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

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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. Here, 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 with 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 with 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. Copyright © 2011 John Wiley & Sons, Ltd.

KEY WORDS   climate change; deuterium excess; land use; Mexico; rooting depth; seasonality; secondary forest; translatory flow

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-analogue 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, 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). The variation generated through the evaporation and mixing of different water sources can be exploited to 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

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hydrologic cycle, and differences in the origin of the precipitation (i.e. convective versus orographic), the type of precipitation (i.e. rain versus 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 the 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 (δ^{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 2 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 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?

STUDY SITE

The study was carried out in two neighbouring 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*, *Quercus 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.*, 2011).

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.*, 2011). The underlying geology is composed of semi-permeable, moderately weathered andesitic breccias interspersed with weathered and fractured andesitic-basaltic rocks (saprolite). The topographical, soil physical, and vegetational characteristics of the study area are provided in greater detail by Muñoz-Villers *et al.* (2011).

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 in 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 and 555 ± 204 mm 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.*, 2011).

METHODS

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 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 collection tube (4 cm wide and 40 cm long) 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 1 to 2 days after the event. During the wet season, a sample was typically taken every 3 to 4 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 min 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, NC, USA).

Fluxes: Plants

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 *Q. lanceifolia*, *Q. ocoteifolia*, *A. latifolia*, and *Clethra mexicana* from the mature forest and *A. jorullensis*, *A. latifolia*, *C. mexicana*, and *M. glaberrima* from the secondary forest. Although we tested the efficacy of Bayesian mixing models to 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 the methods established above, we obtained plant xylem water samples from at least six 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 these 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:

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

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: Soils and streams

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 depths of 5, 15, 30, 50, 70, and 100 cm 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 were 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 2 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 by Muñoz-Villers et al. (in review).

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 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 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). Injected H_2O was reduced to H_2 gas in a hot chromium reactor, and the $^2H/H$ ratio of this gas was then analysed by mass spectrometry. For $\delta^{18}O$ analysis of plant and soil waters, $50\,\mu l$ of water samples was equilibrated in vials with a $0.2\,\%$ CO_2 headspace for 48 h at 21–23 °C. Following equilibration, vials were inserted into a GasBench II (GB, ThermoFinnigan) connected to a Delta Plus XL mass spectrometer (ThermoFinnigan). 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 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 $\delta^{18}O$) were calculated from spectral absorbance in specific wavelengths using off-axis integrated cavity output spectroscopy. We analysed a representative subset (10%) of these samples for spectral interference with the Spectral Contaminant Identifier V1.0 (Los Gatos Research) using the factory preset criteria, and found no evidence of organic contamination (West *et al.*, 2011).

For all methods, isotope ratios are expressed in per mil (‰) as:

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

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 isotope ratio mass spectrometry and isotope ratio infrared spectroscopy 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 totalled 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 totalled 2561 mm, and dry-season rainfall was nearly double that of the previous year, totalling 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, 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 with 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 with 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 and 31.55 mm for the March 2009 and May 2010 plant and soil sampling dates, respectively.

Over the course of the 2-year collection period, precipitation varied by more than 130‰ for δ^2H (range: −122.6‰ to 11.5‰) and 18‰ for $\delta^{18}O$ (range: −17.6‰ to 0.6‰) (Table 1; Figure 1). Mean wet-season precipitation events (δ^2H -38.7 ± 3.1‰ and $\delta^{18}O$ -6.8 ± 0.4‰ SE) were significantly depleted in both δ^2H and $\delta^{18}O$ as compared with dry-season precipitation events (δ^2H -12.6 ± 2.6‰ and $\delta^{18}O$ -4.2 ± 0.3‰ SE) (Wilcoxon rank sum, $n = 38$ dry-season 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; $\delta^2H = -5.67 - 0.08*precip$, $r^2 = 0.63$, $p < 0.0001$) (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 (Rozanski *et al.*, 1993; $\delta^2H = 10 + 8*\delta^{18}O$), the composite local meteoric water line (LMWL) had a higher slope and intercept (2008–2010: $\delta^2H = 18.5 + 8.21*\delta^{18}O$). Moreover, the 2008–2009 LMWL demonstrated a steeper slope compared with 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 with

Table I. 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.

| Month	2008–2009					2009–2010				
	δ^2H (‰)	$\delta^{18}O$ (‰)	d-excess	Precipitation (mm)	Temperature (°C)	δ^2H (‰)	$\delta^{18}O$ (‰)	d-excess	Precipitation (mm)	Temperature (°C)
April	−5.13	−3.03	19.09	39	15.50	−15.07	−3.55	13.34	299	16.44
May	−26.47	−5.72	19.28	375	16.93	−14.47	−3.79	15.83	128	16.11
June	−71.41	−10.93	16.01	735	14.16	−72.93	−10.94	14.55	376	15.93
July	−75.05	−11.03	13.18	637	14.44	−31.63	−5.85	15.20	239	16.00
August	−24.69	−4.94	14.85	456	15.58	−32.37	−5.87	14.58	749	15.53
September	−68.83	−10.74	17.05	675	14.52	−56.83	−9.08	15.82	670	15.66
October	−40.75	−7.54	19.56	341	12.71	−37.18	−7.19	20.34	304	15.65
November	−5.46	−3.82	25.14	85	11.87	−21.93	−4.84	16.78	67	12.87
December	−3.16	−3.04	21.14	74	12.18	−20.47	−5.20	21.17	183	12.70
January	−26.81	−6.18	22.59	61	11.85	−24.29	−5.54	20.05	121	10.19
February	−6.95	−3.66	22.32	74	12.80	−25.70	−5.57	18.86	116	10.63
March	10.19	−1.60	22.99	24	13.11	−0.76	−2.48	19.04	1139	12.98
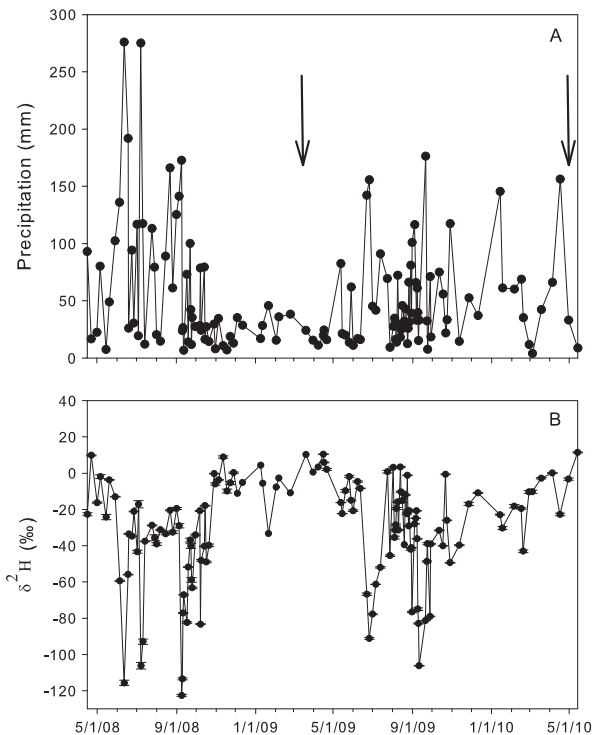
Figure 1. Weekly values for (A) net precipitation and (B) mean δ^2H ‰ (±1 SD) of precipitation samples collected at a tropical montane cloud forest in central Veracruz, Mexico. Arrows indicate soil and plant xylem water isotope sampling dates.
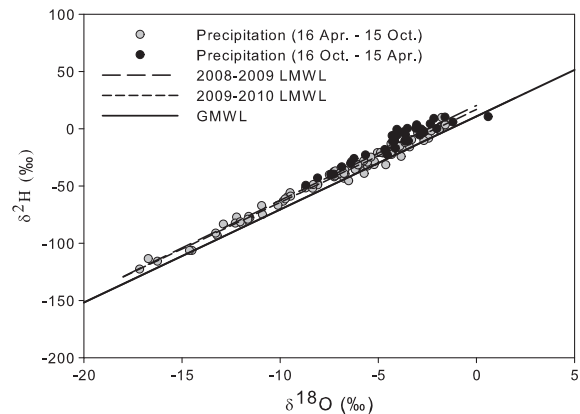

Figure 2. Water isotopes from wet-season and dry-season precipitation, collected between April 2008 and April 2010. The lines represent the 2008–2009 local meteoric water line (LMWL; $\delta^2H = 21.0 + 8.36 * \delta^{18}O$), the 2009–2010 LMWL ($\delta^2H = 16.6 + 8.10 * \delta^{18}O$) and the global meteoric water line (GMWL; $\delta^2H = 10 + 8 * \delta^{18}O$). The long-term LMWL for the area is $\delta^2H = 18.5 + 8.21 * \delta^{18}O$.

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 G).

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
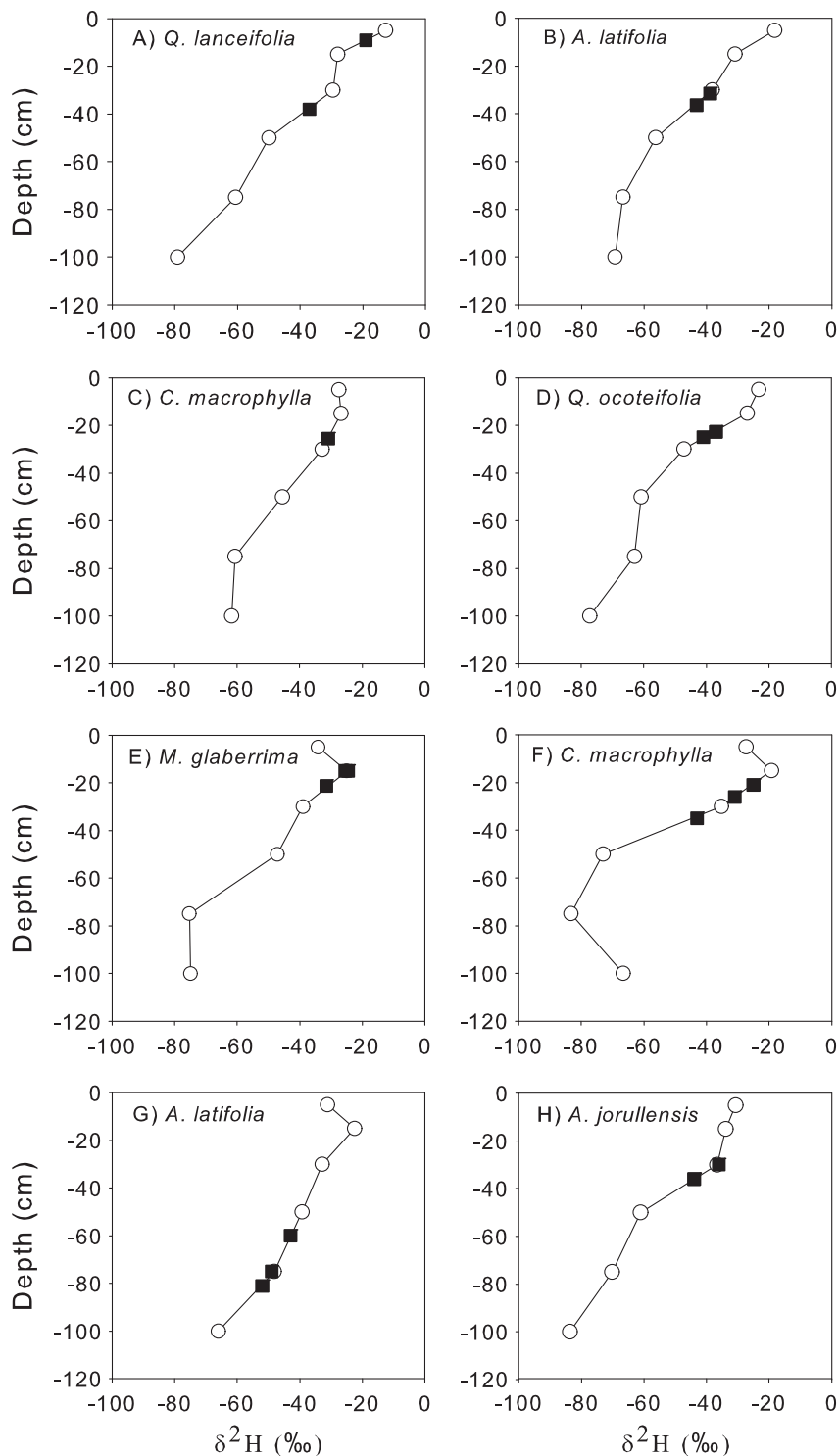
Figure 3. Stable isotopes of water (δ^2H ‰) from bulk soil samples collected at different depths (open circles) below the soil surface in mature (panels A–D) and secondary (panels E–H) tropical montane cloud forests sampled on 1 March 2009. Each line thus represents a distinct soil profile associated with the sampling of stable isotopes of xylem water from nearby trees (filled squares).

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 with 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.
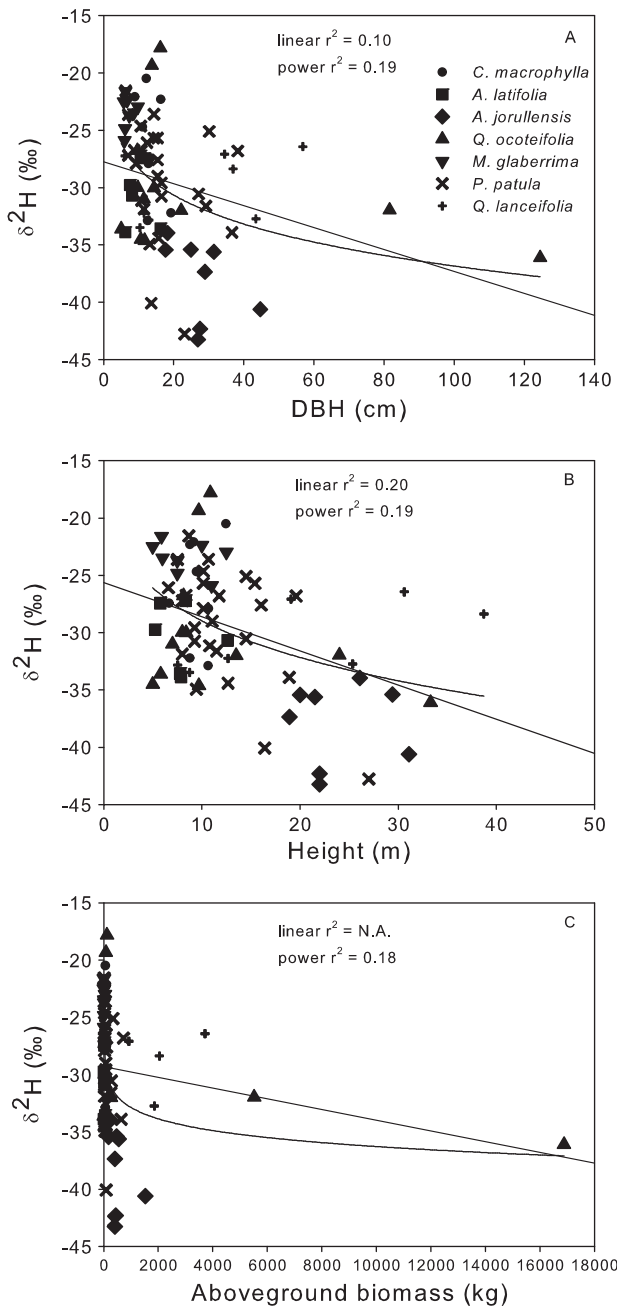
Figure 4. Relationship between δ^2H and measures of tree size including (A) DBH, (B) height, and (C) aboveground biomass, where δ^2H is a proxy for plant functional rooting depth. Lines represent linear (least-squares) and power functions.
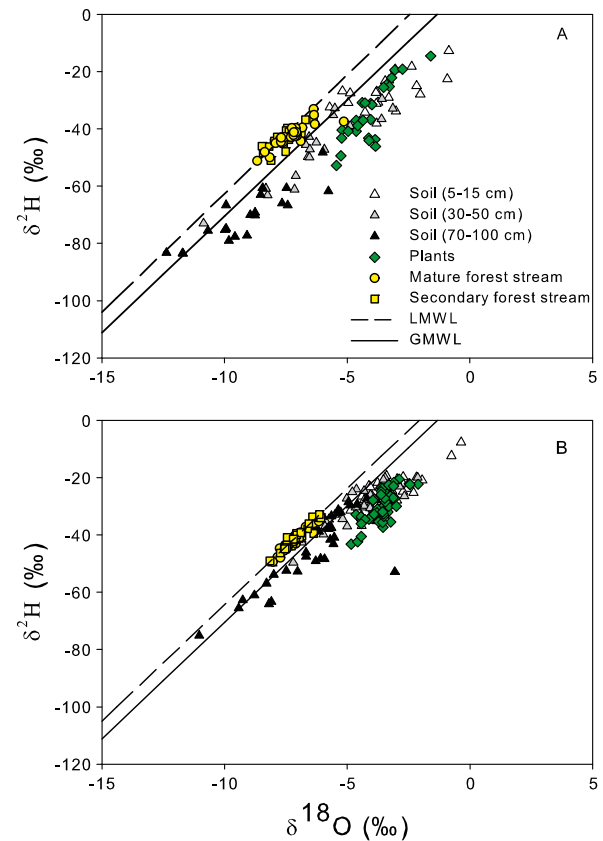

Figure 5. Stable isotopes of water collected from bulk soils, plants, and streams in mature and secondary tropical montane cloud forests. Soil and plant samples were collected in (A) 1–2 March 2009 and (B) 4–5 May 2010. Stream samples, as well as precipitation samples used to generate the LMWL, were collected regularly during their respective hydrologic years.
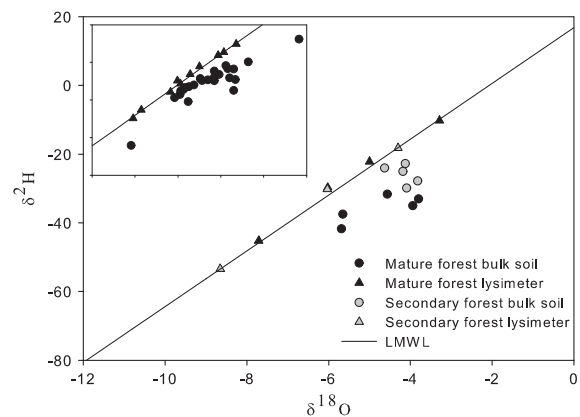

Figure 6. Water stable isotopes from lysimeter and bulk soil samples collected at different depths (5–120 cm) in mature and secondary tropical montane cloud forests. Lysimeter samples represent the accumulation of 7 days of water collected on 5 May 10, while all soil samples were collected nearby on the same date. Inset represents data collected across a larger spatial scale in the mature forest on the same date. The line represents the LMWL for 2009–2010 ($\delta^2H = 16.8 + 8.12\delta^{18}O$).

Pools: Soils and streams

Stable isotope values of bulk soil water consistently fell below the LMWL, 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 did not

undergo 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: $\delta^{18}O$ −8.5 to −6.2‰ and δ^2H −51.0 to −35.3‰ and secondary (range: $\delta^{18}O$ −8.7 to −5.1‰ and δ^2H −51.2 to −33.1‰) forests over the 2 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.*, in review). Further evidence of this mixing is seen in the average stable isotope values of the groundwater samples obtained in the mature forest in 2009 ($n = 3$; $\delta^{18}O$ −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 ($n = 67$; $\delta^{18}O$ −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 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 the re-evaporation of previously condensed water vapour. The deuterium excess values reported herein are consistent with those 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 changes in deuterium excess suggested to occur as a result of the shifting of the Inter-Tropical Convergence Zone in relation to regional terrestrial water sources in that example are not completely analogous to 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 in the tropics (Bowen, 2008). However, variation is likely underestimated due to a recognized lack of reliable long-term sampling. For instance, the GNIP (Global Network of Isotopes in Precipitation) 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 $\delta^{18}O$ 18‰, which are

consistent with the results of 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 the 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 of 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, consistent with the findings of Holwerda *et al.* (2010) and Muñoz-Villers *et al.* (2011).

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 forests.

Despite a small range of plant xylem water stable isotope values (~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 of 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 in our system, it is 8% of the total in 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 undergoes precipitation through the dry season. The maximum dry-season precipitation deficit (rainfall reference evapotranspiration) over the last 4 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 rainforests (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, personal communication). 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 at <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 of 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 of 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 of 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 of the complete displacement of all the standing soil water in the soil profile with additional precipitation infiltration, but rather of some form of preferential flow through the soil that maintains the two distinct pools described above, which has yet to be described in these systems.

However, there is long-standing evidence of 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, 1964). Additional evidence of 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 and Wierenga, 1979; De Smedt *et al.*, 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 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 with 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 suggesting that 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 with the LMWL, 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 the 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 with 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 a quantitative foundation for generating process-based eco-hydrology 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, exert evaporative effects (enrichment) that result 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 with 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 undergo 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 tendency to explain processes that are
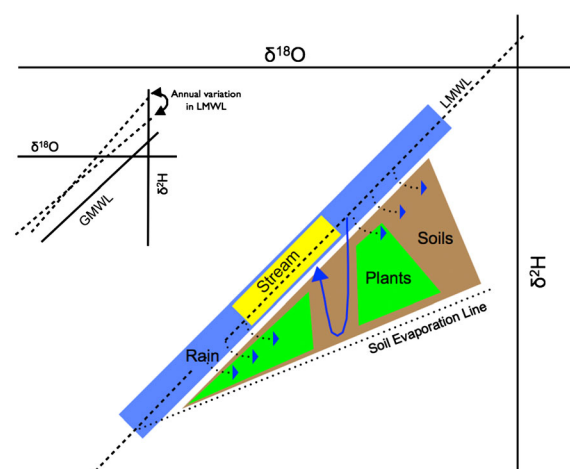

Figure 7. A conceptual model for understanding inputs, fluxes, and pools of water moving through a tropical montane cloud forest as represented in dual isotope space. Inset represents inter-annual variation in the LMWL generated by differences in precipitation inputs.

not synchronized in time and space must be avoided. For instance, in our model, the LMWL 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 undergo 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 modelling.

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