mexico, CWI contributed only 6–8% of the total dry-season rainfall (Holwerda *et al.* 2010; Muñoz-Villers *et al.* 2012).

Less work has examined the indirect effects of fog, mist or clouds on plant water relations, which in some environments may have a similar or even more important influence on tree water balance than CWI. For example, in a seasonal cloud forest in the Canary Islands, Ritter, Regalado & Aschan (2009) found that transpiration rates during fog periods were 30 times lower than during fog-free conditions. In the coastal redwood forests of Northern California, fog not only suppressed transpiration rates, but was also directly absorbed into the leaves (i.e. foliar fog uptake) which caused an increase in leaf water potential in the xylem and temporarily decoupled leaf-level water relations from soil water availability (Burgess & Dawson 2004; Limm *et al.* 2009; Simonin, Santiago & Dawson 2009, see also Breshears *et al.* 2008; Goldsmith, Matzke & Dawson 2013; Oliveira, Dawson & Burgess 2005 for additional examples).

In seasonally dry TMCFs, plants may also experience periods of high evaporative demand. High evaporative demand, especially at night, can cause large amounts of evapotranspiration, which may have a significant impact on leaf water potential and whole plant water use (Dawson *et al.* 2007 and references therein). In one example, night-time transpiration (NT) in one TMCF species contributed about 50% of the total daily water loss (Feild & Holbrook 2000).

While this is not likely to be representative of all TMCFs, NT may be a common occurrence in TMCFs and therefore play an important role in local and regional water balance.

While we may expect high NT in TMCFs when soil moisture availability is usually high, the ability of a species to regulate its stomata may exert a strong influence on NT and result in lower rates than predicted based solely on evaporative demand and soil water availability. TMCF research in Ecuador found that under high vapour pressure deficits (VPDs), stomatal regulation was strong and prevented severe water deficits (Motzer *et al.* 2005). In contrast, TMCF research in Venezuela found that stomatal regulation varied widely across species and ranged from strong to non-existent (Rada, Garcia-Nunez & Ataroff 2009). A broad range in the capacity of different species to regulate their stomata in response to microclimate fluctuations has also been documented for other tropical ecosystems (Sobrado 2003, 2010; Goldstein *et al.* 2008), suggesting a high degree of interspecific and geographic variability that remains poorly understood.

This study examined the relative importance of foliar uptake (FU) and NT in the seasonally dry TMCF of Veracruz, Mexico. While dry-season conditions vary considerably, both fog and high VPD conditions can occur within a single day (Holwerda *et al.* 2010; Muñoz-Villers *et al.* 2012). Therefore, dry-season NT may be significant due to the combination of high levels of soil water availability and high evaporative demand in this region (cf. Muñoz-Villers *et al.* 2012). Although the importance of FU and NT for tree ecophysiology and canopy water fluxes has been independently documented in different species and environments, the combined effects of these processes on tree crown water balance in an ecosystem that experiences both regular fog and high evaporative demand are unknown and may have significant implications for ecosystem functioning. Moreover, because a number of studies suggest that TMCF climate will become warmer and drier with increased variability in the future (Pounds, Fogden & Campbell 1999; Still, Foster & Schneider 1999; Lawton *et al.* 2001; Pounds *et al.* 2006; Karmalkar, Bradley & Diaz 2008), additional information on canopy water balance is critical for understanding how TMCF structure and function may be expected to change. Herein, we were specifically interested in understanding the dry-season crown water relations of the dominant tree species, *Quercus lanceifolia*, to address the following questions: (1) Does this species currently experience dry-season water deficits? (2) Do FU and NT occur in this seasonal TMCF, and if so, at what frequency and spatial variability within the crown? (3) What are the meteorological conditions that govern FU and NT? (4) What is the relative contribution of FU and NT to the overall dry-season, branch-level water balance?

MATERIALS AND METHODS

Study area

This study took place in Central Veracruz, Mexico, on the eastern slopes of the Cofre de Perote volcano in a mature TMCF forest site in 'La Cortadura', a 107 ha reserve (2100 m

a.s.l., 19.4978°N and 97.0419°W). The vegetation is dominated by two oak species, *Q. lanceifolia* and *Quercus ocoteifolia*, as well as *Clethra macrophylla*, *Parathesis melanosticta* and *Alchornea latifolia* (Garcia Franco *et al.* 2008). The soils are Umbric Andosols and have deep profiles (2–3 m), a high organic content and a silt-loamy texture (Campos-Cascaredo 2010). According to the Köppen classification modified by Garcia (1988), the climate between 2000 and 3000 m elevation in this region of Mexico is 'temperate humid' with abundant rainfall during the summer, and average monthly temperatures and annual rainfall between 12 and 18 °C and 2000 and 3000 mm, respectively. At La Cortadura, total average annual rainfall is approximately 3200 mm, of which 80% typically falls during the wet season (Holwerda *et al.* 2010; Muñoz-Villers *et al.* 2012). Fog is a common phenomenon, especially in the dry season (November–April), when it occurs about 20% of the time (Holwerda *et al.* 2010). The dry-season fog events in this region are usually associated with cold fronts that cross the Gulf of Mexico, forming stratus clouds that are then advected inland, delivering rain, drizzle and fog to highland regions (Holwerda *et al.* 2010). Monthly mean temperatures at the site range from 15.4 °C in the wet season to 13.4 °C in the dry season. The highest monthly temperatures occur in April and May while the lowest temperatures are typically in January (Holwerda *et al.* 2010).

Three large *Q. lanceifolia* trees were selected for this study. The height of the selected trees ranged between 20 and 25 m, and their crowns had a lateral extension of approximately 15 m from the main trunk (unpublished data, this study). Climbing ropes were installed along the trunk and throughout the crowns of the study trees to facilitate installation and maintenance of sap flow and micrometeorological sensors in the canopy.

Sap flow

Sap flux velocity (cm h^{-1}) was determined on the study trees using the heat-pulse ratio method (HRM), which allows for detection and quantification of low and reverse flows expected during foggy conditions (Burgess *et al.* 2001). Every 10 min, a pulse of heat was sent to the heater probe and the ratio of the increase in temperature was measured at two points equidistant downstream and upstream from the heat source. In each tree, a sap flow array consisting of a datalogger with multiplexer (CR1000 and AM16/32; Campbell Scientific Inc, Logan, UT, USA) and an external 12 V battery was established. Eight sensor sets were placed throughout the crown of each tree: six sensor sets were placed high in the crown at different ordinal positions, while two sensor sets were installed in the main trunk. Ten meter (m) long cables were connected to the multiplexer, which were in turn connected to the datalogger. The height of the crown varied from tree to tree, while the cable length was fixed at 10 m (Burgess *et al.* 2001). As a result, the height where the main trunk sensor was installed varied as well (from 1 to 8 m). At each sensor set, the compass direction, branch diameter, distance from the trunk and distance from the ground were measured. Sap flow stations were installed in December 2009 and

operated through June 2010. Data were processed to correct for wounding following Burgess *et al.* (2001). Volumetric sap flow (F, L h^{-1}) was determined by multiplying average sap velocity by the sap wood area. Sapwood area for each sample tree stem and branch was estimated using an allometric relationship developed between sapwood depth (which was determined visually) and diameter at breast height (DBH) (Gómez-Cárdenas, unpublished data). The allometric relationship employed was:

$$A_s = 1.5682 dbh^{1.40673},$$

where A_s is in cm^2 and dbh in cm ($r^2 = 0.97$ and $n = 18$). Radial profiles of sap flow in trunks and branches of the individuals sampled in this study were very similar (data not shown), indicating that the sapwood/DBH relationship that Gómez-Cárdenas found in main stems was reasonable to employ in branches.

Probe installation and calibration

A sap flow sensor set consisted of two thermocouple sensors and a centrally positioned heater sensor. Each thermocouple needle was equipped with three thermocouples at depths of 0.5, 1.7 and 3 cm from the base of the needle. Sensors were custom made in the Asbjornsen Lab at the University of New Hampshire. Prior to installation, the bark was removed with a small knife to ensure installation directly into the xylem. The three needles were installed using a metal drill bit guide to ensure that they were installed perpendicular to the trunk and equidistant from one another. The distance between the heater probe and the top and bottom thermocouple needles was 0.6 cm, respectively (Burgess *et al.* 2001). Prior to installation, needles were coated with petroleum jelly to improve heat conduction. Moulding clay and aluminium foil were placed over the exterior portion of the sensors to minimize natural temperature gradients due to direct or indirect radiation.

At the end of the study, all sensor sets were individually calibrated by drilling a hole above and below the sensor sets to sever the xylem, thereby stopping sap flow. Sap flow systems then collected data for three additional days (to ensure that we captured at least one sunny day). Once it was clear that the systems were reading zero flow (based on flat lining of the curve, not the absolute value), the average flow rate for a 24 h flat-line period was used as the 'true zero' rate (Supporting Information Fig. S1). The data for each sensor set were adjusted accordingly. In some cases, sensors were replaced mid-season due to malfunction, which precluded sensor calibration. In these cases, we estimated zero flow using meteorological data (Ambrose, Sillett & Dawson 2009; Ambrose *et al.* 2010).

Sap flow data management

Small gaps in the data or periods with noisy or erroneous data resulting from equipment malfunction were removed from the data set by employing an algorithm such that windows of data (30 consecutive, 10 min periods) were screened and removed if the standard deviation (SD) within

the window was larger than six. We found in prior examination of the data that windows with a SD > 6 resulted from biologically impossible values due to instrumental failure.

Data gaps were filled using a correlation analysis between a series of data from a sensor with gaps and a continuous data series from a nearby sensor set. If a nearby sensor set and a sensor set with a gap had a correlation coefficient (R) of ≥ 0.9, then the nearby sensor data would be filled in for the missing sensor set. Generally, $R \geq 0.94$ were common within tree crowns. After screening and gap filling, only the sensor sets that were $\geq 90\%$ complete were used in the analyses presented (14 of 24 possible sensor sets). All screening and gap filling were completed using the statistical software R (Version 2.13.0, R Development Core Team).

Micro-meteorology

Three leaf wetness sensors (LWS-L; Decagon Devices, Pullman, WA, USA) and three radiation-shielded temperature and relative humidity sensors (HOBO U23 pro v2; Onset Corp., Bourne, MA, USA) were installed near the sap flow sensors in different locations throughout each tree crown and measurements logged every 10 min. A weather station was located in an open pasture at 100–500 m from the sampled trees. This station measured bulk precipitation (ARG100 tipping bucket rain gauge; Environmental Measurements, Herefordshire, UK), incoming solar radiation (CM3 pyranometer; Kipp and Zonen, Delft, The Netherlands), temperature and relative humidity (HMP45; Vaisala, Vantaa, Finland), and wind speed (A100R cup anemometer; Vector Instruments, Denbighshire, UK). Actual vapour pressure and VPD were calculated from relative humidity and the saturation vapour pressure at ambient temperature. Fog presence and density were measured using a Mini Optical Fog Sensor (Optical Sensors, Gothenburg, Sweden), which uses an optical backscatter technique to measure visibility. In accordance with the international definition, we considered conditions to be foggy when visibility was <1000 m (Glickman 2000). Weather station measurements were made every 30 s and the average over each 10 min period was stored using a CR1000 datalogger (Campbell Scientific Inc).

Leaf-level measurements

Leaf water potential and stomatal conductance

Diurnal cycles of leaf water potential (Ψ_{leaf}) and stomatal conductance (g_s) were measured in the three tree crowns on consecutive sunny days at the end of the dry season (May 2010). Measurements were made at pre-dawn (1745–1845 h), mid-morning (0830–0930 h), midday (1130–1230 h) and afternoon (1400–1500 h). Two to three areas of the tree crown were measured during each time period depending on the time it took to re-locate within the tree crown. Between 5 and 10 sunlit leaves were measured for Ψ_{leaf} and g_s for each time period.

To measure Ψ_{leaf}, humid plastic bags were placed over a leaf and then the leaf petiole was cut with a sharp razor. Humidity was increased in the bag by exhaling into it. This

increased the humidity so that additional water would not be lost from the excised leaf prior to measurement but did not induce leaf wetness, which could cause FU. The plastic bag was then sealed and sent directly to the ground for measurement of equalizing pressure in a Scholander-style pressure chamber (Plant Moisture Systems, Corvallis, OR, USA). Measurements of g_s were made on intact leaves near the cut leaf using a steady-state diffusion porometer (SC-1; Decagon Devices).

Isotope tracer experiment

To assess the capacity for FU among common plant species, we measured the uptake of water into leaves by means of a tracer experiment using deuterated water (D_2O). To simulate a leaf wetting event, ab- and ad-axial leaf surfaces were sprayed *in situ* with 99% atom-enriched D_2O and covered loosely with a clear plastic bag for a period of 15 min. Simultaneously, an unsprayed control leaf from the same branch was placed in a clear plastic bag. Experimental leaves were then thoroughly rinsed with tap water and carefully dried with paper towel. Excised control and experimental leaves were placed in separate sealed vials and frozen until processing. A pair of understory leaves was sampled from three separate individuals each of the following species: *A. latifolia*, *Alnus jorullensis*, *C. macrophylla*, *Miconia glaberrima*, *Pinus patula*, *Pinus pseudostrobulus*, *Q. lanceifolia* and *Q. ocoteifolia*.

Water was extracted from leaves using cryogenic vacuum distillation following the methods of West, Patrickson & Ehleringer (2006) and remained frozen in a sealed vial until analysis. Analysis was performed using off-axis integrated cavity output isotope ratio infrared spectroscopy (DLT-100 Liquid Water Isotope Analyzer; Los Gatos Research, Mountainview, CA, USA) at the University of California Davis Stable Isotope Facility. Although laser-based stable isotope analysis methods are sensitive to interference from plant secondary compounds that distil during water extraction (West *et al.* 2010), the very high level of D-enrichment provided by the D_2O far exceeded any possible measured offsets from true stable isotope ratios. To minimize carry-over effects, we analysed only the last 4 out of 15 injections for each sample. Stable isotope ratios are expressed in per mil units (‰; parts per thousand) and 'delta (δ) notation' as: $\delta = (R_{sample}/R_{standard} - 1) \times 1000$, where R denotes the ratio of $^2H/H$. All stable isotope ratios are presented relative to the Vienna Standard Mean Ocean Water.

Differences between control and sprayed leaves within species were analysed using a paired *t*-test. Because of the small sample size, we also performed non-parametric tests to confirm results and found no discrepancy between the two tests. Finally, we applied a linear, two-source mixing model to determine the proportion of water in sprayed leaves originating from the D_2O tracer following Dawson *et al.* (2002):

$$f_A = \frac{\delta_t - \delta_B}{\delta_A - \delta_B},$$

where f_a is the fraction of the total contributed by water from the tracer, δ_A is the stable isotope ratio of water in the tracer,

δ_B is the stable isotope ratio of water in the control leaf, and δ_t is the stable isotope ratio of water in the experimentally sprayed leaf.

Pressure–volume curves

Pressure–volume (PV) curves were measured following the methods and calculations used by Schulte & Hinckley (1985) to determine the water potential at turgor loss, water content at turgor loss, the maximum bulk elastic modulus, the relative water content of the apoplast and the osmotic potential at full turgor. Three to five sunlit branches were cut high in each tree crown and then immediately sent to the ground, placed in black plastic bags and transported to the field laboratory. In the laboratory, branches were re-cut under water and kept covered in black plastic in water overnight to rehydrate. The following morning, branchlets (with three to five leaves) were bench dried; their weight and water potential was measured repeatedly throughout the day. We found no evidence of oversaturation for any of the curves (the so-called 'plateau effect'; see Kubiske & Abrams 1990).

Data analysis

The influence of night-time VPD and the number of hours in a fog/rain event on branch-level F were determined by performing a series of regressions within and across the study trees. Prior to analysis, meteorological data were screened to examine when fog events (either without or with rainfall) resulted in canopy wetness. The beginning of a fog event was determined by a visibility of less than 1000 m (see above), and extended to include rain (if it occurred as part of the fog event) and the complete period of canopy wetness associated with that particular fog/rain event. Because FU occurs when leaves are wet, the end of the event was noted only when canopy leaf wetness sensors were dry again. Only events that began with a dry canopy were included so that the number of hours needed for full canopy wet-up could be determined. The total FU for each event was then calculated (L per event). Sap flow data (absolute value) were square root transformed to achieve normality. In the first set of analyses, the number of hours in a fog/rain event was determined and then regressed against the total FU (L per event). Sap flow during leaf wetness events ($n = 32$) was analysed for each branch ($n = 14$) separately. There were small gaps in data for some branches resulting in a lower sample size in leaf wetness events for particular branches ($n = 27–29$ was common). The relationship across all branches (14 branches pooled) was also analysed. In the second set of analyses, the daily mean night-time VPD (obtained from the meteorological station) was regressed against the total night-time sap flow (L per night). Only sensor sets that had ≤10% missing data were included in this analysis. Firstly, night-time sap flow was plotted for each branch individually for each night-time period ($n = 143$). Branch sap flow values ($n = 14$) were then averaged and regressed against the average night-time VPD. We performed analyses of variance (ANOVAS) to test for possible effects of aspect on dry-season FU and NT. We also

performed three nested, random factor ANOVAS to quantify variation in volumetric sap flow (total F, negative F and night-time F) within ($n = 4$ branches for Tree #1, $n = 6$ branches for Tree #2, $n = 4$ branches for Tree #3) and across different tree crowns ($n = 3$). All analyses were performed using JMP statistical software (SAS Institute, Cary, NC, USA).

Error analysis

An error propagation analysis was performed to determine the magnitude of potential error in the dry-season branch-level water balance components, FU and NT. Sources of error included sensor installation, zero-flow calibration, determination of sap flux velocity by averaging measurements from three thermocouples, gap filling of missing data and the scaling of sap flow values to obtain volumetric flow. An examination of each of these sources of error revealed that the scaling of sap flux rates to sap flow volume introduced error several orders of magnitude greater than any other error source. As a result, other sources of error were excluded from final calculations of an error term. Each type of error is addressed below.

The error associated with scaling sap flux velocity to sap flow volume (σSA) was calculated as the root mean square error of the regression equation for the DBH versus sapwood area for diameters smaller than 20 cm (Gómez-Cárdenas, unpublished data, as described in Methods). Larger diameters were excluded because this study was restricted to branches. The branch-level error in NT resulting from scaling point measurements of sap velocity to whole-branch transpiration was therefore calculated using the following equation derived from Stull (1999):

$$\sigma NT = NT * (\sigma SA / SA),$$

where SA is the sapwood area of each branch.

The branch-level error in FU (σFU) was similarly calculated:

$$\sigma FU = FU * (\sigma SA / SA).$$

Errors due to sensor installation were corrected during data analyses (see above) and therefore were excluded from the error analysis. Zero-flow calibration resulted in very low variation in values ($\ll 1\%$ of sap flow rates) due to care in xylem severing and calibration against meteorological data (i.e. dark periods with negligible wind, high humidity and no leaf wetness, see Ambrose *et al.* 2009, 2010); this source of error was also excluded from the error propagation analysis. The error associated with averaging measurements from the three thermocouples was also excluded as other methods papers suggest that the scale of variation is not relevant to the ecological questions addressed in this paper (Madurapperuma, Bleby & Burgess 2009; Skelton *et al.* 2013).

The final source of error was due to gap filling of data (described in Methods). The standard error term for gap filling was quantified conservatively using the worst fitting

regression used in generating gap-filled data ($r^2 = 0.9$), applied across a complete data set. The root mean square error of this regression was so small (.0012 cm h^{-1}) that it did not affect our results significantly and was therefore excluded from analysis.

The errors for FU and NT are reported for each branch in Table 1.

An additional error analysis was performed to determine the percentage of recorded FU hours in which volumetric sap flow rates, F (L h^{-1}), were significantly below zero. This allowed us to ask the question, what portion of the negative values of F was negative enough to conclude that FU actually occurred during these intervals? The confidence interval (σ) associated with F was calculated for each branch using the following equation derived from Stull (1999):

$$\sigma F = AVG(F) * (\sigma SA / SA),$$

where AVG(F) is equal to the total volume of sap flow across the entire experiment divided by the number of hours from which data were recorded. We report in the Results section the proportion of FU values for which F $\pm$ σF is significantly < zero.

RESULTS

Microclimate

The total rainfall for the 2010 dry season was 696 mm. There were six periods without rain for 5 or more days (Fig. 1a). One of these dry periods was in January (8 d), one was in February (6 d), and the rest were in April and May (one 6 d, one 5 d and two 8 d periods). The average daily temperature during the study period varied from 10 °C in January to 16.6 °C in May (Fig. 1b). Periods with cloudiness and precipitation (low VPD) alternated with periods of high evaporative demand (Fig. 1c). This pattern is typical for the study region and reflects the intrusion of cold fronts and subsequent high pressure events (Holwerda *et al.* 2010; Muñoz-Villers *et al.* 2012). Fog was present for approximately 25% of the dry season and usually preceded rainfall events, although it also occurred during rainfall events (Fig. 1d).

Many of the 73 rain events that took place during the study period were bracketed by periods of fog. When fog preceded or followed a rain event, it created a bridge in canopy wetness across a number of events. Across the study period, there were 36 distinct canopy wet-up events caused by the combination of fog and rain events. These events were evaluated to examine the effects of microclimate on canopy wetness and sap flow. Nearly all periods with fog occurrence were accompanied by rainfall. Fog/rain events varied greatly in length: the shortest event was 2 h long, while the longest event lasted nearly 4 d (average = 39 $\pm$ 27.5 h *SD*). Interestingly, in the absence of rain, fog resulted in canopy wetness only after a lag time of 10 h (Fig. 2a). While there were not many long fog periods without rain in this study, there were a number of shorter fog events (1–5 h duration) that did not result in canopy wetness. On the other hand, when fog was accompanied by rainfall,

Table 1. Dry season branch-level water balance for all branches with <10% missing data

Branch	FU (L)	σ of FU (L)	% FU	%time FU	NT (L)	σ of NT (L)	% NT	% time NT	r^2 (FU)	r^2 (NT)
1_1	−40.9	14.1	12	40	65.8	22.7	19	53	0.93	0.78
1_3	−7.3	2.9	11	36	16.4	6.5	24	60	0.59	NS
1_5	−16.7	5.1	5	30	57.5	17.4	16	61	0.32	0.75
1_8	−76.2	28.3	14	40	101.4	37.6	18	51	0.92	0.71
2_1	−5.0	2.0	4	28	26.7	10.6	20	64	0.59	NS
2_2	−10.5	4.5	4	23	48.3	20.5	19	70	0.28	0.39
2_3	−5.7	2.5	6	28	16.6	7.3	18	63	0.44	0.65
2_5	−42.3	23.4	16	43	42.4	23.5	16	46	0.73	0.58
2_6	−47.4	16.0	11	39	62.6	21.1	14	50	0.61	0.57
2_7	−16.5	5.5	12	40	13.9	4.7	10	49	NS	0.53
3_1	−21.2	8.0	11	34	31.0	11.6	16	57	0.61	0.62
3_2	−19.5	6.5	15	37	21.1	7.1	16	56	0.65	0.42
3_5	−21.4	5.2	4	32	113.6	27.4	20	61	0.91	0.82
3_6	−6.8	3.2	5	31	23.6	11.0	18	61	0.72	0.81
AVG	−24.1	9.1	9.3	34.4	45.8	16.4	17.4	57.4		

The first column of data contains the tree number followed by the branch number. FU (L) is the total amount of water in litres that was recovered during the study period. The σ of FU (L) is the error term associated with the FU estimate determined using error propagation analysis (see the Results section). The % FU indicates the percentage of the total positive flow in litres throughout the study that was recovered via FU. The % time FU is the percentage of hours in the study period (small gaps in data are excluded) that FU was occurring. NT (L) is the total number of litres of water that were lost during the night-time hours in the study while σ of NT (L) was the error term for each branch calculated with error propagation and the % NT was the percentage of the total transpiration that occurred at night. The % time NT is the percentage of night-time hours in the study period (small gaps in data are excluded) when transpiration was occurring. The r^2 (FU) column contains the regression coefficients for branch-level regression analyses between the number of hours in a fog event and the amount (total L) of FU. The r^2 (NT) column contains the regression coefficients of branch-level regression analyses between the average night-time VPD and the total night-time transpiration (L per night). For a graphical display of the results for the last two columns, please see Figs 4 and 5 and Supporting Information Figs S2 and S3.
FU, foliar uptake; NT, night-time transpiration.

canopy wetness occurred in the same hour as the rain started, or in the following hour (Fig. 2b).

Foliar uptake

Under foggy and rainy conditions, *F* was often suppressed and sap flow reversals were common (Table 1, Fig. 2). General patterns of FU varied depending on the duration of fog periods and whether fog was accompanied by rain (Fig. 2a,b). While FU was common in all branches ($n = 14$), the total amount of FU in the dry season varied (Table 1). FU took place an average of 34.4% of dry-season hours (SE +/−1.6, range: 28–43%, Table 1). An error analysis indicated that for at least 95% of these hours, branches underwent negative flow after the inclusion of an error term (see Error Analysis section above). The relationship between the number of hours in a rain/fog event and the amount of FU (L per event) across all branches was also significant (Fig. 4, $r^2 = 0.91$, $P \leq 0.01$). Of the 14 branches included in this analysis, 12 demonstrated a significant relationship between the length of the fog event and the amount of FU when analysed individually ($r^2 = 0.39$–0.82, all $P \leq 0.01$, Table 1 and Supporting Information Fig. S2). The random-factor ANOVA for negative sap flow data (i.e. FU) indicated that 17.6% of the variation in the model was attributed to branch-level variation in sap flow while 25% of the variation in FU was attributed to tree-level variation. These results differ from the random-factor ANOVA for all sap flow data that indicated a

very minor effect (0.05%) of 'tree' on average sap flow rates. Further, FU (L h^{-1}) was not significantly affected by the compass direction (east/west, *f*-ratio = 4.37, *P* = 0.07). In contrast, the percentage of the dry-season hours that any branch underwent FU *was* significantly affected by its compass direction. Branches facing in an easterly direction underwent FU 38% of the dry-season hours while branches facing west underwent FU an average of 28% of the dry-season hours (*f*-ratio = 6.53, *P* = 0.01).

Results of the tracer experiment provided independent evidence for FU in our main study species, *Q. lanceifolia*, as well as in seven other common tree species in this TMCF. At the outset of the treatment, the δ^{2}H of the deuterated water tracer was 1077‰, while the δ^{2}H of the rinse water was −35‰. For all of the species we studied, the leaves we sprayed with a deuterium-treated water demonstrated a significantly higher leaf water δ^{2}H compared with control leaves ($n = 3$ individuals spp.$^{-1}$, $P < 0.05$), indicating that all species are capable of FU. Moreover, the application of the mixing model demonstrated that water from the deuterated tracer accounted for 5.7–22.2% of the water subsequently extracted from the sprayed leaves (Table 2). These values were similar to volumetric recovery rates using sap flow techniques (Table 1).

Night-time transpiration

Trees experiencing high night-time VPDs (0.5–1.5 kPa) often transpired. NT occurred, on average, 57.4% of all night-time
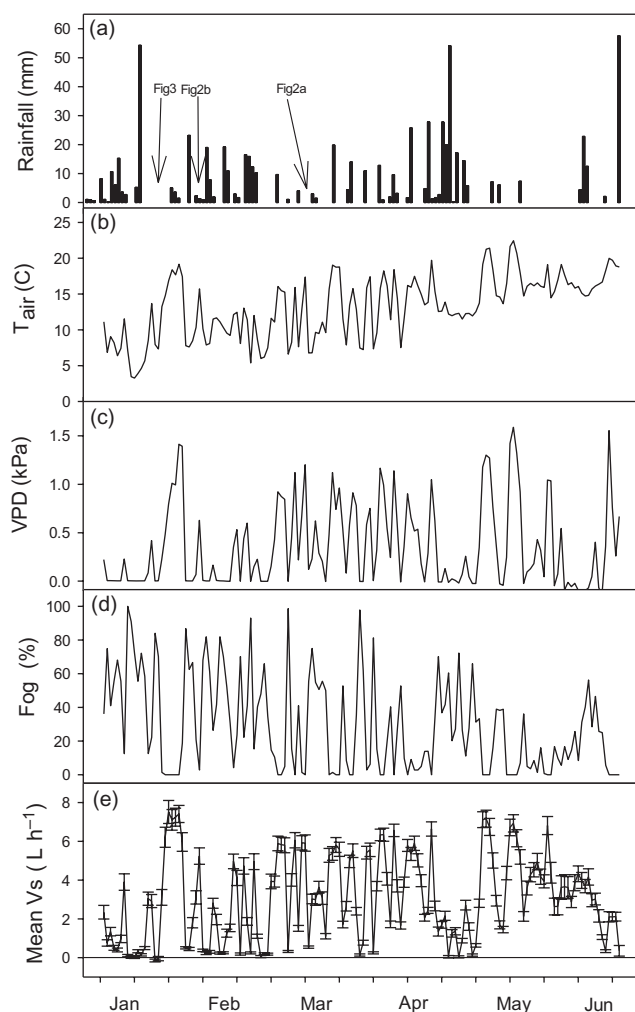
Figure 1. Daily rainfall totals (a), daily mean air temperature (b), mean nightly vapour pressure deficit (c), fog occurrence (d) and daily sap flow averages (e) over the course of the study period in 2010 measured on the study trees and at a meteorological station near the study trees. Arrows in the top panel refer to periods of time shown in Figs 2 and 3. The transition from the dry season to the wet season occurred in early June. Error bars indicate the standard error of the mean.

hours (SE +/–1.8, range: 46–70%, Table 1, Fig. 3). There was a strong positive relationship between night-time VPD and NT averaged across all branches ($n = 14$ branches, $r^2 = 0.87$, $P = 0.01$, Table 1, Supporting Information Fig. S2a–o). Of the 14 branches included in this analysis, 13 demonstrated a significant relationship when analysed individually ($r^2 = 0.28$–0.93, $P < 0.05$; Table 1 and Supporting Information Fig. S3). The random-factor ANOVA for night-time V_s indicated that none of the variation in NT was due to the effect of tree (0% variation of 'tree' in the model) while 21.5% of the variation in NT was due to branch-level variation. We did not find a significant effect of aspect on rates of NT (data not shown).

Integrated branch-level water balance

To determine the relative contribution of FU and NT to the branch-level, dry-season water balance, we calculated the
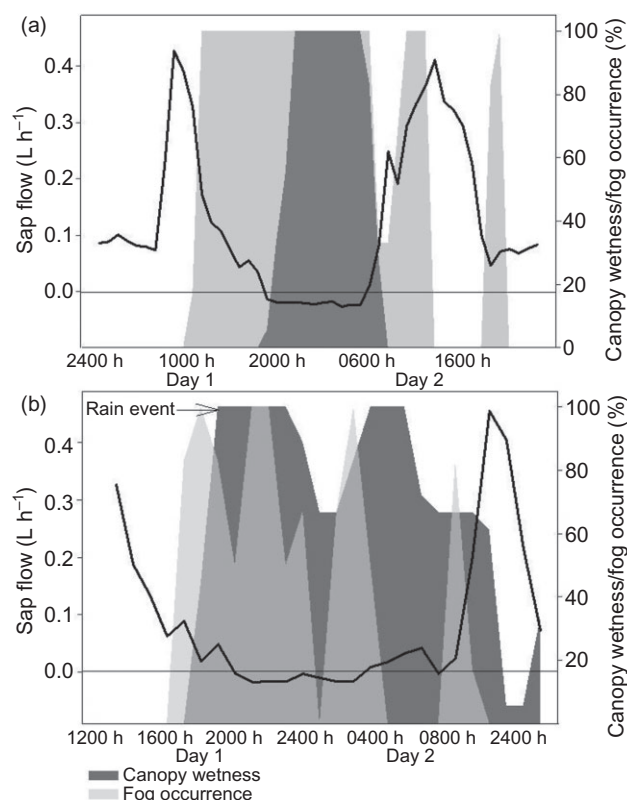
Figure 2. (a, b) Sap flow for two foggy periods in the dry season. In both panels, the dark shading represents the percent canopy wetness while the light shading represents the percent fog occurrence. The black line represents the hourly sap flow for Oak1. For the period of time in the upper panel (4–5 March), there was no rainfall and canopy wetness occurred 10 h after the percent fog occurrence was 100. In the lower panel (2–3 February), there was a rain event shown with the arrow. In this case, the canopy wetness occurred at the same time as the rain event. In both panels, sap flow is negative during most of the period when the canopy is wet. Oaks 2 and 3 show a similar pattern.

total dry-season transpiration and partitioned water flux into daytime and night-time hours, as well as into positive and negative flow rates. As FU and NT rates varied across branches, and rates of FU also varied across trees (see

Table 2. Results of a linear, two-source mixing model demonstrating the percent of water originating from foliar uptake in eight common plant species in the seasonally dry tropical montane cloud forests of Veracruz, Mexico

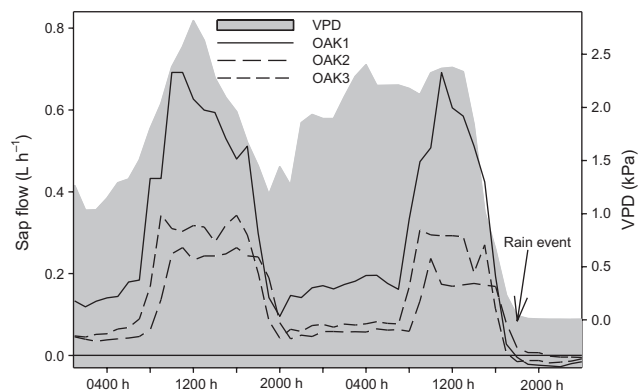| Species	Leaf H_2O originating from tracer (%)
Alchornea latifolia	6.7–12.4
Alnus jorullensis	19.9–22.2
Clethra macrophylla	6.3–13.7
Miconia glaberrima	6.3–13.6
Quercus lanceifolia	9.8–12.9
Quercus ocoteifolia	8.3–15.5
Pinus patula	6.7–8.6
Pinus pseudostrobulus	7.1–17.0

Figure 3. Sap flow for the three oak trees for a 2 d dry period (23–24 January). The shaded area represents the hourly mean vapour pressure deficit (VPD). During this period, there was 100% visibility, dry canopies and no rainfall until the end of the period where an arrow indicates the time of a small rainfall event. High night-time VPD resulted in positive night-time flow rates on both the 23rd and the 24th. The VPD dropped with the rainfall event the night of the 24th and the sap flow quickly dropped as well.

two-way random-factor analysis above), the water balance also varied among branches within trees. On average, 9.3% (± 1.2% SE) of the total branch-level transpired water was recovered via FU, which represented 24 L per branch (± 5.5 L SE, range: 5–76 L, Table 1). NT was also highly variable within and among trees; however, on average, NT accounted for 17.4% of the total dry-season transpiration (± 0.9% SE, range: 13.4–35.2%), which represented a total loss of 45.8 L (± 8.4 L SE, range: 16–113 L) over the entire study period (5 months). This water loss was almost twice the amount of water recovered via FU (Table 1).

Leaf-level physiological measurements

Diurnal courses of Ψ_{leaf} were conducted in the three tree crowns at the end of an 8 d dry period in May; this was the longest period without rain during the 2010 dry season. Ψ_{leaf} ranged from an average of −0.2 MPa at pre-dawn to −1.2 MPa at midday. We consider this midday value to be the seasonal minimum leaf water potential ($\Psi_{leafmin}$), because other 8 d dry periods were either in January, when temperatures were lower, or in late May following the largest rainfall event of the 2010 dry season. During the same dry spell, diurnal courses of g_s were measured in each of the three tree crowns, and ranged from 72.48 mmol m^{-2} s^{-1} at pre-dawn (± 6.35 SE) to 259 mmol m^{-2} s^{-1} at midday (± 11.7 SE); the midday rate was maintained for most of the early afternoon (3 h). Analyses of PV curves conducted on crown branchlets indicate that the Ψ_{leaf} at the turgor loss point for *Q. lanceifolia* averaged −1.63 MPa. Additional parameters obtained from the PV curve analysis for *Q. lanceifolia* were as follows: osmotic potential at full saturation (Ψpsat = 0.07 MPa), the water content at the turgor loss point (0.94), the maximum bulk elastic modulus (ε = −10.5 MPa) and the relative water content of the apoplast (Ra^* = 0.32).

DISCUSSION

The occurrence and significance of foliar fog uptake

In this seasonal TMCF in Mexico, the presence of fog and mist led to leaf surface wetness and FU in a dominant canopy species, *Q. lanceifolia*. Despite the relatively low contribution of fog to total water inputs at our site (Holwerda *et al.* 2010; Muñoz-Villers *et al.* 2012), FU occurred frequently throughout the dry season and was an important factor in determining the dry-season water relations and water balance in this oak species. We also found that seven other tree species possessed the capacity to absorb water directly through leaf surfaces, adding to a growing body of research demonstrating the ubiquity of FU in TMCFs and other ecosystems (Goldsmith, Matzke & Dawson 2013). This suggests that FU is also an important community level characteristic at our site and may be an important plant functional response to the dominant climatic conditions of TMCFs in general. On average, FU accounted for the recovery of 9.3% of the total branch-level transpiration. Although this recovery represented a relatively small proportion (24.1 L) of the total dry-season branch-level water loss (260 L), it is likely important in helping trees maintain a favourable canopy water status, especially in the annual dry season. Improved canopy water status via FU can have clear benefits to the plants. In a Costa Rican TMCF, Goldsmith, Matzke & Dawson (2013) found that leaf wetting events improved plant water status across a number of different species. It is likely that FU, which occurred 34% of the dry-season hours, also improved leaf water status and influenced the high dry-season water potentials measured in this study. Because these trees are both tall and have broad canopies, the most distal leaves can be located 35–40 m from soil water sources. By absorbing water directly through leaf surfaces, trees may temporarily be decoupled from soil water and therefore more quickly recover from periods of high VPD than if they had to take up water through the soil-plant-atmosphere continuum, as also noted in coastal Redwoods (Burgess & Dawson 2004; Simonin *et al.* 2009).

The temporary decoupling of trees from the soil water pool may play a role in determining the hydrology observed in this TMCF. Goldsmith *et al.* (2012) found that the hydrology of this TMCF can be broken down into two major pools: one pool that consists of more tightly bound shallow soil water, and a second pool that is highly mobile and consists of precipitation that quickly infiltrates through the site's porous soil and later contributes to the stream water. Stable isotope ratios of stem xylem water from four common tree species in that study, including *Q. lanceifolia*, were consistent with the use of the shallow water pool (20–60 cm), suggesting that plants are able to maintain a functional water-balance strategy without accessing deeper sources of water. The ability of trees to meet their water demand, especially during extended periods without rain or high VPD, may depend on their ability to take up water through their leaves and thus decrease their use of shallow soil water. An increase in cloud base heights or decrease in precipitation,

as projected in climate change models for tropical montane regions, would likely lead to reduced opportunity for FU and, hence, a greater demand on soil water (Still *et al.* 1999; Foster 2001; Lawton *et al.* 2001; Van der Molen *et al.* 2006; Barradas *et al.* 2010). Such a shift could have significant implications for regional hydrology, potentially decreasing stream flow and availability of water throughout the watershed.

The occurrence and significance of NT

Our findings indicated that NT was also frequent in the study trees and contributed 14–24% of the total branch-level water transpired during the dry season. These findings are well within the range of previous studies examining NT across a number of habitats (Bucci *et al.* 2004; Dawson *et al.* 2007; Goldstein *et al.* 2008; Novick *et al.* 2009). In this study, *Q. lanceifolia* had high rates of water loss due to high evaporative demand during night-time hours (see NT values, Table 1 and Fig. 3). NT has been hypothesized to be adaptive under low nutrient availability (Scholtz *et al.* 2006), although this is not likely a factor at this site because the soil organic matter is high (Campos-Cascaredo 2010).

The present results suggest that projected decreases in rainfall or an increase in cloud base height (Lawton *et al.* 2001; Karmalkar *et al.* 2008) could have large physiological consequences because increases in NT and decreases in FU could lead to increased water stress in the oak species studied herein. Alternatively, it is possible that this oak species does have the ability to increase stomatal regulation, but that we did not detect such regulation in night-time *F* due to the consistently high soil water content during this unusually wet study period (L.E. Muñoz-Villers, unpublished data). To our knowledge, there are only two other studies showing NT in a cloud forest. Data from a wetter cloud forest in Hawaii found comparable rates of NT (Table 1 here and in Dawson *et al.* 2007). In a more recent paper in the Brazilian cloud forest, high rates of NT as well as stomatal control under higher VPDs were also documented. Other species of *Quercus* demonstrate strong stomatal control under drought conditions (Cavender-Bares, Sack & Savage 2007; Otieno *et al.* 2007; Haldimann, Galle & Feller 2008), although none of these studies were conducted in TMCFs.

Despite high dry-season water loss, *Q. lanceifolia* leaves maintained leaf water potentials above the turgor loss point. Nonetheless, $\Psi_{leafmin}$ was low, especially considering the wetter-than-average dry-season conditions (Holwerda *et al.* 2010; Muñoz-Villers *et al.* 2012). The $\Psi_{leafmin}$ for canopy leaves was −1.2 MPa; PV curves revealed Ψ_{leaf} at turgor loss of −1.63 MPa. Under climatic warming and drying related to global and/or regional climate change, we would predict a decline in crown-level water potential, which, in turn, could lead to an increased probability of water stress. Even under current precipitation patterns, increases in cloud base height could lead to increases in crown-level VPD which could also lead to decreases in $\Psi_{leafmin}$.

The effect of canopy micrometeorology on FU and NT patterns and variability

Within an hour of canopy wet-up, rates of *F* decreased and FU began (Figs 2 & 3). In the absence of rain, fog events also resulted in canopy wetness, but only after considerable time lags (Fig. 2a). Time lags between fog occurrence and canopy wetting have previously been demonstrated at shorter time scales (Burgess & Dawson 2004); the longer wetting-up times found in this study may also be attributed to differences in the fog interception efficiency occurring as a function of leaf type (broadleaf versus needle leaf). As the liquid water content of fog in central Veracruz is known to be high (García-García & Montañez 1991), the low wind speeds prevailing at the study site must also be a factor of some importance (Holwerda *et al.* 2010; cf. Shuttleworth 1977). While long lag times in canopy wet-up reduce the time that trees can take advantage of foliar fog water inputs, fog was generally accompanied by rainfall and thus even short precipitation events quickly wet up the canopies (Figs 2b & 3), which then remained wet and underwent FU in the presence of fog even without rain.

One of the novel aspects of the results herein is the linkage between fog/rain events, associated canopy wetness conditions, and the observed rates and quantities of branch-level FU. We found that the duration of canopy wetness events (induced by fog and/or rain) had a significant effect on total FU (L per event). Very short events led to negligible recovery rates in trees, while events spanning multiple days led to recovery rates of up to 1.4 L of branch-level water (Fig. 4). In this study, FU and recovery were assessed in small branches, so individual values appear small. However, if FUs were summed across entire tree crowns, it could lead to the recovery of hundreds of litres of water during each leaf wetness event. Furthermore, during crown wetting events there is no
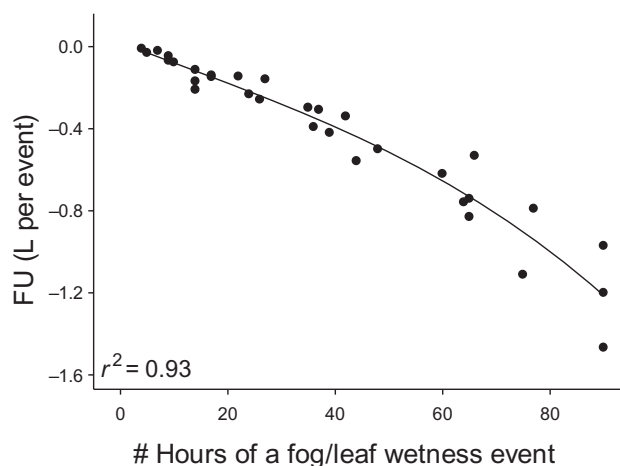

Figure 4. The relationship between the number of hours of a leaf wetness event induced by fog and/or rain and the foliar uptake (FU) (L per event). This figure shows the average relationship for all branches in the three oak canopies. The r^2 for this relationship is shown in the lower part of the panel. Values on the Y-axis are negative to indicate reverse flow.
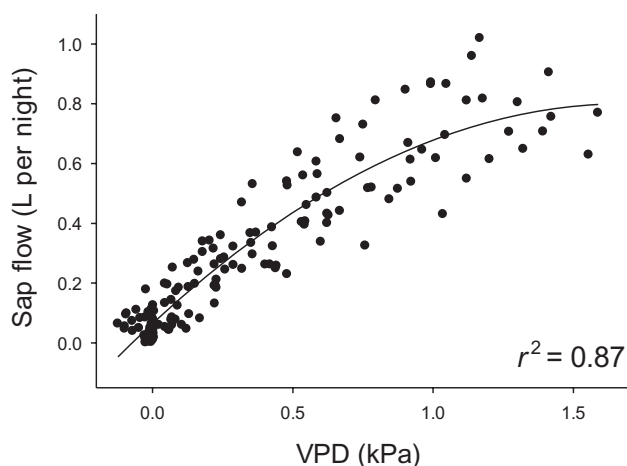
Figure 5. The relationship between night-time vapour pressure deficit (VPD) and night-time transpiration (NT). Each data point is the total number of litres transpired in a given night averaged across all branches. The r^2 for this relationship is shown in the lower part of the panel.

transpiration, so regardless of the magnitude of impact FU, leaf wetting events play a central role for the water relations of trees in this forest. FU over long periods of time may cause decoupling of the canopy water balance from soil water and result in improved leaf water status solely from fog inputs.

We also found a strong relationship between NT and night-time VPD. This relationship has been documented across a number of temperate and tropical habitats, and is generally associated with species having poor stomatal regulation (Bucci *et al*. 2004; Motzer 2005; Dawson *et al*. 2007). Excess water loss at night could lead to a reduction in leaf water content and greater tissue water deficits or even stress. Furthermore, watershed and ecosystem models do not generally consider NT when quantifying water budgets, which in this TMCF ecosystem may lead to a considerable underestimation of stand-level transpiration.

While relationships across branches were generally robust (Figs 4 & 5), we did find a fair amount of variability in rates of FU and NT among branches and trees (Table 1; cf. supplemental figures). This suggests that the within-crown microenvironment is spatially heterogeneous. Variability in forest canopy and/or tree crown microclimate can be caused by a number of factors, including gap formation (Chazdon & Fetcher 1984), shifts in canopy structure (Caldwell *et al*. 1986; Cardelus & Chazdon 2005) and changes in epiphyte loads (Freiberg 2001; Stuntz, Simon & Zotz 2002). Although these factors may also contribute to variation in forest canopy microclimate in our study location, we did not detect significant spatial environmental heterogeneity in temperature and RH within the individual crowns of our study trees. This may indicate that differences in sap flow rates across branches are due to factors such as leaf age and self-shading rather than microclimate *per se*. The crowns of these trees are rather open, which may promote efficient mixing of the within-canopy air. It is also possible that there was biologically meaningful heterogeneity in climatic variables within the tree crowns that were not detectable with our instruments and/or sampling design.

The relative contribution of FU and NT to the dry-season, branch-level water balance

FU and NT were common phenomena in the study trees. FU occurred on average over 34.4% of the total hours measured, while NT occurred 57.4% of the dry-season night-time hours. Although the rates and magnitudes of both FU and NT were low, there was still a measurable effect of these two processes on dry-season, branch-level water balance. NT accounted for an average of 17.4% of all dry-season water transpired, amounting to an average of 45.8 L of water lost per branch in the dry season. While study trees experienced water loss at night, they were able to recover some water through FU. Branches recovered an average of 24.1 L of water over the course of the dry season, which translated into a 9.3% recovery rate. If foggy conditions follow a period with high VPD, FU may buffer the leaf-level effects of high evaporative demand. While FU may enable quick rehydration of leaf tissues, this rehydration did not completely compensate for water lost at night via NT, which was almost two times greater than the water recovered. This is the first study that we are aware of that determines the relative contribution of FU and NT to canopy water balance in a seasonally fog-affected system. While the contribution of fog to the annual water budget is low in this forest (Holwerda *et al*. 2010, Muñoz-Villers *et al*. 2012), as compared with other fog-affected systems (e.g. de-Val *et al*. 2006; Hildebrandt *et al*. 2007; Giambelluca *et al*. 2011), our findings indicate that even this low fog incidence (and accompanying drizzle) can have significant effects on the water relations of trees and partially offset the water losses due to NT.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Zero flow calibration example for Oak2 branch 5. Towards the end of the experiment, a hole was drilled above and below the sensor sets to physically restrict flow. This average flow was taken as the 'true zero' and all sap flow values were calibrated to this true zero which was performed on each sensor set.

Figure S2. (a–m). The relationship between the number of hours of fog and leaf wetness events and the FU (L per event) for branches of the three oak trees. The values on the Y-axis are negative to indicate reverse flow. The number on the upper right corner of each figure refers to the tree number followed by the branch number. Below this identification number is the r^2 value. Analyses were completed using square-root transformations. Raw data are shown here for ease of viewing because the relationships were similar. Branch 2_2 was not plotted because there was no significant relationship in this branch. For all other branches, the relationship was significant at $P < 0.0001$.

Figure S3. (a–l). The relationship between night-time VPD (kPa) and night-time volumetric branch sap flow (L per night). Each dot represents a nightly total of water loss (L). The number on the upper left corner of each figure refers to the tree number followed by the branch number. Below the identification number is the r^2 value for that branch. Analyses were completed using the square-root transformation of night-time sap flow data. Raw data are shown here for ease of viewing. Branches 1_3 and 3_1 were not plotted because there was no significant relationship for these branches. For all other branches, the relationship was significant at $P < 0.0001$.