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Stable Isotope Characterization of the Ecohydrological Cycle at a Tropical Treeline Site

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Abstract

We investigated the seasonal variation in pools of water available to mature trees growing at high elevation in a tropical environment. The study focused on the dominant tree species (*Pinus hartwegii*) at about 3800 m a.s.l. on Nevado de Colima, Mexico, where climate is typical of the North American Monsoon System. Stable isotope ratios of hydrogen and oxygen in water extracted from soil, xylem, and leaves were measured through a cycle of two dry and two wet seasons in 2003–2004. Isotopic ratios were also measured in accumulated precipitation, a few single precipitation events, and in spring water over the two-year period. Based on evidence from water, stable isotopes in soil, and xylem samples, trees utilized water from relatively shallow soil depths, which are representative of current conditions, rather than tapping groundwater, which is more representative of long-term trends. While the stable isotope signature in environmental waters showed a slightly different pattern before and during the monsoon, the more pronounced differences in leaf water isotopes between the two seasons, due to drought stress, will lead to a clear seasonal isotopic signal in tree ring cellulose. This study represents a unique snapshot of water cycling in a tropical treeline ecosystem, where our understanding of ecohydrological pathways is limited. This type of analysis is also useful for proper calibration of stable isotopic signals in tree ring records.

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Introduction

Global change research has placed increased emphasis on studying hydrologic cycles (IPCC, 2001). Concentrations of water vapor are highly dependent on the functional processes of the terrestrial biosphere, specifically transpiration and photosynthesis in plants (Ainsworth and Long, 2005), so understanding the movement (cycling) of water through plants is a critical part of these studies. Water cycling in the terrestrial biosphere can be investigated using stable isotopic ratios (IAEA, 2002). Water arrives at the terrestrial biosphere as precipitation, and returns to the atmosphere through direct evaporation at the soil surface and through transpiration of terrestrial plants. Each of these processes imparts a different isotopic signal, through distinct fractionation processes, on the water returning to the atmosphere and on the water used for tree growth (Dawson and Ehleringer, 1998). Isotopic fractionation of water takes place during direct evaporation from soil, and during gas exchange at the leaf surface (transpiration). In both cases, the heavy isotopes are less likely to enter the vapor phase, leaving them in higher concentrations in the water left behind. Fractionation does not take place in the root water uptake or in xylem transport in the stem. The timing and distribution of water availability during the growing season greatly influences the stable isotope composition of tree ring cellulose (Pendall, 2000; Jaggi et al., 2003).

The North American Monsoon System (NAMS; Vera et al., 2006) regulates summer precipitation over Central America, Mexico, and the American Southwest, reaching as far north as Las Vegas and the Great Basin (Adams and Comrie, 1997; Magana et al., 1997, 1999; Barlow et al., 1998). The percentage of total annual precipitation due to the NAMS peaks at 65% in northwestern Mexico, decreases to 50% in southwestern Arizona and southeastern New Mexico, and is further reduced moving northward (Douglas et al., 1993; Higgins et al., 1999), with corresponding influences on water resources and plant growth in these regions. In addition, an out-of-phase relationship exists between NAM precipitation and warm-season rainfall in the Great Plains/Northern Tier region (Higgins et al., 1997, 1998, 1999). The potential for seasonal forecasting of North American summer precipitation has been underscored by a possible mechanistic link with sea surface temperatures (Castro et al., 2001; Mitchell et al., 2002; Mo and Juang, 2003). Another connection has also been proposed between snow cover in western North America and the NAM (Ellis and Hawkins, 2001; Lo and Clark, 2002). Based on instrumental records, both the onset and total amount of NAM rainfall have oscillated over time, with a tendency towards increased variability in recent years (Higgins and Shi, 2000). Given the interplay between ocean, atmosphere, and land that characterizes the NAMS, it is necessary to place modern instrumental observations (which are limited to a few decades) within a longer temporal perspective in order to fully represent the range of NAM variability and improve our understanding of underlying processes and driving forces at interannual to interdecadal scales.

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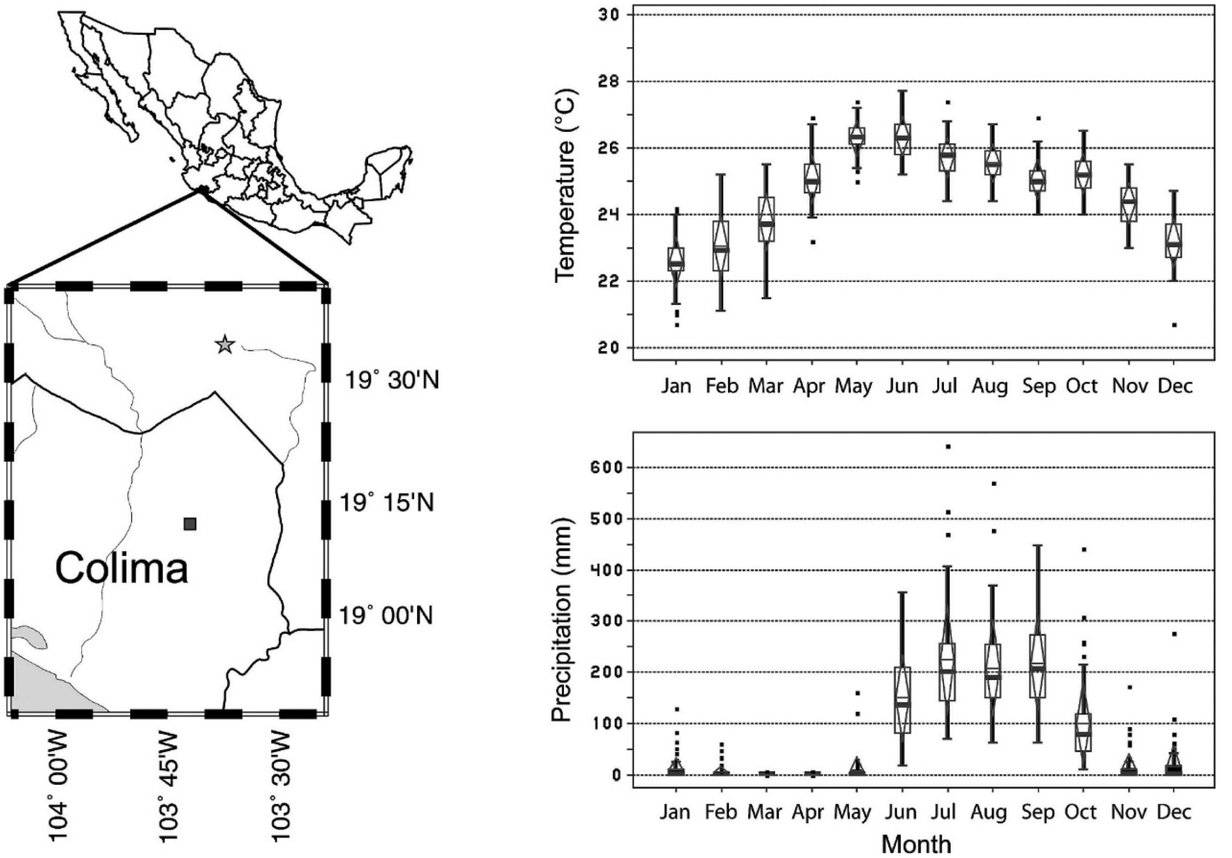

FIGURE 1. (Left) Map of Mexico with expanded section showing the location of Nevado de Colima (star) and Colima City (square). (Right) Climatic box-plot diagram of total monthly precipitation and mean monthly temperature at Colima City (source: Global Historical Climate Network, v. 2, http://www.ncdc.noaa.gov/oa/pub/data/ghcn/v2/ghcnftp.html).

Stable isotope analysis of tree rings has been proposed as an ideal tool to quantify past climatic changes in tropical regions, especially for trees without annual growth rings (Evans and Schrag, 2004; Poussart et al., 2004). Stable isotopes signals can also provide important information about moisture cycling and growth dynamics in trees that have datable annual rings (Roden and Ehleringer, 2007). For the North American Monsoon, a lingering question is the source of precipitated moisture, either from the Pacific or the Atlantic Ocean (Berbery, 2001). The stable isotopic signature of water (i.e., δ^{18}O for oxygen, and δD for hydrogen) in the eastern tropical Pacific and western tropical Atlantic may be different enough (Wright, 2001) to allow for a corresponding stable isotope signal in tree ring cellulose. Stable isotopes correlate with periods of increased intensity of the Asian Monsoon in China (Aucour et al., 2002). Reconstructing the temperature and relative humidity record from stable isotopic signals in tree ring samples (Schleser et al., 1999; Roden et al., 2000) benefits from a comprehensive isotopic description of the eco-hydrological cycling of water, from the atmosphere to the soil, then from the ground to the tree, and finally back to the atmosphere.

While various studies have used stable isotopes to trace water sources and plant water use (Thorburn and Walker, 1993; Dawson, 1998), relatively few have been undertaken in the tropics and even fewer at high elevation (Field and Dawson, 1998; Stratton et al., 2000; Drake and Franks, 2003). Our study was conducted in a tropical high elevation ecosystem that is subject to alternating dry (before the monsoon) and moist (during the monsoon) conditions. The working hypothesis was that differences in water isotopic composition related to the dry/wet seasons could be measured throughout the atmosphere-soil-tree continuum. We then sampled multiple components of the water cycle to test for differences before and during the monsoon. Hydrogen and oxygen stable isotopic ratios from precipitation, groundwater, soil, xylem, and needle water were used to characterize the end of the dry season (March) and the end of the monsoon (November). Such investigation of isotopic ratios in the ecohydrological cycle, although not routinely performed, helps understanding the mechanism for a distinguishable climatic signal being recorded in tree ring cellulose.

Materials and Methods

STUDY SITE

The study area was located on Nevado de Colima (19°34.778′N, 103°37.180′W), in western central Mexico, at an approximate elevation of 3800 m a.s.l. (Fig. 1). This site has been used for investigating the dendroecology of tropical treelines (Biondi, 2001; Biondi et al., 2003) as well as the relationship between treeline location and temperature in the tropics (Körner and Paulsen, 2004). Nevado, an extinct volcano, is the westernmost peak in the Mexican transvolcanic belt (Gonzáles et al., 2002). Its summit lies about 5 km north of Volcán de Fuego, an active volcano. The vegetation at this high elevation tropical site is dominated by an open forest composed of nearly pure stands of *Pinus hartwegii* interspersed with bunch grasses (*Calamagrostis tolucensis*). The soils are volcanic in origin with relatively shallow organic horizons, high porosities, and high water contents (20–

TABLE 1

Isotope sampling sites at Nevado de Colima.

Site ID	Location	Distance from Weather Station	Aspect	Elevation (m)
S3	19°34.685′N, 103°37.125′W	100 m SE	SW	3795
S4	19°34.819′N, 103°36.708′W	1500 m NW	NE	3817
S5	19°35.155′N, 103°37.164′W	1000 m W	WNW	3828

35% water content by volume at 10–30 cm depths). Three sampling sites were selected within a 3-km radius from an automated meteorological station, whose records were described by Biondi et al. (2005).

The climate of the study area (Figs. 1 and 6A) is a monsoon-type climate with a distinct summer wet season (June–October) interspersed with a slightly drier period in August and a prolonged dry season (November–May). Annual rainfall averages 1000–1200 mm, with daily precipitation reaching a maximum of 164 mm, based on data collected between 22 May 2001 and 24 March 2004 (Biondi et al., 2005). The dry winter season is characterized by cold high pressure cells coming from the north, occasionally accompanied by cold fronts producing snow falls at higher altitudes (Galindo Estrada et al., 1998). Minimum and maximum 30-minute temperatures at our weather station for the period 22 May 2001 to 24 March 2004 are −13°C and 18°C, respectively (Biondi et al., 2005). Water samples were taken from multiple components of the ecohydrological cycle, i.e. from precipitation, a natural spring, shallow and deep soil water, tree xylem, and needles. This was done twice a year, i.e. shortly before the monsoon (March) to get an accumulated record of the fall/winter/spring precipitation, and again just after the summer rains (November) to get a record of the monsoon precipitation.

PRECIPITATION AND GROUNDWATER

Due to the remote nature and difficult access of the study area, discrete sampling of precipitation events was not possible. Bulk precipitation samples were obtained using two collectors, which were added in 2003 to our automated weather station (in operation since 2001). These collectors are of a simple design that has been used widely by the U.S. Geological Survey (Claussen and Halm, 1994), and consist of a funnel collection area converging into a restricted opening to the collection vessel below. A layer of mineral oil in the collection vessel is used to further reduce evaporation. In November 2004 we also collected several single-event, liquid and frozen precipitation samples. Groundwater samples were collected twice a year from a natural spring that discharges 500 m NW of the meteorological station. Since there are no apparent surface water sources, we assumed the spring was fed mostly by groundwater.

Rainfall samples during the 2004 and 2005 monsoon were collected from the Centro Universitario de Investigaciones en Ciencias del Ambiente at the Universidad de Colima (19°12.6′N, 103°48.3′W, ~400 m a.s.l., mean annual total precipitation 1275 mm). These samples were intended to show the complex evolution of the isotopic signature of the monsoon from storm to storm (Fig. 6B). The two collection sites, i.e. the Universidad de Colima and the weather station on Nevado de Colima (NDC) are only 40 km apart from each other, but the NDC site is 3400 m higher in elevation. In a monsoon dominated system, relatively close to the Pacific Ocean, rapid elevation and temperature changes are expected to play a large role in isotopic evolution (Clark and Fritz, 1997). The change in isotopic ratios with altitude is largely a function of condensation temperature, as well as rainout due to orographic lifting, and is approximately linear (Poage and Chamberlain, 2001). However, many of the data points used for Poage and Chamberlain's (2001) globally averaged lapse rate of $-2.8‰\ \mathrm{km}^{-1}$ are at higher latitudes or at higher elevation, hence places where much of the precipitation falls as snow. To better reflect our site conditions, and also take into account the effects of secondary evaporation at our low latitude site, we adopted the lapse rate calculated from a study in Oman (Stanger, 1986), i.e.

$$\Delta\delta^{18}\mathrm{O}_{\mathrm{precipitation}} = -0.002(\Delta\mathrm{elevation}), \qquad (1)$$

which is equivalent to an approximate collapse rate for δ^{18}O of $2‰\ \mathrm{km}^{-1}$ of elevation. This relationship was used to compare the δ^{18}O values measured for single storms in Colima city with those from bulk collectors at Nevado de Colima. A similar comparison (Colima city vs. Nevado) was done for δD by assuming that the linear relationship between δ^{18}O and δD at Colima city is maintained as the precipitation moves up the mountain. Based on that assumption, we then found that

$$\Delta\delta\mathrm{D}_{\mathrm{precipitation}} = -0.014(\Delta\mathrm{elevation}), \qquad (2)$$

A comparison of deuterium excess (Clark and Fritz, 1997) in isotopic ratios scaled for elevation with those obtained from the bulk collectors confirmed our assumption. Average deuterium excess was 15.2‰ for bulk-collected monsoon precipitation, and it was 15.6‰ for the elevation scaled values weighted by the amount of precipitation. Furthermore, the event-based precipitation collected in the city, scaled for elevation, and the pre-monsoon bulk precipitation collected on the mountain (representing the rather small fraction of precipitation that falls during the winter), were used to calculate a regional Local Meteoric Water Line (LMWL) for the site.

SOIL, XYLEM, AND LEAF WATER

Sampling sites were located on different aspects relative to each other, and within several hundred meters of treeline (Table 1). Soil samples were collected during March and November of 2003 and 2004. Soil pits were dug to 30 cm at all three sampling sites, and discrete samples were taken from the 15 and 30 cm horizons. In March 2003, a third soil pit was sampled adjacent to the weather station (for purposes of calibrating the soil moisture sensor) and an additional sample was collected from 50 cm depth. Samples were placed in glass jars and sealed in the field. Xylem samples were extracted from trees at each collection site. Two cores, ~20 mm long and 12 mm in diameter, were taken from each tree at breast height on opposite sides of the tree and parallel to the slope contours. The bark was removed from these cores in the field, and the xylem samples were placed in glass vials and sealed. Leaf (needle) samples were taken from the cored tree or from a tree adjacent to it and of similar size. Two leaf samples were taken from each tree, one on the north and one on the south side of the tree. Needles were cut, placed in glass vials, and sealed in the field. An effort was made to collect all samples from a given

TABLE 2

Mean isotopic values of environmental waters collected on Nevado de Colima.

			2003			
Pre-monsoon	$\delta^{18}O_{VSMOW}$ (‰)	Std Dev	# of Analyses	δD_{VSMOW} (‰)	Std Dev	# of Analyses
Xylem	−15.7	0.7	11	−112	3.0	11
Precipitation	−6.3	2.1	2	−46	10.8	2
Spring	−15.5	0.1	2	−110	0.1	2
Soil 15 cm	−16.8	0.6	4	−125	6.3	4
Soil 30 cm	−18.4	1.5	3	−134	12.0	3
Soil 50 cm	−19.5	—	1	−147	—	1
Leaf	9.9	2.8	10	−39	4.8	10
Monsoon						
Xylem	−18.5	1.2	9	−131	8.3	9
Precipitation	−12.6	1.2	5	−89	11.7	5
Spring	−15.1	—	1	−104	—	1
Soil 15 cm	−18.9	1.5	3	−134	12.3	3
Soil 30 cm	−24.8	0.9	3	−174	6.6	3
Leaf	−9.4	0.6	12	−83	8.0	12

			2004			
Pre-monsoon	$\delta^{18}O_{VSMOW}$ (‰)	Std Dev	# of Analyses	δD_{VSMOW} (‰)	Std Dev	# of Analyses
Xylem	−14.7	1.2	9	−107	7.2	9
Precipitation	−10.9	0.5	2	−75	3.4	2
Spring	−15.5	—	1	−109	—	1
Soil 15 cm	−11.8	1.2	3	−84	1.8	3
Soil 30 cm	−13.8	2.3	3	−98	19.2	3
Leaf	3.3	2.1	12	−27	5.1	12
Monsoon						
Xylem	−12.6	0.4	9	−88	2.7	9
Precipitation	−12.9	0.1	2	−87	0.1	2
Spring	−15.2	—	1	−109	—	1
Soil 15 cm	−13.0	0.9	3	−90	3.7	3
Soil 30 cm	−11.6	1.3	3	−79	9.2	3
Leaf	16.7	6.2	12	−7	15.7	12

site at the same time of the day because isotopic ratios, especially in the leaves, can change quickly during the diurnal evapotranspiration cycle. All samples were frozen as soon as possible after collection.

WATER EXTRACTION

Extraction of water from soil, xylem, and leaves, with subsequent isotopic analysis of those waters, was done at the Nevada Stable Isotope Laboratory of the University of Nevada, Reno. Water extraction for all samples was done using the toluene azeotropic distillation process (Revesz and Woods, 1990; Ingraham and Shadel, 1992; Thorburn et al., 1993). Water-δD analyses were performed in continuous flow mode, using an Eurovector elemental analyzer interfaced to a Micromass IsoPrime stable isotope ratio mass spectrometer, following the method of Morrison et al. (2001). Water $\delta^{18}O$ analyses were performed in dual inlet mode, using a Micromass MultiPrep preparation device interfaced with a Micromass IsoPrime stable isotope ratio mass spectrometer, following the CO_2-H_2O equilibration method of Epstein and Mayeda (1953). Isotope values use the delta notation, where the isotopic composition of a given material is compared to a global standard as given by

$$\delta = \left(\frac{R_s}{R_{st}} - 1\right) * 1000 \qquad (3)$$

where δ is the isotope ratio (δD for hydrogen, $\delta^{18}O$ for oxygen), R_s is the molar ratio of heavy to light isotope in the sample, and R_{st} is the ratio of heavy to light isotopes in the standard. Results are expressed relative to the Vienna Standard Mean Ocean Water (VSMOW). Sample precision and accuracy is ±0.1‰ for $\delta^{18}O$ and ±1‰ for δD (values are ± one standard deviation)

Results

A summary of all stable isotopic measurements is given in Table 2. Monsoon and pre-monsoon sample values over the two years of measurement are plotted in Figure 2 except for leaf water, which is plotted in Figure 3. A regression line (Sokal and Rohlf, 1981) was fit to the isotopic data that characterize the ecohydrologic cycle (i.e. the bulk precipitation, soil, stem, and spring water samples) for both years of measurement, with the following results:

$$\begin{array}{lllll} \text{Pre-monsoon}: & \delta D = 7.44\,\delta^{18}O + 3.3 & N = 43 & R^2 = 0.97, \\ \text{Monsoon}: & \delta D = 7.17\,\delta^{18}O + 2.5 & N = 40 & R^2 = 0.98. \end{array}$$

The *p*-values for the slope parameters are much less than 0.001, but the estimated intercepts are not significantly different from zero (*p*-values > 0.25). The difference between the two slope coefficients was highly significant according to an *F*-test procedure (Sokal and Rohlf, 1981, p. 505).

These equations can be compared to the Global Meteoric Water Line (GMWL; Fig. 2); i.e. $\delta D = 8.13\,\delta^{18}O + 13.9$, which represents the average relationship between hydrogen and oxygen in global precipitation (Rozanski et al., 1993). The slopes are similar between the GMWL and the lines fit through the

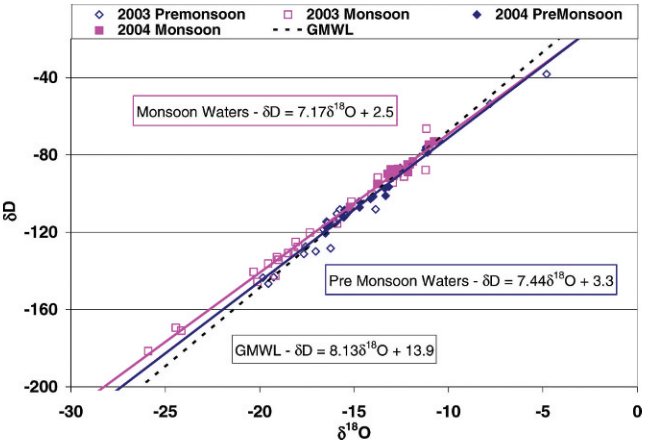

FIGURE 2. Stable isotopic composition of precipitation, soil water, xylem water, and groundwater (from a spring) at the Nevado de Colima study area. A separate regression line was fit to isotopic values before and during the monsoon to summarize and compare them. Also plotted is the GMWL, or Global Meteoric Water Line (Rozanski et al., 1993).

environmental waters collected on the mountain, suggesting minimum secondary evaporation or other fractionation (though the slope is significantly shallower during the warm season, likely indicating some secondary evaporation). However, the intercepts are very different between the GMWL and these seasonal relationships. In part this is caused by shallowing of the slopes (<8) on the intercept. This is also a measure of deuterium excess, with the higher intercept indicating evaporation at higher relative humidity (Clark and Fritz, 1997). Such patterns are consistent with a tropical moisture source. Statistically significant differences between pre-monsoon and monsoon slopes may be due to the amount effect, i.e. more intense precipitation during the monsoon vs. the infrequent post-monsoon storms (Gonfiantini et al., 2001). In fact, deuterium excess values are higher for all bulk monsoon samples relative to pre-monsoon samples, suggesting more intense precipitation and rainout of air masses.

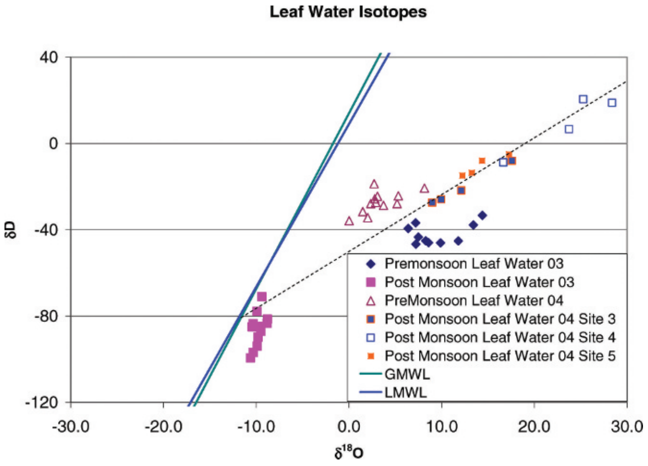

FIGURE 3. Oxygen and hydrogen stable isotopic ratios in leaf tissues collected at the Nevado de Colima study area in 2003 and 2004. Also plotted are the Global Meteoric Water Line (GMWL, see Fig. 2), the Local Meteoric Water Line (LMWL, see Fig. 4), and an evaporation profile (dashed line).

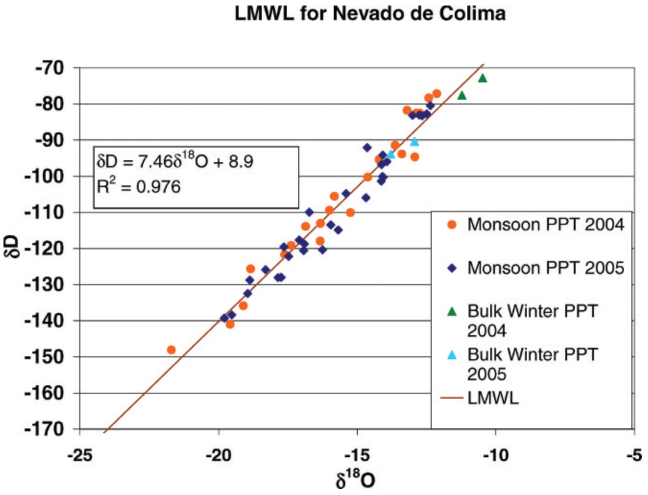

FIGURE 4. Isotopic ratios of samples collected from individual storms in 2004 and 2005 at the Universidad de Colima ("Centro Universitario de Investigaciones en Ciencias del Ambiente"; elevation ~400 m), and scaled for the elevation difference between that site and the study area at Nevado de Colima (~3800 m). Also plotted are bulk precipitation samples from the study area. A Local Meteoric Water Line (LMWL) was obtained by fitting a linear regression equation to the stable isotope data.

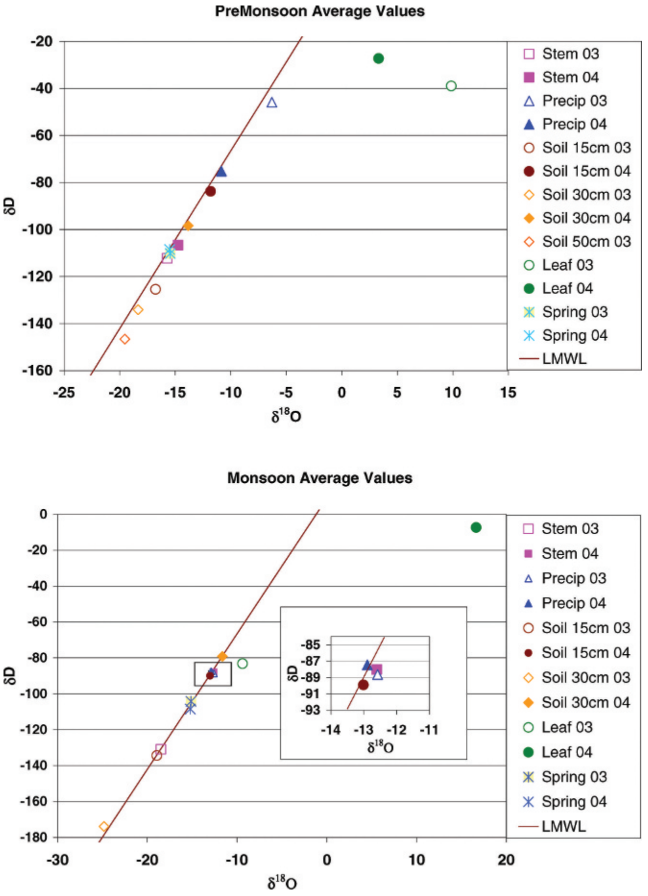

FIGURE 5. Averages of isotopic measurements taken from different components of the water cycle over the two years of monitoring at the Nevado de Colima study area.

TABLE 3

Stable isotope measurements of waters collected on Nevado de Colima[1].

2003					
Pre-monsoon			Monsoon		
Sample ID	$\delta^{18}O_{VSMOW}$ (‰)	δD_{VSMOW} (‰)	Sample ID	$\delta^{18}O_{VSMOW}$ (‰)	δD_{VSMOW} (‰)
3AL	7.9	−40	3AL	−9.9	−94
3BL	6.8	−36	3BL	−9.1	−84
3CL	7.6	−44	3CL	−10.2	−96
3DL	7.6	−34	3DL	−9.5	−91
4AL	10.3	−43	4AL	−8.4	−80
4BL	9.0	−43	4BL	−9.3	−82
4CL	8.7	−42	4CL	−8.4	−78
4DL	13.8	−35	4DL	−9.9	−81
5AL	14.8	−30	5AL	−9.4	−87
5BL	12.2	−42	5BL	−9.5	−75
3AS	−15.9	−110	5CL	−9.0	−68
3CS	−15.8	−108	5DL	−10.0	−82
3DS	−13.9	−108	3AS	−18.0	−128
4AS	−15.5	−112	3BS	−18.1	−125
4BS	−15.3	−109	3CS	−19.0	−134
4CS	−16.3	−116	4AS	−15.9	−116
4DS	−16.1	−115	4BS	−18.2	−130
5AS	−16.4	−115	4CS	−19.6	−136
5BS	−15.4	−111	5AS	−19.1	−133
5CS	−16.5	−115	5BS	−20.1	−146
5DS	−16.0	−115	5CS	−18.5	−131
SPR03	−15.5	−110	SPR11	−15.1	−104
SPR05	−15.4	−110	PPTA1	−14.1	−100
PPTA	−7.8	−54	PPTA2	−13.0	−94
PPTB	−4.8	−38	PPTB1	−11.2	−88
SM15	−16.2	−128	PPTB2	−12.4	−91
SM30	−19.3	−143	PPTS	−13.7	−92
SM50	−19.5	−147	PPTL	−11.2	−66
3S15	−17.6	−127	3S15	−17.3	−120
3S30	−19.8	−144	3S30	−24.2	−171
4S15	−16.3	−116	4S15	−20.3	−141
4S30	−16.7	−118	4S30	−24.4	−169
5S15	−17.0	−130	5S15	−19.2	−143
5S30	−17.7	−131	5S30	−25.9	−182
2004					
Pre-monsoon			Monsoon		
Sample ID	$\delta^{18}O_{VSMOW}$ (‰)	δD_{VSMOW} (‰)	Sample ID	$\delta^{18}O_{VSMOW}$ (‰)	δD_{VSMOW} (‰)
3AL	0.0	−36	3AL	12.1	−22
3BL	3.1	−25	3BL	17.6	−8
3CL	1.5	−32	3CL	10.0	−26
3DL	2.7	−19	3DL	9.0	−27
4AL	3.7	−29	4AL	23.8	7
4BL	2.8	−26	4BL	16.7	−9
4CL	5.3	−24	4CL	28.4	19
4DL	2.4	−28	4DL	25.3	21
5AL	5.2	−28	5AL	17.3	−5
5BL	8.1	−21	5BL	14.4	−8
5CL	2.9	−27	5CL	13.3	−14
5DL	2.0	−34	5DL	12.2	−15
3AS	−14.7	−104	3AS	−13.1	−87
3BS	−14.1	−103	3BS	−13.1	−92
3CS	−16.5	−117	3CS	−12.7	−90
4AS	−16.3	−117	4AS	−12.9	−91
4BS	−13.3	−101	4BS	−12.1	−89
4CS	−15.6	−112	4CS	−12.2	−85
5AS	−14.0	−102	5AS	−12.8	−88
5BS	−13.4	−97	5BS	−11.9	−83
5CS	−14.7	−107	5CS	−12.7	−87
SPR	−15.5	−109	SPR	−15.2	−107
PPTA	−11.2	−78	PPTA	−12.9	−88
PPTB	−10.5	−73	PPTB	−12.8	−87

TABLE 3

Continued.

2004					
Pre-monsoon			Monsoon		
Sample ID	$\delta^{18}O_{VSMOW}$ (‰)	δD_{VSMOW} (‰)	Sample ID	$\delta^{18}O_{VSMOW}$ (‰)	δD_{VSMOW} (‰)
3S15	−11.2	−76	3S15	−13.2	−90
3S30	−12.6	−87	3S30	−10.8	−73
4S15	−11.1	−79	4S15	−13.8	−95
4S30	−12.5	−88	4S30	−11.0	−75
5S15	−13.2	−97	5S15	−12.1	−85
5S30	−16.5	−121	5S30	−13.1	−90

[1] 3AL, 3BL, 3CL, 3DL: Leaf water samples from Site 3, trees A–D.
4AL, 4BL, 4CL, 4DL: Leaf water samples from Site 4, trees A–D.
5AL, 5BL, 5CL, 5DL: Leaf water samples from Site 5, trees A–D.
3AS, 3BS, 3CS, 3DS: Stem (xylem) water samples from Site 3, trees A–D.
4AS, 4BS, 4CS, 4DS: Stem (xylem) water samples from Site 4, trees A–D.
5AS, 5BS, 5CS, 5DS: Stem (xylem) water samples from Site 5, trees A–D.
SPR: Spring (groundwater) samples.
PPTA, PPTB: Precipitation sample from bulk collector A, B.
PPTS, PPTL: Precipitation sample collected solid (frozen), liquid.
3S15, 3S30: Soil water sample from Site 3 collected at 15 cm, 30 cm.
4S15, 4S30: Soil water sample from Site 4 collected at 15 cm, 30 cm.
5S15, 5S30: Soil water sample from Site 5 collected at 15 cm, 30 cm.
SM15, SM30, SM50: Soil water sample collected from the meteorological (weather) station at 15 cm, 30 cm, 50 cm.

ISOTOPIC RATIOS IN PRECIPITATION AND GROUNDWATER

There was generally good agreement between isotopic ratios from the two precipitation bulk collectors in samples taken before and during the monsoon (Table 3). The greater depletion in hydrogen isotopes (−42.8‰) than in oxygen ones (−6.3‰) between the pre-monsoon and the monsoon samples in 2003 suggests a divergence from the LMWL, but does not indicate that any evaporation took place. The accuracy of the bulk precipitation measurements was tested in November 2004 by collecting single event samples on the mountain, which was found to have isotopic composition similar to the bulk collectors (Table 3).

Storm-based water samples were collected at the Universidad de Colima during the 2004 and 2005 monsoon seasons (Figs. 4 and 6B). We analyzed those samples and also scaled them for the higher elevation following the methods of Poage and Chamberlain (2001). In doing this we assumed that the dominant isotopic fractionation between the two sites was due to temperature decrease with altitude (Table 4). The lower elevation Colima city site receives more precipitation on average than the high elevation site, indicating that rainout is also a factor. A LMWL computed from the waters collected at Colima was

$$\delta D = 7.54\,\delta^{18}O + 3.8 \quad N = 54 \quad R^2 = 0.97,$$

while the LMWL from the scaled values and inclusive of the bulk collectors was

$$\delta D = 7.55\,\delta^{18}O + 8.0 \quad N = 56 \quad R^2 = 0.97.$$

Slopes and intercepts were statistically significant (p-values $\leq$ 0.005), and the two slope coefficients were not significantly different (p-value = 0.33; Sokal and Rohlf, 1981, p. 505). From this scaling, and the resulting LMWL agreement between the individual storms and the bulk collectors, it follows that the bulk collectors were indeed capturing the mean value of precipitation for the monsoon season.

Isotopic ratios from the natural spring were very similar in pre-monsoon and monsoon samples ($\delta^{18}O$ was −15.5 ± 0.5; Table 3). This could be explained by water reaching the spring with a certain time lag, so that the resulting isotopic ratios represent a long-term average of precipitation and soil waters with varying degrees of evaporation. Isotopic signatures of soil water samples at relatively shallow depths (about 15 cm below ground) tend to overlap with those of stem and spring water, while they differ at greater depths (about 30–50 cm below ground). This is more noticeable for δD values, whose change from pre-monsoon to monsoon periods was also evident in stem water samples (Table 3).

STABLE ISOTOPES IN SOIL AND TREE WATER

Isotopic values for soil water profiles in 2003 and 2004 are given in Table 3. In 2003 and 2004, monsoon water from the 15 cm horizon had a mean $\delta^{18}O$ value of −18.9‰ and −13‰, respectively (Table 2 and Fig. 5). The mean values from the 30 cm horizon were −24.8‰ and −11.6‰. In the same years, the pre-monsoon mean $\delta^{18}O$ values were −16.8‰ and −11.8‰ for the 15 cm horizon, and −18.4‰ and −13.8‰ for the 30 cm horizon.

The xylem water showed a pattern similar to that of the soil water in 2003 and 2004, with a mean value of −18.5‰ and −12.6‰ for the monsoon samples, and −14.7‰ and −15.7‰ for the pre-monsoon samples. Leaf water showed large changes, with mean $\delta^{18}O$ before the monsoon of 9.9‰ in 2003 and 3.3‰ in 2004, while for monsoon samples the mean $\delta^{18}O$ was −9.4‰ in 2003 and 16.7‰ in 2004 (Fig. 3).

Discussion

LOCAL METEORIC WATER LINE

Temperature of the source water, and atmospheric path taken by the moisture, are expected to determine the isotopic content of the precipitation. An effective way to summarize the isotopic signature of precipitation is the LMWL (Fig. 4), which has never before been calculated for this region. Because the isotopic regression lines before and during the monsoon (Fig. 2) were

TABLE 4

Isotopic composition of monsoon water samples collected at Colima city.

2004				Values corrected for elevation	
Sample ID	Date Collected	$\delta^{18}O_{VSMOW}$ (‰)	δD_{VSMOW} (‰)	$\delta^{18}O_{VSMOW}$ (‰)	δD_{VSMOW} (‰)
CMP1-04	5/31/2004	−0.9	−3	−7.7	−50
CMP2-04	6/2/2004	−12.5	−85	−19.3	−131
CMP3-04	6/7/2004	−15.8	−115	−22.6	−161
CMP4-04	6/14/2004	−8.4	−59	−15.2	−105
CMP5-04	6/16/2004	−7.1	−50	−13.9	−96
CMP6-04	6/24/2004	−7.1	−55	−13.9	−101
CMP7-04	7/2/2004	−3.7	−32	−10.5	−78
CMP8-04	7/6/2004	−4.2	−31	−11.0	−77
CMP9-04	7/12/2004	−5.0	−32	−11.8	−79
CMP10-04	7/12/2004	−10.3	−78	−17.1	−124
CMP11-04	7/17/2004	−3.5	−20	−10.3	−66
CMP12-04	7/20/2004	−6.0	−47	−12.8	−93
CMP13-04	7/21/2004	−5.4	−37	−12.2	−84
CMP14-04	7/22/2004	−9.9	−73	−16.7	−119
CMP15-04	7/27/2004	−8.1	−56	−14.9	−102
CMP16-04	8/7/2004	−4.4	−28	−11.2	−75
CMP17-04	8/16/2004	−3.6	−20	−10.4	−66
CMP18-04	8/17/2004	−2.9	−14	−9.7	−60
CMP19-04	8/18/2004	−4.0	−19	−10.8	−65
CMP21-04	8/31/2004	−7.6	−51	−14.4	−97
CMP22-04	9/27/2004	−3.2	−15	−10.0	−62
CMP23-04	9/29/2004	−6.6	−43	−13.4	−89
CMP24-04	9/29/2004	−9.6	−63	−16.4	−109
Weighted Mean		−7.9	−56	−14.7	−102

2005				Values corrected for elevation	
Sample ID	Date Collected	$\delta^{18}O_{VSMOW}$ (‰)	δD_{VSMOW} (‰)	$\delta^{18}O_{VSMOW}$ (‰)	δD_{VSMOW} (‰)
CMP1-05	6/22/2005	−5.4	−43	−12.5	−91
CMP2-05	6/23/2005	−4.7	−33	−11.8	−81
CMP3-05	6/29/2005	−15.0	−108	−22.1	−157
CMP4-05	6/30/2005	−10.3	−75	−17.4	−123
CMP5-05	7/3/2005	−8.5	−65	−15.6	−113
CMP6-05	7/4/2005	−9.1	−63	−16.1	−111
CMP7-05	7/5/2005	−7.7	−58	−14.8	−106
CMP8-05	7/7/2005	−6.4	−52	−13.5	−100
CMP9-05	7/8/2005	−3.4	−20	−10.5	−68
CMP10-05	7/11/2005	−4.8	−31	−11.9	−79
CMP11-05	7/13/2005	−3.1	−17	−10.2	−66
CMP12-05	7/13/2005	−3.8	−20	−10.8	−68
CMP13-05	7/17/2005	−8.2	−59	−15.3	−107
CMP14-05	7/19/2005	−8.6	−65	−15.7	−113
CMP15-05	7/20/2005	−10.6	−76	−17.6	−124
CMP16-05	7/21/2005	−9.7	−69	−16.8	−118
CMP17-05	7/29/2005	−7.0	−57	−14.1	−105
CMP18-05	7/31/2005	−7.7	−56	−14.7	−104
CMP19-05	8/5/2005	−6.2	−42	−13.2	−90
CMP20-05	8/11/2005	−4.9	−38	−12.0	−86
CMP21-05	8/14/2005	−8.4	−57	−15.5	−105
CMP22-05	8/19/2005	−4.9	−34	−12.0	−82
CMP23-05	8/23/2005	−6.7	−50	−13.8	−99
CMP24-05	8/25/2005	−4.9	−37	−11.9	−85
CMP25-05	9/27/2005	−4.8	−37	−11.9	−85
CMP26-05	9/28/2005	−9.6	−66	−16.7	−114
CMP27-05	9/29/2005	−16.1	−115	−23.1	−163
CMP28-05	9/30/2005	−3.3	−20	−10.3	−68
CMP29-05	10/5/2005	−3.5	−20	−10.6	−68
CMP30-05	10/6/2005	−7.9	−55	−14.9	−103
CMP31-05	10/7/2005	−7.5	−47	−14.6	−95
CMP32-05	10/8/2005	−5.4	−29	−12.5	−77
Weighted Mean		−7.5	−54	−14.3	−100

similar, the LMWL is a reasonable summary for the site, even though it may be somewhat biased toward summer monsoon rainfall, which has accounted for 83–91% of the annual precipitation during the period of measurement (Fig. 6). We have included the bulk winter precipitation for 2004 and 2005 collected up on the mountain to help offset this bias. The calculated slope of 7.55 is less than the GMWL slope (8.13), which seems to indicate there is at least some secondary evaporation taking place, and it is closer to the pre-monsoon (7.44) than to the monsoon (7.17) slopes computed from environmental waters collected on the mountain (Fig. 2).

SOIL AND XYLEM WATER

The water that falls on Colima City, and at the study site, changes in isotopic composition in going from the atmosphere to the soil and then the xylem. In a simplified model, two processes can control the $\delta^{18}O$ and δD of soil water: precipitation, which adds meteoric water of a certain composition, and evaporation of water from the soil, which results in the $\delta^{18}O$ and δD enrichment of these waters (Barnes and Allison, 1988). The patterns observed at the study area at different times all show enrichment in the soil water consistent with an evaporation profile (Barnes and Turner, 1998). These profiles are formed when water near the surface, which has been subject to evaporation, is pushed farther down into the soil profile due to piston flow of water from above.

There is no fractionation when water is transferred from the soil to the tree (Wershaw et al., 1966), so xylem water isotopes are representative of the soil horizon where most of the root system lies. Depending on soil properties, trees may utilize water from various depths (Kramer and Boyer, 1995), but we recognize that information on rooting structure is needed to compare results from different studies. In this study, isotopic values of pre-monsoon stem water were generally consistent with those of deep soil water, while the monsoon stem water samples tended to follow isotopic patterns found in shallow soil water. This relationship was seen in the 2003 monsoon data, and in both 2004 sampling events (Fig. 5). For the 2003 pre-monsoon samples, stem water was more enriched than water from the shallow soil horizon, probably because of isotopically heavier spring precipitation (Table 3).

LEAF WATER

The water travels up the xylem to the leaf where there is some exchange (and thus fractionation) with atmospheric water vapor while the stomata are open. Also, water is evaporated at the leaf surface, causing further fractionation (Dawson et al., 2002). These processes follow complex physical pathways, and the exact fractionation dynamics have been the subject of several recent studies (Roden et al., 2000; Yakir and Sternberg, 2000; Barbour and Farquhar, 2003; Barbour et al., 2004; Farquhar and Cernusak, 2005).

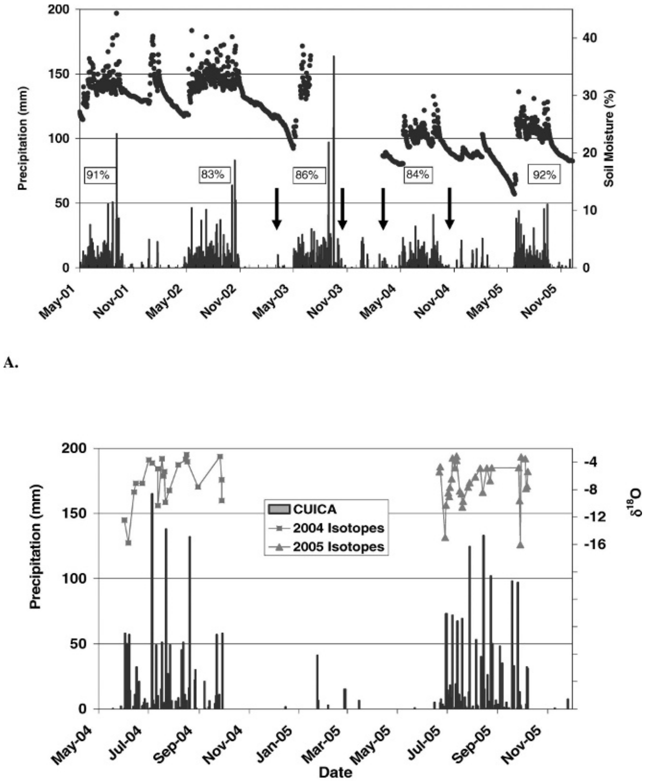

FIGURE 6. (A) Daily precipitation (bars) and volumetric soil moisture (dots) as measured at the Nevado de Colima site from 2001 to 2005 (see Biondi et al., 2005, for details). Percentages indicate the monsoon precipitation (June to October) with respect to the total precipitation for the water year (June to May). Arrows indicate the timing of the isotopic sampling events at the Nevado de Colima study area. (B) Stable isotopic composition of monsoon precipitation events, and precipitation amounts, as collected in Colima City during the monsoon, 2004–2005.

There was great variability in stable isotopic ratios of needle water at our study area (Fig. 3). Leaf water mean $\delta^{18}O$ was depleted by 19‰ during the monsoon sampling relative to the dry season during 2003. In 2004 the isotopic pattern of leaf water was reversed, with a mean enrichment of about 13‰ during the monsoon relative to the dry season (Fig. 5). These large differences were likely due to micrometeorological conditions, based on results obtained in a parallel study (Monnar, 2007) and in other diurnal experiments (Farquhar and Cernusak, 2005) Although we did not have the equipment for directly measuring gas exchanges in the field, it is well known that leaf water is enriched at the leaf surface through transpiration, and subsequently, through photosynthesis (Yakir and Sternberg, 2000). If we considered not the mean values, but the values from leaf water at the individual sites and at the individual trees (Table 3, Fig. 3), it was clear that the monsoon leaf values fell into site specific groups. This suggested that conditions at individual sites influenced leaf water at the time of sampling. The 2003–2004 winter, which was unusually dry at the site (Fig. 6A), may have played a part in the results we obtained by increasing evaporative demand from the soil and at the leaf surface. In addition, the 2004 monsoon season was the driest of the five monsoon seasons we measured, and it ended rather abruptly without the usual series of tropical storms that come through in October (Fig. 6A).

Leaf waters from pre-monsoon samples collected in 2003 and 2004 followed an evaporation profile from the GMWL (Fig. 3). Monsoon values for 2004 are even further along this evaporation profile and show some extremely enriched values. This can be explained by the different climatic conditions the trees were experiencing during the 2003 and 2004 monsoons (Fig. 7). The 30-day period preceding the collection of monsoon samples in 2003 was characterized by several multi-day intervals of increased precipitation, including at least seven days with precipitation greater than 5 mm, and two days exceeding 100 mm. In comparison, the 30 days prior to the 2004 monsoon sampling were characterized by very little precipitation, with no daily rainfall above 5 mm. While we do not have soil moisture measurements for the 2003 monsoon samples due to an equipment

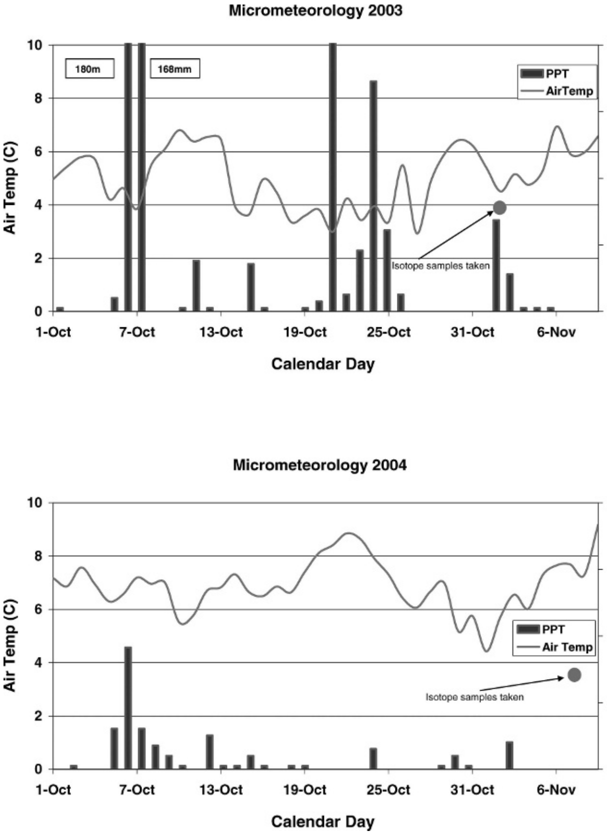

FIGURE 7. Daily total precipitation and mean air temperature during the ~30 days prior to collecting monsoon isotopic samples in 2003 and 2004. These data were included among those summarized and discussed by Biondi et al., 2005.

malfunction (see Biondi et al., 2005, for details), the 2004 monsoon soil moisture was amongst the lowest we have measured, exceeded only by the 2005 pre-monsoon, another arid period at the site (Fig. 6A).

There are two sources of signals that can be preserved in the cellulose isotopes, i.e. the seasonal change in water supply or source water for the tree, and the changing conditions at the leaf surface. Photosynthates produced in the leaves, and later transported elsewhere in the tree for cellulose production, carry with them a specific signal based on the raw materials (water and CO_2) and on the environment the leaf is experiencing at the time of production. Thus, both varying depth of source water and the timing of growth need to be considered in modeling the system. The preservation of isotopic records in tree cellulose depends on understanding the average leaf water transpiration with respect to leaf water content during the production of photosynthates. Leaf water enrichment through transpiration at the leaf surface is affected mostly by relative humidity, temperature, wind, photosynthetically active radiation or light status, water status of the tree, and available water in the soil (Cernusak et al., 2002; Gan et al., 2003). Our investigation of isotopic ratios in the hydrological cycle, although not routinely performed for dendroclimatic calibration of cellulose stable isotopes, will help understanding the mechanism for a distinguishable climatic signal being recorded in tree ring cellulose at this tropical treeline site.

Conclusion

Stable isotopic ratios highlighted a marked difference in pre-monsoon and monsoon water cycling. Environmental waters in March (pre-monsoon) and November showed a clear difference in needle isotopic ratios, and provided evidence for a link between xylem and soil samples at various depths. Overall, trees utilize water from relatively shallow soil depths, which are representative of current conditions, rather than tapping groundwater, which is more representative of long-term trends. An ongoing parallel study at the same site has determined that while the tree dormant season coincides with the dry season, rapid onset of radial growth in the spring is initiated before the monsoon moisture arrives (Biondi et al., 2005). From the point of view of understanding tree ring isotopic values, we would then expect incorporation of the dry season isotopic profile (winter moisture sources) into the earlywood, while monsoon moisture would instead dominate in the latewood. Paleoclimatic reconstructions tend to work even without a complex understanding of the systems involved, but at the same time confidence in the reconstructions is improved when the underlying mechanisms have been demonstrated to be valid in at least some case studies. The large changes in leaf water isotopes suggest the site is also suitable for stable isotopic analysis on a seasonal to sub-seasonal scale, although additional studies are needed on how temporal variability in leaf water fractionation is affected by micrometeorology at the leaf surface.

One of the most interesting results of this study was the large contrast in monsoon leaf water isotopes between the two years. In a year (2003) with abundant precipitation prior to monsoon sampling, leaf water isotopes were barely enriched relative to source water, whereas in a year (2004) with much less precipitation prior to monsoon sampling, leaf water was very enriched, and comparable to pre-monsoon leaf water, which represents the dry season. These differences can be attributed to diffusional limitation of water exchange due to reduced stomatal opening, a consequence of drought stress. Considering that most tree radial growth takes place during the monsoon, in synchrony with water availability, but radial expansion also extends into the dry season that follows the monsoon (Biondi et al., 2005), the ^{18}O isotopes in fixed photosynthate (hence tree ring cellulose) are expected to vary substantially from season to season and from year to year. From the perspective of reconstructing past climate from tree rings, annual isotopic variation should therefore reflect plant moisture stress, possibly linked to some metric of monsoon precipitation variability. This is in contrast to an alternative hypothesis that variations in ^{18}O of tree ring cellulose would reflect variation in the origin of the monsoon water, either from the Pacific or the Atlantic Ocean.

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