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Plant-Water Relations in Seasonally Dry Tropical Montane Cloud Forests

By

Gregory Rubin Goldsmith

A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy

in

Integrative Biology

in the

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of the

University of California, Berkeley

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Professor Todd Dawson, Chair
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Plant-Water Relations in Seasonally Dry Tropical Montane Cloud Forests

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by

Gregory Rubin Goldsmith

Abstract

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Seasonal changes in water availability have been demonstrated to play a fundamental role in determining plant survival-mortality dynamics in a number of different ecosystems. Tropical montane cloud forests are often considered to be aseasonal environments that do not experience significant water deficits; however, there is growing recognition that many of these rare ecosystems experience one or more dry seasons annually. Moreover, many tropical montane cloud forests are projected to experience decreases in dry season precipitation and increases in dry season temperature as a function of climate change. While the regular presence of clouds may mitigate plant water stress occurring during the dry season, clouds are also projected to decrease in frequency, intensity and cover. At present, little is known about the plant-climate interactions in tropical montane cloud forests.

In order to improve our understanding of the effects of climate change on tropical montane cloud forests, I studied how seasonal changes in water availability affect plant functioning using observational and experimental approaches at a number of different scales:

In *Chapter 1,* I present the results from a study on tropical montane cloud forest ecohydrology. Despite longstanding recognition of the unique nature of hydrologic cycling in tropical montane forests, comprehensive and comparative studies remain limited. I studied the intra- and inter-annual variation in the inputs, pools and fluxes of water in a seasonally dry tropical montane cloud forest near Veracruz, Mexico using hydrogen and oxygen stable isotope ratios (δ^2H and δ^{18}O, respectively) in water. There was significant seasonal variation in the δ^2H and δ^{18}O ratios of precipitation inputs driven by differences in the origin and size of storm events. This variation facilitated the separation of different pools of water, revealing the presence of two separate soil water pools, one highly mobile pool contributing to streams and a second less mobile pool being used by plants. At the peak of the dry season, the predominant deciduous and evergreen tree species were accessing shallow soil water from this second, less mobile pool. The results provide a foundation upon which to better understand the coupling between the hydrology and ecology of tropical montane cloud forests now and given projected scenarios of climate change.

In *Chapter 2,* I present the results from a study on the effects of a sustained decrease in water availability on the growth and physiology of tropical montane cloud forest plant seedlings. Research on the functional response of tropical plants to seasonal changes in water availability

has largely focused on tropical lowland ecosystems. I conducted an experimental dry-down of seedlings from four common tropical montane plant species in the genus *Ocotea* at a common site near Monteverde, Costa Rica. Despite only a small decrease in soil water availability, plants subjected to the experimental dry down demonstrated species-specific reductions in both physiology and growth. The results indicate that water is likely to play a strong role in tropical montane cloud forest plant functioning.

In *Chapter 3,* I present the results from a study on the interactions between clouds and tropical montane cloud forest plants, with a focus on the prevalence and significance of foliar water uptake. Foliar water uptake, the direct uptake of water accumulated on leaf surfaces into leaves, is a common phenomenon in ecosystems that experience frequent fog or cloud immersion, but has not been studied in tropical montane cloud forests. I quantified cloud cover patterns in two neighboring, seasonally dry tropical montane cloud forests near Monteverde, Costa Rica using remote sensing data. I then correlated these patterns with ground-based observations of leaf wetting occurring due to the physical impaction of cloud moisture on leaf surfaces. During the dry season, when rainfall is reduced and leaf wetting due to clouds is the primary source of water in the ecosystem, leaf wetting events resulted in foliar water uptake in all species studied. While all the species demonstrated the capacity to improve their leaf water potential as a result of foliar water uptake, this capacity differed between the two forests and among the species studied within a forest. The results indicate that changes in the frequency, intensity, and duration of cloud cover projected to occur as a function of regional warming will not affect all species or forests equally.

In *Chapter 4*, I present the results from a study on the effects of leaf wetting events and the resultant foliar water uptake on leaf water pressure-volume relations. Research on foliar water uptake has almost exclusively focused on the implications of additional water for leaf water potential and its impacts on photosynthetic performance. However, the study of pressure-volume relations can provide important insights into whether foliar water uptake alters traits responsible for the movement and conservation of water in leaves. I compared the pressure-volume relations of leaves rehydrated through both xylem and foliar water uptake compared to xylem alone in four tropical montane plant species near Monteverde, Costa Rica. Rehydration through both pathways caused inconsistent differences among species; however, one species demonstrated a decrease in the modulus of elasticity and an increase in capacitance that may be a function of differences in leaf anatomy. The results indicate that changes in pressure-volume traits associated with leaf wetting and foliar water uptake may have consequences for plant functioning and suggest that future research should more explicitly consider the role of leaf wetting in plant-water relations.

Taken together, these studies suggest that the annual dry season experienced by the tropical montane cloud forests studied herein result in plant water deficits that impact plant functioning. However, these plant water deficits can be mitigated by the presence of clouds. The species-specific nature of many of the results indicate that projected changes in dry season water availability may alter plant survival-mortality dynamics and thus affect the species composition of tropical montane cloud forests in the future.

DEDICATION

To my parents, Roz and Marc, for their love and support.

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ACKNOWLEDGEMENTS

CHAPTER 1

Stable isotopes reveal linkages among ecohydrological processes in a seasonally dry tropical montane cloud forest

ABSTRACT

Despite their critical role as freshwater resources and their vulnerability to anthropogenic pressures, our knowledge of the ecohydrology of tropical montane cloud forests remains limited. We use a dual stable isotope approach (δ^2H and δ^{18}O) to trace water inputs, fluxes, and pools through a seasonally dry tropical montane cloud forest in central Veracruz, Mexico. We found strong seasonal variation in the isotope composition of precipitation inputs (δ^2H ~130‰ and δ^{18}O ~18‰), with significantly more depleted wet season values as compared to dry season values. These seasonal differences are subject to inter-annual variation, as reflected by shifts in the local meteoric water line. Even at the peak of the dry season, stable isotope values of plant xylem water from six different deciduous and evergreen species occurring in the understory and canopy of mature and secondary forests were all consistent with the use of shallow soil water (20-60 cm). Alternative limiting factors, such as nutrient distribution along the soil profile, may be strongly contributing to plant rooting patterns. Stable isotope values of soil water were evaporatively enriched compared to precipitation inputs, whereas stream values plotted with precipitation on the local meteoric water line. In contrast to current hydrologic models, this indicates the presence of two distinct soil water pools, one highly mobile pool of precipitation that contributes to streams and a second less mobile pool of soil water that contributes to plant fluxes. This comprehensive perspective can provide the basis for generating process-based models of ecohydrology in the future.

INTRODUCTION

The study of ecohydrology is critical to our understanding of tropical montane cloud forest ecosystem functioning and associated ecosystem services (Hamilton *et al.* 1995, Bruijnzeel *et al.* 2011). These rare and often highly biodiverse ecosystems remain seriously threatened by anthropogenic pressures such as land-use and climate change (Toledo-Aceves *et al.* 2011). Demand for natural resources and land conversion has led to the widespread and dramatic degradation and loss of tropical montane cloud forests worldwide (Bruijnzeel *et al.* 2011), such that regenerating and secondary forests will play an increasingly important role in the future. Additionally, tropical montane cloud forests are predicted to be especially vulnerable to climate change, experiencing novel, non-analog climates within this century (Williams *et al.* 2007). The best available research suggests that tropical montane cloud forests will likely experience drier, warmer climates with increased variability in the future (Pounds *et al.* 1999, Still *et al.* 1999, Lawton *et al.* 2001, Pounds *et al.* 2006, Karmalkar *et al.* 2008). Such changes may both directly and indirectly affect the ecohydrology of tropical montane cloud forests and additional knowledge is critical for the conservation of these ecosystems and the services they provide.

Despite the great need to better understand the ecohydrology of tropical montane cloud forests, the linkages among meteorological inputs, plant water fluxes and catchment hydrological pools remain poorly understood. This is a consequence of the paucity of data in these regions, as well as a past focus on quantifying only single components of the hydrologic cycle (e.g. rainfall and cloud water interception, or transpiration, or streamflow; Bruijnzeel *et al.* 2010). Stable isotopes provide a powerful tool for understanding the movement of water through catchments and ecosystems (Kendall and McDonnell 1998). By exploiting variation generated through the evaporation and mixing of different water sources, they can serve as tracers for the origin of water inputs, the flux of water within the system, and differentiation among different pools of water. Precipitation is the predominant form of input into the hydrologic cycle and differences in the origin of the precipitation (i.e. convective vs. orographic), the type of precipitation (i.e. rain vs. fog) and the effects of its movement across the landscape (i.e. elevation and latitude) all influence isotopic composition and create important variation over time and space (see review by Ingraham 1998). Transpiration is one of the predominant fluxes within the hydrologic cycle and the (general) lack of isotopic fractionation of water upon uptake by plants permits the identification of water sources used by any number of different plants occurring among different ecosystems over time (Dawson and Ehleringer 1998). Waters in soils, bedrock, and streams are among the predominant pools in the hydrologic cycle and changes in these pools can reveal differences in flow paths of water with varying precipitation inputs (Buttle 1994). Stable isotopes of water have not historically been applied as often in tropical ecosystems, presumably due to either a real or perceived lack of variation in water inputs from tropical sources (Bowen 2008).

Here we apply a dual stable isotope approach ($\delta^{18}O$ and δ^2H) to trace the inputs, fluxes, and pools that link the ecohydrologic cycle of a seasonally dry, tropical montane cloud forest in eastern Mexico. To better understand inputs, we first establish variation in seasonal precipitation and associated source effects over the course of two years (April 2008 – April 2010). To better understand fluxes within this ecosystem, we then use stable isotopes of water from plants and soils to establish the depth of dry season plant water use for different plant species of different sizes between mature and secondary forests. Finally, we join the precipitation, stream, soil and plant stable isotope data to establish the pools of water in these headwater catchments and their

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associated sources. Combined, we expected that the data would give us a perspective with which to provide a conceptual model for the flow of water through the ecosystem. Our primary research questions were: (1) Do seasonal differences in the origin of precipitation events lead to large differences in stable isotope composition of water inputs into the ecosystem? (2) Does reduced dry season water availability lead to the partitioning of water resources among plant species, as well as a shift to deeper soil water resources? And (3) Is the stable isotope composition of soil water consistent with precipitation and stream pools?

METHODS

Study Site

The study was carried out in two neighboring headwater catchments located in the tropical montane cloud forest belt on the eastern slopes of the Cofre de Perote volcano in central Veracruz, Mexico (19.45°N and 97.04°W; ~2100 m a.s.l.). The area partially belongs to the 107 ha "La Cortadura" Forest Reserve of the municipality of Coatepec. The mature forest catchment (25.6 ha) is dominated by evergreen and semi-deciduous broadleaf tree species including *Quercus ocoteifolia, Q. lanceifolia, Alchornea latifolia,* and *Clethra macrophylla*, with an abundance of *Miconia glaberrima* in the understory. The secondary forest catchment (11.9 ha) is characterized by a complex land-use history that includes conversion to pasture for cattle grazing and a wildfire that occurred approximately 20 years ago. It is dominated by the deciduous broadleaf tree species *Alnus jorullensis*, although a number of the mature forest species listed above are beginning to return. Both catchments are steep (20° - 45°) with deeply incised valleys, and are drained by first-order, perennial streams (Muñoz-Villers *et al.* 2012a).

Soils at the site are Umbric Andosols derived from volcanic ashes with silt loam textures (Campos-Cascaredo 2010). Soil profiles are deep and well developed, characterized by low bulk densities ($0.25 - 0.45$ g cm^{-3}), high porosities (~72%) and high capacity for residual water content (0.08 cm cm^{-3}) (Muñoz-Villers *et al.* 2012a). The underlying geology is composed of semi-permeable, moderately weathered, andesitic breccias interspersed with weathered and fractured andesitic-basaltic rocks (saprolite). Topographic, soil physical and vegetation characteristics of the study area are provided in greater detail in Muñoz-Villers *et al.* (2012a).

Climate at this latitude is strongly influenced by the trade winds and the subtropical high pressure belt (Metcalfe 1987). Between November and April, the proximity of the subtropical high leads to stable, dry weather conditions. However, during this time of year the study region is also affected by the regular passage of cold fronts that produce light rains and/or drizzle for 1−3 days per event (Báez *et al.* 1997). With the northward movement of the Inter-Tropical Convergence Zone (ITCZ) in the summer, the region comes under the influence of easterly trade winds that bring humid conditions with frequent showers and thunderstorms (Báez *et al.* 1997). Hence, the climate can be divided into two distinct seasons: a relatively dry season (November−April) and a wet season (May−October). Rainfall measurements at this site started in July 2005. Average rainfall for the four hydrological years between November 2005 and November 2009 was 3183 ± 306 mm per year, with 2628 ± 379 mm and 555 ± 204 mm falling during the wet and dry seasons, respectively. Hence, dry season rainfall is on average about 20% of the annual rainfall (L.E. Muñoz-Villers and F. Holwerda, unpublished data). Cloud water interception (i.e. fog and wind-driven drizzle) by the mature and secondary cloud forests was estimated to be ≤2% of the annual rainfall (Holwerda *et al.* 2010). Monthly mean temperatures

range between about 12 °C in January and about 16 °C in May, and the reference evapotranspiration (Allen *et al.* 1998) is approximately 850 mm per year (Muñoz-Villers *et al.* 2012a).

Inputs: Precipitation

To quantify precipitation inputs, rainfall was measured beginning in July 2005 in a small (0.5−1.0 ha) pasture area at 2128 m a.s.l., located in between the mature and secondary cloud forests, using a tipping bucket rain gauge (0.2 mm per tip) (Casella CEL, New Hampshire, USA). At the same location, rainfall was collected for stable isotope analysis using a 4 cm diameter by 40 cm long collection tube fitted with a 9.5 cm diameter funnel. The collection tube contained a float to prevent evaporation. In addition, the tube was inserted into a 7.5 cm diameter PVC pipe wrapped with bubble foil insulation to protect the water against direct sunlight and minimize temperature variations. The PVC tube was partly buried so that the opening of the funnel was at about 30 cm above the ground. The length of the sampling interval varied between 1 and 34 days, depending on rainfall amount and frequency. Samples of isolated rain events during the dry season were usually taken one to two days after the event. During the wet season, a sample was typically taken every three to four days, and daily during very rainy periods to prevent overflowing of the gauge. Here we do not try to distinguish between precipitation inputs from rain versus the capture of cloud moisture by vegetation, as this 'occult precipitation' contributes very little to the overall water budget (Holwerda *et al.* 2010).

Temperature was measured at a climate station located at the same pasture using a combined temperature and relative humidity sensor (HMP45, Vaisala, Finland). Measurements were made every 30 s and average data were recorded every 10 minutes using a data logger (CR1000, Campbell Scientific, Utah, USA). Monthly mean temperatures were calculated from the daily mean temperatures, which in turn were calculated as the average of the daily minimum and maximum temperatures.

To establish which atmospheric processes may be governing the seasonal variation in stable isotope values of precipitation, we tested a number of relationships between climate and precipitation isotopes. This included a general linear model (least-squares) of volume-weighted, monthly mean values of precipitation as a function of monthly mean temperature and monthly precipitation. We also calculated the deuterium excess (*d*-excess) of all precipitation collections as $d = \delta^2H - 8*\delta^{18}O$ (Dansgaard 1964). We then compared deuterium excess values of precipitation using a two-way ANOVA with season, year, and an interaction term between season and year as factors ($\alpha = 0.05$). All statistical analyses were carried out in JMP 8.0.2 (SAS Institute, Cary, North Carolina, USA).

Fluxes: Plant

To establish the functional rooting depth of different plants at our sites, xylem water and bulk soil water (see below) samples were obtained during the middle of the 2009 dry season (1-2 March). Xylem samples were taken from three individuals of each species in each forest type using an increment borer inserted into the bole at 1.3 m above ground. We sampled *Quercus lanceifolia, Quercus ocoteifolia, Alchornea latifolia,* and *Clethra mexicana* from the mature forest and *Alnus jorullensis, Alchornea latifolia, Clethra mexicana,* and *Miconia glaberrima* from the secondary forest. Although we tested the efficacy of Bayesian mixing models to

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quantitatively determine functional rooting depth, we found that they were unable to distinguish sufficiently among sources in the absence of suitable informed priors (i.e. root biomass or nutrient distributions). An assessment of depth of plant water use was thus made by directly associating the stable isotope value of plant xylem water with the soil depth at which the same stable isotope value of soil water occurred

To establish if there was a relationship between plant size and functional rooting depth, we sampled again during 4-5 May 2010 (end of dry season). Following methods established above, we obtained plant xylem water samples from at least 6 individuals of each species. For each individual, we also measured diameter at breast height (DBH) and height. Using additional xylem core samples, we established wood density following the methods of Chave *et al.* (2003). From this data, we derived a measure of aboveground biomass for each individual following the equation provided by Brown *et al.* (1989) for tropical trees >10 cm DBH:

$$AGB = \exp\left(-2.409 + 0.952\ln\left(\rho D^2 H\right)\right) \tag{1}$$

where *AGB* is aboveground biomass (in kg), ρ is the oven-dry wood specific gravity (in g cm^{-3}), *D* is the diameter at 1.3 m height (in cm), and *H* is the tree height (in m).

Pools: Soil and Stream

To establish the extent of isotope variation in our soil water pools, bulk soil water samples were collected during each plant sampling. Bulk soil water samples were obtained at 5, 15, 30, 50, 70, and 100 cm depths below the soil surface by establishing an open-face soil pit within 5 m of each species collected, then inserting a soil corer horizontally into the pit at the desired soil depth to avoid any potential evaporation occurring where the soil was exposed.

To establish if there were differences between more and less mobile soil water pools, soil water was collected during the 4-5 May 2010 plant and soil water sampling from porous cup lysimeters (more mobile) using a suction of about 60 kPa (Soil Moisture Equipment Corp., California, USA). At the top and middle of each catchment, a lysimeter was installed at each one of four different depths (30, 60, 90, and 120 cm), whereas a lysimeter was installed at each one of three different depths (30, 60, and 90 cm) at the bottom of the catchment where soils are shallower. We present spatially paired lysimeter and soil data from the upper catchment in the mature and secondary forests, as well as unpaired data from across the entire catchment for the mature forest alone.

To establish the extent of isotope variation in stream water pools, grab samples of baseflow were collected every two weeks at the streamflow gauging station of each catchment between 16 April 2008 and 17 April 2010. During the 2009 wet season, groundwater samples were taken from wells located near the stream (< 40 m) in the bottom third of the catchment, since these were the ones that showed a permanent water table through the wet period. The wells were pumped dry and allowed to recharge before a sample was taken. Soil-lysimeter and ground water sampling locations and methods are provided in greater detail in Muñoz-Villers *et al.* (2012b).

Isotope Analysis

All samples of precipitation, plant, soil, stream, and lysimeter water for isotope analysis were collected in 30 ml borosilicate glass vials with polycone sealing caps to prevent evaporation. All samples were refrigerated until their extraction and analysis.

Plant and soil water samples were extracted using cryogenic vacuum distillation following the methods of West *et al.* (2006). We used isotope ratio mass spectrometry (IRMS) for δ^2H analysis of plant and soil waters. Microliter quantities of water were injected into an H/Device (ThermoFinnigan, Bremen, Germany) coupled to a Delta Plus mass spectrometer (ThermoFinnigan, Bremen, Germany). Injected H_2O was reduced to H_2 gas in a hot chromium reactor and the $^2H/H$ ratio of this gas was then analyzed by mass spectrometry. For $\delta^{18}O$ analysis of plant and soil waters, 50 µl water samples were equilibrated in vials with a 0.2 % CO_2 headspace for 48 hours at 21-23°C. Following equilibration, vials were inserted into a GasBench II (GB, ThermoFinnigan, Bremen, Germany) connected to a Delta Plus XL mass spectrometer (ThermoFinnigan, Bremen, Germany). The GasBench II was modified with a 10-port injection valve, allowing a 0.2% CO_2 reference injection to follow each sample CO_2 injection.

We used isotope ratio infrared spectroscopy (IRIS) for δ^2H and $\delta^{18}O$ analysis of precipitation, stream and lysimeter water. Microliter quantities of water were injected into a vaporization chamber and then passed into an infrared absorbance cavity on a DLT-100 Liquid Water Isotope Analyzer (Los Gatos Research, California, USA). Isotope ratios (δ^2H and $\delta^{18}O$) are calculated from spectral absorbance in specific wavelengths using OA-ICOS. We analyzed a representative subset (10%) of these samples for spectral interference using Spectral Contaminant Identifier V1.0 (Los Gatos Research) using the factory preset criteria and found no evidence for organic contamination (West *et al.* 2011).

For all methods, isotope ratios are expressed in ‰ as:

$$\delta^N E = \left(\frac{R_{sample}}{R_{standard}} - 1 \right) * 1000 \qquad (2)$$

where N represents the heavy isotope of element E and R is the ratio of the heavy to light isotope ($^2H/H$ or $^{18}O/^{16}O$). Two calibration standards were used to adjust the delta values relative to V-SMOW and then validate between IRMS and IRIS methods. The long-term precision of the instruments is no greater than 1.1‰ for δ^2H and 0.15‰ for $\delta^{18}O$ (1σ). All sample extraction and analysis was performed at the Center for Stable Isotope Biogeochemistry at the University of California Berkeley and the Hillslope and Watershed Hydrology Lab at Oregon State University.

RESULTS

Inputs: Precipitation

In 2008-2009, a strong dry season began at the end of October 2008 and persisted through the end of March 2009 (Table 1; Figure 1). In the wet season preceding the onset of the dry season, there was 3192 mm of precipitation, whereas dry season precipitation totaled 434 mm and the average event size was 7.8 ± 8.8 mm (*SD*). In 2009-2010, the dry season was not as pronounced. Wet season precipitation totaled 2561 mm and dry season rainfall was nearly double that of the previous year, totaling 776 mm. The average event size during the 2009-2010 dry season (8.7 ± 11.2 mm) was only slightly higher than that observed for the 2008-2009 dry season; however,

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the total number of rainfall events was nearly double (89 vs. 55 in the 2008-2009 dry season). The average event rainfall intensity (calculated as total event rainfall divided by event duration) was three times higher for the wet season (3.7 mm h^{-1}) compared to the dry season (1.1 mm h^{-1}), reflecting the dominance of convective rainfall from cumulus and cumulonimbus clouds during the wet season as compared to low intensity rainfall from stratus clouds associated with the passage of cold fronts during the dry season. As a measure of moisture conditions at the time of plant and soil sampling, antecedent precipitation indices were calculated for the 15 days prior to both dates following the methods of Viessman et $al.$ (1989). The 15-day antecedent precipitation indices were 10.26 mm and 31.55 mm for the March 2009 and May 2010 plant and soil sampling dates, respectively.

Over the course of the two year collection period, precipitation varied by more than 130‰ for δ^2H (range: -122.6‰ to 11.5‰) and 18‰ for δ^{18}O (range: -17.6‰ to 0.6‰) (Table 1; Figure 1). Mean wet season precipitation events (δ^2H -38.7 ± 3.1‰ and δ^{18}O -6.8 ± 0.4‰ SE) were significantly depleted in both δ^2H and δ^{18}O as compared to dry season precipitation events (δ^2H -12.6 ± 2.6‰ and δ^{18}O -4.2 ± 0.3‰ SE) (Wilcoxon Rank Sum, n = 38 dry and 96 wet season observations, p <0.001). Large magnitude, isotopically depleted precipitation events occurred several times at the beginning and at the end of the wet seasons (Figure 1); these events originated from broad synoptic easterly depressions with significant rainout of heavy isotopes prior to landfall. This is also noted in an amount effect, whereby monthly precipitation demonstrated a significant, negative relationship with volume-weighted monthly mean isotope values (Table 1; δ^2H=-5.67 – 0.08*precip, r^2=0.63, p <0.0001) (Dansgaard 1964, Rozanski et $al.$ 1993). However, there was no relationship between isotope values of precipitation and mean monthly temperature, or the interaction between temperature and precipitation (p > 0.1).

Relative to the global meteoric water line (GMWL, Rozanski et $al.$ 1993; δ^2H=10 + 8*δ^{18}O), the composite local meteoric water line had a higher slope and intercept (2008-2010; δ^2H=18.5 + 8.21δ^{18}O). Moreover, the 2008-2009 LMWL demonstrated a steeper slope compared to the 2009-2010 LMWL (Figure 2). This was likely driven by high, dry season deuterium excess values in 2008-2009. Deuterium excess was significantly higher in 2008-2009 (18.6 ± 0.6‰) than in 2009-2010 (16.2 ± 0.5‰; F$_{1,126}$= 11.8, p <0.001) and significantly higher in the dry season (21.6 ± 0.9‰) as compared to the wet season (15.6 ± 0.4‰; F$_{1,126}$= 62.1, p < 0.0001). There was no significant interaction between season and year (F$_{1,126}$= 1.4, p > 0.2).

Fluxes: Plants

Bulk soil water sampled in March 2009 demonstrated a consistent pattern of increasing isotopic depletion with depth from the soil surface (Figure 3). Evaporative enrichment of shallow soil water is well documented as a function of excess evaporation from the shallowest soil layers relative to deeper soils (Allison et $al.$ 1983). However, variation among profiles at small scales (i.e. within the mature forest) is evident and likely reflects soil heterogeneity (Dawson et $al.$ 1998). Between forest types and among all species, nearly all plant species demonstrated plant xylem water stable isotope values consistent with water use from shallow soil depths (20-40 cm deep; Figure 3). Individuals of *A. latifolia* occurring in the secondary forest were alone in demonstrating stable isotope values consistent with slightly deeper soil depths; however, *A. latifolia* occurring in the mature forest did not demonstrate this pattern (Figure 3B and 3G).

There was a significant relationship between tree size (diameter and height) and δ^2H of tree xylem water samples for both linear and power functions, as well as a significant relationship between tree size (aboveground biomass) and δ^2H of tree xylem water samples for the power function ($p < 0.05$; Figure 4). We use water isotopes of δ^2H here as a proxy for depth of water use by roots, with more negative (depleted) values signifying deeper water use. The relationship with DBH (Figure 4A) was better described by the power function ($r^2 = 0.19$) as compared to the linear function ($r^2 = 0.10$), whereas for height it was similarly described by both the power function ($r^2 = 0.19$) and the linear function ($r^2 = 0.20$). The relationship with aboveground biomass ($r^2 = 0.18$), as described by the power function, was similar in strength to other size predictors. The removal of the outlying individual of *Q. ocoteifolia* with high aboveground biomass does not qualitatively change the results, which are only considered across species because insufficient sample size precluded the examination of individual species.

Pools: Soils and Streams

Stable isotope values of bulk soil water consistently fell below the local meteoric water line, with shallower soil samples demonstrating more enriched values (Figure 5). For both sampling dates, stable isotope values of plant xylem water clustered with values from shallow soil waters. In contrast to bulk soil water samples, stable isotope values of water collected from low-tension suction lysimeters for the week prior to soil sampling fell along the local meteoric line (Figure 6). Furthermore, the stable isotope values of water in the lysimeters demonstrated more depleted values with increasing soil depth. Although water in the lysimeters had not experienced evaporative enrichment, it still changed as a function of the different sources of precipitation that predominate at each respective soil depth.

Despite the large variation in the stable isotope values of precipitation inputs, stable isotope values of stream water catchments demonstrated low variation (Figure 5). Moreover, the range of stable isotope values between the mature (range: δ^{18}O -8.5 to -6.2‰ and δ^2H -51.0 to -35.3‰ and secondary (range: δ^{18}O -8.7 to -5.1‰ and δ^2H -51.2 to -33.1‰) forests over the two hydrologic years was very similar. This reflects complete mixing of water occurring within the large subsurface reservoir of the catchments delivered to the streams, with no evidence of evaporative enrichment (Muñoz-Villers *et al.* 2012b*)*. Further evidence for this mixing is seen in the average stable isotope values of the groundwater samples obtained in the mature forest in 2009 (*n* = 3; δ^{18}O -7.67 ± 0.10‰ and δ^2H 44.56 ± 1.32‰ SE), which closely resemble the 2009-2010 hydrologic year average stable isotope values for stream water across both catchments (*n* = 67; δ^{18}O -7.17 ± 0.10‰ and δ^2H -41.71 ± 0.42‰ SE)

DISCUSSION

Inputs: Isotopic variation of precipitation in the Tropics

Stable isotope values of precipitation demonstrated strong seasonal variation that differed between years. Isotopically depleted, wet season precipitation originating from large, easterly storm events appears to be subject to an amount effect, driven by the successive rainout of heavy isotopes prior to landfall (Dansgaard 1964*,* Rozanski *et al.* 1993). In contrast, isotopically enriched dry season precipitation originating from northwestern cold front storms appears to be

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more subject to local recycling, as indicated by significantly higher deuterium excess values (Gat 2000). This inter-annual variation in deuterium excess (~2.5‰) contributed strongly to differences in the LMWL during the two hydrologic years sampled. Deuterium excess reflects kinetic fractionation processes occurring during either the evaporation of water at lower humidity or re-evaporation of previously condensed water vapor. The deuterium excess values reported herein are consistent with others previously observed in tropical montane ecosystems (Rhodes *et al.* 2006, Scholl *et al.* 2007). As with the research of Rhodes *et al.* (2006) in the Costa Rican cordillera, we interpret that precipitation derived from re-evaporated sources (e.g. lowland evapotranspiration occurring west of the study site) likely forms a significant proportion of the observed dry season precipitation. However, the annual change in deuterium excess suggested to occur as a result of the shifting of the ITCZ in relation to regional, terrestrial water sources in that example are not completely analogous the ecosystem herein; the precise weather patterns and sources of re-evaporated water underlying these observations are cause for future study.

Global analyses of precipitation isotopes generally find low intra-annual variation (Bowen 2008) in the Tropics. However, variation is likely underestimated due to a recognized lack of reliable, long-term sampling. For instance, the Global Network of Isotopes in Precipitation (GNIP) database contains only two stations for Mexico, both of which ceased functioning in 1988 (IAEA/WMO 2006). Nevertheless, we found intra-annual differences of δ^2H 130‰ and δ^{18}O 18‰, which is consistent with the limited body of research from other regions in the mountainous Neotropics (Scholl *et al.* 1996, Rhodes *et al.* 2006, Lachniet and Patterson 2009, Scholl *et al.* 2009). As with previous observations, this appears to be driven by an amount effect associated with the source of the precipitation as noted above, as opposed to a temperature effect (Dansgaard 1964, Rozanski *et al.* 1993, Bowen 2008). Due to sampling design, we cannot rule out an elevation effect; additional research in the mountainous regions of the Neotropics is necessary to further our general understanding in this area (Scholl *et al.* 1996). Given both the observed variation and even a basic understanding of the physical processes driving differences, stable isotopes of precipitation provide the basis for understanding ecohydrologic inputs to tropical montane cloud forests, as well as to how they might be altered by land-use and climate change.

Fluxes: Leveraging isotopic variation for improved ecohydrological understanding

Stable isotope values of plant xylem water were consistent with plant water use from shallow soil depths. Previous research on depth of water use by plants in the Tropics has revealed differences among seasonally dry lowland forest ecosystems, including soil water use generally between 20 cm and 1 m (Jackson *et al.* 1995, Meinzer *et al.* 1999, Oliveira *et al.* 2005, Hartsough *et al.* 2008, Liu *et al.* 2010). However, both deep roots and deep water use by plants have also been reported (>1 m; Nepstad *et al.* 1994, Moreira *et al.* 2000). The most pronounced differences have been among different species within an ecosystem, where research has suggested soil water resource partitioning among species based on water use strategy (Jackson *et al.* 1995, Meinzer *et al.* 1999). We found little evidence for variation in inferred depth of water use among the six deciduous and evergreen species sampled. Moreover, given that the plant xylem water value reflects a volume-weighted mixing of soil water uptake, species-specific variation is even more likely to be minimal. Land-use differences do not appear to be affecting a strong change in ecohydrologic function, as is consistent with the findings of Holwerda *et al.* (2010) and Muñoz-Villers *et al.* (2012a).

Depth of plant water use also did not vary with the strength of the dry season or among forest types. Our initial plant and soil sampling (March 2009) occurred during an average dry season (Holwerda *et al.* 2010), when shifts to deeper soil water use would be most likely. Seasonal shifts in depth of water use have been inferred in other tropical ecosystems where decreasing surface soil water availability results in a shift to more stable, deeper soil water sources over the course of the dry season (Romero-Saltos *et al.* 2005). As noted below, the only apparent difference in depth of plant water use was between individuals of *A. latifolia* occurring between mature and secondary forest.

Despite a small range of plant xylem water stable isotope values (approx. δ^2H 30‰) and the further inference that depth of water use is directly correlated with δ^2H, we did find a negative relationship between our metrics of plant size and δ^2H across all species. Curiously, height and DBH were equally good predictors of rooting depth, as opposed to aboveground biomass, which has previously been demonstrated to correlate strongly with maximum rooting depth in water-limited systems (Schenk and Jackson 2002a). Moreover, a strong positive power function between DBH and δ^2H has previously been demonstrated, indicating that small plants establish deeper taproots to avoid soil water deficit (Meinzer *et al.* 1999). Our results find weak evidence for deeper depth of water use with increasing height and diameter, which may be a result of additional factors driving rooting patterns (i.e. physical anchoring, nutrient distribution, etc.).

Consistent use of shallow soil water by plants, irrespective of species, season, and land-use history, likely reflects patterns of water and nutrient availability in the ecosystem. Plants locate roots in order to access both water and nutrients. There is tremendous variation in the degree and severity of seasonality in tropical ecosystems. Previous work in seasonally dry tropical systems has focused on locations with less dry season precipitation and higher transpiration demand. For instance, while dry season precipitation averages approximately 20% of the total our system, it is 8% of the total on Barro Colorado Island, Panama (Meinzer *et al.* 1999) and 15% of the total in the eastern state of Pará, Brazil (Moreira *et al.* 2000). By contrast, the tropical montane cloud forest studied herein experiences precipitation through the dry season. The maximum dry season precipitation deficit (rainfall – reference evapotranspiration) over the last four years was only 24 mm, indicating that it remains fairly wet during the dry season (F. Holwerda, unpublished data). Moreover, cloud cover and lower temperatures mitigate transpiration demand. As such, water does not seem to be a strong limiting factor in this ecosystem.

In contrast, soil nutrients may be a stronger limiting factor and therefore a greater driver of root distributions. If this is the case, variation between the mature and secondary forests caused by differing land-use histories may explain the apparent differences in the depth of water use by *A. latifolia.* Weathered, old soils in the Tropics generally have thin organic layers. Nitrogen and phosphorous, two primary limiting macronutrients, have been demonstrated to have the highest concentration at soil depths <20 cm in other tropical montane rain forests (Cavelier 1992, Silver *et al.* 1994). For instance, both percent nitrogen and available phosphorous decline below the B horizon (~40 cm) at our site (D. Geissert, Pers. Comm). Relatively high water availability and nutrient concentrations near the soil surface are also consistent with measurements of root biomass distribution. The mean soil depth of 50% of all root biomass in tropical forests has previously been calculated as <20 cm, with 95% of all root biomass occurring at <95 cm soil depth (Schenk and Jackson 2002b). In tropical montane cloud forests, fine root biomass is highest <20 cm soil depth (Cavelier 1992, Vance and Nadkarni

1992). Our results, when contextualized with further information on seasonality, nutrient availability and root biomass distribution, are not surprising.

Pools: A two pool system

Stable isotope values of plant xylem water were not consistent with direct use of precipitation or stream water, but rather with soil water that was evaporated from precipitation inputs before plants used it. Moreover, stable isotope values of stream and ground water were not consistent with an evaporated soil water pool, but rather with values of precipitation. This provides evidence for two distinct pools of subsurface water; one highly mobile precipitation pool that quickly infiltrates through the site's highly porous soil and eventually contributes to the stream and a second, soil bound soil water pool derived from precipitation that is modified by evaporative processes and is then taken up by plants. This second, soil bound pool of water does not appear to be contributing significantly to stream or groundwater pools. Further evidence for these two distinct pools is provided by differences in the stable isotope values of water collected from low-tension lysimeters (highly mobile, unbound water) and bulk soils (less mobile, bound water).

The significance of the presence of two distinct pools of subsurface water has recently been highlighted by the research of Brooks *et al.* (2010). Working in a small, forested watershed in an ecosystem exposed to a moderate, Mediterranean climate, they found similar evidence for two distinct pools of subsurface water. In their ecosystem, Brooks and colleagues posit that precipitation arriving early in the wet season gradually refilled an empty soil water pool; this precipitation then persisted throughout the wet season despite additional inputs and was used by plants in the dry season. Brooks *et al.* (2010) further hypothesized that precipitation from the early rainy season remained "tightly bound" in smaller soil pores, whereas additional precipitation remained distinct from water in the smaller pores by draining rapidly through larger pores in gravity-driven preferential flow. Brooks *et al.* (2010) then contrast their findings with translatory flow, one of the predominant paradigms in ecohydrology and a principle tenant in the design of hydrologic models. Translatory flow posits that precipitation infiltrating the soil displaces existing soil water, pushing it deeper into the subsurface and eventually into the stream (Hewlett and Hibbert 1967). As with the research of Brooks *et al.* (2010), we find no evidence for the complete displacement of all the standing soil water in the soil profile with additional precipitation infiltration, but rather for some form of preferential flow through the soil that maintains the two distinct pools described above but has yet to be described in these systems.

However, there is longstanding evidence for the presence of two distinct soil water pools resulting from incomplete displacement of existing soil water pools. Hewlett and Hibbert (1967) do not imply the complete (100%) displacement of all water with additional inputs. The primary experimental data they cite in establishing the mechanism of translatory flow demonstrated that water with a tritium tracer added to a soil column at field capacity displaced 67% of the water in the column prior to the emergence of any tracer and 87% of the water prior to the peak of the tracer (Horton and Hawkins 1965). Additional evidence for incomplete displacement of soil water by additional infiltration inputs has long been established by tracer experiments measuring solute diffusivity and attributed to 'immobile' water pools (Biggar and Nielsen 1962, De Smedt 1979, De Smedt and Wauters 1986). Moreover, as has previously been noted (Buttle 1998), analytical solutions for the immobile water fraction can be obtained from both soil water retention curves (Bengtsson *et al.* 1987) and from isotope tracers (Gvirtzman and Magaritz 1986,

Bengtsson *et al.* 1987), with resulting quantitative differences attributed to differences in soil structure. Additional stable isotope research has also previously confirmed the presence of water pools with varying mobility associated with matric potential differences arising from soil structure (Landon *et al.* 1999). Finally, the differentiation of soil water pools as compared to the precipitation inputs and groundwater has been observed in a number of studies on plant water use, as well as in conceptual models (Williams and Ehleringer 2000, Gat and Airey 2006, Querejeta *et al.* 2007, Wang *et al.* 2010); however, the ecological focus of these studies has not incorporated streams. Whether or not current hydrologic models assume translatory flow and the complete mixing of soil water at any given depth, there is a large body of long-standing evidence that suggests translatory flow does not completely displace existing soil water pools.

Our results constitute an independent data set from a distinct ecosystem that can be compared and contrasted to the findings of Brooks *et al.* (2010). Although both ecosystems demonstrate similar soil profile patterns that are enriched as compared to the local meteoric water line, the mechanism generating this pattern likely differs. As with Brooks *et al.* (2010), we find that evaporation alone cannot fully account for all of the variation in soil water values, especially at deeper depths, where values must reflect inputs from highly depleted precipitation events occurring at either the onset or conclusion of the rainy season in both 2008 and 2009. Also, a gradual recharge of the soil water pool with successive early season rain events, as proposed by Brooks *et al.* (2010), is not likely to account for the soil profiles we found (Figure 3). It is possible; however, that large magnitude, depleted events occurring late in the rainy season serve to 'reset' the soil and that evaporatively enriched dry season precipitation events, as well as soil surface evaporation, then create the pattern observed. Finally, although both ecosystems demonstrate stable isotope values of plant xylem water that are enriched compared to the LMWL, the extent of enrichment differs. Relative to bulk soil water values, the stable isotope values of plant xylem water presented by Brooks *et al.* (2010) are more enriched than most of the soil water they measured (perhaps further evaporated). In contrast, our values lie entirely between the precipitation and bulk soil water values we obtained. As additional comprehensive studies emerge, these comparisons can serve to provide a framework for an improved understanding of ecohydrological processes.

A vision for future isotope ecohydrology in the Tropics

In addition to serving as a benchmark upon which to measure change in the future, our study and the application of additional studies using stable isotopes can serve as quantitative foundation for generating process-based ecohydrology models. In Figure 7, we consider the flow of water through the ecosystem in dual isotope space. We hypothesize that water entering the system is subject to shifts in isotopic composition as a function of seasonality. These varied precipitation inputs, once within the soil, experience evaporative effects (enrichment) that results in a soil water pool 'space.' This enriched soil water, some of which remains in a soil bound pool within pores of the soil matrix, serves as the primary source of water for plants (as compared to the rapidly infiltrating water discussed below). We imply nothing about how tightly the water is held within this soil bound pool, only that xylem water values are consistent with the soil bound pool and thus must be the location of plant roots. Stable isotope values of xylem water thus closely overlap the soil water pool 'space.' However, additional precipitation inputs entering the soil experience little or no evaporation as they rapidly infiltrate through more mobile (presumably larger) soil channels and eventually enter into the stream. This second pathway

allows rainfall events to effectively bypass the soil bound water pool used by plants prior to any significant mixing. Such a conceptual model provides a basis for understanding ecohydrology, as each component is driven by quantifiable physical mechanisms (e.g. evaporation, infiltration). Thus, each component can be compared over time and space and among different systems, ultimately leading to model parameterization.

We note that the addition and mixing of water isotopes in a continuous three-dimensional space over time is extremely complicated and the temptation to explain processes that are not synchronized in time and space must be avoided. For instance, in our model, the local meteoric water line reflects a year of precipitation inputs, whereas the stable isotope values of plant xylem water are often (although this is not necessarily always true) representative of only one or a few time points. Thus, explanations regarding the processes governing water movement through an ecosystem, which would then become components of any process-based model, must be applied on matching scales. Despite this, the application of stable isotopes for the understanding of ecohydrology is a growing field with promise for a quantitative understanding of how water moves through ecosystems both now and in the future.

CONCLUSIONS

Tropical montane ecosystems are predicted to experience unprecedented rates of climate change in the foreseeable future, even exceeding that predicted for lowland tropical ecosystems (Williams *et al.* 2007). Our dual isotope approach provided a comprehensive view into the coupled inputs, fluxes and pools of water associated with ecohydrologic cycling in a mature and secondary tropical montane cloud forest. Although we found evidence of strong intra- and inter-annual variation in precipitation inputs and their associated isotopic composition, there was little variation in the inferred depth of plant water uptake between forest types and among species in the dry season. The shallow soil water pool utilized by plants differed in isotopic composition from precipitation, stream baseflow and soil-lysimeter water pools. This coupled understanding of the movement of water through this ecosystem provides an example of how stable isotopes can be used in the Tropics, information about how such systems may be altered by future land-use and climate change, and a foundation for new advances in modeling.

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Table 1. Monthly volume-weighted mean stable isotope precipitation and deuterium excess values, as well as net monthly precipitation and average monthly temperature collected at a tropical montane cloud forest in central Veracruz, Mexico for the 2008-2009 and 2009-2010 hydrologic years.

				2008-2009	
Month	δ^2H ‰	δ^{18}O ‰	*d*-excess	Precipitation (mm)	Temperature (°C)
April	-5.13	-3.03	19.09	39	15.5
May	-26.47	-5.72	19.28	375	16.93
June	-71.41	-10.93	16.01	735	14.16
July	-75.05	-11.03	13.18	637	14.44
August	-24.69	-4.94	14.85	456	15.58
September	-68.83	-10.74	17.05	675	14.52
October	-40.75	-7.54	19.56	341	12.71
November	-5.46	-3.82	25.14	85	11.87
December	-3.16	-3.04	21.14	74	12.18
January	-26.81	-6.18	22.59	61	11.85
February	-6.95	-3.66	22.32	74	12.8
March	10.19	-1.6	22.99	24	13.11

				2009-2010	
Month	δ^2H ‰	δ^{18}O ‰	*d*-excess	Precipitation (mm)	Temperature (°C)
April	-15.07	-3.55	13.34	299	16.44
May	-14.47	-3.79	15.83	128	16.11
June	-72.93	-10.94	14.55	376	15.93
July	-31.63	-5.85	15.2	239	16
August	-32.37	-5.87	14.58	749	15.53
September	-56.83	-9.08	15.82	670	15.66
October	-37.18	-7.19	20.34	304	15.65
November	-21.93	-4.84	16.78	67	12.87
December	-20.47	-5.2	21.17	183	12.7
January	-24.29	-5.54	20.05	121	10.19
February	-25.7	-5.57	18.86	116	10.63
March	-0.76	-2.48	19.04	139	12.98
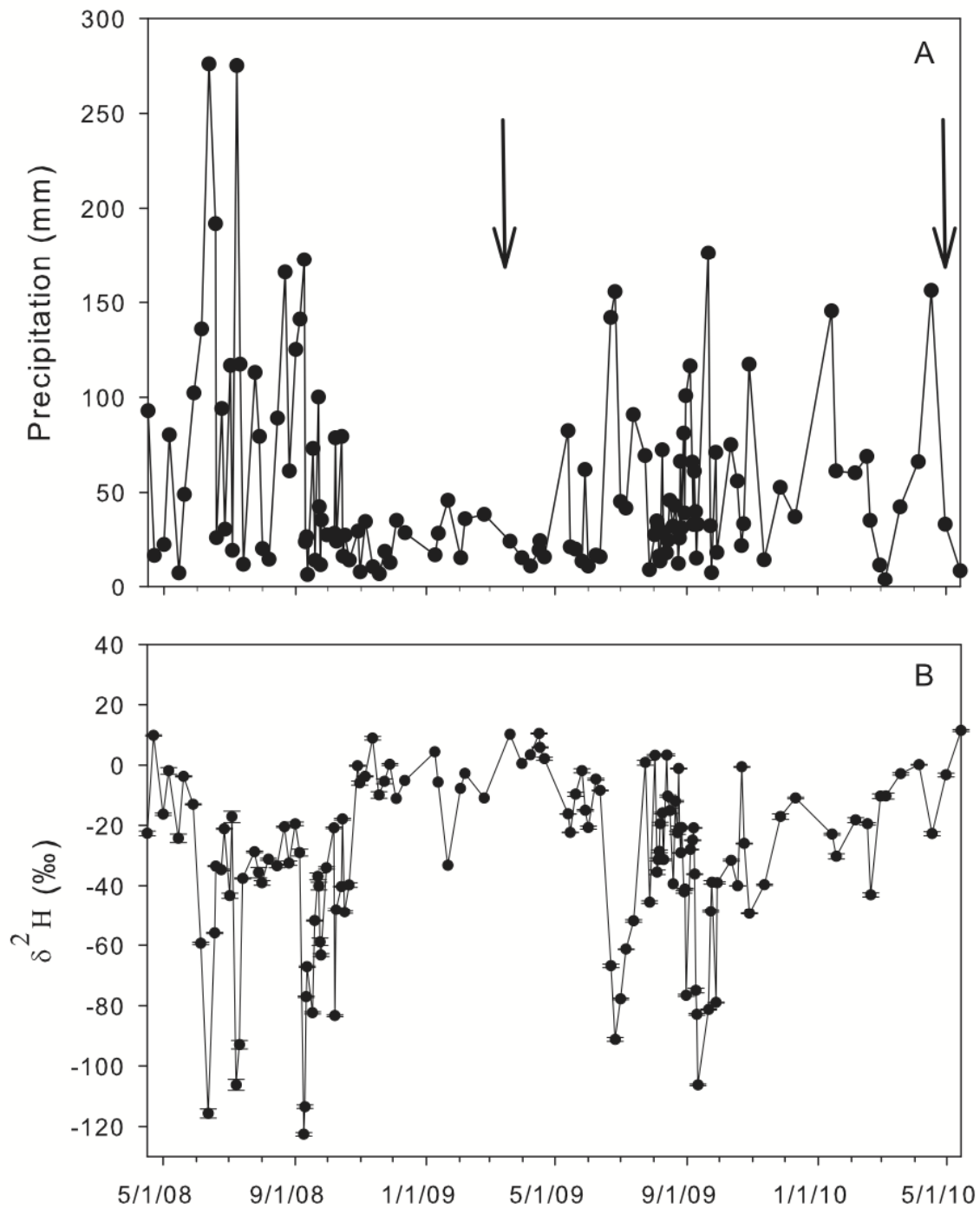
Figure 1. Weekly values for A) net precipitation and B) mean δ^2H ‰ ($\pm$ 1 SD) of precipitation samples collected at a tropical montane cloud forest in central Veracruz, Mexico. Arrows indicate soil and plant xylem water isotope sampling dates.
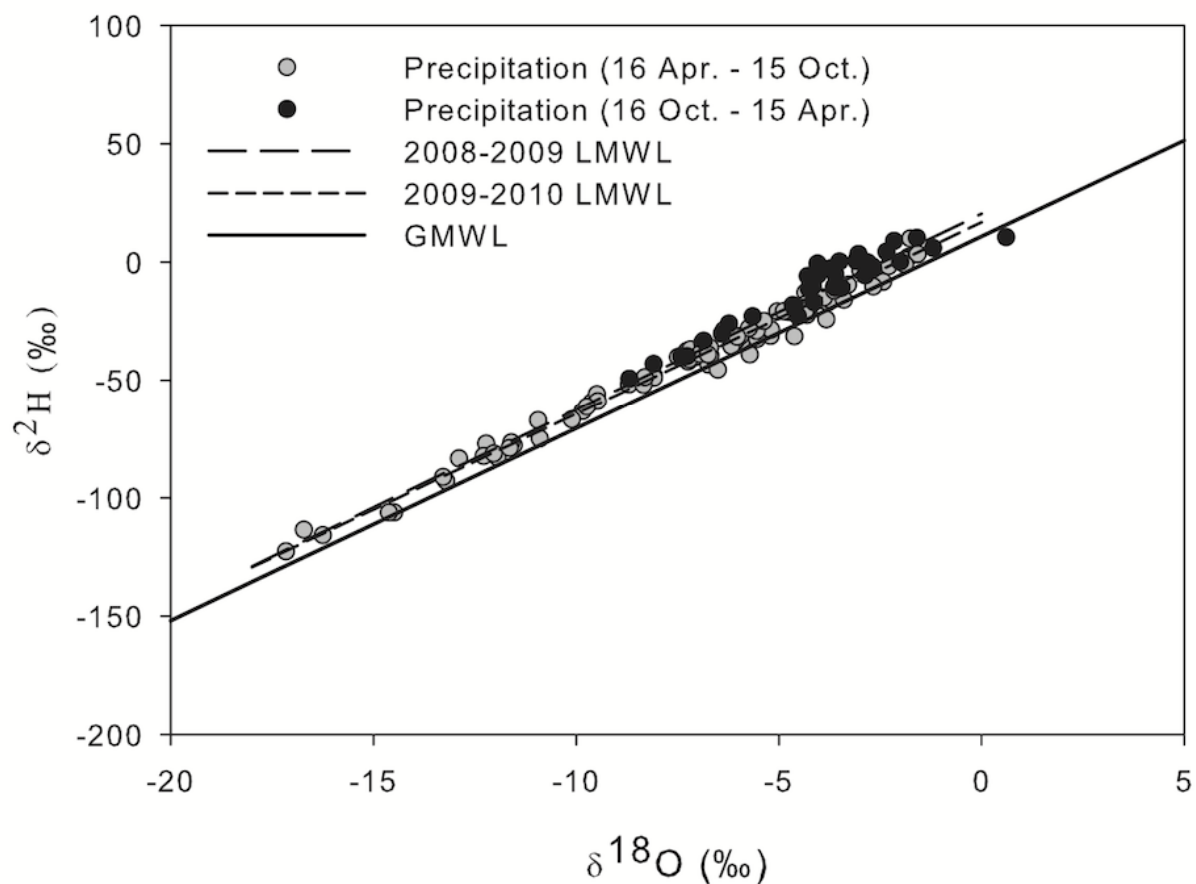
Figure 2. Water isotopes from wet and dry season precipitation, collected between April 2008 and April 2010. The lines represent the 2008-2009 local meteoric water line (LMWL; $\delta^2H = 21.0 + 8.36\delta^{18}O$, the 2009-2010 LMWL ($\delta^2H = 16.6 + 8.10\delta^{18}O$) and the global meteoric water line (GMWL; $\delta^2H = 10 + 8\delta^{18}O$). The long-term LMWL for the area is $\delta^2H = 18.5 + 8.21\delta^{18}O$.
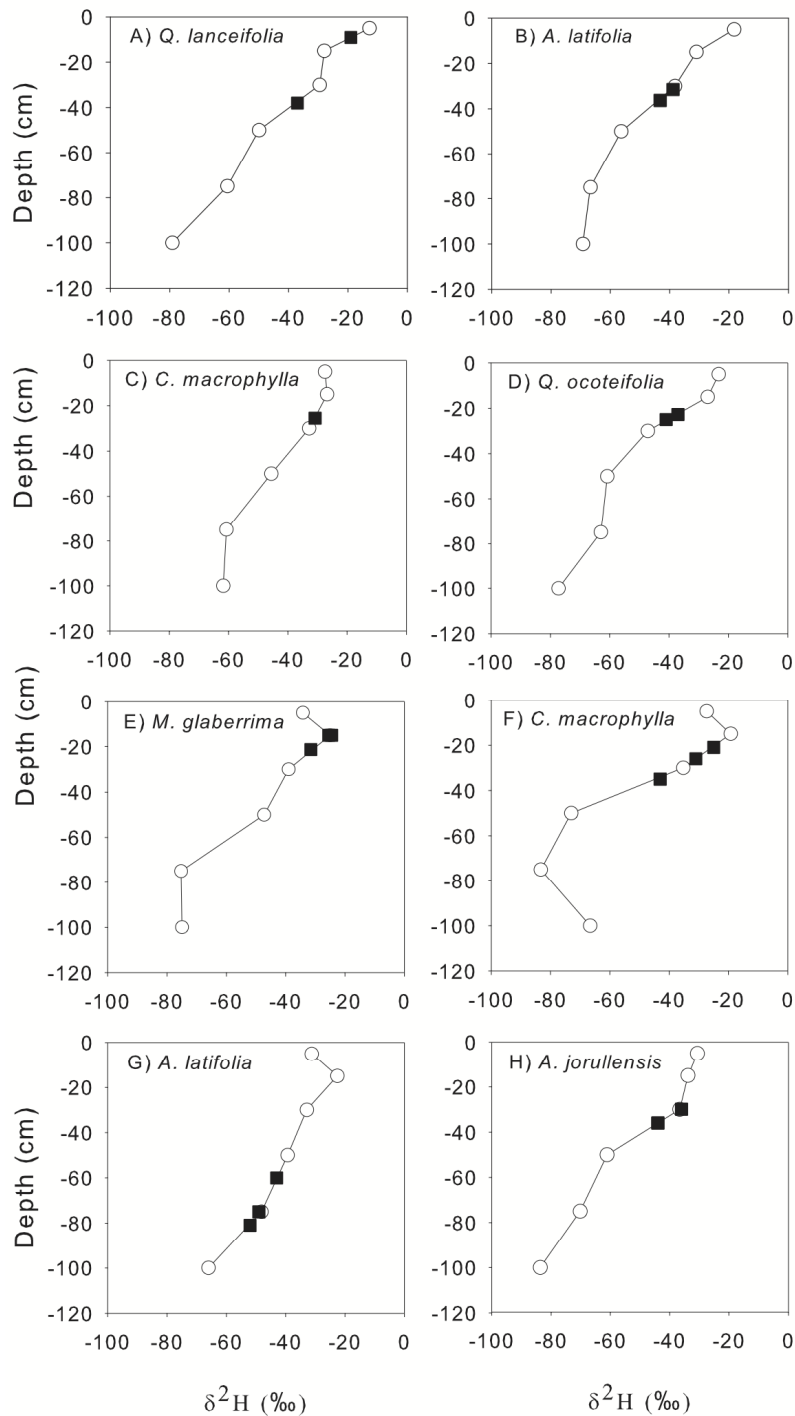
Figure 3. Stable isotopes of water (δ^2H ‰) from bulk soil samples collected at different depths (open circles) below the soil surface in mature (panels A-D) and secondary (panels E-H) tropical montane cloud forests sampled on 1 March 2009. Each line thus represents a distinct soil profile associated with the sampling of stable isotopes of xylem water from nearby trees (filled squares).
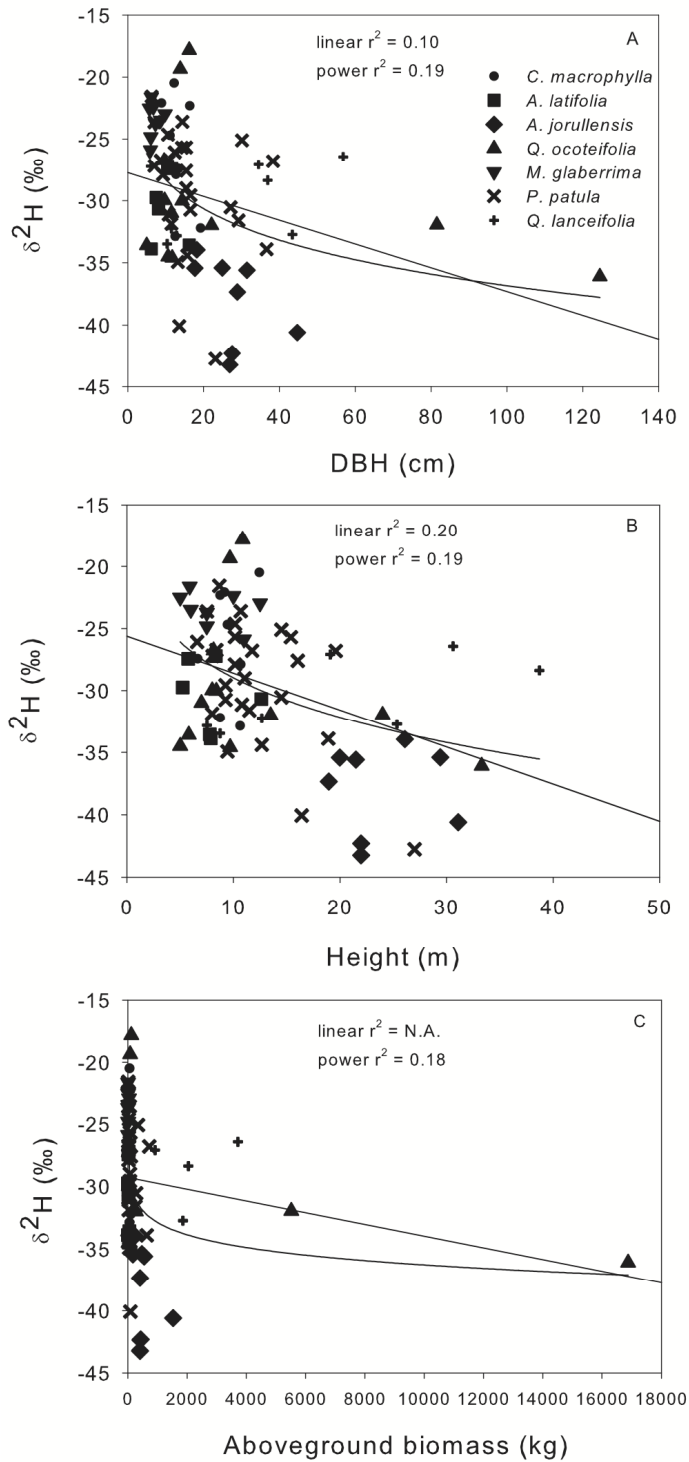
Figure 4. Relationship between δ^2H and measures of tree size including A) DBH, B) height and C) aboveground biomass, where δ^2H is a proxy for plant functional rooting depth. Lines represent linear (least-squares) and power functions.
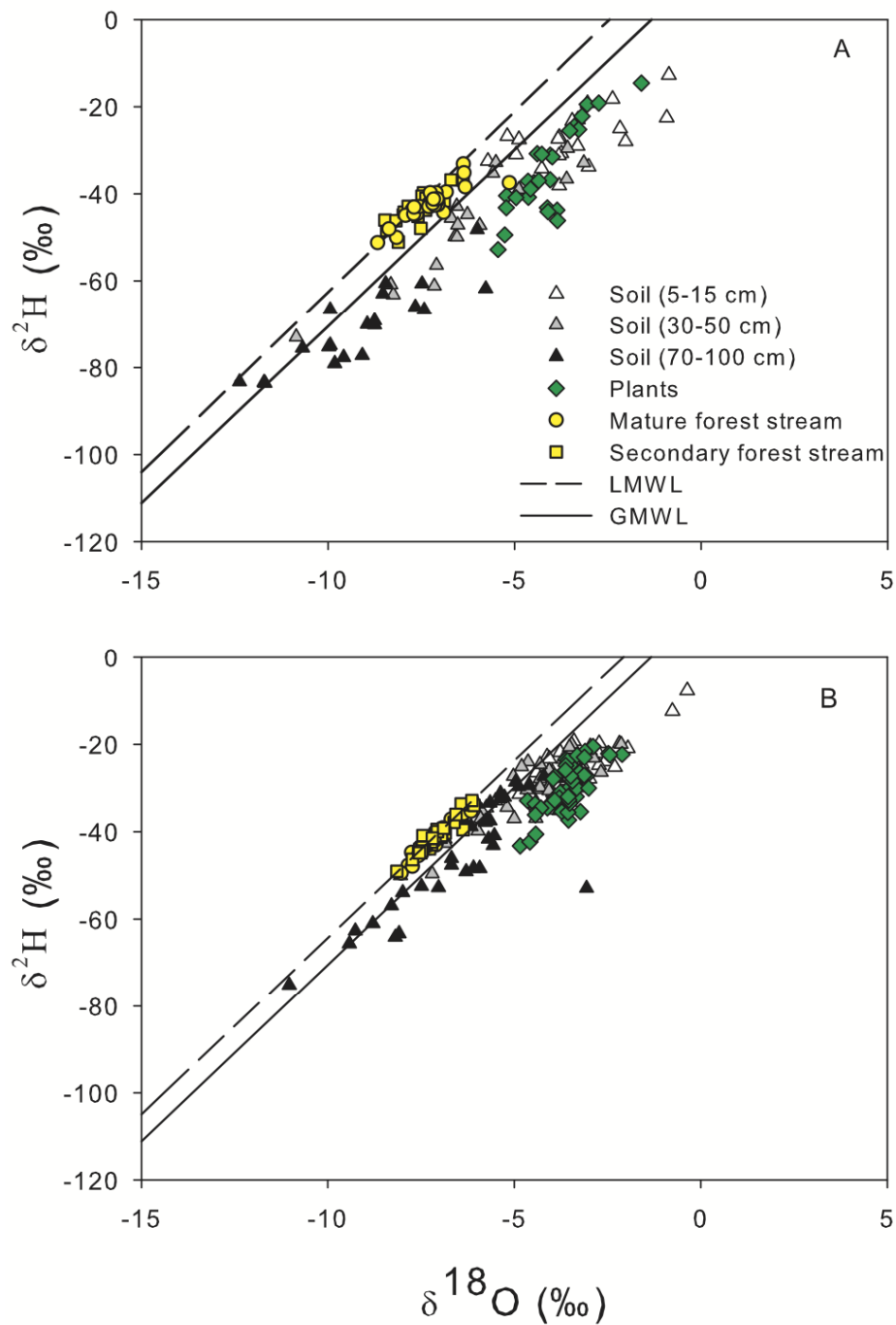
Figure 5. Stable isotopes of water collected from bulk soils, plants, and streams in mature and secondary tropical montane cloud forests. Soil and plant samples were collected in A) 1-2 March 2009 and B) 4-5 May 2010. Stream samples, as well as precipitation samples used to generate the local meteoric water line, were collected regularly during their respective hydrologic years.
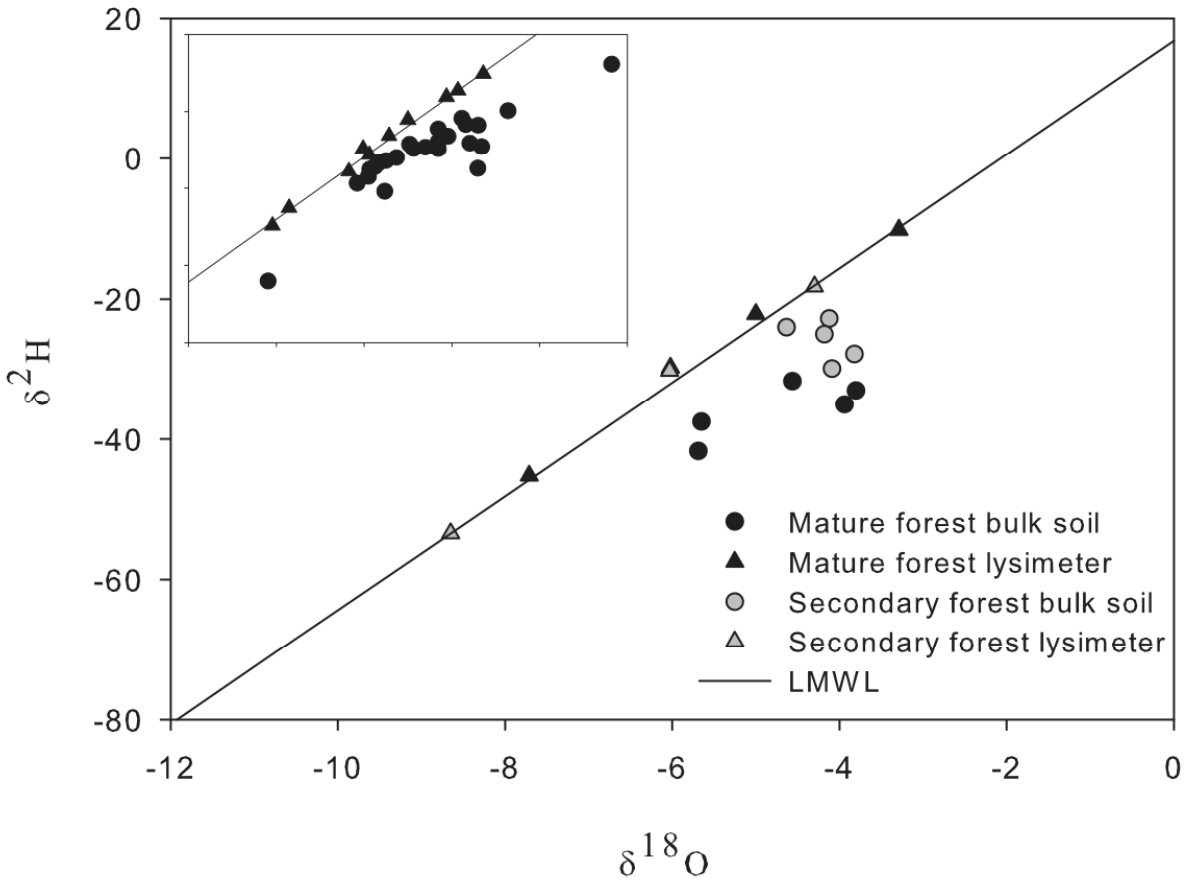
Figure 6. Water stable isotopes from lysimeter and bulk soil samples collected at different depths (5-120 cm) in mature and secondary tropical montane cloud forests. Lysimeter samples represent the accumulation of 7 days of water collected on 5 May 10, while all soil samples were collected nearby on the same date. Inset represents data collected across a larger spatial scale in the mature forest on the same date. The line represents the local meteoric water line for 2009-2010 ($\delta^2H=16.8 + 8.12\delta^{18}O$).
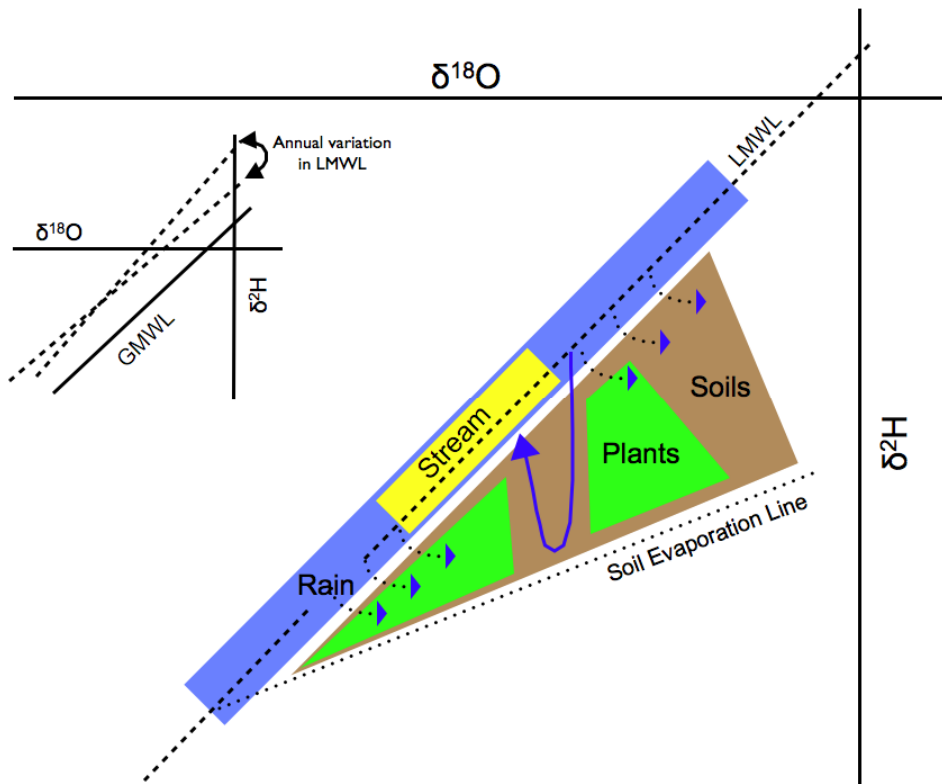
Figure 7. A conceptual model for understanding inputs, fluxes and pools of water moving through a tropical montane cloud forest as represented in dual isotope space. Inset represents inter-annual variation in the local meteoric water line generated by differences in precipitation inputs.

CHAPTER 2

Effects of experimental drought on tropical montane plant growth and physiology

ABSTRACT

Research on tropical plant response to seasonal changes in water availability to date has focused on lowland ecosystems; however, there is a critical need for information on plants from other tropical ecosystems. Recent modeling studies project that tropical montane ecosystems will experience greater rates of climate change than tropical lowland ecosystems, with strong decreases in dry season precipitation and increases in dry season temperature. To better understand tropical montane plant functional response to climate, I examined the impacts of a sustained reduction in water availability on the physiology and growth of tropical montane plant seedlings in the genus *Ocotea* using an experimental dry-down at a common site. I hypothesized that reduced water availability would result in increased plant water deficit, decreased gas exchange, and decreased growth and that these responses would differ among species occurring in environments of contrasting dry season severity. Despite only a small decrease in soil water potential (~0.2 Mpa), plants subject to experimental dry-down reduced their leaf stomatal conductance by up to 40% compared to plants in the control treatment. Additionally, plants in the experimental dry-down demonstrated significant decreases in height and new leaf growth. Results were species-specific and no significant patterns emerged among species from contrasting environments. Results from the experimental dry-down suggest that projected decreases in dry season precipitation are likely to have significant effects on the physiology and growth of the seedlings of the species examined. In tropical lowland ecosystems, dry season severity has been demonstrated to shape plant species distributions as a result of species-specific responses to reduced water availability. Our results provide evidence of how similar responses may shape current and future tropical montane plant distributions.

INTRODUCTION

Many tropical forests experience one or two drought periods annually, as well as more severe intermittent droughts (Walsh and Newberry 1999). Droughts of varying magnitude and duration, ranging from very short (Engelbrecht *et al.* 2006), to more typical (Poorter and Hayashida-Oliver 2000, Markesteijn *et al.* 2010), and ultimately to rare, extreme events (Cao 2000, Gilbert *et al.* 2001) have all been demonstrated to impact plant functioning. In particular, the negative effects of drought on seedling water status has been demonstrated to result in reduced growth and increased mortality (Turner 1990, Veenendaal *et al.* 1995, Burslem *et al.* 1996, Poorter and Hayashida-Oliver 2000, Gilbert *et al.* 2001, Marod *et al.* 2002, Engelbrecht *et al.* 2005, Slot and Poorter 2007). Moreover, because this coupling of seedling water status, growth and mortality is often species-specific, it leads to differences in survival-mortality dynamics among species experiencing the same drought conditions (Engelbrecht and Kursar 2003). These differences in drought sensitivity thus shape tropical forest species' composition at both local (Comita and Engelbrecht 2009, Paine *et al.* 2009) and regional spatial scales (Engelbrecht *et al.* 2005, Engelbrecht *et al.* 2007, Baltzer *et al.* 2008).

Despite the acknowledgement of the importance of drought in tropical forests, research on tropical plant response to seasonal changes in water availability to date has focused almost exclusively on lowland forest ecosystems (references as above, but see Dalling and Tanner 1995). However, tropical montane ecosystems are projected to experience rates of change exceeding those of lowland ecosystems and resulting in non-analog climates before the end of the century (Williams *et al.* 2007). In the mountains of Central America, the most striking projections center on decreases in dry season precipitation and increases in dry season temperature (Karmalkar *et al.* 2008). This increase in the severity of the dry season will thus decrease plant available water while simultaneously increasing plant water demand. There is a critical need for basic information on plant functional response to drought in tropical montane ecosystems, particularly in the context of current climate change projections.

Understanding plant response to seasonal changes in water availability in tropical montane ecosystems requires that investigations acknowledge some additional complexities. For example, in addition to high biodiversity and rates of endemism, montane ecosystems commonly demonstrate strong, compressed climate gradients with changes in elevation. While this gradient is most often cited with respect to decreases in temperature with increasing elevation as a function of adiabatic lapse rates (Colwell *et al.* 2008), mountains are also often subject to gradients in water availability due to orographic precipitation patterns and the effects of rain shadows (Clark *et al.* 2000, McCain and Colwell 2011). Striking patterns of turnover in species composition with elevation appear to correlate with the climatic changes along these gradients (Gentry 1988, Haber 2000, Barone *et al.* 2008). In fact, research suggests that in some mountains, species overlap between two neighboring life zones may be only 20% over the scale of a few hundred meters of elevation change (Haber 2000). Such complexities provide the opportunity to further investigate potential physiological mechanisms underlying plant distributional patterns across environmental gradients (Janzen 1967), providing valuable information about plant distributions under current climate regimes, as well as context for how they might be expected to change in the future.

Here I report the results of an investigation into the role of reduced water availability on the functional ecology of seedling and juvenile tropical montane plants using an experimental dry-down and accompanying field observations. I chose two co-occurring, mid elevation and two

co-occurring, lower elevation species of *Ocotea*, a common and widespread montane plant genus, for our experiments. This design allowed us to begin investigating the effects of seasonality for plants occurring in different climates, while controlling for traits shared among close relatives. Our premise was that the experimental response information I would gather would provide new insights into the putative physiological responses of these closely related tree species to projected climate change and that differential responses would also have implications for future species distributions. Our specific hypotheses were that 1) experimental dry-down of tropical montane plant seedlings would reduce growth as compared to plants in a watered, control treatment, as a result of declines in physiological functioning (e.g. reduced stomatal conductance) and 2) mid elevation species would demonstrate greater sensitivity to reduced water availability as compared to lower elevation species, as a result of differences in seasonality of their native growing environment.

METHODS

Study Site and Species

This study was conducted in tropical pre-montane wet forest on the Pacific slope of the Cordillera de Tilarán mountains near Monteverde, Costa Rica (10°18.287'N, 84° 50.132'W; 1268 m elev.). Climate in the area is generally characterized by three predominant seasons, which are largely controlled by the movement of the Intertropical Convergence Zone (ITCZ); a wet season (May-October), a transitional 'windy-misty' season (November-January), and a dry season (February-April). During the dry season, the predominant weather pattern derives from strong northeast trade winds in the Caribbean, which carry moisture over the Continental Divide. As a result, there is a significant gradient in dry season climate corresponding with distance from the Continental Divide, and thus elevation, along the leeward Pacific slope (Clark *et al.* 2000). Forests at high elevations (~1600 m) continue to experience significant cloud immersion and measurable precipitation during the dry season, whereas forests at lower elevations (~1100 m) experience minimal cloud immersion and little measurable precipitation (Goldsmith *et al.* in review).

To study the effects of seasonal changes in water availability on plant functioning, I chose evergreen tree species from *Ocotea* (Lauraceae), one of the most predominant genera in the region. As with species within many other genera, *Ocotea* species change rapidly with changes in elevation (Haber 2000). This facilitated the use of two common tropical pre-montane forest species (*Ocotea floribunda* and *Ocotea* "los llanos") and two common tropical montane forest species (*Ocotea monteverdensis* and *Ocotea whitei*).

Field Observations

To place the dry season conditions experienced by plants *in situ*, I made monthly measurements of plant available soil water at lower and mid elevation sites from February through May 2010. To do so, pre-dawn Ψ_L was measured on 5 saplings each of *O. floribunda* and *O. los llanos* at a lower elevation site (1284 m), as well as 5 saplings each of *O. monteverdensis* and *O. whitei* at a mid elevation site (1450 m). As no differences emerged between species at a given site, all individuals of both species were grouped together for the purpose of analysis.

Experimental Design

To investigate species response to reduced water availability, I collocated potted seedlings at a single site and performed an experimental dry down. Seeds of two lower elevation species (*Ocotea floribunda* and *Ocotea* "los llanos") and seeds from two mid elevation species (*Ocotea monteverdensis* and *Ocotea whitei*) were germinated from field collections in late 2007 and subsequently transferred from bags to 22 L pots in January 2010. Plants were potted in common, native organic soil. Following transplant, ~30 plants of each species were re-located from a shaded nursery to an open-side shade house fitted with black neutral shade cloth. Plants were randomly arranged and placed at least 1 m away from the edge of the shade house to minimize edge effects.

Beginning on 19 March 2010, I withheld water from half the individuals of each species (dry-down treatment), while maintaining the remaining individuals of each species above 20% soil moisture as a control (control treatment; effectively near field capacity). The early onset of the rainy season necessitated the periodic use of a clear plastic roof, which was removed following precipitation events; plants received no additional water as a result of this precipitation. Treatments were maintained for 50 days, after which the dry-down treatment received water (on 7 May) to monitor recovery. Due to some mortality, each species in each treatment began the experiment with between 14 and 16 individuals.

Environmental Monitoring

To further confirm the correspondence between dry season climate and elevation, I monitored bulk precipitation (S-RGB tipping bucket, Onset Corporation, Bourne, MA), photosynthetically active radiation (PAR; S-LIA sensor, Onset Corporation, Bourne, MA), and vapor pressure deficit (VPD; S-THB temperature and relative humidity sensor, Onset Corporation, Bourne, MA) in open fields at three different elevations during the 2009 dry season using meteorological stations with data loggers (Hobo MicroStation, Onset Corporation, Bourne, MA).

Temperature and relative humidity were monitored in the shade house and in ambient conditions at 20 min intervals using data loggers (U23, Onset Corporation, Bourne, MA). Ambient and shade house light was measured using 15 sec average measurements from a handheld PAR sensor (LI-250A, Li-Cor, Lincoln, NE). I present averages of PAR at 13:00 on days when physiological measurements were made. Volumetric soil water content was monitored weekly in each potted seedling using a handheld probe with 20 cm rods (Hydrosense, Campbell Scientific, Logan, UT).

To relate volumetric soil water content (θ) and soil water potential (Ψ_s), I generated a soil water retention curve on soil samples at varying levels of soil water content using a filter paper equilibration approach developed by Deka *et al.* (1995) and Power *et al.* (2008). This method permits the rapid and accurate generation of a soil water retention curve without intensive or destructive sampling. However, the filter paper method does not accurately describe the very high Ψ_s that occurs at high volumetric soil water content, so I augmented and validated these data with field data using pre-dawn leaf water potential (Ψ_L) measurements collected at random from plants over the course of the dry-down. Pre-dawn Ψ_L measurements serve as a proxy for Ψ_s provided that plant Ψ_L equilibrates with soil overnight in the absence of nighttime transpiration (Sellin 1999, Donovan *et al.* 2003). There is no evidence for nighttime transpiration in these

species (Goldsmith; unpublished data). Although I explored functions of varying complexity (see Warren *et al.* 2005), the data were best described with a modified quadratic function as follows:

$$\psi = \frac{a\theta + b - \sqrt{(a\theta + b)^2 - 4a\theta bc}}{2c} + d \qquad\qquad Eq\ 1.$$

Where a, b, c and d are parameters determined by the function. The function was fit to the combined field and filter paper data (n = 24 observations) using Excel (Microsoft Corporation, Redmond, WA). Thereafter, the equation was used to model Ψ_s from periodic volumetric soil water content measurements.

Growth Measurements

Plant growth response to reduced water availability was measured for a variety of traits at the beginning and end of the experimental treatment. These included stem height measured to the dominant apical meristem, number of expanding leaves, number of mature leaves, number of leaves demonstrating necrosis, number of leaves demonstrating > 50% herbivory, and number of leaves demonstrating > 50% yellowing.

Physiological Measurements

Plant physiological response to reduced water availability was assessed by quantifying midday stomatal conductance (g_s), which correlates strongly with photosynthetic capacity (Wong *et al.* 1979). Measurements were made on 8 individuals of each species in control and dry-down treatments between 12:30 and 14:30 using a steady-state porometer (SC-1, Decagon Devices, Pullman, WA). Diurnal measurements demonstrate no evidence of midday suppression of transpiration in these species (Goldsmith, unpublished data). Measurements were performed at the start, middle, and end of the experimental treatment on days with full, midday sun. I performed additional midday measurements after re-watering the dry-down treatment (9 March).

       Midday Ψ_L was measured on 3 individuals of each species in control and dry-down treatments between 12:30 and 14:30 using a Scholander pressure chamber (Soil Equipment Corporation, Santa Barbara, CA) at the beginning and again at the end of the experimental treatment. Leaves were wrapped in plastic film, excised, and immediately measured. Balancing pressure was recorded at the moment when   xudates appeared at the cut surface using a dissecting scope at 40x. On the day after re-watering, pre-dawn Ψ_L was measured (prior to 06:00 on 8 March).

Statistical Analysis

Due to differences among species, I calculated the relative growth rates (RGR) of stem height and number of mature leaves as: RGR = $(\ln[PT_{t2}] - \ln[PT_{t2}])/(t_2 - t_1)$, where *PT* is the plant trait of interest and t_1 and t_2 are the beginning and end of the experimental treatment in days. Differences in the RGR of stem height, the RGR of the number of mature leaves, proportion of expanding leaves, proportion of leaves demonstrating necrosis, proportion of leaves demonstrating > 50% herbivory, proportion of leaves demonstrating > 50% yellowing, plant Ψ_L and g_s were analyzed using two-way ANOVA (Standard Least Squares, with treatment, species

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and a treatment x species interaction). Two-way ANOVA was the most appropriate analysis because repeated measurements on the same individual were not always possible for all metrics and because data on differences between proportional data at two time points are not subject to the classic problems arising from the analysis of proportional data (e.g. equal variances) (Warton and Hui 2011). Due to limited statistical power, I did not include elevation as a factor. The relationship between RGR in stem height and g_s among species in the dry-down at the conclusion of the experiment was analyzed using a linear regression model (Standard Least Squares). Therein, g_s and species were used as the predictors and RGR as the response; an interaction term (g_s x species) was not significant and therefore not included in the final model. Data met all assumptions of normality and homogeneity of variance. All analyses were performed with JMP v8.0 (SAS, Cary, NC).

Results

Synoptic Climate

Mean monthly PAR and VPD (measured between 06:00 and 18:00) increased with decreasing elevation (Table 1). Average monthly precipitation decreased with decreasing elevation; however, the average event size (± SD) did not vary widely among the low (3.5 ± 5.7 mm), middle (4.8 ± 9.4 mm) and high elevation sites (4.9 ± 7.2 mm) sites. The maximum number of consecutive days without measurable precipitation increased with decreasing elevation.

Field Observations

Pre-dawn Ψ_L, the index used for soil water availability, decreased over the course of the dry season at the mid-elevation site, while remaining relatively steady at the lower elevation site (Figure 1). Differences in soil texture may result in lower water retention at the mid elevation site and thus greater seasonal flux in soil water availability. The early onset of the rainy season facilitated increased pre-dawn leaf water potential at both sites at the time of sampling in May.

Environmental Monitoring

Shade cloth resulted in an average 92% reduction in PAR as compared to ambient conditions and is thus similar to light availability in the understory (Goldsmith; unpublished data). Average measurements (± SE) of PAR (149 ± 5 µmol m^{-2} sec^{-1}, n = 20 each day), temperature (24.6 ± 0.3 °C, n = 7 each day), and relative humidity (68.8 ± 0.7, n = 7 each day) were stable across the four days when physiological measurements were made. The quadratic function used to model the relationship between θ and Ψ_s provided a strong fit (r^2 = 0.88; fit parameters: a = 0.166, b = 1.881, c = 0.99, d = -1.977) with good agreement between field and experimental data (Figure 2). Across all species in the dry-down treatment, soil water potential decreased from an average (± SE) of -0.11 Mpa ± 0.01 to -0.31 Mpa ± 0.02 after 50 days (Figure 3). This corresponds to an average 66% reduction in volumetric soil water content. *O. monteverdensis* tended to dry slightly more slowly than other species. Over the duration of the experimental treatment, the average soil water potential across all species in the control treatment was -0.11 Mpa ± 0.01.

Growth

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At the outset of the experiment, species differed significantly in height (Wilcoxon Rank Sum; χ^2 = 78.7, df = 3, p < 0.0001) and number of mature leaves (Wilcoxon Rank Sum; χ^2 = 37.0, df = 3, p < 0.0001). *O. floribunda* (15.17 ± 0.86 cm) and *O. los llanos* (15.93 ± 0.88 cm) were shorter than *O. monteverdensis* (28.13 ± 0.89 cm) and *O. whitei* (28.64 ± 0.91 cm). *O. whitei* (22.1 ± 11.2) had more leaves than *O. monteverdensis* (15.6 ± 8.7) and *O. los llanos* (12.7 ± 5.2), while *O. floribunda* had the fewest (9.0 ± 2.4).

Plants in the dry-down treatment demonstrated significantly reduced relative growth rates of stem height compared to the control treatment plants (p = 0.004, Figure 4a). Additionally, there were significant differences in growth among species (p < 0.0001, Table 2). There were no apparent patterns between mid and lower elevation species. The dry-down treatment also resulted in variable and species-specific effects on leaf patterns (Table 2). Comparing the proportion of new, expanding leaves at the start and end of the experiment, plants in the dry-down treatment had significantly fewer expanding leaves compared to the control treatment (p = 0.022, Figure 4b). This difference was driven by significant reductions in *O. floribunda* and *O. monteverdensis,* whereas *O. los llanos* and *O. whitei* showed no change in the mean proportion of expanding leaves between control and dry-down treatments. As such, there was no significant difference among species (p = 0.601). There were no significant differences in the relative growth rate of the number of mature leaves, proportion of leaves impacted by herbivores, and proportion of yellowing leaves between the start and end of the experiment. However, plants in the dry-down treatment demonstrated a tendency towards less leaf herbivory than those in the control treatment (Figure 4c) and plants in the control treatment appeared more palatable over the course of the experiment. Plants in both treatments experienced an increase in leaf necrosis between the start and the end of the experiment (Figure 4d). While there was no significant difference in necrosis between plants in control and dry-down treatments (p = 0.263), there was a significant species effect driven by relatively high signs of necrosis in *O. floribunda* (p = 0.001). In the dry-down treatment, there was a noticeable trend towards increased leaf necrosis in *O. monteverdensis* and *O. whitei*. There were no apparent differences in leaf growth between mid and lower elevation species.

Physiology

Midday stomatal conductance was statistically indistinguishable between treatments at the outset of the experiment (Figure 5); however, by the conclusion of the experiment, plants in the dry-down treatment had significantly lower midday g_s compared with the control treatment (Table 3; Figure 5). On average, there was an approximately 1.1% day^{-1} reduction in g_s, such that g_s in the dry-down treatment averaged 40.1% lower than that of the control treatment just prior to re-watering. This pattern was not as clear in *O. whitei,* which also had a lower intrinsic stomatal conductance than all other species over the course of the experiment (Figure 5d). Intrinsic rates of g_s in the control treatments were highest in *O. floribunda* and *O. monteverdensis,* followed by *O. los llanos* and finally *O. whitei.* Following re-watering, there was no significant difference in midday g_s between the dry-down and control treatment (p = 0.4). However, there was a significant difference among species whereby *O. whitei* continued to demonstrate significantly lower g_s than all other species (p < 0.05, Tukey's HSD). Species identity (f = 4.41, p = 0.01), but not relative growth rates of stem height (f = 3.14, p = 0.09), was significantly related to g_s measured on the final day of the experiment in the dry-down treatment (n = 31 plants).

Midday leaf water potential was statistically indistinguishable between treatments at the outset of the experiment (Figure 6a); however, the dry-down treatment induced significantly more negative midday Ψ_L in all species by the conclusion of the experiment (Table 3; Figs 6b, 6d). Mean midday Ψ_L in the dry-down treatment (-0.92 Mpa ± 0.05 SE) was nearly double that of the control treatment (-0.58 Mpa ± 0.11 SE). Following re-watering, pre-dawn leaf water potential was statistically indistinguishable between the control and dry-down treatments (Table 2; Figure 6c). There were no apparent differences in physiology between mid and lower elevation species.

DISCUSSION

Using an experimental dry-down, I assessed the effects of a sustained reduction in water availability on tropical montane plant growth and physiology. Despite the seemingly small decrease in soil water potential, plants from three of the four species demonstrated reductions in growth and significant down regulation in water use compared to plants in the control treatment. Our study provides evidence that even mild water stress can markedly impact tropical montane plants. I discuss the implications of our findings in light of how they may impact plants under projected scenarios of climate change.

Reduced Water Availability Impacts Growth and Physiology

As hypothesized, reduced water availability resulted in reduced growth, as well as a decline in water use. Changes in growth and physiology (g_s) occurred despite only a small decline in soil water potential, which averaged only -0.31 Mpa at the conclusion of the experimental dry-down. The average soil water potential measured at the end of the experimental dry-down was thus less negative than the average soil water potential experienced at either the low (-0.46 Mpa) or mid elevation (-0.78 Mpa) sites at the peak of the dry season; however, the magnitude of change observed in the dry down experiment was similar to seasonal changes observed in the field. Nevertheless, the experimental dry-down demonstrated that species-specific reductions in growth and physiology result from mild water deficit; had I relied solely on field observations to assess plant responses, I may have had difficulty in assigning causality because of additional and possibly confounding effects caused by other abiotic factors (e.g. vapor pressure deficit).

Plants subjected to experimental dry-down demonstrated significant decreases in g_s, with some species reducing g_s by up to 40% compared to plants in the control treatment with accompanying decreases in leaf water potential. Research on tropical lowland species has demonstrated clear reductions in g_s in response to reduced water availability over time (Bonal and Guehl 2001, Slot and Poorter 2007), as well as decreases in xylem and leaf water potentials (Veenendaal *et al.* 1995, Burslem *et al.* 1996, Markesteijn *et al.* 2010). Additionally, some species demonstrated significant decreases in both height and new leaf growth. Research regarding changes in growth in response to reduced water availability has been more equivocal, although there is a general trend towards decreased height growth and new leaf expansion. The lack of clear response in other studies is likely due to additional and potentially confounding factors, such as differences in the timing of relatively fixed leaf flushes or height growth (Wright 1991, Poorter and Hayashida-Oliver 2000, Yavitt and Wright 2008), or changes in above- and below-ground biomass allocation associated with reduced water availability (Burslem *et al.* 1996).

A range of different responses to the experimental dry-down were detected among the four congeneric species investigated. For instance, compared to other species, *O. whitei* demonstrated only a small response to reduced water availability. Rather, it maintained lower basal rates of growth and physiology, indicating a more conservative strategy. Nevertheless, *O. whitei* did demonstrate small decreases in midday leaf water potential and increased leaf necrosis, which may have become more pronounced if further drying had been induced. In contrast, *O. floribunda* and *O. monteverdensis,* which had higher basal rates of growth and physiology, demonstrated strong responses to reduced water availability. Both demonstrated reduced height growth and produced fewer new leaves, while reducing g_s and demonstrating decreased leaf water potential. The response of *O. los llanos* fell between these two groups, demonstrating physiological declines, but no changes in growth. Previous research in the lowland tropics has established a continuum of strategies associated with tropical tree seedling drought performance (Engelbrecht and Kursar 2003, Kursar *et al.* 2009). Even with a mild water deficit, I demonstrate that differences emerge among species within a functional response category (i.e. drought tolerance *sensu* Kursar *et al.* 2009). The species-specific responses I observed could thus influence differential survival/mortality dynamics among seedlings from different tropical montane plant species. Although our observations are confined to seedlings and are thus, in a quantitative sense not necessarily applicable to other life history stages, seedlings are generally considered a vulnerable life history stage (Tyree *et al.* 2003).

Based on ideas first put forth by Janzen (1967), I originally hypothesized that species occurring where there is less climatic seasonality (e.g. higher elevations), would demonstrate greater sensitivity to reduced water availability compared to species occurring where there is greater climatic seasonality (low elevations). Specifically, Janzen (1967) proposed that species occurring in climatically stable environments in the Tropics would exhibit a narrower range of physiological tolerances than species occurring in more climatically unstable environments. From this proposal has emerged the hypothesis that the uniformity of climate at any given elevation on a tropical mountain and the lack of climatic overlap with other elevations promotes niche differentiation and speciation (see review by Ghalambor *et al.* 2006). For instance, the genus *Ocotea* has more than 20 species occurring across the elevation gradient used in this study. Specifically, distributions of *O. floribunda* and *O. los llanos* are centered at lower elevations and distributions of *O. monteverdensis* and *O. whitei* are centered at higher elevations (Haber 2000, Goldsmith, pers. obs.). Our results indicate that higher elevations in Monteverde are not necessarily synonymous with less seasonality, as the rain shadow is likely not the only influence on seasonal water availability (e.g. the possible role of soil texture on soil water availability). Ultimately, no clear pattern of growth or physiological response between mid and lower elevation species emerged; site-independent differences among species appear to be much stronger. Slot and Poorter (2007) report similar results, concluding that seedling response to experimentally induced drought was more tightly linked to species-specific characteristics than to dry or moist forest origin. Working on a climate gradient across the Isthmus of Panama, Engelbrecht *et al.* (2007) demonstrated that differential sensitivity of seedlings to drought shaped distribution patterns. Species with higher drought sensitivity tended to occur on the wetter side of the isthmus; on a local scale, species with higher drought sensitivity tended to occur on wet slopes as opposed to dry plateaus. While our results do not clearly support these findings, additional research designed to test tolerance limits and induce mortality across a variety of tropical montane species will likely prove more informative to our understanding of how seasonal water availability contributes to species distributions.

Re-watering Facilitates Rapid Physiological Recovery

Although episodic precipitation events can occur throughout the dry season, such events are often insufficient to induce a measurable change in soil water content. The average precipitation event is often insufficient in magnitude to exceed canopy or litter storage capacity. As a result, there is a general trend towards decreasing soil water potential over the course of the dry season. However, some precipitation events may be large enough to temporarily increase soil water availability and thus improve physiological functioning. For the experiment presented here, re-watering facilitated rapid physiological recovery in all species. Pre-dawn leaf water potentials of plants in the dry-down treatment were indistinguishable from those in the control treatment only 12 hrs after water addition. Although I waited an additional day prior to measuring stomatal conductance in re-watered plants, these results also indicated rapid recovery of functioning in all species. Given the low magnitude of the water deficit induced by the dry-down, it seems likely that plants did not experience a catastrophic reduction in hydraulic functioning that would thus impair recovery (Tyree *et al.* 2003).

CONCLUSIONS

Implications of a changing climate

Climate modeling studies for the region project an increase in dry season temperature and a decrease in dry season precipitation, with an accompanying increase in the variability of both (Karmalkar *et al.* 2008, Karmalkar *et al.* 2011). Moreover, observational studies have recorded an increasing number of consecutive dry season days without rain over the last three decades (Pounds *et al.* 1999, Pounds *et al.* 2006). These changes are accompanied by an upslope shift in the base elevation of cloud cover driven by rising sea surface temperatures and the effects of lowland deforestation (Still *et al.* 1999, Lawton *et al.* 2001). A decrease in cloud cover increases the severity of a drought, as the presence of clouds reduces transpiration demand and serves as an alternative source of plant available water in the absence of direct rainfall (Goldsmith *et al.* 2012). Taken together, the best available evidence suggests that changes in temperature, precipitation, and clouds will result in longer and more severe dry periods. In this context, the experimental dry down imposed provides a first effort to understand the functional response of tropical montane plants to a prolonged drought. While the maximum number of consecutive days without measurable precipitation at the low elevation site during the 2009 dry season was only 17 days, the site received just 1.2 mm during the month of April; droughts of a similar duration to the one imposed in this experiment are likely to occur under projected scenarios of climate change. Our results suggest that projected changes in the frequency and magnitude of dry season precipitation and cloud events in the tropical mountains of Costa Rica are likely to have significant effects on the physiology and growth of juvenile *Ocotea* spp. As climate information for tropical montane ecosystems improves and is incorporated into new data- and model-driven research, complementary studies focused on plant functional response to environment will be critical for understanding the future of these rare and highly diverse ecosystems.

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Table 1. Precipitation, the maximum number of consecutive days without precipitation, photosynthetically active radiation (PAR), and daytime vapor pressure deficit (VPD) measured between 1 February and 1 May 2009 at three elevations on the Pacific slope of the Cordillera de Tilarán mountains near Monteverde, Costa Rica. PAR and VPD were measured between 06:00 and 18:00. Where appropriate, data represent monthly means ± SD.

Site	Precipitation (mm)	Maximum # of consecutive days without precipitation	PAR (mmol m-2 sec-1)	Daytime vapor pressure deficit (Kpa)
Lower elevation (1015 m)	29 ± 29	17	1178 ± 82	0.84 ± 0.36
Mid elevation (1415 m)	69 ± 74	8	892 ± 66	0.37 ± 0.12
High elevation (1544 m)	100 ± 78	7	340 ± 129	0.14 ± 0.06

Table 2. Summary statistics for two-way ANOVA of plant growth characteristics between control and experimentally-imposed dry-down treatments in four species of *Ocotea*. Numbers represent the F Ratio, the degrees of freedom, and significance as indicated by *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

Trait	Species	Treatment	Species x treatment
RGR of stem height	$14.33_{3,110}$***	$8.66_{1,110}$**	$1.44_{3,110}$
Proportion of expanding leaves	$0.71_{3,110}$	$5.64_{1,110}$*	$2.92_{3,110}$*
RGR of mature leaves	$1.18_{3,110}$	$0.68_{1,110}$	$0.75_{3,110}$
Proportion of herbivorized leaves	$1.04_{3,110}$	$3.28_{1,110}$	$0.11_{3,110}$
Proportion of necrotic leaves	$5.63_{3,110}$**	$1.35_{1,110}$	$0.82_{3,110}$
Proportion of yellowing leaves	$2.92_{3,110}$*	$1.02_{1,110}$	$0.55_{3,110}$

Table 3. Summary statistics for two-way ANOVA of stomatal conductance and leaf water potential between control and experimentally-imposed dry-down treatments in four species of *Ocotea.* Numbers represent the F Ratio, the degrees of freedom, and significance as indicated by *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

	Time point	Species	Treatment	Species x treatment
g_s	*Start*	$2.37_{3,56}$	$0.07_{1,56}$	$0.17_{3,56}$
	End	$3.62_{3,56}$*	$17.77_{3,56}$***	$0.08_{1,56}$
	Re-watering	$6.56_{3,56}$***	$0.63_{1,56}$	$0.87_{3,56}$
Ψ_L	*Start*	$1.31_{3,16}$	$0.55_{1,16}$	$1.08_{3,16}$
	End (midday)	$1.48_{3,16}$	$7.78_{1,16}$*	$1.47_{3,16}$
	Re-Watering (predawn)	$1.45_{3,16}$	$0.07_{1,16}$	$0.16_{3,16}$
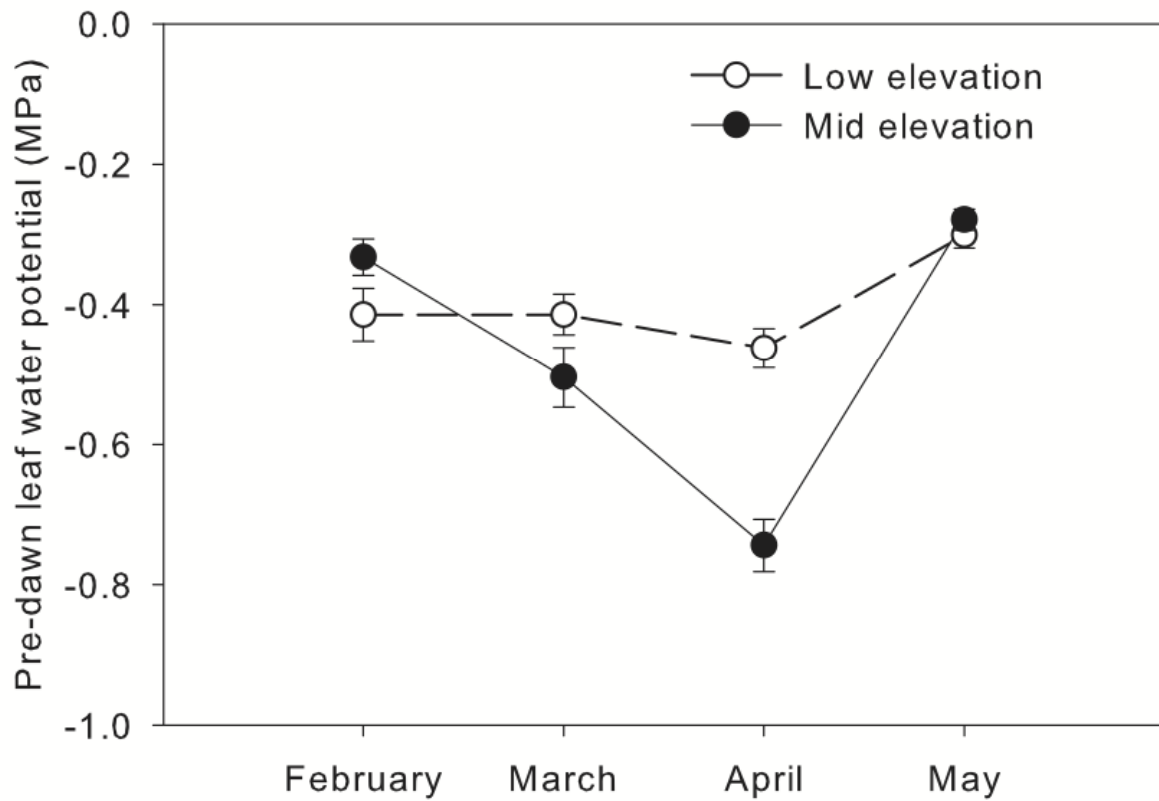
Figure 1. Predawn leaf water potential measured at a low (1284 m) and a mid elevation (1450 m) site on a monthly basis during the dry season in 2009. Measurements were made on five individuals each of two species of *Ocotea* at each site. Species are pooled by site. Data represent means ± 1 SE. The dotted line represents the mean soil water potential at the conclusion of the experimental dry down.
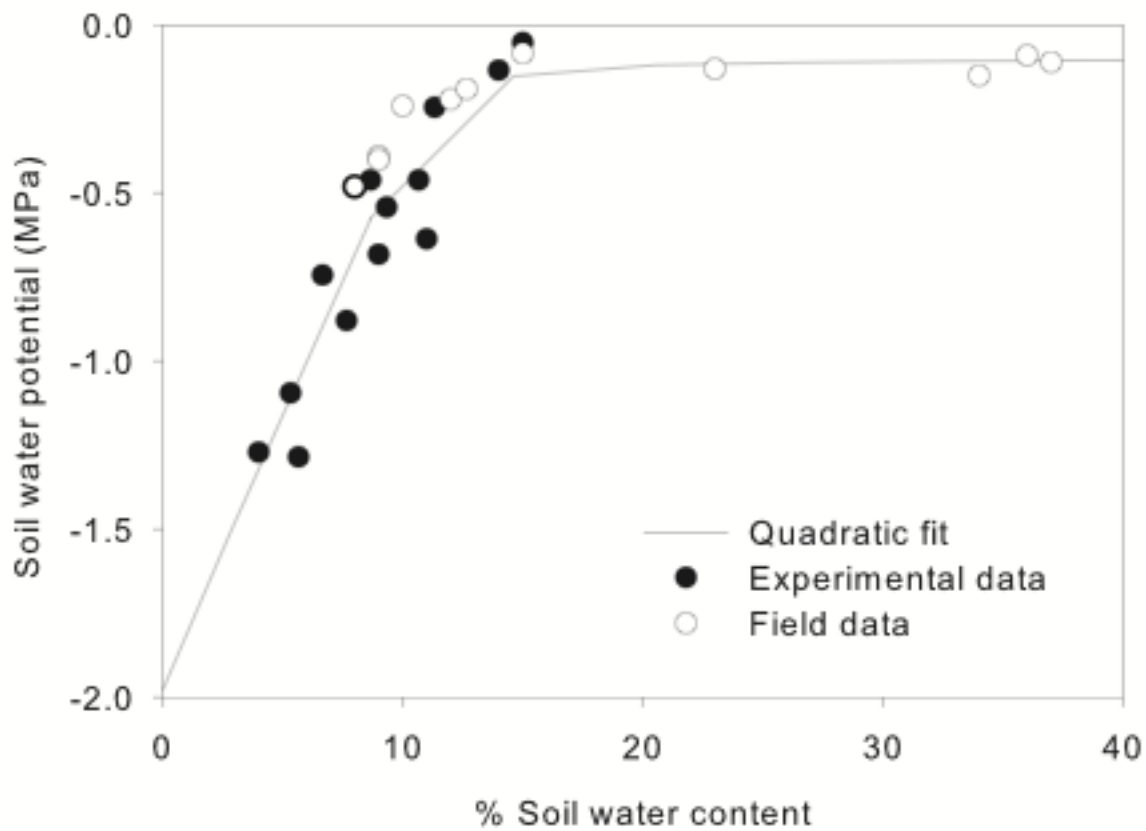
Figure 2. Relationship between soil water potential and soil water content. Experimental data (see methods) represented in black circles and field data in white circles. The line represents a quadratic function fit to all data ($r^2 = 0.88$).
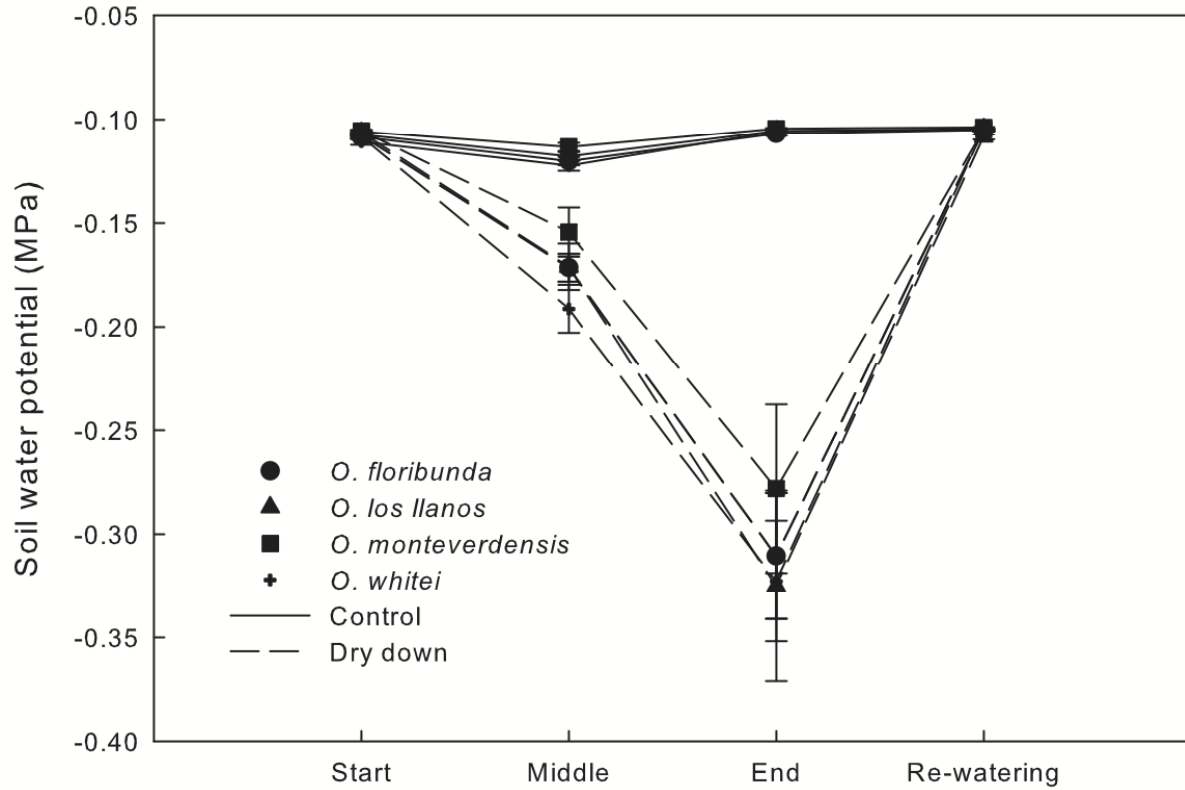
Figure 3. Soil water potential in control and experimentally-imposed dry-down treatments in four species of *Ocotea* (n = 14-16 individuals species[-1] treatment[-1]). Data represent means ± 1 SE.
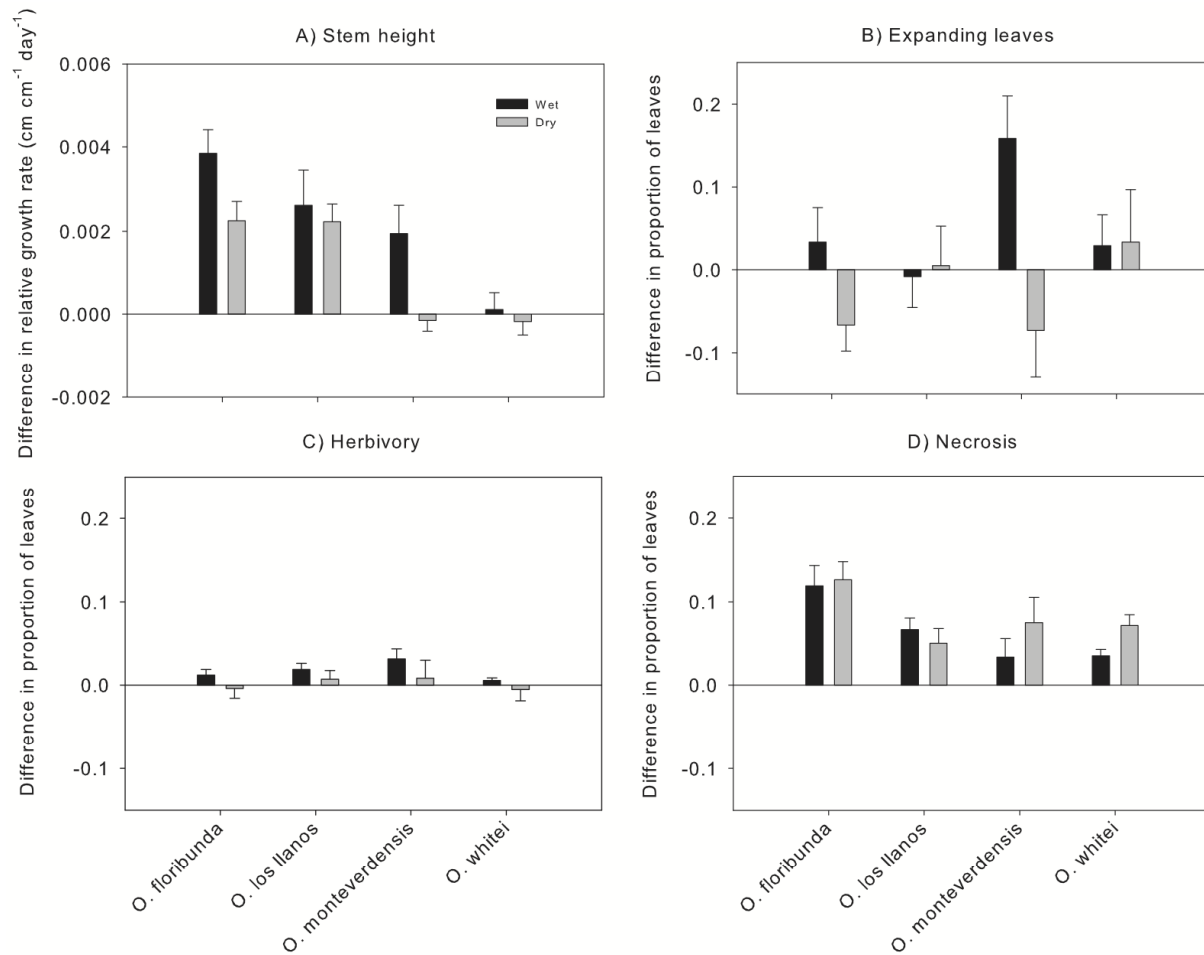
Figure 4. Differences in plant growth measures between control and experimentally-imposed dry-down treatments in four species of *Ocotea* (n = 14 -16 individuals species $^{-1}$ treatment^{-1}). Differences between the start and the end of the experimental treatment in relative growth rate of stem heights (a), proportion of new, expanding leaves (b), proportion of leaves experiencing herbivory (c), and proportion of leaves experiencing necrosis (d). Data represent means ± 1 SE.
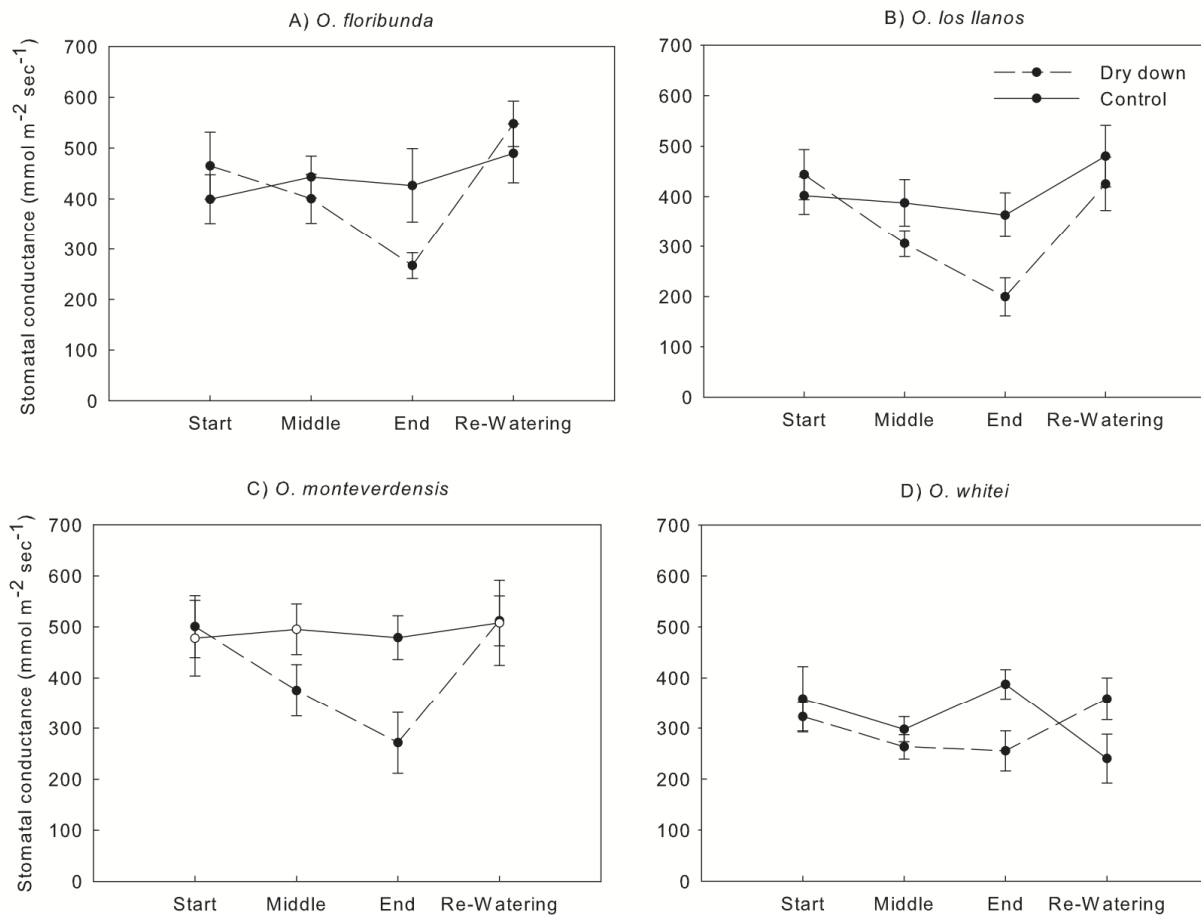
Figure 5. Differences in midday stomatal conductance between control and experimentally-imposed dry-down treatments in four species of *Ocotea* over the course of the experiment (n = 8 individuals species $^{-1}$ treatment^{-1}). Data represent means ± 1 SE.
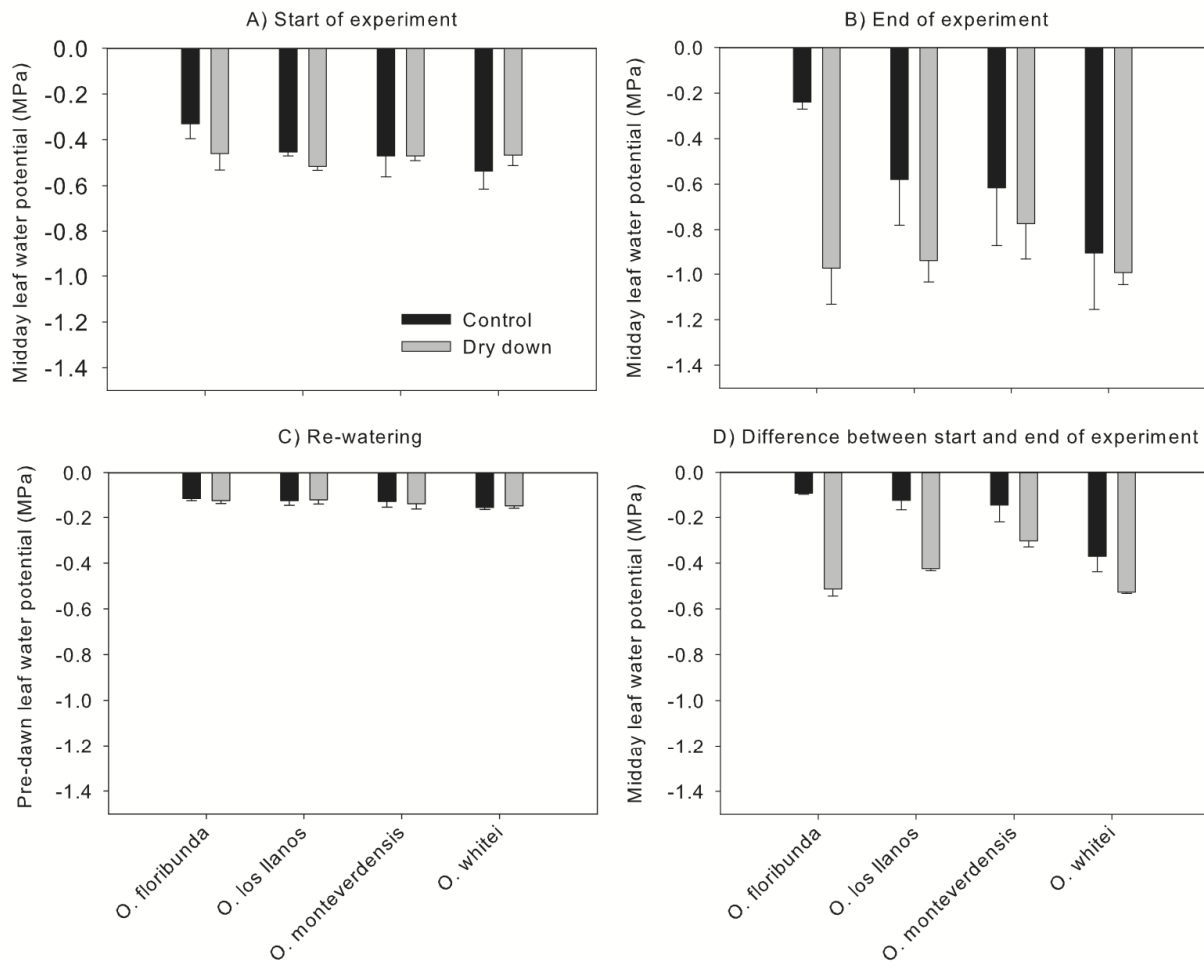
Figure 6. Differences in leaf water potential between control and experimentally-imposed dry-down treatments in four species of *Ocotea* (n = 3 individuals species^{-1} treatment^{-1}). Measurements taken at (a) midday at the start of the experiment, (b) midday at the end of the experiment and (c) before dawn immediately following re-watering. Data represent means ± 1 SE.

CHAPTER 3

The incidence and implications of clouds for cloud forest plant water relations

ABSTRACT

Although clouds are the most recognizable and defining feature of tropical montane cloud forests, little research has focused on how clouds affect plant functioning. We used satellite and ground-based observations to study cloud and leaf wetting patterns in contrasting tropical montane and premontane cloud forests. We then studied the consequences of leaf wetting for the direct uptake of water accumulated on leaf surfaces into the leaves themselves. During the dry season, the montane forest experienced higher precipitation, cloud cover, and leaf wetting events of longer duration than the premontane forest. Leaf wetting events resulted in foliar water uptake in all species studied. The capacity for foliar water uptake differed significantly between the montane and premontane forest plant communities, as well as among species within a forest. Our results indicate that foliar water uptake is common in these forest plants and improves plant water status during the dry season.

INTRODUCTION

Tropical montane cloud forests (TMCF) are among the planet's most rare and endangered ecosystems (Bruijnzeel *et al.* 2010, Ponce-Reyes *et al.* 2012). While there is no universally agreed upon definition of the frequency, duration, or extent to which a tropical forest must be immersed in clouds to be defined as a TMCF, it is generally agreed upon that these forests experience a high frequency of immersion in clouds extending to the ground (i.e. fog; Bruijnzeel *et al.* 2011, Scholl *et al.* 2011). The locations that meet such criteria are exceptional; TMCF occupies just 1.4 % of the world's tropical forest area (Scatena *et al.* 2010). Unfortunately, TMCF is extremely vulnerable to a host of anthropogenic pressures (Bruijnzeel *et al.* 2010). In particular, evidence suggests that rising land and sea surface temperatures will affect cloud patterns (Pounds *et al.* 1999, Still *et al.* 1999, Lawton *et al.* 2001, Karmalkar *et al.* 2008), with associated implications for maintaining ecosystem diversity, functioning, and services (Martínez *et al.* 2009). As such, there is a critical need for an improved understanding of clouds and their effects on cloud forest plant functioning.

As summarized below, clouds can play a unique and varied role in cloud forest plant ecology, primarily affecting plant functioning by 1) altering leaf energy balance and suppressing plant transpiration, 2) adding water to the soil through drip from the canopy, and 3) the uptake of water accumulated on leaf surfaces directly into leaves.

Clouds alter microclimate by changing energy balance and thus reducing vapor pressure deficit (VPD) and photosynthetically active radiation. In turn, this decreases plant water demand. The suppression of leaf-level transpiration has been observed in a number of cloud-affected ecosystems, including TMCF (Burgess and Dawson 2004, Reinhardt and Smith 2008, Gotsch *et al.* in review).

Cloud water droplets moving horizontally through cloud-affected ecosystems can be intercepted by and accumulate on plant surfaces, often resulting in leaf wetting. When the plant canopy storage capacity is exceeded, soil water availability can be increased through the resultant drip (Gomez-Peralta *et al.* 2008). Although measuring this "occult" (hidden), horizontal precipitation is difficult, additional inputs into TMCFs have already been observed to increase water availability well beyond that of vertical precipitation alone (Giambelluca and Gerold 2011). Such inputs can be particularly valuable during the dry season experienced by many TMCFs, when measurable precipitation decreases, but clouds may persist (Vogelmann 1973, Holder 2004).

Finally, the uptake of intercepted water on leaf surfaces into leaves, a phenomenon referred to as foliar water uptake (foliar uptake), has previously been demonstrated in plants from a range of other dew and cloud-affected ecosystems (Stone 1957, Boucher *et al.* 1995, Munné-Bosch *et al.* 1999, Martin and von Willert 2000, Gouvra and Grammatikopoulos 2003, Limm *et al.* 2009), as well as where rainfall is intercepted by plant canopies, but does not always increase soil water availability (Breshears *et al.* 2008). Although leaf wetting events are often considered to negatively affect plant performance (Brewer and Smith 1997), there are now many examples where improvements in plant water status can result from foliar uptake and lead to improved photosynthetic performance and even growth (Boucher *et al.* 1995, Simonin *et al.* 2009). The effect of foliar uptake on plant performance can be especially important in seasonally dry ecosystems where, in the absence of rainfall reaching the soil, water from dew or cloud immersion accumulating on leaves may be the most readily available water source (Dawson 1998). Despite this, the prevalence and significance of foliar uptake in TMCF is unknown and

there have been no comparisons between cloud forests that vary in both their abiotic (e.g. cloud cover and resultant leaf wetting events) and biotic (e.g. plant species composition) characteristics.

We studied cloud cover and leaf wetting patterns, and their relationship with foliar uptake, at neighboring tropical montane and premontane cloud forests. We expected that differences in seasonal climate patterns and species composition between the two forests would allow us to compare and contrast the effects of varying cloud cover and leaf wetness on plant functioning (Haber *et al.* 2000). Our objectives were to 1) describe and compare cloud cover patterns and leaf wetness for each forest, 2) determine the prevalence of foliar uptake among plant species in each forest, and 3) compare the capacity for foliar uptake among study species and between the two forest communities. While we demonstrate that foliar uptake is widespread and coupled to leaf wetting events commonly occurring due to clouds, we also demonstrate that forests and the individual species within them do not necessarily benefit equally from this unique phenomenon.

METHODS

Study Site

Field study was conducted from 2009 – 2011 along the Pacific slope of the Cordillera de Tilarán mountains near Monteverde, Costa Rica. We studied a relatively aseasonal, old growth lower montane wet forest (*montane*; 10°17.959'N, 84°47.460'W, 1563 m) and a highly seasonal, old growth premontane wet forest (*premontane*; 10°17.918'N, 84°48.563'W; 1409 m). Forest descriptions are provided by Haber *et al.* (2000). Synoptic climate is summarized by Clark *et al.* (2000).

Cloud Cover Using Remote Sensing

To characterize cloud cover patterns, we surveyed daily remote sensing products available between 2002-2012 as provided by the Moderate Resolution Imaging Spectroradiometer aboard NASA's *TERRA* and *AQUA* satellites. We employed a Level 2 cloud mask product (MOD35), which uses a series of threshold algorithms to identify cloud cover for each pixel (1 km^2) in a field of view, thus providing a standardized means by which to compare spatial and temporal patterns (Ackerman *et al.* 1998, Platnick *et al.* 2003). Due to satellite orbits and the field site latitude, four daily cloud mask products are available in the following time windows: 09:25 – 11:05, 12:20 - 13:55, 21:15 – 22:50, and 00:25 – 02:10.

Cloud mask products were accessed from NASA's EOSDIS website, geo-referenced with a Level 1 geo-location product (MOD03), and checked for QA/QC (EOSDIS 2009). The MOD35 product algorithm categorizes pixels as confident clear (confidence > 0.99 of clear skies), probably clear (0.99 ≥ confidence > 0.95), probably cloudy (0.95 ≥ confidence > 0.66), and confident cloudy (confidence ≤ 0.66). Due to its typical use for identifying cloud-free scenes, the cloud mask product is clear-sky conservative; it is more likely to falsely identify scenes with clouds than scenes without clouds. Therefore, we focused on querying the pixels covering each study forest (ca. 2 km apart) to determine whether or not each time point was designated as confident cloudy or not.

Environmental Characterization and Monitoring

To characterize rainfall patterns, bulk precipitation was measured in 2011 by the Monteverde Institute near the premontane forest via manual rain gauge and by the Tropical Science Center near the montane forest via tipping bucket rain gauge. To characterize microclimate, leaf wetness, temperature and relative humidity were monitored at each forest. Leaf wetness was monitored between 5 February and 10 April using three leaf wetness sensors painted matte white and attached at a slight incline to understory branches facing different aspects (Model 237, Campbell Scientific; Logan, Utah, USA). Leaf wetness was determined to occur only when all three sensors were below the manufacturer-specified measurement threshold. Temperature and relative humidity were monitored between 1 March and 31 March using an HMP35C probe (Vaisala Instruments; Helsinki, Finland) and used to calculate VPD. All measurements were recorded once a minute for 5 min at 15 min intervals, so as to derive an average value, using CR10X data loggers (Campbell Scientific; Logan, Utah, USA). Volumetric soil water content was measured weekly between 1 March and 31 March in 10 random locations using the "Hydrosense" model handheld sensor with 20 cm probes (Campbell Scientific; Logan, Utah, USA). The relationship between mean monthly proportion of cloudiness observations and bulk precipitation at each forest in 2011 was evaluated using linear regression. To determine whether cloud cover observations can serve as a proxy for leaf wetness, the relationship between leaf wetness and cloud cover observations was evaluated using a logistic regression where leaf wetness was a binary response (1 = wet, but see below) and cloudiness (1 = cloudy), time of day (06:01 - 18:00 or 18:01 – 06:00; 1 = night), and their respective interaction were binary predictors. As observations of leaf wetness and cloud cover may be slightly decoupled, models were constructed using single observations of leaf wetness before, at, and after the single cloud observation, as well as multiple observations of leaf wetness in time windows (0.5, 1, 2, and 3 hrs) centered around the single cloud observation. Where there is a time window, the response is the proportion of observations registering leaf wetness relative to the total number of observations. Each model at each site used between 187-190 cloud observations. Differences in leaf wetness, VPD, and soil water content between forests were evaluated via t-tests. The relationship between the daily number of hours registering leaf wetness and the mean daily VPD was evaluated using ANCOVA where VPD was a response and leaf wetness and forest were predictors. These and all other statistics were conducted using R 2.14.12 (R Development Core Team 2012).

Sap Flow

To characterize foliar uptake *in situ*, we measured sap flow on small branches of woody plants using the heat ratio method, which is particularly effective for measuring low flow rates and reverse flow (Burgess *et al.* 2001). We used the sensor assembly of Clearwater *et al.* (2009), where small sensors are affixed externally to the surface of a small branch. Implementation of this method followed Roddy and Dawson (2012). Briefly, every 15 min, starting temperatures were recorded downstream (proximal to terminal leaves) and upstream (distal), a 4 s heat pulse was applied, and new average temperatures were recorded for 40 s following a 60 s waiting period. Heat pulse velocity (V_h) was calculated as:

$$v_h = \frac{k}{x} \ln\left(\frac{\delta T_1}{\delta T_2}\right) \quad \text{cm s}^{-1} \qquad\qquad \text{Equation 1}$$

where k is thermal diffusivity (cm^2 s^{-1}; see below), x is distance from the heat pulse to the temperature sensors (cm), and δT_1 and δT_2 are the downstream and upstream temperature changes (°C). We determined k by measuring the time to reach maximum temperature rise following a heat pulse applied daily at 05:00, when no sap flow is occurring. We calculated k as:

$$k = \frac{x^2}{4 t_m} \quad \text{cm}^2 \text{ s}^{-1} \qquad\qquad \text{Equation 2}$$

where t_m is time to maximum temperature (s) and x is as above. Given the small variation in time to maximum temperature rise among species (53 ± 6 s SD), we applied this average to all calculations. Our calculation of k (2.83×10^{-3} cm^2 s^{-1}) was in strong agreement with previous empirical determinations (Burgess et $al.$ 2001). A reference velocity ($v_h = 0$) was determined for each sensor at times when sap flow is assumed to be zero by averaging values between 24:00 and 05:00 at high humidity (>95%) and no leaf wetness (Rosado et $al.$ 2012). This was the only method of determining zero flow we could successfully and repeatedly apply; attempts to stop flow by severing the stems above and below the sensor caused movements of the thermocouples that led to massive errors in determining zero flow. As such, this method could not be used on our small stems.

We measured sap flow for 3 days in March 2011 on 2 small branches of each individual plant of 6 common woody plant species occurring in the understory at each forest. We focused on the understory to facilitate sampling logistics and used transect-based, plant abundance data to guide our species' choice (Appendix 1; R.O. Lawton and W.A. Haber, $unpublished$ $data$). Mean stem diameter at the premontane forest was 3.5 ± 0.7 mm (SD) and 4.2 ± 0.5 mm at the montane forest. At 12:00 on the second day of sap flow measurements, we continuously sprayed water on the leaves of one of the two branches from each individual for 3 hrs. During this time, water potential (Ψ_L) was measured on wetted leaves of each plant at the conclusion of the experiment, as well as on control leaves before and at the conclusion of the experiment (n = 3 leaves at each time point). Wetted leaf surfaces were dried just prior to the measurement. Ψ_L was measured with a pressure chamber (Soilmoisture Equipment Corporation; Santa Barbara, California, USA). We were unable to measure Ψ_L for $Sorocea$ $trophoides$ and $Tabernaemontana$ $longipes$ due to latex.

While monitoring sap flow, rainfall was measured via tipping bucket rain gauge (Onset Corporation, Bourne, Massachusetts, USA). For experimentally wetted leaves of each species, the mean heat pulse velocity over the entire experiment was compared relative to zero using a one-tailed, single sample t-test. Mean Ψ_L measured on wetted leaves at the conclusion of the experiment was compared to mean Ψ_L measured on control leaves via t-test. Finally, the relationship between mean heat pulse velocity over the course of the experiments and the difference in Ψ_L control and wetted leaves at the conclusion of the experiments, as well as between mean heat pulse velocity in the first hour of the experiments and the initial Ψ_L of control leaves was evaluated across both forests by linear regression.

Foliar Uptake Capacity

To characterize differences in foliar uptake between the two forests, we measured what we define as the "capacity for foliar uptake" in the 12 most common woody plant species occurring in the understory from each forest (Appendix 1). For each species, we measured 3 leaves each from 3 individuals. To measure uptake capacity, branchlets were collected in late afternoon, re-cut under water, and rehydrated overnight. Following rehydration, a single leaf was excised from the branchlet and measured for Ψ_L. Pressure in the chamber was then slowly increased to -1.0 MPa and maintained for 1 min to induce water deficit. We chose -1.0 MPa based on typical dry season *in situ* leaf water deficits (Goldsmith, *unpublished data*). Leaves were then submerged in water for 1 hr, similar to Limm *et al.* (2009). Petioles were sealed with parafilm and left above water to prevent water entry. Following submersion, leaves were dried and immediately measured for Ψ_L. Capacity for foliar uptake was measured as improvement in Ψ_L following submersion following adjustment for initial Ψ_L. Differences between the two forests, as well as differences among species within a forest were evaluated using a nested ANOVA, where forest was treated as a fixed factor with species and individual treated as nested, random factors.

RESULTS

Synoptic Climate

During 2011, the cloud mask determination was made between 255 and 263 times each for the four daily products (e.g. images) at each forest, resulting in ≥ 17 products month^{-1} for analysis (average across all sites and times was 21.4 products month^{-1}). Cloud mask determinations are not always possible due to incomplete spatial coverage. Both forests demonstrated intra-annual patterns in cloud cover consistent with regional seasonality (Figure 1). During the wet season (May-October), the proportion of daytime (0.89 ± 0.09 *SD*) and nighttime (0.76 ± 0.15 *SD*) observations of cloudiness month^{-1} at the montane forest was virtually identical to daytime (0.89 ± 0.09 *SD*) and nighttime (0.77 ± 0.09 *SD*) observations at the premontane forest. However, differences developed over the course of the misty season (November-January). During the dry season (February-April), the proportion of daytime (0.52 ± 0.11 *SD*) and nighttime (0.31 ± 0.25 *SD*) observations of cloudiness month^{-1} was higher at the montane forest than the daytime (0.36 ± 0.16 *SD*) and nighttime (0.21 ± 0.16 *SD*) observations at the premontane forest. The intra-annual pattern of cloud cover observed in 2011 is consistent with monthly observations made from 2002-2012 (Figure 2).

Over the course of 2011, the montane forest received 3494 mm of bulk precipitation, with 6.0% (208 mm) of the total recorded during the dry season. The premontane forest received 2983 mm, with 3.1% (92 mm) of the total recorded during the dry season. Additionally, the monthly bulk precipitation totals at the montane forest exceeded those at the premontane forest in 11 of the 12 months. There was a significant linear relationship between the average monthly proportion of cloudiness observations and bulk precipitation at both the montane ($r^2 = 0.32$, *p* < 0.001, f = 486x, intercept = 0) and premontane forest ($r^2 = 0.52$, *p* < 0.001, f = 459x, intercept=0).

Coefficient estimates (odds ratios) indicate that cloud observations and time of day are significant predictors of leaf wetness at both forests (Figure 3). The relationships did not vary strongly with differing time windows; however, the confidence intervals became narrower as the time window increased, likely due to an increasing number of wetness observations. The

inclusion of the interaction term had a strong effect on coefficient estimates at the premontane forest, changing their sign, but not at the montane forest. This may indicate that at the premontane forest, there is little unexplained variation after the interaction term has been taken into account. The models with no interaction term provide additional insight: at the montane forest, both cloudiness and time of day are significant predictors of leaf wetness, with time of day a stronger predictor. The pattern is similar at the premontane forest, but the odds ratios are much lower. The weaker relationship at the premontane forest, as well as the significant interaction, are apparent when comparing additional details for models fit to both forests at a time window of 0.5 hrs (Table 1). The AUC, a measure of accuracy treating the logistic model as a binary classifier where 0.5 would indicate random guess and 1.0 would indicate perfection, is higher at the montane than the premontane forest (Agresti 2002). The AIC, a measure of goodness of fit for comparison of models within a forest where lower numbers indicate better fit, is lower for the premontane forest model that incorporates the interaction (Agresti 2002).

Environmental Monitoring

During March 2011, the mean number of hours day^{-1} registering understory leaf wetness at the montane forest (12.8 ± 1.3) was significantly higher than at the premontane forest (2.8 ± 0.8) (Figure 4A; t = 6.37, df = 50.98, $p < 0.0001$). There was a daily pattern to leaf wetness at the montane forest, with a higher frequency of daytime wet observations (10:00 – 20:00), whereas the premontane forest demonstrated an even distribution of wet observations (Figure 4B). The premontane forest had fewer (15 total) and shorter (6.0 ± 1.6 hrs) leaf wetting events compared to the montane forest, which had more (44 total) and longer (9.0 ± 2.1 hrs) events. Mean daily VPD was significantly lower in the montane (0.05 ± 0.01 kPa) than the premontane (0.17 ± 0.01 kPa) forest (Figure 4C; t = -6.96, df = 53.57, $p < 0.0001$). There was a negative relationship between the mean daily VPD and the daily number of hours registering leaf wetness, even when considering the significant effect of forest ($F_{2,59} = 46.19$, $p < 0.001$, $r^2 = 0.60$). The volumetric soil water content was significantly higher at the montane (48 ± 7 %) compared to the premontane (23 ± 7 %) forest (t = -2.64, df = 6.00, $p < 0.04$).

Sap Flow

While monitoring sap flow, both forests experienced nighttime leaf wetness events that persisted for several hours (Figure 5). The premontane forest received measurable rainfall only once during this time, with 0.8 mm recorded between 06:00 and 08:00 on the first morning of monitoring. This corresponds with the first leaf wetting event and the most negative heat pulse velocities recorded. There was no additional measurable rainfall at either forest, such that all leaf wetting events were likely caused by cloud interception or very light rainfall.
   Negative velocities (reverse flow) were observed in all species at each forest and were tightly coupled to leaf wetting events (Figure 5). These observations were confirmed by the experimental wetting of one of the two branches monitored on each species, which again resulted in negative velocities in all species (Table 2). While negative velocities were often recorded soon after experimental wetting commenced, they did not always persist over the entire 3 hr treatment, such that mean heat pulse velocities in experimentally wetted branches were significantly less than zero in only 4 of 12 cases ($p < 0.05$; Table 2). Mean Ψ_L measured on experimentally wetted branches following the treatment was significantly less negative than mean Ψ_L measured on

nearby control branches in 4 of the 6 montane species and 1 of the 4 premontane species (n = 3, $p < 0.05$). The species that did not demonstrate high negative velocities in response to experimental wetting: *Meliosma idiopoda, Chione sylvicola, Matayba oppositifolia, Guarea rhopalocarpa,* and *Guatteria oliviformis*, were the same ones that did not demonstrate differences in Ψ_L between before and after experimental wetting. Across all species at both forests, Ψ_L improved as a function of increasing reverse sap flow rates resulting from experimental leaf wetting (Figure 6; f = -0.237x + 0.073, n = 10, f = 9.89, $p < 0.02$, $r^2 = 0.55$). However, there was no significant relationship between initial Ψ_L and mean heat pulse velocity in the first hour of the experiment (n = 10, f = 1.49, $p = 0.26$).

Foliar Uptake Capacity

The mean capacity for foliar uptake was significantly higher (f $_{1,183}$ = 70.43, $p < 0.0001$) in the montane (0.67 ± 0.02 MPa) compared to the premontane (0.55 ± 0.12 MPa) forest and there were significant differences among plant species within a forest (Figure 7; f $_{22,183}$ = 6.72, $p < 0.0001$).

DISCUSSION

We characterized the contrasting patterns of cloud cover at premontane and montane tropical cloud forests, as well as the associated relationship with leaf wetness. Leaf wetting events were tightly coupled to foliar uptake by all of the common species in the two forests. However, there were significant differences in the capacity for foliar uptake among species and between the two forest communities.

Characterizing Clouds and Leaf Wetness

We used a standardized remote sensing method to provide a quantitative approach to describing cloud cover. Cloud cover at each forest demonstrated consistent and long-term, monthly patterns related to that of precipitation, with a decrease occurring over the course of the misty (November-January) and dry (February-April) seasons, as well during the summer veranillo (little summer) that typically occurs in August. Consistent with the progression of daytime cumulus cloud cover over Costa Rica, both forests experienced more daytime cloudy observations than nighttime cloudy observations during the dry season (Nair *et al.* 2003).

Although the forests are only ca. 2 km apart (ca. 1 pixel), there were strong differences in dry season cloud cover and leaf wetness. The premontane forest is further leeward (west) and at a lower elevation and therefore subject to a greater rain shadow and likely to less cloud immersion. The premontane forest experienced a lower average number of hours of leaf wetness per day, as well as a shorter average duration of each individual leaf wetness event compared to the montane forest. The premontane forest also demonstrated a weaker relationship between leaf wetness and cloud observations, likely due to reduced cloud immersion that results from the lower elevation. The remote sensing products analyzed herein do not give any indication about cloud immersion (Nair *et al.* 2008, Welch *et al.* 2008). Nevertheless, our results indicate that cloud observations derived from remote sensing are valuable in that they are significant predictors of leaf wetness at both the montane (ca. 3.4-6.1x more likely to experience wetness given clouds) and premontane (ca. 1.4-2.2x more likely) forests. During the dry season, the montane forest more frequently experiences the conditions that can result in foliar uptake.

The observed patterns of cloudiness, leaf wetness, and VPD have a number of implications for leaf, plant, community, and ecosystem water balance, particularly during the dry season when vertical precipitation inputs are reduced. Most notably, our observations indicate that the high cloud cover frequency is likely to suppress leaf level transpiration and result in leaf wetting events that can result in foliar uptake.

Prevalence of Foliar Uptake

We monitored sap flow to survey the prevalence of foliar uptake resulting from the leaf wetness' patterns described above. Although this is the most direct and only real-time technique currently available to measure foliar water uptake, its application has been limited to date (Burgess and Dawson 2004). Reverse sap flow was observed in response to leaf wetting in all the species we surveyed in both forests. Moreover, the capacity to improve Ψ_L through foliar uptake was observed in all 24 species that were experimentally assessed, indicating that foliar uptake is ubiquitous in both the tropical premontane and montane cloud forest plant communities. These results, the first from a TMCF, add to a growing list of species identified as capable of foliar uptake. Research since 1950 has identified at least 70 species as being capable of foliar uptake, representing >85% of all those studied (Appendix 2). Foliar uptake is likely to be a widespread phenomenon, and seemingly occurs independent of phylogeny, morphology, or growth habit (Dawson 1998, Limm and Dawson 2010); however, additional information on its physiological significance is needed.

Significance of Foliar Uptake

We used both sap flow and water potential measurements to study the significance of foliar uptake among plant species and between forests. Foliar uptake improved plant water status in the laboratory and the field, with greater rates of reverse sap flow resulting in greater water deficit reductions. While foliar uptake consistently improves leaf water status, there were significant differences in the magnitude of improvement among species. Moreover, the montane plant community demonstrated a significantly higher capacity for foliar uptake compared to the premontane plant community. In contrast, previous research has focused on characterizing foliar uptake either within plant species communities (Gouvra and Grammatikopoulos 2003, Limm *et al.* 2009), or across a single species' range (Limm and Dawson 2010). Our results indicate that not all species or forests are likely to benefit equally from the leaf wetting events that could reduce leaf water deficit through foliar uptake. Where foliar uptake occurs in other ecosystems, the reduction in leaf water deficit can result in improved carbon gain (Simonin *et al.* 2009), growth (Boucher *et al.* 1995), and survival (Stone 1957). Thus, foliar uptake is likely to be particularly important during the dry season, when there is reduced soil water availability (Goldsmith *et al.* in review). As such, foliar uptake would more likely benefit the drier, premontane forest. However, leaf wetting events in the premontane forest are less frequent and the plant community is unable capitalize on them physiologically to the same extent as the montane forest plant community.

Mechanisms and Pathways for Foliar Uptake

It has been hypothesized that foliar uptake should occur given a gradient toward more negative Ψ_L (e.g. from the saturated leaf surface to the unsaturated matrix inside the leaf) (Rundel 1982). However, no relationship between initial Ψ_L and reverse sap flow rates was detected. Previous research has also indicated that Ψ_L does not appear to correspond with the magnitude of foliar uptake (Burgess and Dawson 2004, Limm and Dawson 2010, but see Breshears *et al.* 2008). Detecting this water potential gradient is likely limited primarily by the lack of appropriate techniques.

The observed differences in capacity for foliar uptake among plant species and between forests may be a function of differences in the leaf traits that facilitate uptake. Previous research has identified the cuticle (Yates and Hutley 1995, Limm and Dawson 2010), trichomes (Franke 1967) and hydathodes (Martin and von Willert 2000) as possible pathways for water entry into the leaf. Although it is an obvious additional pathway, evidence suggests that films of water are unable to enter stomatal pores (Schönherr and Bukovac 1972). In the absence of specialized traits (e.g. trichomes and hydathodes), diffusion of water through the cuticle is the most likely pathway. Cuticular permeability is a function of chemical composition and micro-architecture (Jetter *et al.* 2000), traits that are in turn modified by age and environmental degradation (Shepherd and Griffiths 2006). If the cuticle were the pathway, this could provide a potential explanation for observed differences. For instance, if the more seasonal, drier environment at the premontane forest favored plants with more desiccation-resistant cuticles, this would also result in a decrease in foliar uptake capacity.

Regardless of the mechanisms and pathways by which water enters the leaf, our results indicate strong differences in plant capacity to use leaf wetting events to improve plant water relations. Such findings should be considered in the context of global climate change.

Climate Change Projections

Tropical mountains are projected to experience high rates of climate change in the future (Williams *et al.* 2007, Karmalkar *et al.* 2008, Karmalkar *et al.* 2011). Mean dry season surface air temperature along the Pacific slope of Costa Rica, where Monteverde is located, is projected by regional climate models to increase 3.8ºC, leading to a non-analog climate before 2100 (Karmalkar *et al.* 2011). Moreover, these changes are projected to be associated with a strong increase in dry season temperature variability. Similarly, dry season precipitation is projected to decrease by 14%, with an accompanying decrease in variability (Karmalkar *et al.* 2011). Finally, evidence suggests that changes in surface temperatures are associated with an increasing number of consecutive dry season days (Pounds *et al.* 1999, Pounds *et al.* 2006), as well as an increase in cloud base height (Still *et al.* 1999, Lawton *et al.* 2001, Nair *et al.* 2003). If these projections come to bear, cloud forest plants may experience increased water demand simultaneous to a decrease in precipitation, cloud immersion, and leaf wetting events leading to foliar uptake.

CONCLUSIONS

Although clouds are the most recognizable and defining feature of TMCF, there is still a critical need for information regarding how clouds affect plant functioning. We demonstrated that cloud cover and associated leaf wetting events vary seasonally, as well as over very small distances. We then focused on foliar uptake, one means by which clouds could affect plant functioning. Foliar uptake was demonstrated to be tightly coupled to leaf wetting events associated with cloud

cover, leading to improved water relations. However, there were both species and forest differences in foliar uptake. Thus, if climate change affects cloud cover and associated leaf wetting events, not all plant species or communities will respond equally.

ACKNOWLEDGEMENTS

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Table 1. Model statistics for logistic regression models predicting leaf wetness as a function of a cloud observation (inferred as cloudy or clear from remote sensing products, where cloudy = 1) and the time of day (day or night, where night = 1) for tropical montane and premontane forests near Monteverde, Costa Rica, as measured between 5 February and 10 April 2011. An additional model at each forest includes an interaction between the cloud observation and time of day as an additional predictor (i.e. the additional probability associated with a simultaneous cloudy-night observation). Leaf wetness observations are binned over a 0.5 hr time window centered on the cloud observation time (e.g. 2-3 leaf wetness observations for each cloud observation). The log(odds ratio) states the change in probability of leaf wetness as a function of the cloud observation, the time of day, or the interaction of the two. The AUC (Area Under Curve) is provided as an additional evaluation metric, as AIC (Akaike Information Criterion) measures cannot be compared across sites.

Site	Interaction	Parameter	log(odds ratio)	95% CI	p-value	AUC	AUC 95% CI	AIC	Δ AIC	Akaike weight
Montane	Yes	Intercept	-4.96	(-7.07 - -2.89)	p<0.001	0.77	(0.71-0.84)	460.6	1.4	0.33
		Cloud observation	2.06	(0.57 - 3.55)	p<0.01					
		Time of day	2.39	(0.96 - 3.78)	p<0.001					
		Interaction	-0.42	(-1.44 - 0.69)	ns					
	No	Intercept	-4.26	(-5.36 - -3.23)	p<0.001	0.77	(0.71-0.84)	459.2	0	0.67
		Cloud observation	1.52	(1.02 - 2.03)	p<0.001					
		Time of day	1.86	(1.41 - 2.34)	p<0.001					
Premontane	Yes	Intercept	-0.68	(-4.06 - 2.95)	ns	0.68	(0.58-0.78)	309.6	0	0.91
		Cloud observation	-2.28	(-5.37 - 0.21)	ns					
		Time of day	-1.28	(-3.31 - 0.64)	ns					
		Interaction	1.85	(0.42 - 3.51)	p<0.05					
	No	Intercept	-4.66	(-6.19 - -3.25)	p<0.001	0.67	(0.56-0.77)	314.1	4.6	0.09
		Cloud observation	0.81	(0.16 - 1.44)	p<0.05					
		Time of day	1.09	(0.48 - 1.74)	p<0.001					

Table 2. Heat pulse velocities (cm hr^{-1}) measured on control and experimentally wetted branches of an individual plant for different species located in tropical montane and premontane forests near Monteverde, Costa Rica. Data represent means (± 1 *SD*) and ranges (in parentheses). Results in bold are significantly less than zero as measured by a one-tail, single sample t-test ($p <$ 0.05).

Species	Montane	
	Control	Wetted
O. meziana	1.42 ± 0.30 (1.15 - 2.24)	-0.16 ± 0.34 (-0.62 - 0.56)
G. rhopalocarpa	1.00 ± 0.46 (0.17 - 1.94)	0.05 ± 0.21 (-0.30 - 0.49)
I. costaricensis	1.15 ± 0.38 (0.54 - 1.81)	**-0.30 ± 0.31 (-0.91 - 0.16)**
G. oliviformis	0.66 ± 0.20 (0.38 - 1.07)	-0.01 ± 0.26 (-0.28 - 0.62)
P. elata	1.04 ± 0.21 (0.72 - 1.36)	**-0.37 ± 0.11 (-0.58 - -0.23)**
P. amalago	1.09 ± 0.26 (0.79 - 1.67)	**-0.49 ± 0.17 (-0.76 - -0.12)**

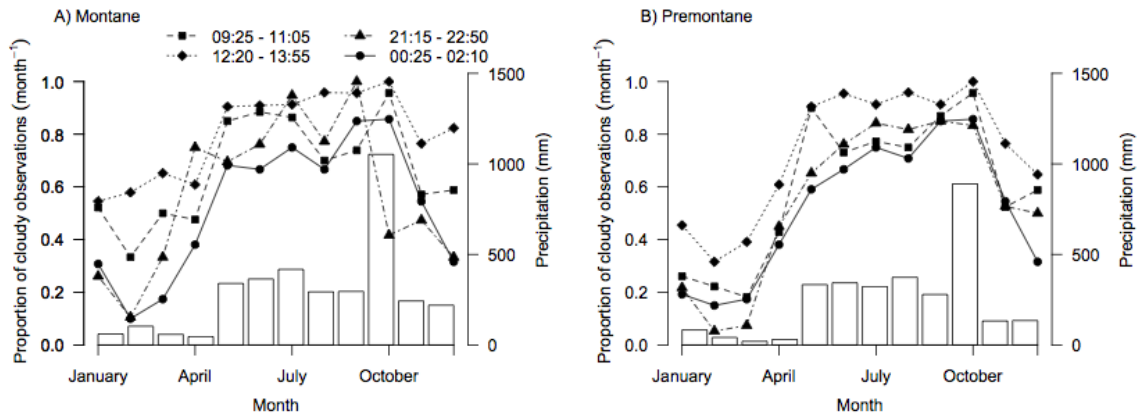| Species	Premontane	
	Control	Wetted
M. idiopoda	1.01 ± 0.24 (0.69 - 1.57)	0.24 ± 0.24 (-0.21 - 0.57)
S. tuerckheimii	0.49 ± 0.15 (0.23 - 0.75)	**-0.23 ± 0.23 (-0.72 - 0.19)**
T. longipes	0.84 ± 0.37 (0.28 - 1.42)	-0.09 ± 0.44 (-1.09 - 0.47)
C. sylvicola	1.45 ± 0.24 (1.10 - 1.81)	0.05 ± 0.22 (-0.36 - 0.41)
S. trophoides	1.15 ± 0.41 (0.60 - 1.85)	-0.23 ± 0.62 (-1.46 - 0.50)
M. oppositifolia	1.05 ± 0.35 (0.69 - 1.89)	0.18 ± 0.55 (-0.94 - 0.96)

Figure 1. Monthly patterns of cloudy observations (lines with symbols) and precipitation (bars) determined for tropical A) montane and B) premontane tropical montane cloud forests near Monteverde, Costa Rica for 2011.
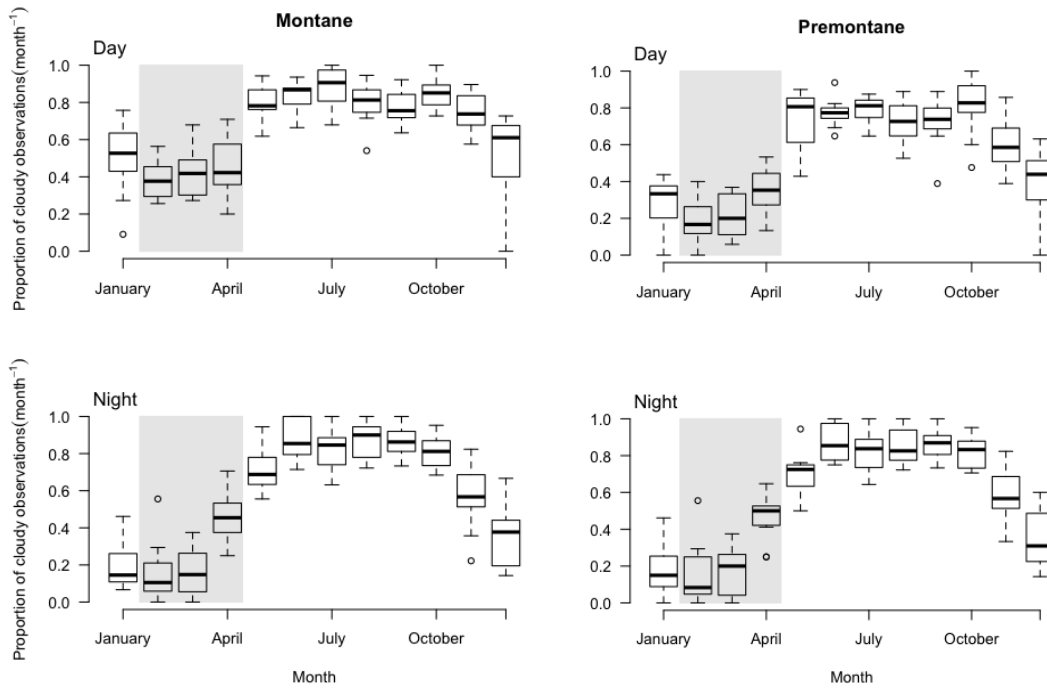
Figure 2. Mean monthly proportion of day (09:25-11:05) and night (21:15-22:50) cloudy observations determined between 2002-2012 for tropical montane and premontane tropical montane forests near Monteverde, Costa Rica. Data is generated from remote sensing products collected by the MODIS aboard the *Terra* satellite. Gray-shaded box represents dry season period of interest.
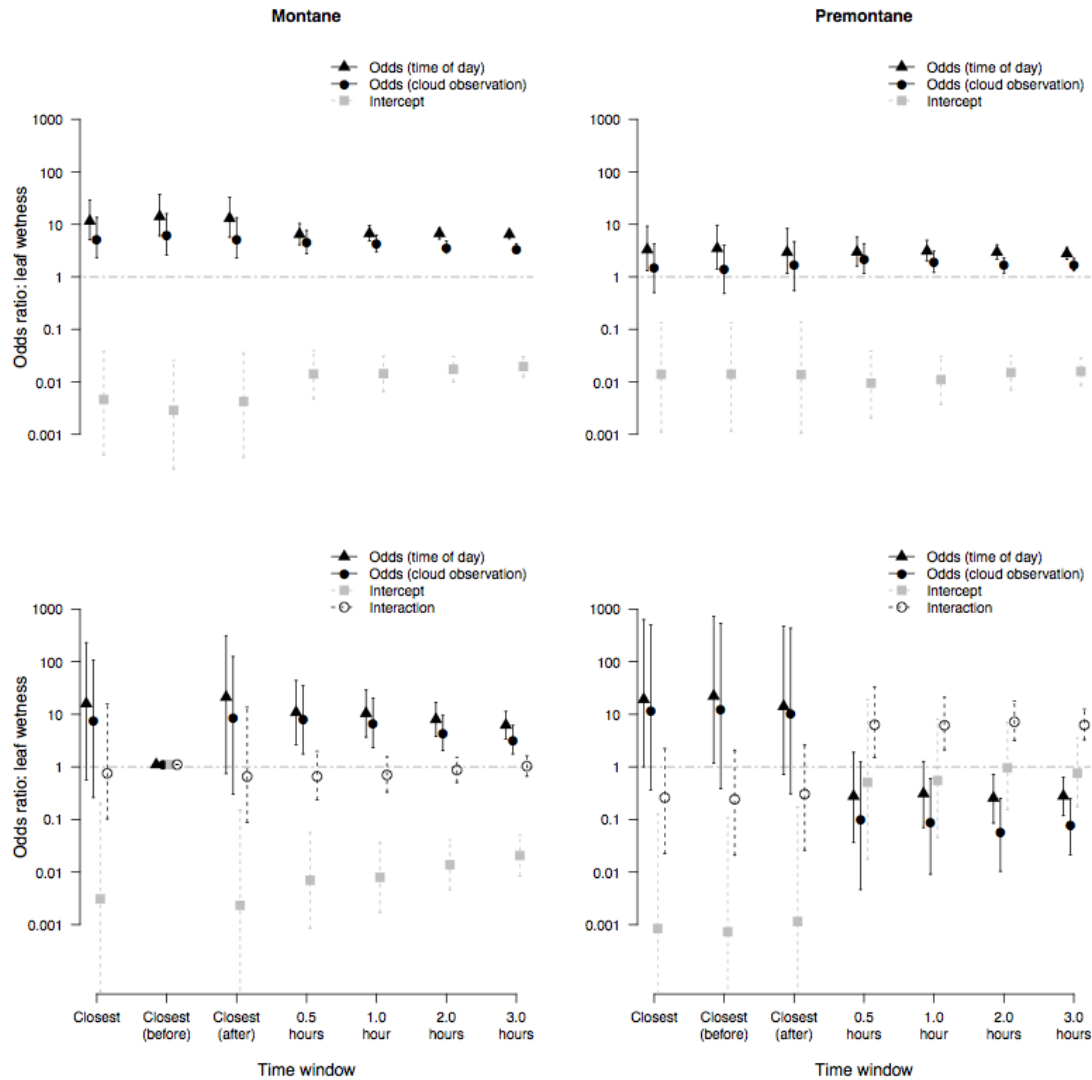
Figure 3. Model statistics for logistic regression models predicting the probability of leaf wetness as a function of a cloud observation (inferred as cloudy or clear from remote sensing products, where cloudy = 1) and the time of day (day or night, where night = 1) for tropical montane and premontane forests near Monteverde, Costa Rica, as measured between 5 February and 10 April 2011. The x-axis represents a series of models constructed by pairing a single cloud observation with the closest temporal leaf wetness observation before, at, or after the cloud observation. Additional models were made by pairing a single cloud observation with multiple observations of leaf wetness in time windows (0.5 hrs, 1 hr, 2 hrs, and 3 hrs) centered around the cloud observation. Where there is a time window, the response is the proportion of observations registering leaf wetness relative to the total number of observations. For example, for a 1 hr time window, a single cloud observation would be paired with 5-6 leaf wetness observations, represented as a proportion of wet/total observations. The y-axis represents the odds ratio, plotted on a log scale. The odds ratio is the change in probability of leaf wetness as a function of the cloud observation, the time of day, or the interaction of the two. An odds ratio of 1.0 is equal to a 0.5 probability of leaf wetness. The lower panels include an interaction between the cloud observation and time of day as an additional predictor (i.e. the additional probability associated

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with a simultaneous cloudy-night observation). In the lower left plot, the odds ratios for the coefficients using the "Closest (before)" time window have been set to 1, as the inference failed to converge after many iterations of the model-fitting algorithm, an indication of an extremely flat likelihood surface.
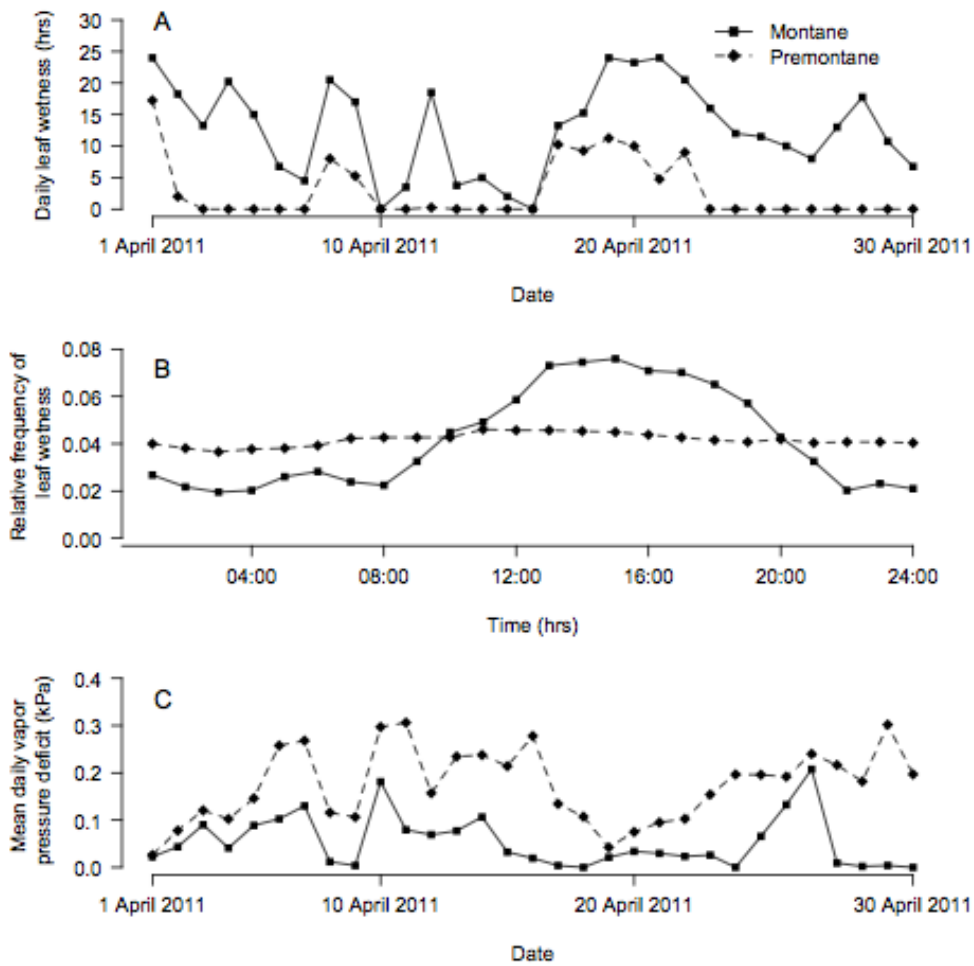
Figure 4. Microclimatic measures determined in the understory of tropical montane and premontane cloud forests near Monteverde, Costa Rica between 1 March and 31 March 2011. Measures include A) the number of hours day^{-1} registering leaf wetness, B) the relative frequency of leaf wetting events hour^{-1}, and C) the mean daily vapor pressure deficit.
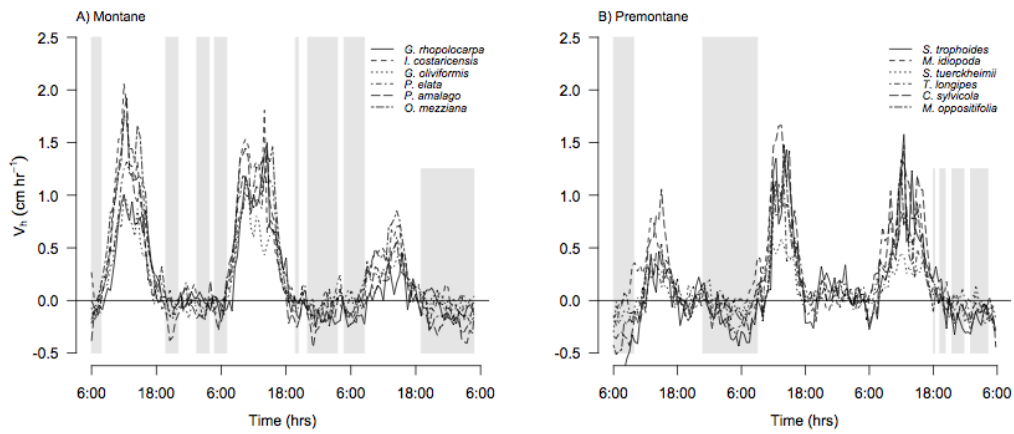
Figure 5. Sap flow (V_h) monitored *in situ* on a single individual of 6 different species in the understory of tropical A) montane forest and B) premontane cloud forests near Monteverde, Costa Rica. Light gray bars represent leaf wetting events.
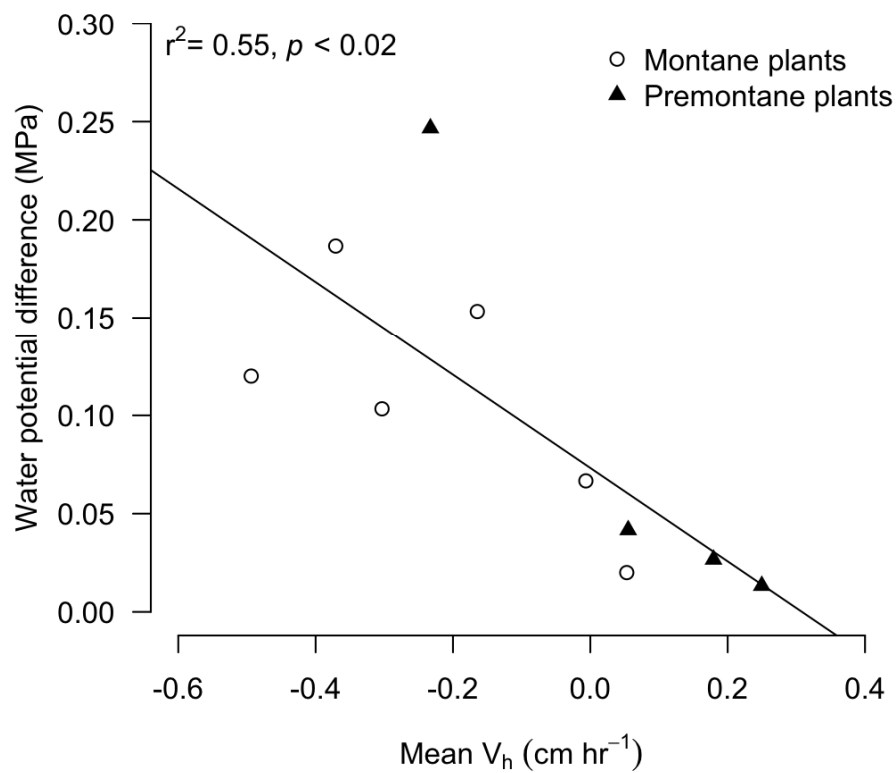
Figure 6. The difference in leaf water potential between control and experimentally wetted leaves as a function of the mean heat pulse velocity (V_h) as measured in 3 hr experiments in tropical montane and premontane cloud forests near Monteverde, Costa Rica.

Figure 7. Improvement in mean leaf water potential measured in 12 common understory species in tropical montane and premontane cloud forests near Monteverde, Costa Rica following 1 hr of experimental wetting. Results represent means ± 1 *SE*.
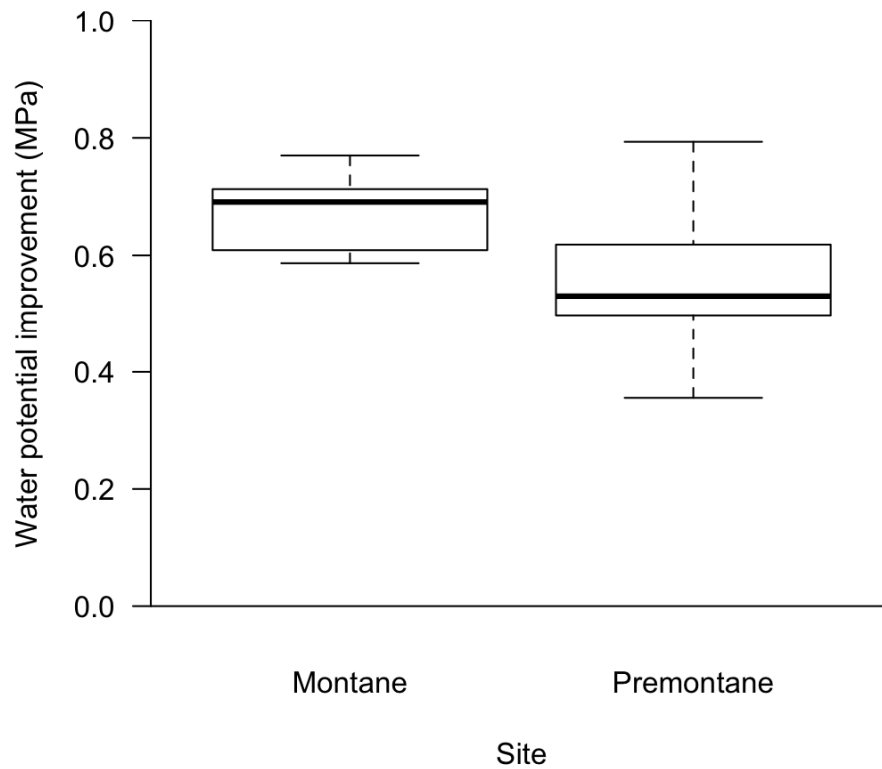
Appendix 1. Species used for sap flow and foliar water uptake capacity measurements.

Montane		Premontane	
Species	Family	Species	Family
Conostegia pittieri †	Melastomataceae	*Beilschmiedia brenesii †*	Lauraceae
Guarea rhopalocarpa *†	Meliaceae	*Cestrum "panamense" †*	Solanaceae
Guatteria oliviformis *†	Annonaceae	*Chione sylvicola **	Rubiaceae
Ilex costaricensis *†	Aquifoliaceae	*Dendropanax querceti †*	Araliaceae
Maytenus recondita †	Celastraceae	*Matayba oppositifolia *†*	Sapindaceae
Meliosma vernicosa †	Sabiaceae	*Meliosma idiopoda *†*	Sabiaceae
Nectandra smithii †	Lauraceae	*Mortoniodendron guatemalense †*	Tiliaceae
Ocotea "small leaf" †	Lauraceae	*Myrcia "fuzzy leaf" †*	Myrciaceae
Ocotea meziana *†	Lauraceae	*Neea "oblanceolate" †*	Nyctaginaceae
Ocotea tonduzii †	Lauraceae	*Ocotea floribunda †*	Lauraceae
Piper amalago *	Piperaceae	*Ocotea whitei †*	Lauraceae
Pouteria exfoliata †	Sapotaceae	*Picramnia "stuckey" †*	Simaroubaceae
Pouteria fossicola †	Sapotaceae	*Psychotria sylvivaga †*	Rubiaceae
Psychotria elata *	Rubiaceae	*Solanum tuerckheimii**	Solanaceae
		Sorocea trophoides*	Moraceae
		Tabernaemontana longipes*	Apocynaceae

* indicates use for *in situ* measurement of sap flow.
† indicates use for measurement of capacity for foliar water uptake.

Appendix 2. Plant species capable or incapable of foliar water uptake as identified through a survey of peer-reviewed literature published since 1950.

Author(s)	Year	Spp. capable of foliar uptake	Spp. incapable of foliar uptake
Brezeale *et al.*	1950	*Solanum lycopersicum*	
Brezeale *et al.*	1951	*Zea mays*	
Stone	1957	*Pinus ponderosa*	
Leyton & Armitage	1968	*Pinus radiata*	
Went & Babu	1978	*Citrullus vulgaris*	
Went & Babu	1978	*Cucumis melo*	
Suarez & Gloser	1982	*Panicum maximum*	
Suarez & Gloser	1982	*Paspalum notatum*	
Grammatikopoulos & Manetas	1994	*Phlomis fruticosa*	*Nerium oleander*
Grammatikopoulos & Manetas	1994	*Verbascum mallophorum*	*Olea europea*
Grammatikopoulos & Manetas	1994	*Verbascum spesiosum*	*Hedera helix*
Yates & Hutley	1995	*Sloanea woodsii*	
Boucher *et al.*	1996	*Pinus strobus*	
Feild & Dawson	1998	*Didymopanax pittieri*	
Munne-Bosch & Alegre	1999	*Melissa officinalis*	
Munne-Bosch *et al.*	1999	*Lavandula stoechas*	*Rosmarinus officinalis*
Martin & von Willert	2000	*Crassula clavata*	*Crassula cordata*
Martin & von Willert	2000	*Crassula elegans*	*Crassula hirtipes*
Martin & von Willert	2000	*Crassula expansa*	*Crassula perforata*
Martin & von Willert	2000	*Crassula grisea*	*Crassula rogersii*
Martin & von Willert	2000	*Crassula pellucida*	*Crassula setulosa*
Martin & von Willert	2000	*Crassula sarmentosa*	
Martin & von Willert	2000	*Crassula sladenii*	
Martin & von Willert	2000	*Crassula spathulata*	
Martin & von Willert	2000	*Crassula tetragona*	
Gouvra & Grammatikopoulos	2003	*Cymbalaria muralis*	
Gouvra & Grammatikopoulos	2003	*Parietaria judaica*	
Gouvra & Grammatikopoulos	2003	*Phagnalon graecum*	
Gouvra & Grammatikopoulos	2003	*Reichardia picroides*	
Gouvra & Grammatikopoulos	2003	*Hyoscyamus albus*	
Burgess & Dawson	2004	*Sequoia sempervirens*	
Breashears *et al.*	2008	*Juniperus monosperma*	
Limm *et al.*	2009	*Polystichum munitum*	*Umbellularia californica*
Limm *et al.*	2009	*Pseudotsuga menziesii*	*Oxalis oregana*
Limm *et al.*	2009	*Arbutus menziesii*	
Limm *et al.*	2009	*Sequioa sempervirens*	
Limm *et al.*	2009	*Gaultheria shallon*	
Limm *et al.*	2009	*Vaccinium ovatum*	
Limm *et al.*	2009	*Polypodium californicum*	
Limm *et al.*	2009	*Notholithocarpus densiflorus*	

CHAPTER 4

Leaf wetting alters leaf water pressure-volume relations in certain tropical montane plant species

ABSTRACT

The accumulation of precipitation, fog and dew on leaf surfaces, commonly referred to as leaf wetting, is generally considered to negatively impact the performance of leaves.
However, leaf wetting can also positively impact the performance of leaves through the direct uptake of water that has accumulated on leaf surfaces and is subsequently taken-up into leaves. I studied the effects of this phenomenon, referred to as foliar water uptake, on leaf water pressure-volume relations in woody tropical montane plant species occurring near Monteverde, Costa Rica. Among the four species studied, there were inconsistent differences in pressure-volume relations parameters between leaves rehydrated through both xylem and foliar uptake compared to through xylem alone. However, the species *Cestrum panamense* demonstrated a decrease in the bulk modulus of elasticity and an increase in capacitance (storage) when rehydrated through both xylem and foliar uptake. Moreover, leaves of *C. panamense* rehydrated through both xylem and foliar uptake demonstrated a slower rate of decline in leaf water potential compared to leaves rehydrated through xylem alone. In a preliminary consideration of the mechanisms driving the observed differences, I hypothesize that anatomical properties that may be unique to *C. panamense* leaves permit the entry of excess water into apoplastic spaces that are not accessible via rehydration through the xylem. This excess water increases capacitance and thus slows the rate at which leaf water potential decreases. The observed changes in pressure-volume relations occurring as a function of foliar uptake could have consequences for net photosynthesis and even growth; however, additional research is necessary. These results underscore the importance of considering plant rehydration through different pathways – through the xylem or through both the xylem and leaf surfaces – in studies of plant-water relations where there are regular leaf wetting events.

INTRODUCTION

Leaf wetting is generally considered to have negative impacts on plant functioning by reducing photosynthesis and slowing gas exchange, promoting the growth of pathogens and increasing the deposition of pollutants (Huber and Gillespie 1992, Ishibashi and Terashima 1995, Cape 1996). However, a growing body of research has determined that leaf wetting that occurs during dew, fog and rain events may also positively impact plant functioning through a phenomenon referred to as foliar water uptake (Burgess and Dawson 2004, Breshears *et al.* 2008, Limm *et al.* 2009, Goldsmith *et al.* 2012). Foliar water uptake, the movement of water accumulated on leaf surfaces into leaves, can improve leaf water potential (Ψ_L) and may also have positive impacts on photosynthesis, growth, and even survival (Stone 1957, Boucher *et al.* 1995, Simonin *et al.* 2009). A recent review of the literature determined that >85% of the plant species surveyed to date were capable of performing foliar water uptake, suggesting that it is likely to be a more widespread and common phenomenon than previously recognized (Goldsmith *et al.* 2012).

Research on foliar uptake resulting from leaf wetting has not yet examined the impacts on plant or leaf water relations beyond considering the effects of additional water on Ψ_L. However, the study of leaf water pressure-volume relations may provide additional insight into whether foliar uptake alters specific traits associated with the movement and conservation of water in leaves. Pressure-volume relations demonstrate how Ψ_L varies as a function of changes in leaf water content (Tyree and Hammel 1972, Shulte and Hinckley 1985, Bartlett *et al.* 2012). This information can then be employed to understand the variation in leaf-level strategies used to maintain Ψ_L in support of photosynthesis (e.g. osmotic potentials and cell wall stiffness), as well as the point at which photosynthesis is no longer sustainable (e.g. turgor loss point). These traits in turn vary across broad gradients in plant water availability, demonstrating their broader role in plant functioning (Bartlett *et al.* 2012).

I studied the effects of foliar uptake on the leaf water pressure-volume relations of four woody species occurring in seasonally dry tropical premontane and montane cloud forests near Monteverde, Costa Rica, where the patterns of leaf wetting and the associated incidence of foliar uptake have recently been described (Goldsmith *et al.* 2012). In particular, I asked whether rehydration through both xylem and foliar uptake would result in changes in pressure-volume relations compared to rehydration through xylem alone.

METHODS

Study Site

This study was conducted in old growth lower montane wet forest (*montane*; 10°17.959'N, 84°47.460'W, 1563 m elevation) and old growth premontane wet forest (*premontane*; 10°17.918'N, 84°48.563'W; 1409 m elevation) on the Pacific slope of the Cordillera de Tilarán mountains near Monteverde, Costa Rica. General site descriptions following the same nomenclature are provided by Haber *et al.* (2000). In addition to high annual rainfall (>2000 mm), the region is subject to significant cloud immersion (Clark *et al.* 2000, Nair *et al.* 2008). As a result, leaf wetting events, occurring due to either rainfall or the impaction of cloud water droplets on leaf surfaces, are very common throughout the year (Goldsmith *et al.* 2012).

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Pressure-Volume Relations

To characterize the effects of leaf wetting on leaf-water relations, I compared the pressure-volume relations of leaves that were rehydrated through xylem and leaf wetting (two potential pathways) to leaves rehydrated through xylem alone (one pathway). Samples were collected from understory individuals of the woody premontane species *Cestrum panamense* (Solanaceae) and *Meliosma idiopoda* (Sabiaceae), as well as the woody montane species *Guarea rhopalocarpa* (Meliaceae) and *Coussarea austin-smithii* (Rubiaceae). Both observational and experimental methods have established that these species are all capable of foliar water uptake (Goldsmith *et al.* 2012).

Two branchlets with fully flushed leaves were collected from 4 individuals of each species after 18:00, re-cut under water, and rehydrated overnight while covered with a black plastic bag. Rehydration time was thus standardized at ca. 12 hrs. One of the two samples from each individual was also exposed to continuous wetting by means of an ultrasonic fog-generating machine (Chaoneng Electronics, Nanhai, Guangdong, China). Pressure-volume curves were generated by repeatedly making paired measurements of Ψ_L and leaf weight following intervals of bench drying (n = 9-12 points curve^{-1}). The surface of experimentally wetted leaves were carefully and completely dried immediately before initiating the measurements. Samples where rehydration had obviously not occurred (e.g. initial $\Psi_L < -0.2$ MPa) were discarded. Pressure-volume curve parameters, including leaf osmotic potential at full saturation (Ψ_P^{100}), relative water content at turgor loss point (RWC$_{TLP}$), water potential at turgor loss point (Ψ_{TLP}), bulk modulus of elasticity (ε), and relative capacitance were derived following the methods of Sack and Pasquet-Kok (2011). Visual inspection of each pressure-volume relationship revealed no evidence of any plateau effect associated with excessive rehydration (Parker and Pallardy 1987; Kubiske and Abrams 1990; Kubiske and Abrams 1991). Differences between pressure-volume curve parameters derived from experimentally wetted and control plants were evaluated using paired t-tests. Statistical analyses were carried out in R 2.15.1 (R Core Team 2012).

To further investigate the effects of leaf wetting on different leaf-water relations parameters, we also measured Ψ_L in leaves rehydrated through both xylem and leaf wetting compared to leaves rehydrated through xylem alone, in this case as a function of time. Methods were identical to those used for pressure-volume relations, except that 2 branchlets from only 3 individuals of each species were collected due to the necessity of carrying out parallel measurements on experimentally wetted and control branchlets. The surface of experimentally wetted leaves were dried immediately before initiating the measurements, which were carried out at 20 min intervals for 1 hr. Due to the small sample size, the experiment is presented for illustrative purposes only and no statistics were applied.

RESULTS

Mean values of pressure-volume traits determined for control leaves of all four species were in the range of previous values compiled for tropical moist forests in the review of Bartlett *et al.* (2012) (Figure 1). Pressure volume-relations measured on leaves of *C. panamense* rehydrated through both xylem and leaf wetting; however, demonstrated a significantly lower ε (defined as Δ turgor pressure / Δ RWC) as compared to leaves rehydrated through xylem alone (Table 1; Figure 2). This difference is most notable in the departure between the curves for the two treatments just prior to the Ψ_{TLP} (Figure 2). Although a similar quantitative difference in ε

between the two treatments was also observed in *M. idiopoda* and *G. rhopalocarpa*, no visual difference was observed. It is important to note that in these cases we must be cautious because the derivation of this parameter can be overly sensitive to the initial measurements based on the extent of rehydration for individual species. In *C. panamense* and *M. idiopoda* there was also a non-significant increase in capacitance (defined as Δ RWC / Δ Ψ_L) in leaves rehydrated through both xylem and leaf wetting, as well as a significant increase for *G. rhopalocarpa*. Finally, leaves of *M. idiopoda* rehydrated through both xylem and leaf wetting demonstrated a slight, but significantly higher increase in $\Psi_P{}^{100}$ as compared to leaves rehydrated through xylem alone. Leaves of *C. panamense* and *G. rhopalocarpa* rehydrated through both xylem and leaf wetting tended to demonstrate smaller changes in Ψ_L over time than leaves rehydrated through xylem alone, whereas *M. idiopoda* and *C. austin-smithii* demonstrated similar changes in Ψ_L over time between the two treatments (Figure 3).

DISCUSSION

Most research incorporating leaf water pressure-volume (PV) relations has only considered rehydration through the xylem, the dominant hydraulic pathway that plants use to obtain water for maintenance of tissue hydration and leaf transpiration. Here, I consider an expansion of the pressure-volume hydration protocol to include both the xylem hydration pathway and direct foliar water uptake pathway as possible avenues for increasing leaf tissue water content to a fully hydrated state. Foliar water uptake occurring as a result of leaf wetting did show biologically significant impacts on leaf pressure-volume relations; however, the results were not consistent across the four species studied. I believe that these inconsistencies may reflect differences in leaf anatomy among the species investigated.

Foliar Uptake Alters Pressure-Volume Relations

Among the species studied, the most novel and striking results were observed for *C. panamense*, which demonstrated a decrease in ε and a related increase in capacitance (a smaller change in Ψ_L per unit water loss) in leaves subjected to rehydration through both xylem and leaf wetting compared to xylem alone. This may have occurred if for *C. panamense*, water taken in through leaf surfaces is stored in apoplastic spaces (between cells or in cell walls) that would not normally be accessible as a result of leaf tissue rehydration through the xylem alone. This additional, perhaps excess, water appears to have increased tissue elasticity (ε), perhaps as a result of either additional water pushing against the cell walls or additional water within the cell walls themselves; either way, the outcome is a significant change in the 'hydrostatic' property of the tissue when rehydrated through both pathways. This additional water may also serve as a new "capacitor" within the leaf itself because these leaves experienced a slower decline in Ψ_L than plants rehydrated through xylem alone. The slower rate of dehydration may be due to some combination of the observed increase in capacitance or an increase in resistance to leaf water loss (Nobel and Jordan 1983, Kramer and Boyer 1995). Some plants rehydrating through foliar uptake have previously been observed to demonstrate slower rates of dehydration (Kerr and Beardsell 1975, Grammatikopolous and Manetas 1994, Gouvra and Grammatikopoulos 2003). Nevertheless, my results indicate that the effects of foliar water uptake on pressure-volume relations are not ubiquitous among species and that additional information on the traits driving the differences is of interest.

Differences in the presence, size, abundance and arrangement of different leaf anatomical structures may account for the observed differences in pressure-volume relations induced by rehydration through foliar water uptake. Experimental approaches to understanding leaf hydration kinetics have found evidence for distinct pools of water within a leaf, which were accounted for based on differences in leaf anatomy (Tyree *et al.* 1981, Morse 1990, Zwieniecki *et al.* 2007, Blackman and Brodribb 2011). In leaves of *C. panamense*, pools of water in the epidermal and/or spongy mesophyll tissues may serve as hydraulic capacitors that provide on-demand water for transpiration that buffers against rapid changes in Ψ_L imposed by low water supply or high water demand (Zwieniecki *et al.* 2007). Evidence for a wide range of different leaf anatomical strategies is provided for by the range of values observed in pressure-volume parameters for both the species studied here and in those examined across tropical moist forests (Bartlett *et al.* 2012). Future research would benefit from pairing measures of foliar uptake with measures of leaf anatomy across a diverse array of species.

Implications of Increased Capacitance

The maintenance or increase of Ψ_L through foliar water uptake has previously been demonstrated to facilitate photosynthesis at otherwise unsustainable rates (Grammatikopoulos and Manetas 1994, Munné-Bosch and Alegre 1999, Simonin *et al.* 2009). This is particularly important for plants that inhabit seasonally dry ecosystems such as the tropical montane cloud forests studied herein, where transient leaf wetting events from clouds or light precipitation ameliorate leaf water deficits when soil water availability is low (see Chapter 3). The increase in capacitance through foliar water uptake may also facilitate enhanced photosynthesis by improving Ψ_L and water content. Capacitance is hypothesized to serve in maintaining Ψ_L when plant water supply and demand fluctuates over the course of the day, thus enabling sustained transpiration (Sack and Tyree 2005). Moreover, capacitance is a fundamental component of leaf hydraulic conductance, which is positively related with photosynthesis (Sack *et al.* 2003, Brodribb *et al.* 2005). However, previous research has found no direct relationship between leaf capacitance and stomatal conductance (Brodribb *et al.* 2005). Moreover, the capacitance represented by a plant's total leaf area is generally considered to be a very small component of a plant's daily water use (Sack and Tyree 2005, Gotsch *et al.* in review). Whether or not the increase in capacitance that results from foliar water uptake is able to facilitate more than a transient increase in the water available to maintain photosynthesis remains to be determined.

CONCLUSIONS

Studies demonstrating that leaf wetting events leading to foliar uptake can affect water and carbon relations have largely originated from ecosystems where dew or fog is a significant component of the hydrologic cycle (Munné-Bosch and Alegre 1999, Gouvra and Grammatikopoulos 2003, Limm *et al.* 2009, Goldsmith *et al.* 2012). While there is no reason to expect that leaf wetting events resulting from rainfall in other ecosystems would not result in similar effects, studies of water relations almost exclusively focus on xylem as the sole means of plant rehydration. Our results suggest that in certain species, foliar uptake may serve to alter water relations and that future studies should explicitly consider the role of leaf wetting in plant functioning.

ACKNOWLEDGEMENTS

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Table 1. Pressure-volume relations measured in leaves rehydrated through xylem (control) compared to leaves rehydrated through xylem and foliar water uptake (treatment), including osmotic potential at full saturation (Ψ_P^{100}), relative water content at turgor loss point (RWC_{TLP}), water potential at turgor loss (Ψ_{TLP}), bulk modulus of elasticity (ε) and capacitance. Results represent means (± 1 *SE*) and numbers in bold are significantly different as measured by a paired t-test ($p < 0.05$).

|	*C. panamense*		*M. idiopoda*		*G. rhopolocarpa*		*C. austin-smithii*	
Parameter	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
Ψ_P^{100} (MPa)	-0.98 ± 0.03	-0.79 ± 0.12	**-0.45 ± 0.07**	**-0.53 ± 0.07**	-1.64 ± 0.18	-1.61 ± 0.19	-0.61 ± 0.05	-0.72 ± 0.02
RWC_{TLP} (%)	96.5 ± 1.0	94.4 ± 0.7	98.7 ± 0.6	97.2 ± 0.8	95.3 ± 0.8	91.9 ± 1.7	92.7 ± 1.2	93.4 ± 0.8
Ψ_{TLP} (MPa)	-1.08 ± 0.04	-0.95 ± 0.12	-0.55 ± 0.07	-0.65 ± 0.08	-1.9 ± 0.2	-1.9 ± 0.2	-0.77 ± 0.05	-0.90 ± 0.03
ε (MPa)	**29.7 ± 4.4**	**13.2 ± 2.7**	51.2 ± 12.8	18.8 ± 2.0	35.0 ± 2.9	22.0 ± 2.0	8.5 ± 1.3	10.8 ± 1.6
Capacitance (RWC MPa^{-1})	0.03 ± 0.01	0.071 ± 0.01	0.02 ± 0.01	0.04 ± 0.01	**0.03 ± 0.01**	**0.04 ± 0.01**	0.10 ± 0.01	0.08 ± 0.01
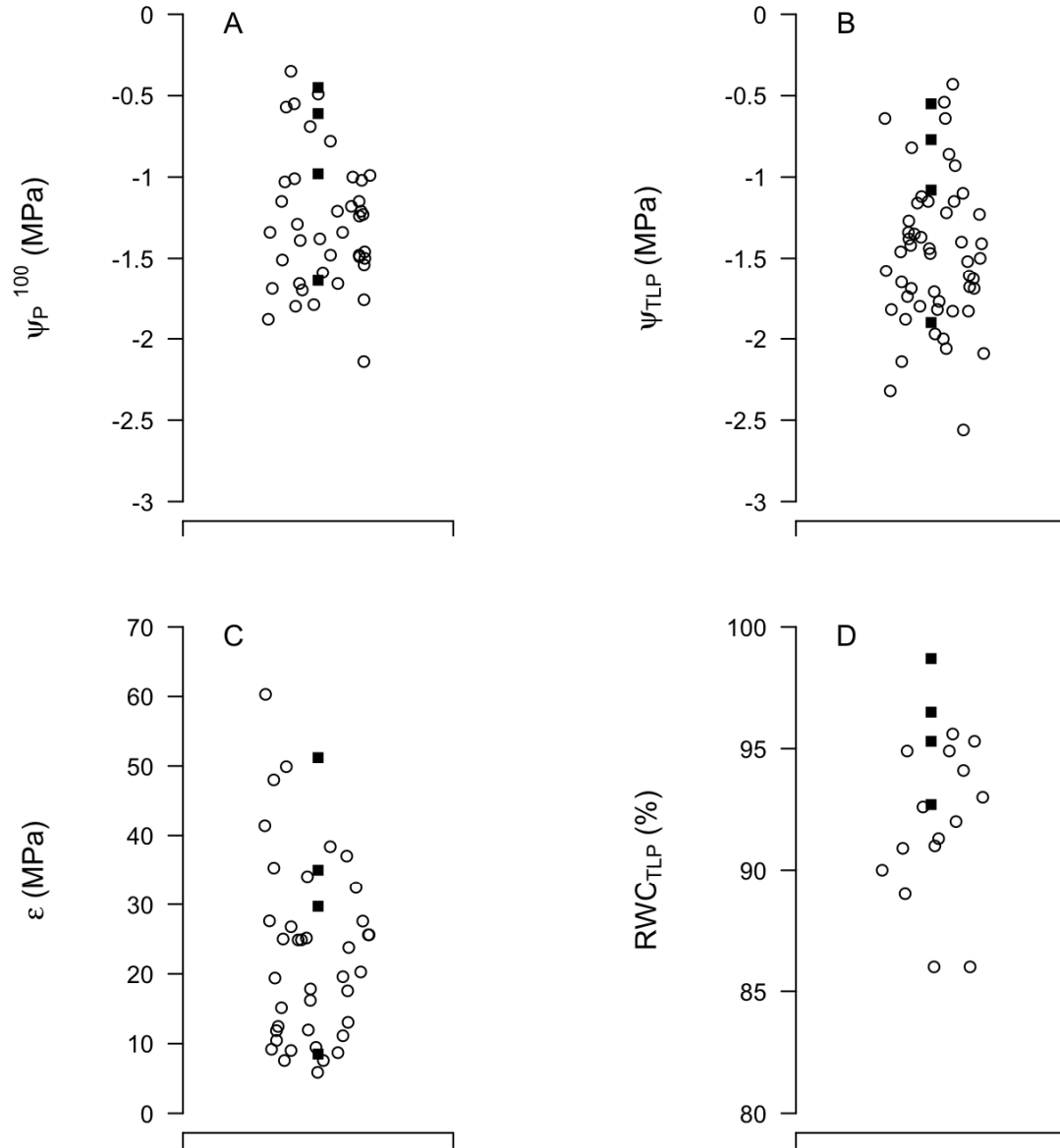
Figure 1. Mean values for pressure-volume parameters presented on a jitter plot (false x-axis), including (A) osmotic potential at full saturation (Ψ_P^{100}), (B) water potential at turgor loss (Ψ_{TLP}), (C) bulk modulus of elasticity (ε) and (D) relative water content at turgor loss point (RWC$_{TLP}$) for tropical moist forest species (open circles) and tropical montane cloud forest species studied herein (closed squares). Tropical moist forest species are as compiled by Bartlett *et al.* (2012).
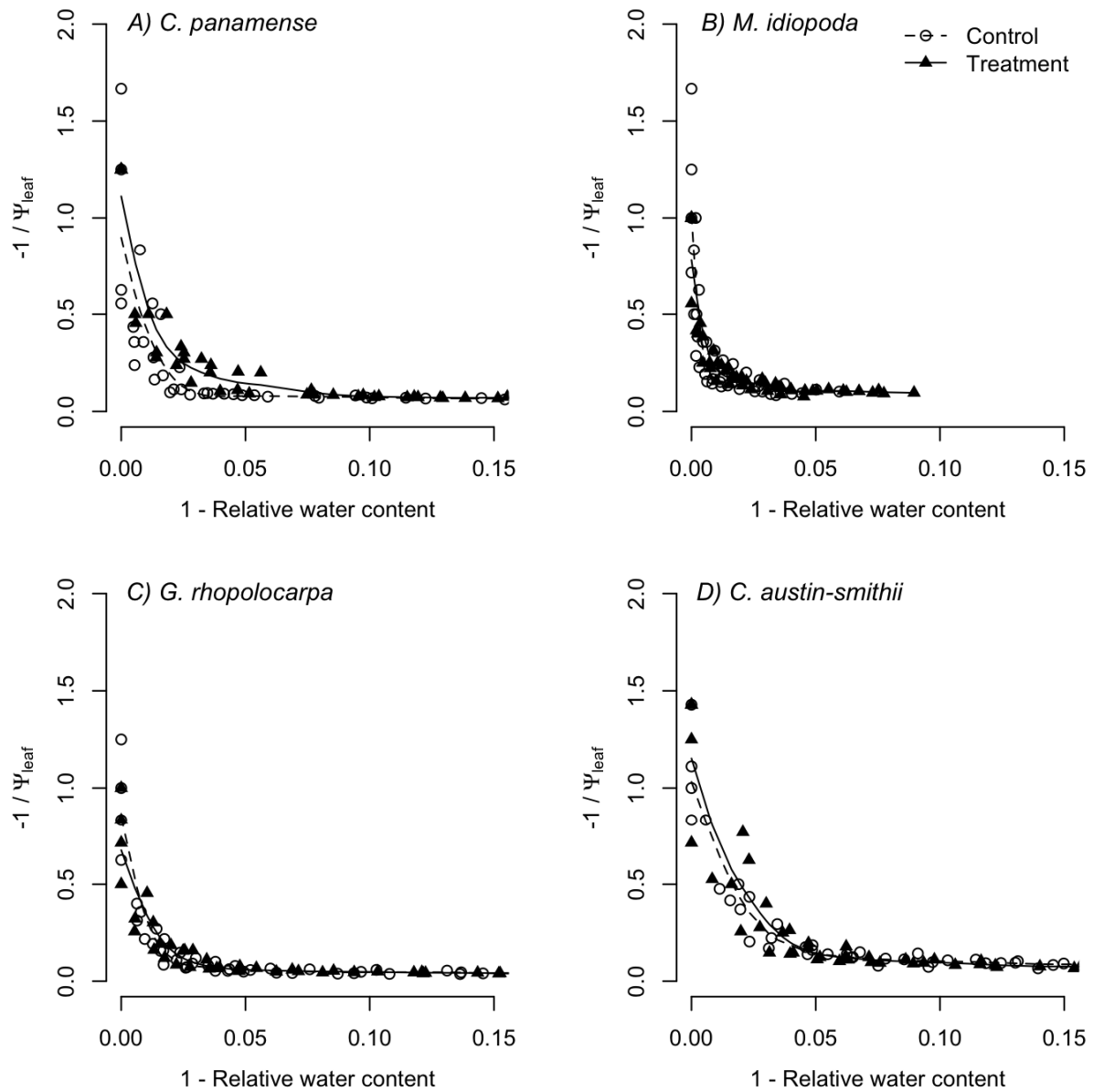
Figure 2. Pressure-volume water relations measured in leaves of (A and B) two tropical premontane and (C and D) two tropical montane cloud forest plant species rehydrated through both xylem and leaf wetting (treatment) or through xylem alone (control) (n = 4 individuals of each species). Curves represent smooth splines fit through all data for each treatment.
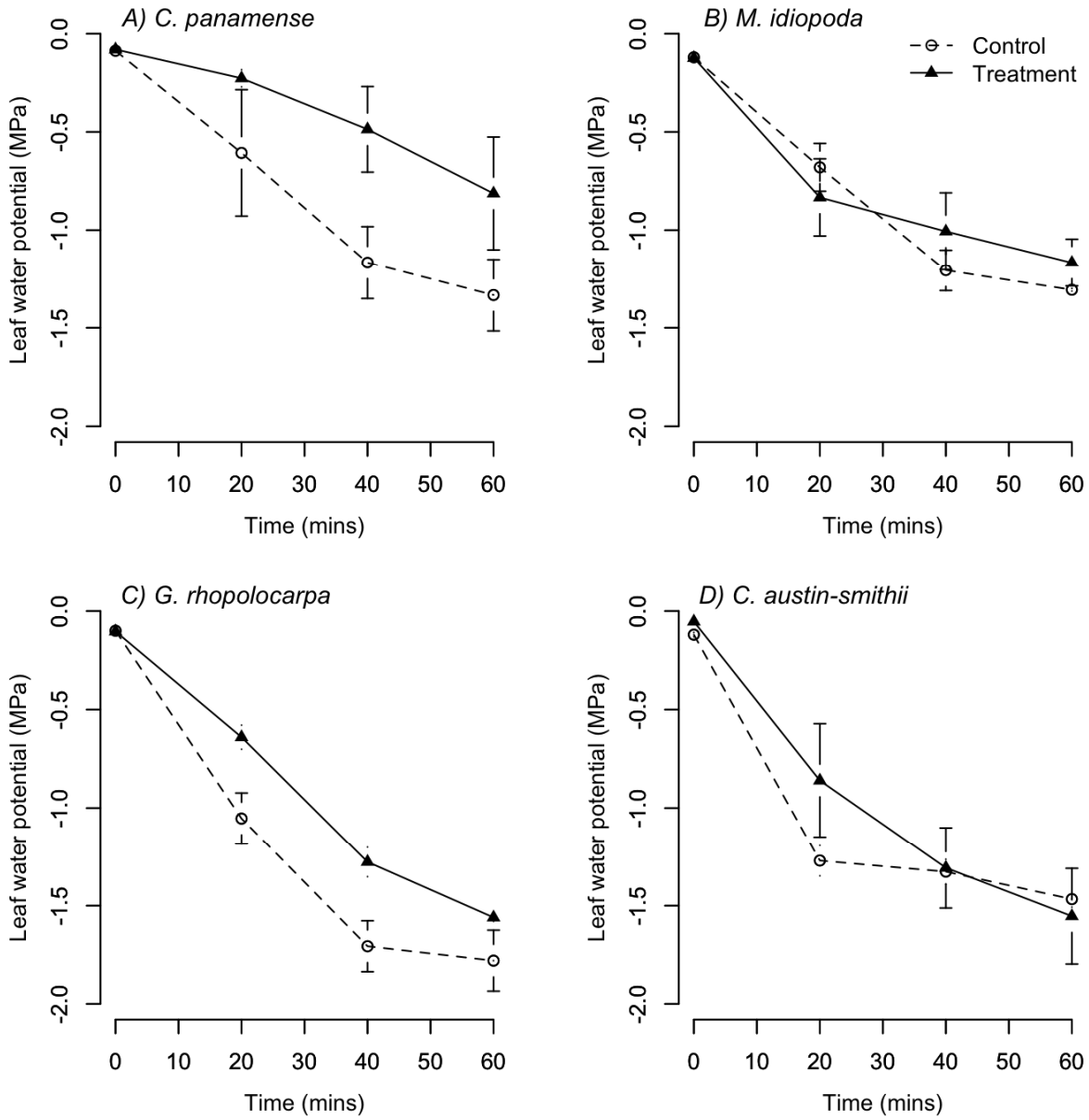
Figure 3. Leaf water potential measured over time in leaves of (A and B) two tropical premontane and (C and D) two tropical montane cloud forest plant species rehydrated through both xylem and leaf wetting (treatment) or through xylem alone (control) (n = 3 individuals of each species). Results represent means ± 1 *SE*.