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A stable isotope-based approach to tropical dendroclimatology

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Abstract—We describe a strategy for development of chronological control in tropical trees lacking demonstrably annual ring formation, using high resolution $\delta^{18}O$ measurements in tropical wood. The approach applies existing models of the oxygen isotopic composition of alpha-cellulose (Roden et al., 2000), a rapid method for cellulose extraction from raw wood (Brendel et al., 2000), and continuous flow isotope ratio mass spectrometry (Brenna et al., 1998) to develop proxy chronological, rainfall and growth rate estimates from tropical trees lacking visible annual ring structure. Consistent with model predictions, pilot datasets from the temperate US and Costa Rica having independent chronological control suggest that observed cyclic isotopic signatures of several permil (SMOW) represent the annual cycle of local rainfall and relative humidity. Additional data from a plantation tree of known age from ENSO-sensitive northwestern coastal Peru suggests that the 1997-8 ENSO warm phase event was recorded as an 8‰ anomaly in the $\delta^{18}O$ of α-cellulose. The results demonstrate reproducibility of the stable isotopic chronometer over decades, two different climatic zones, and three tropical tree genera, and point to future applications in paleoclimatology. *Copyright © 2004 Elsevier Ltd*

1. INTRODUCTION

A particular gap in the network of high resolution proxy observations used for paleoclimate and paleoenvironmental studies is in the terrestrial tropics (World Data Center-A for Paleoclimatology, 2003; Fig. 1). One potential source of annual-resolution paleoclimate data lies in dendroclimatology, the science linking tree-rings to environmental conditions (Fritts, 1976). This approach requires large numbers of individuals of a single species to be sampled, statistically processed, and assembled into a site average. Yet, although exciting successes have recently been reported (Stahle, 1999; Stahle et al., 1998; D'Arrigo et al., 2001a; Biondi, 2001; Morales et al., 2001; Worbes, 2002), dendrochronology remains unestablished in much of the tropics, for several reasons. Although there are tropical tree species which exhibit annual ring structure (Worbes, 2002; Jacoby and D'Arrigo, 1990), other species or the environment do not appear to encourage annual ring formation (Fig. 2). Once target species are identified, finding the necessarily large sample population of annual ring-forming trees within the diversity of tropical forests (Wilson, 1988) makes species characterization and dataset development difficult. For these reasons and others, attempts to develop reliable paleoclimatic time serie from tropical trees using classical ring dating methods (Stokes and Smiley, 1968) have proceeded slowly.

Here we present a strategy to develop chronometric estimates in tropical trees lacking demonstrably annual ring structure, using high resolution stable isotopic measurements in tropical woods. We call this approach "tropical isotope dendrochronology." The approach exploits recent advances in mechanistic modeling of the oxygen isotopic composition of the α-cellulose component of wood, and a rapid protocol for extracting the α-cellulose component of wood from very small-samples in combination with online, continuous flow mass spectrometric techniques for necessary high-throughput analyses. The approach has the potential advantage of generality across species, and hypothetically permits development of proxy paleo-rainfall/humidity and growth rate data as an additional output. We describe the approach and lay out the primary methodological assumptions in Section 2. We describe methods and data in Section 3 and results in Section 4. A synthesis of the results and the prospects for dendroclimatology from isotopic data developed from tropical trees is in Section 5.

2. HYPOTHESIS

The basic premise of this study is that most tropical locales experience *precipitation* seasonality, even if they do not experience the *temperature* seasonality which is primarily responsible for the reliable formation of tree-rings in the extratropics (Fritts, 1976, Fig. 2). Hence, we seek to detect seasonality in the amount of tropical convective rainfall, as mirrored in the stable isotopic composition ($\delta^{18}O$) of the annual cycle in tropical convective rainfall (Fig. 3). Following process modeling studies of the isotopic composition of tree-ring cellulose in extratropical trees, we expect to find that the seasonality of the isotopic composition of tropical rainfall, primarily modified by evaporative effects at the leaf, is resolved by intraseasonal-resolution measurements of the $\delta^{18}O$ of the α-cellulose component of contemporaneously-formed wood. In this manner we seek to establish chronology within tropical wood samples we wish to employ for paleoclimatic study. Once chronology is established, we can proceed to the calibration and development of proxy rainfall/humidity and growth estimates from tropical trees.

In a recent set of careful greenhouse, field and modeling studies, Roden and colleagues showed that the oxygen isotope composition of the α-cellulose component of wood depends

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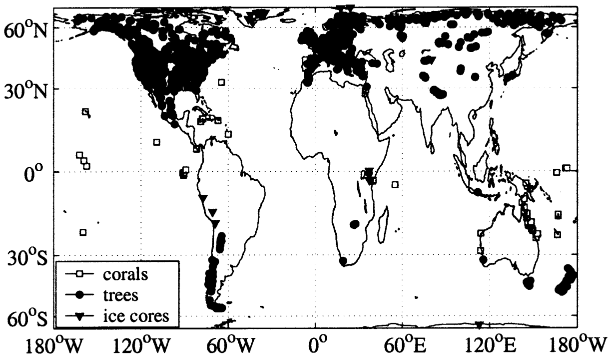

Fig. 1. Equal-area map of locations of high resolution coral, tree-ring and ice core paleoclimate data currently in the NGDC World Data Center-A for Paleoclimatology electronic database (http://www.ngdc.noaa.gov/paleo/). Plots as of June 2003.

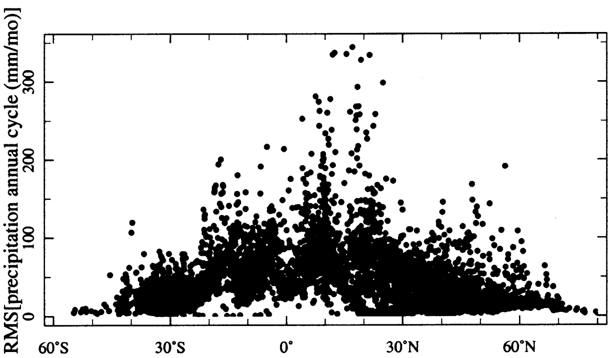

Fig. 3. Amplitude of the precipitation seasonal cycle at stations having at least 30 years of monthly observations, as a function of latitude, from Peterson and Vose (1997). Many of the highest amplitude seasonal cycles are in the terrestrial tropics.

primarily on the oxygen isotopic composition of source waters and evaporative enrichment at the leaf where photosynthate is produced. The Roden-Lin-Ehleringer (RLE) model (Roden and Ehleringer, 1999a,b, 2000; Roden et al., 2000) gives us the mechanistic underpinning to resolve time in tropical trees lacking annual rings:

$$\delta^{18}O_{cx} = f_o(\delta^{18}O_{wx} + \epsilon_o) + (1 - f_o)(\delta^{18}O_{wl} + \epsilon_o)$$

where $\delta^{18}O_{cx}$ is the isotopic composition of the tree-ring cellulose, $\delta^{18}O_{wx}$ is the $\delta^{18}O$ of tree xylem water, $\delta^{18}O_{wl}$ is the $\delta^{18}O$ of leaf water, and ϵ_o is the isotopic fractionation factor for the enzyme-mediated exchange or addition of oxygen to synthesized cellulose (+27‰) (Roden et al., 2000). The dimensionless constant weighting term f gives the proportion of cellulose oxygen which undergoes exchange with stem or leaf water (≈0.42; (Roden et al., 2000)). The $\delta^{18}O$ of stem water does not undergo fractionation during uptake, and is therefore equal to the $\delta^{18}O$ of the mixture of ground and meteoric waters that are taken up by the tree through its roots. The $\delta^{18}O$ of leaf water, on the other hand, is modified by evaporation in the leaf prior to photosynthetic production of sucrose subsequently used to make xylem α-cellulose. The modification of leaf water $\delta^{18}O$ is modeled as in Flanagan et al. (1991): fractionation of leaf water is due to equilibrium exchange with atmospheric water vapor and kinetic fractionation due to diffusion through stomata and in the leaf boundary layer. Subsequent analysis with the Roden and Ehleringer (1999a) dataset has shown that the Flanagan model should be damped to account for a Péclet effect, relative to the $\delta^{18}O$ of source waters: high rates of transpiration advect unfractionated xylem water into the leaf, which counteracts evapotranspirative isotopic enrichment of leaf water (Barbour et al., 2004).

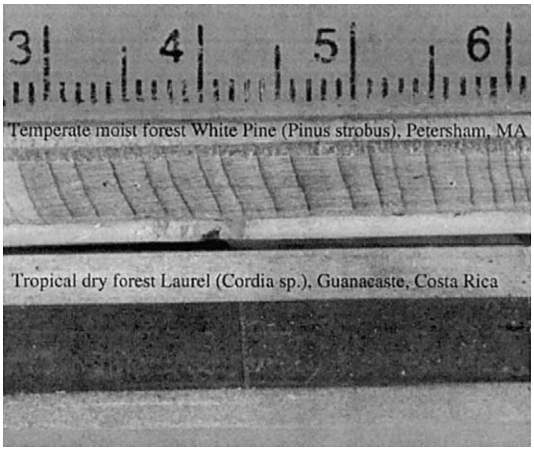

Fig. 2. Photographs of 5 mm-diameter increment core sections taken from extratropical and tropical tree species. Top: Harvard Forest (Petersham, MA, USA) Pinus strobus (White Pine). Bottom: Costa Rican dry forest Cordia sp. (Laurel). The scale is in centimeters. Both cores are mounted onto blond wood core-holders. Rings are clearly visible in the P. strobus core, but the Costa Rican Cordia sp. is a uniform, dark color throughout.

The RLE model suggests that seasonal patterns in isotopic composition of tropical trees may be empirically used to determine time (Figure 4). In much of the tropics, precipitation and relative humidity fields show marked and correlated seasonality (Peixoto and Oort, 1992). Seasonality of the hydrological cycle in turn is expressed in the stable isotopic composition of precipitation and leaf water through the fractionating processes of condensation and evaporation. The $\delta^{18}O$ of precipitation from tropical convective systems over a range of time scales is largely explained by the "amount effect" (Gat, 1996). In this model, the process of precipitation preferentially removes ^{18}O-labeled water from an air mass, leaving subsequent precipitation isotopically depleted. Consequently we expect that heavy precipitation falling at the height of the rainy season will reflect decreased $\delta^{18}O$, relative to that of lighter precipitation falling at the outset of the rainy season, as a consequence of the amount effect (Fig. 4). Since the amount effect is effectively driven by amount of precipitation, we expect its influence on xylem $\delta^{18}O$ of α-cellulose via the RLE model to be strongest during the rainy season, and of lesser importance during the dry season (Fig. 4).

The seasonal cycle in relative humidity also affects the oxygen isotopic composition of plant waters, through the preferential evaporative loss of $\delta^{18}O$ labeled water to the atmosphere. We expect increased moisture convergence during the rainy season to minimize the evaporative enrichment of leaf water $\delta^{18}O$ by reducing the intercellular-extracellular and intercellular-environmental relative humidity gradients (Fig. 4)

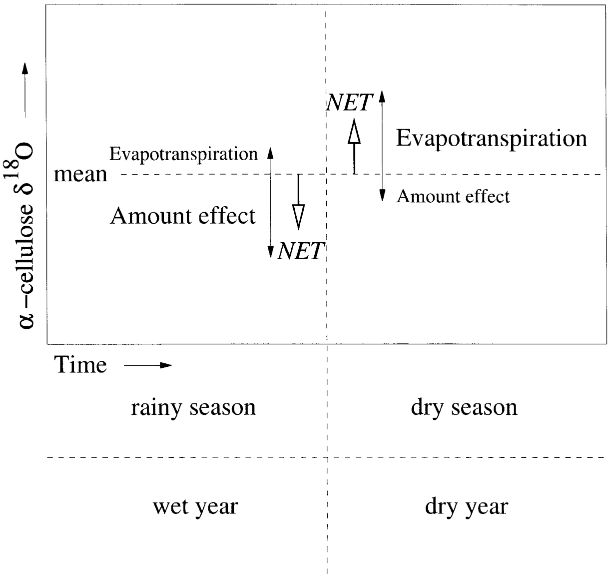

Fig. 4. Schematic diagram of expected isotopic effects in tropical tree α-cellulose as a function of the annual cycle and interannual variations of tropical convective rainfall and evapotranspiration (see text for description).

(Roden et al., 2000, their equation 2). During the dry season, we expect lower relative humidity to increase leaf water evaporative enrichment, resulting in increased leaf water δ^{18}O. Since leaf water enrichment is driven by relative humidity gradient, we expect its influence on xylem δ^{18}O of α-cellulose via the RLE model to be strongest during the dry season, and of lesser importance during the rainy season (Fig. 4).

RLE also predicts that oxygen isotopic measurements should permit the study of interannual climate variability in tropical trees from critical regions (Fig. 4). Analogous to the seasonal cycle hypothesis outlined above, unusually rainy years should be reflected by α-cellulose δ^{18}O values offset lower with respect to the climatological seasonal cycle, and unusually dry years should be reflected by α-cellulose δ^{18}O values offset higher with respect to the climatological seasonal cycle. For example, consider the effects of the El Niño-Southern Oscillation (ENSO) phenomenon in the terrestrial tropical Pacific basin. In the rainforests of the western tropical Pacific, the ENSO warm phase brings catastrophic drought due to anomalously high atmospheric subsidence over the region (Trenberth and Caron, 2000). This should result in anomalously high δ^{18}O values in α-cellulose formed during the growing season. Conversely, wetter than normal ENSO cold phase conditions should be evident in more isotopically-depleted α-cellulose. In northwestern coastal Peru, the opposite effects should be observed: heavy amounts of isotopically-depleted precipitation falls during ENSO warm phase events, while low amounts of precipitation falling during the ENSO cold phase are relatively isotopically enriched. Analogous to the annual cycle, we expect that evaporative effects should somewhat counteract the rainfall-driven amount effect during rainy years, and should dominate during dry years (Figure 4). In this manner high resolution δ^{18}O measurements from tropical trees from ENSO-sensitive regions should be useful for estimating paleoclimatic rainfall and/or humidity anomalies associated with the phenomenon.

3. DATA AND METHODS

3.1. Sampling

We test the strategy described here using pilot data series from a range of tropical ecosystems and tree genera from the Neotropics. Our first priority is to verify the age model hypothesis described in section 2; a secondary priority is detect an interannual climate signal in the proxy data. Sampling rationale, site climatology, and age model development and testing are described briefly below and summarized in Table 1.

3.1.1. Temperate forest, central Massachusetts

Samples collected in 1999 were intended to demonstrate within-increment core reproducibility and to observe the annual cycle in high resolution isotopic measurements within a known growing season. Climatological temperature varies smoothly from −4.8°C in January to 21.6°C in July. Climatological precipitation is greatest in boreal summer at 96–105 mm/month, and is minimum during winter at 77–90 mm/month. Ring structure is clear (Fig. 2, top) within these short increment core samples from young trees, and although the samples have not been rigorously cross-dated (Stokes and Smiley, 1968) via massive replication, intercomparison of three core samples indicates the data obtained is from the period 1906–1925. Isotopic data are plotted on an age model linearly interpolated over the observed May-October growing season (O'Keefe and Johnson, 2003). To assess intra-ring reproducibility of high resolution α-cellulose δ^{18}O measurements, four splits of each wood sample were independently processed and analyzed.

3.1.2. Dry forest, Liberia, northwestern Costa Rica

To test the stable isotope chronometer in samples lacking ring structure but from a region with marked rainfall seasonality (Figs. 2, 7), we collected a single increment core in March 2000 from a timber log in a woodyard in Quebrada Grande, Costa Rica (10°N, 85°W, ~300 m). Core orientation was chosen based on observable ray structure in the wood. Although the age of these samples is unknown, the cored log must

Table 1. Data sets reported in this study.

Site	Lat.	Long.	Elev.	Environment	Genus & species	Age information
Petersham, MA, USA	42°N	72°W	186 m	temperate	*Pinus strobus*	ring structure
Liberia, Costa Rica	10°N	85°W	300 m	dry tropical	*Cordia sp.*	radiocarbon
La Selva, Costa Rica	10°N	84°W	40 m	wet tropical	*Hyeronima alchorneoides*	plantation
Piura, Peru	5°S	81°W	49 m	dry tropical	*Prosopis sp.*	plantation

have been alive when felled, because it retained bark and fresh leaves. Based on estimated growth rates for samples from 0–180 mm depth, we chose to measure isotopic composition at half the depth resolution for samples from 180–308 mm depth, by analyzing only every other prepared 200 μm resolution α-cellulose sample. Rainfall in the region is highly seasonal, ranging from up to 400 mm/month in boreal fall to trace levels in boreal spring. Based on these climatological data and on phenological observations in the Santa Rosa National Park nearby (M.V. Gutierrez, Universidad de Costa Rica; Tschinkel (1966)), an age model for May–December growth has been applied to the depth series data. The age model makes the explicit assumption that isotopic maxima occur at the beginning of the growing season, which is at the end the dry season, in April. Isotopic minima are expected to occur during the height of the rainy season, in October–December.

To independently test the stable isotope-derived chronology, we made radiocarbon measurements at hypothesized 10-year intervals using α-cellulose samples from 1–2 year sampling intervals. Samples of α-cellulose were combusted with Cu/CuO catalyst and cryogenically cleaned of water before being sent to the Woods Hole and Lawrence Livermore National Laboratory AMS facilities for stable carbon and radiocarbon analysis using standard techniques. Radiocarbon dates were calibrated using CALIB 4.3 (Stuiver and Reimer, 1993).

3.1.3. Rain forest, La Selva, eastern Costa Rica

To provide proof-of-concept for the stable isotope chronometer in a rainforest climate, a single increment core sample was taken from a 16-year old plantation tree from the “Canada Plots” at the Organization for Tropical Studies’ (OTS) La Selva Biological Research Station (10.4°N, 84°W, 40m) (Butterfield and Espinosa, 1997). No ring structure is visible in the core. Rainfall in the region is seasonal, ranging from 170 mm/month in boreal spring to over 500 mm/month in a broad and variable rainy season lasting from June through December. ENSO warm phase events are associated with positive summertime (July–October) rainfall anomalies (Waylen et al., 1996). Based on climatological rainfall data and the model presented in Section 2, isotopic minima were assigned to July of each year, and intrannual ages were estimated for each data point via continuous, piecewise linear interpolation. No seasonal growth hiatus was assigned for each year.

3.1.4. Dry forest, Piura, northwest coastal Peru

To test the ability of high resolution stable isotope measurements to record climate anomalies in a tropical environment, we sampled a 10-year old plantation *Prosopis* sp. specimen from Piura, Peru (5°S, 81°W, 49 m). Plantation saplings were irrigated during the first year, but not thereafter. Some ring structure is visible in the wood sections, but it is unclear whether rings are annual or represent episodic growth. Climatological rainfall is very low and irregular, ranging only to a maximum of 25–35 mm/month in boreal spring (Peterson and Vose, 1997). Moderate to strong ENSO events bring anomalously heavy rainfall during boreal winter and early spring to this very dry region (Aceituno, 1988; Kalnay et al., 1996); inter-annual rainfall anomalies associated with the 1997–8 ENSO warm phase event reached 800–1000 mm over the period December 1997 through February 1998 alone (Xie and Arkin, 1997). A preliminary age model for the isotopic data is based on the known total age of the sample and speculative interannual growth rate variations.

3.2. Micro-sampling

Samples were taken either by 5 mm diameter increment borer or by removing sections from felled trunks. In the case of section material, 5 mm × 5 mm cross-section transects were sawn out of the section along lines estimated as parallel to growth rays visible in the wood for subsequent sampling. Sampling for processing to α-cellulose was performed using an American Optical rotary microtome using either resharpened or disposable knives, and an advance of 20 μm per crank rotation. Depending on wood density and grain, 10 microtome slices (200 μm sampling interval) was sufficient to produce 600 μg to 1.2 mg of wood for processing. This sampling protocol was based on conservative minimal sample α-cellulose processing and analysis mass requirements. We assumed a cylindrical solid wood core sample, minimum sampling depth $d = mnx/f\rho\pi r^2$, where m = required α-cellulose sample mass for mass spectrometry, n = number of desired analysis replications, x = number of time-series measurements required to resolve the seasonal cycle, f = fractional yield of α-cellulose from raw wood, (ρ = wood density, and r = core radius. For typical values, $m = 100$ μg, $n = 2$ replicates, $x = 6$ samples/year, $f = 0.3$, $r = 2.5$ mm, $\rho = 0.4$–1.2 g/cm^3), $d = 0.17$–0.51 mm annual growth increment is necessary to resolve the annual cycle. Our sample set apparently never approached these values, but work involving trees growing as slowly as is typical in old-growth temperate forests may approach these limits.

3.3. α-Cellulose Preparation

Wood samples were processed following a Brendel procedure (Brendel et al., 2000) modified for speed and small sample processing as follows (Table 2). All chemistry was performed in a fume hood. Batches of 40 finely-shaved or ground wood samples of 0.4–1 mg mass were placed in 1.5 mL polyethylene microcentrifuge tubes, into which was pipetted 120 μl 80% acetic acid and 12 μl 69% nitric acid. Capped sample tubes were placed in an aluminum heating block at 120°C for 20–30 minutes, removed and allow to cool. 400 μl 100% ethanol was pipetted into the sample tubes and capped tubes were centifuged 5 min. at 13,000 rpm. The following rinse steps were then made in capped centrifuge tubes, each rinse preceeded by removal of supernatant and each followed by centrifuging for 5 min. at 13,000 rpm: (1) 300 μl distilled deionized water; (2) 150 μl 100% Ethanol. A final rinse in Acetone and removal of supernatant was followed by drying in a warm oven for 30 min. and overnight storage in a vacuum desiccator. A full description of materials and the modified Brendel procedure described here is available on request from the corresponding author. The published and modified Brendel α-cellulose extraction procedures were compared by isotopic analysis of an in-house, homogenized standard wood powder (“Brazilian wood”) of known isotopic composition. To test the precision and accuracy

Table 2. Comparison of α-cellulose extraction protocols.

Protocol	Ehleringer et al. [2000]	Brendel et al. [2000]	Modified Brendel [2001]
Number samples	10	56	80
Sample size	250 mg	10–100 mg	200 μg–2 mg
Delignification time	6–7 days	30 min.	30 min.
Bleaching time	5 hrs.–4 days	—	—
α-cellulose extraction time	8–10 hrs.	7 hrs.	3 hrs.
Percent yield α-cellulose	35	35	35
Total samples/day	~1	56	160

of the modified Brendel procedure, 1–2% of samples processed over the course of this study were Brazilian wood standards. In addition, M. Lott of the Biosciences Dept., University of Utah made an intercomparison of the δ^{18}O of α-cellulose produced via the Brendel and Jayme-Wise (Ehleringer et al., 2001) methods.

3.4. Isotopic Analysis

100–150 μg α-cellulose samples were loaded within silver capsules into an autosampler capable of 32 sequential analyses per run. Online pyrolysis of samples to CO was performed in a ThermoFinnigan Thermal Conversion/Elemental Analyzer (TC/EA) at 1450°C. Isotopic composition of the CO product was determined on a ThermoFinnigan Delta Plus XL continuous flow isotope ratio mass spectrometer coupled to the TC/EA via a ConFlo II open split interface (ThermoQuest, 1999). Post-processing of isotopic data included corrections based on pre- and post-sample standard CO measurements for every sample; quality control for stability of standard peaks, background baseline and linear sample voltage range; and batch correction based on 5–10% in-run measurements on an in-house standard cellulose powder (granular cellulose obtained from J.T. Baker). Data are reported on the Standard Mean Ocean Water (SMOW) scale. Internal reproducibility of the mass spectrometer was typically 0.1‰ (SMOW) or less. External reproducibility of oxygen isotopic measurements, based on several hundred measurements of a laboratory standard cellulose powder, is 0.3‰ (SMOW).

4. RESULTS

4.1. Modified Brendel α-cellulose Extraction Method

Modification of the Brendel α-cellulose processing protocol permitted up to 160 wood samples to be processed daily to α-cellulose by a trained undergraduate research assistant. Processing yield was measured on Brazilian wood to be 30–35%, in agreement with results from Brendel et al. (2000) and similar to theoretical yield and yield using classical methods (Ehleringer et al., 2001). Measurement precision obtained for 40 replicate Brazilian wood analyses using the modified Brendel method was 0.37‰. Intercomparison of isotope data derived via the modified Brendel extraction method and the Brendel et al. (2000) protocol show no significant difference in mean δ^{18}O. T-tests between isotopic analyses of Brendel and Jayme-Wise α-cellulose processing products show no significant difference between mean values. Median reproducibility of isotopic measurements, as estimated by high-resolution intra-ring replication of α-cellulose extraction and δ^{18}O analyses on the Harvard Forest white pine samples, is 0.51‰. Overall, we found that δ^{18}O measurement precision is most dependent on sample homogenization prior to α-cellulose extraction and δ^{18}O analysis, which is consistent with the conclusions of the Ehleringer group. External isotopic standard analytical precision, based on over 200 replicate analyses of an internal cellulose standard (J.T. Baker) is 0.3‰, near limits of the online pyrolysis unit used to make the δ^{18}O measurements (ThermoQuest, 1999).

4.2. Temperate Forest, Central Massachusetts

δ^{18}O data obtained from Harvard Forest white pine samples are shown in Fig. 5. Based on observations of clear ring structure and rapid growth, we estimate that age model error is ±1 year. The data have an average annual cycle of about 2.5‰, with low isotopic values in the early part of the growing season, high intermediate values, and low values at the end of the growing season. Early and late-season minima in δ^{18}O composition are consistent with cooler early growing season temperatures and moisture delivered via midlatitude frontal storms. Mid-summer maxima in δ^{18}O of α-cellulose are consistent with summertime temperatures and tropical/subtropical moisture flux from the Gulf of Mexico and the subtropical North Atlantic (IAEA/WMO, 2003). On interannual time scales, comparison of the isotopic data with rainfall and temperature data from Amherst, MA for 1905–1915 (results not shown) suggests that evaporative enrichment of leaf water δ^{18}O associated with the integrated May–September rainfall deficit is responsible for interannual variability in mean seasonal δ^{18}O of α-cellulose at this location, consistent with earlier reports for Mohonk, NY (Lawrence and White, 1984).

4.3. Dry Forest, Liberia, Northwestern Costa Rica

δ^{18}O data obtained from the *Cordia sp.* specimen from the northwestern sector of Costa Rica are shown in Fig. 6a. Data from the dry forest region represents our first test of the isotopic chronometer in tropical woods with no visible ring structure (Figs. 2, 6). Although ring structure is absent, the high resolution measurements (up to 5 analyses per millimeter) reveal a range of 7‰ (Fig. 6a) and an average cyclicity of about 3‰ amplitude (Fig. 6c). Application of the proposed age model hypothesis, consistent with climatological rainfall (Fig. 6b). results in identification of 33–40 isotopic "years" (Fig. 6d). We were unable to independently confirm the stable isotopic chronometer with radiocarbon data, due to an unknown and signif-

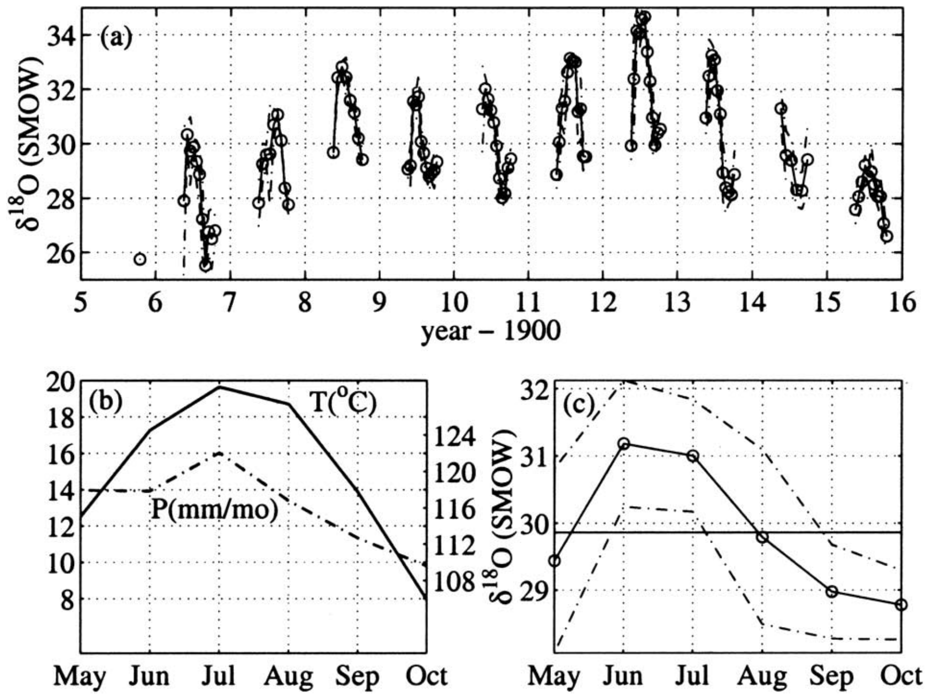

Fig. 5. Harvard Forest white pine δ^{18} data. (a): Age-modeled δ^{18} data. Open circles give inter-sample means; inter-sample replication $+1\sigma$ is shown by solid lines. (b): Climatological temperature (left scale, °C) and precipitation (right scale, mm/mo) over the May-October growing season. δ^{18} climatology (open circles) referenced to data series mean (solid line); dashed lines give $+1\sigma$ uncertainty.

icant source of fossil carbon (radiocarbon results not shown). However, if the stable isotope chronometer hypothesis is applicable, we speculate that the average age model error is ±5 years, due to the lack of visible ring structure in the wood and the imperfect nature of the presumed annual cycles in some sections of the record (Fig. 6a).

The range of the observed δ^{18}O cyclicity, 3–5‰, is approximately equal to the annual range of observed rainfall δ^{18}O values (IAEA/WMO, 2003) for stations from Honduras, Costa Rica and Panama. Although we have no direct evidence that the tree experienced annual growth cessation as in the hypothesized age model, since growth rings are absent (Fig. 2), the isotopic "climatology" (Fig. 6c) is consistent with prior observations suggesting that growth of Cordia is correlated with rainfall amount (Loján, 1965, Tschinkel, 1966). Figure 6e shows the growth rate estimates derived from construction of the age model. Mean radial growth rate is 8.1 ± 3.3 mm/yr. We observe no correlation between annual growth rate and annual mean δ^{18}O. Growth rates are similar to the results of Loján (1965), who reported incremental annual diameter growth (approximately twice radial growth) at sites near Turrialba, Costa Rica, to range from 10–40 mm/year.

Figure 6f shows July–October integrated rainfall amount for the 0.5° × 0.5° gridbox centered on 85.25°W, 10.25°N from New et al. (2000). Small negative rainfall anomalies are observed for this season during ENSO warm-phase events (Fig. 6b) consistent with results of Waylen et al. (1996). However, differences between normal-year and ENSO-year α-cellulose δ^{18}O "climatologies" are not significant (Fig. 6c), and there is no consistent link between isotopic anomalies and low JASO rainfall associated with ENSO warm-phase events in 1966, 1973, 1977, 1983, 1986, and 1997 (Figs. 6d, 6f).

4.4. Rain Forest, La Selva, Eastern Costa Rica

δ^{18}O data obtained from the *Hyeronima alchorneoides* (Piln) specimen from the eastern Costa Rican rain forest are presented in Fig. 7. 16 isotopic cycles of 4–6‰ are visible in the data series at intervals ranging from 4–18 mm. The results give an age model which is in agreement with the known age of the plantation samples. However, the isotopic cycles observed are not uniform. Hence, alternate age models having from 14–19 cycles may be postulated. Therefore, due to the irregularity of the hypothesized annual isotopic cycle observed in the data, we estimate age model uncertainty to be ±2 years.

Analysis of the annual rainfall pattern for the climatological and canonical ENSO year at La Selva (Fig. 7b) suggests there is a positive June–September (JJAS) rainfall anomaly during ENSO warm phase events, with a 50% increase in June–August precipitation relative to climatology. However, strong interannual variability renders this difference statistically insignificant. Consistent with this result, cellulose δ^{18}O climatologies for normal years show minima in July, and a small magnification of the annual cycle in ENSO years which is not statistically significant (Fig. 7c).

Intraseasonal and interannual variability is evident in the time series of monthly precipitation and JJAS summed precipitation (Fig. 7d–f). Highest JJAS rainfall totals are found in 1994, 1991, 1986, and 1997, and correspond to low cellulose δ^{18}O values. 1990–1991, a wet period, corresponds to a muted

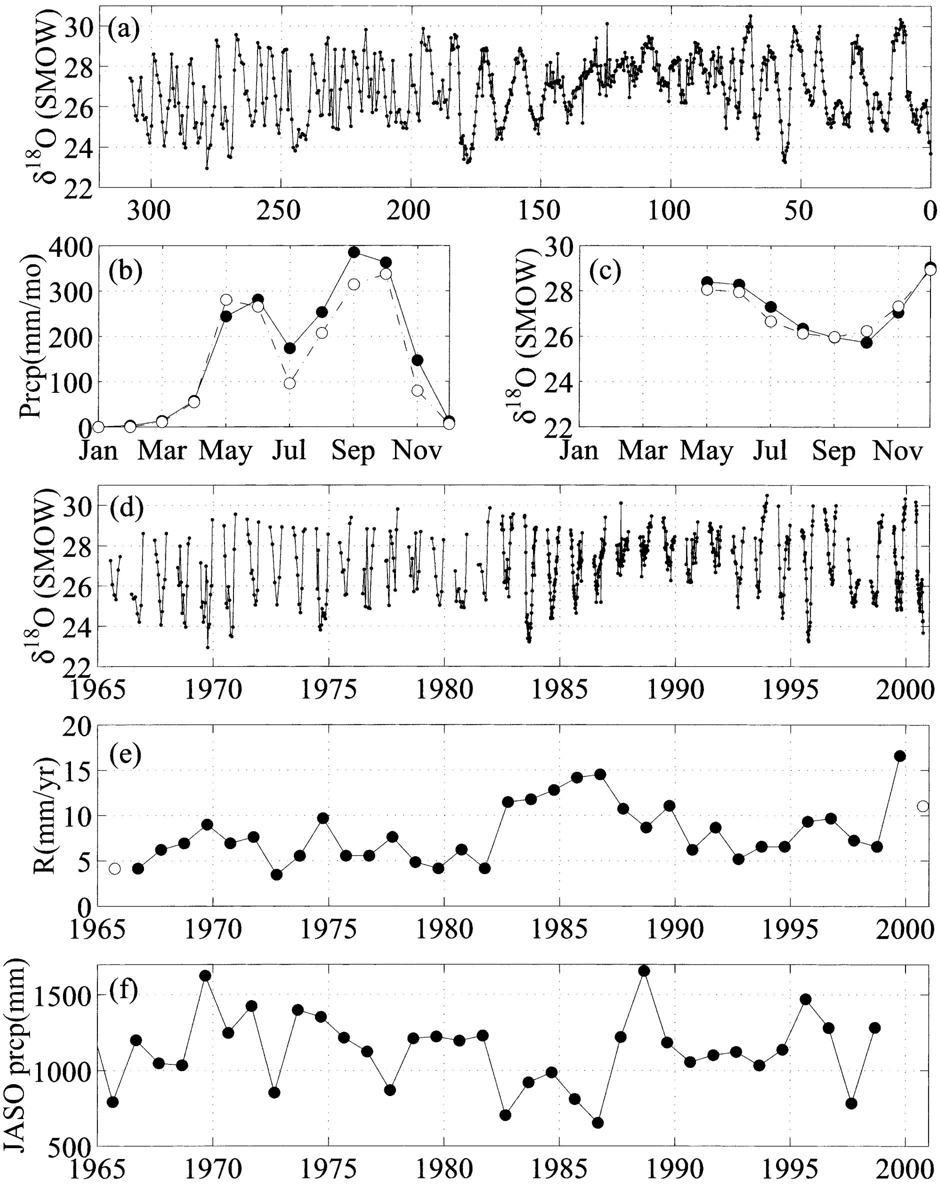

Fig. 6. Costa Rica Liberia region *Cordia sp.* δ^{18}O data. (a): data series plotted vs. depth from tree exterior (mm). (b): Precipitation climatologies for normal years (filled circles) and ENSO years (1965–6, 1969–70, 1972–3, 1976–7, 1982–3, 1986–7, 1991–3, 1997–8; open circles); data from New et al. (2000). (c): Average δ^{18}O climatologies for normal and ENSO years, as in (b), based on δ^{18}O data for 33 inferred years of high resolution δ^{18}O data, interpolated to monthly resolution. (d): Age-modelled δ^{18}O data series. (e): inferred annual growth rates (mm/yr) given age model plotted in (d); 1967 extrapolation shown as filled bar. (f): July–October integrated rainfall, from New et al. (2000).

annual cycle and low δ^{18}O values, and is consistent with a rainy dry season in winter 1990–1991. A similar effect can be seen in 1997, which resulted in a muted annual cycle with loss of a pronounced dry season signal. Conversely, 1995–1996 is a dry period, with two of the drier JJAS precipitation totals in the period, consistent with high δ^{18}O values through that period. A similar example can be seen in 1987–1988. Growth rates for this tree, estimated using the age model, vary interannually by up to 10 mm/year (Fig. 7e), and are similar to results from the dry forest (Fig. 6). However, growth rates are not well-correlated with rainfall amount or δ^{18}O of wood. For example, the two wettest JJAS seasons in the interval of study (1994, 1991) both correspond to low growth years, but the highest growth rate year (1998) also corresponds to a low rainfall year.

4.5. Dry Forest, Piura, Northwestern Coastal Peru

Isotopic data from northwestern coastal Peru provide our first test of the approach to developing a paleo-raingage indexing ENSO warm phase activity. The isotopic data show little evidence of cyclicity on the depth scales seen in the other pilot datasets (Figure 8). However, because the analyzed samples are from a plantation tree, we know the total time span covered by the data series is 10 years. We exploit this information to

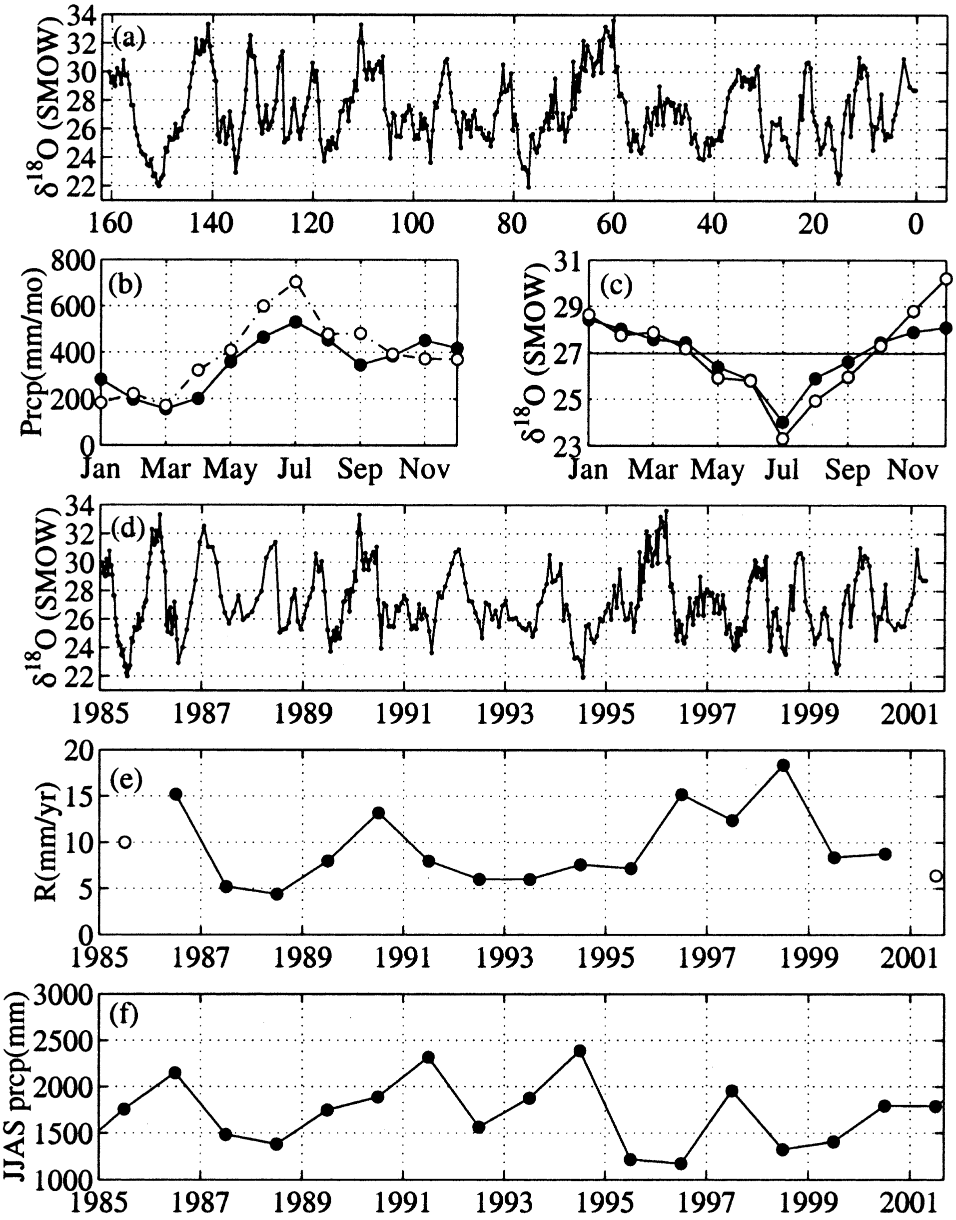

Fig. 7. Costa Rica La Selva *Hyeronima sp.* δ^{18}O data. (a): δ^{18}O data series plotted vs. depth from tree exterior (mm). (b): Precipitation climatologies for normal years (filled circles) and ENSO years (1957–8, 1965–6, 1969–70, 1972–3, 1976–7, 1982–3, 1986–7, 1991–2, 1997–8, 2002–3; open circles); data from Brenes (2003). (c): δ^{18}O climatologies for normal and ENSO (1986–7, 1991–2, 1997–8) years, as in (b), based on 15 inferred years of δ^{18}O data interpolated to monthly resolution. (d): Age-modelled δ^{18}O data series. (e): Inferred annual growth rates (mm/yr) given age model plotted in (d); estimates for 1985 and 2001 shown as open circles. (f): June-September integrated rainfall, from Brenes (2003).

develop a simple age and growth rate model for the δ^{18}O data. The average growth rate for this specimen is about 3.4 mm/yr over ten years. If we assume that the 8–10‰ isotopic excursion evident in the middle of the data series is the result of ENSO-related rainfall anomalies which occurred between January and March of 1998 in northwest coastal Peru (Xie and Arkin, 1997), then the tree grew in radial dimension by about 8 mm during 1998. This also implies that the annual growth rate was 2 mm/year prior and 3 mm/yr subsequent to the 1997–8 ENSO event (Fig. 8).

5. DISCUSSION

5.1. Chronometric Control in Trees Lacking Clear Annual Rings

Pilot results from a broad range of tropical environments and ecosystems suggest that we can use high resolution δ^{18}O measurements, as predicted by application of the RLE model to tropical environments, to infer chronology in wood samples lacking annual rings. The pilot dataset developed from Harvard Forest White Pine demonstrates that intra-ring isotopic season-

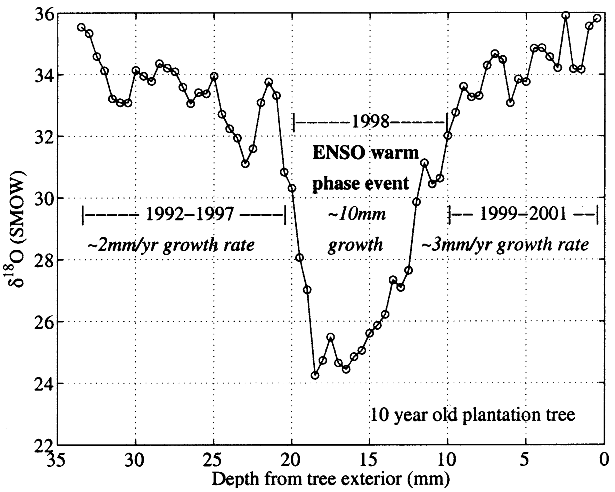

Fig. 8. Piura, Peru *Prosopis sp.* $\delta^{18}O$ data. Data series plotted vs. depth from tree exterior (mm). Interpretation as shown and discussed in paper.

ality shows a replicated and pronounced annual cycle within independently-dated rings. Results from a strongly seasonal dry tropical forest *Cordia sp.* tree from the northwestern Costa Rican dry forest give an average annual isotopic cycle which is qualitatively consistent with combined rainfall amount and evapotransiration effects (Gat, 1996, Fig. 4) and represent the first successful test of this approach to tropical dendrochronology. However, independent age control was not achieved due to problems with radiocarbon analyses. Data developed from a known-age plantation Hyeronima alchorneoides sample from the northeastern Costa Rican rain forest illustrates a semi-regular average annual isotopic climatology which is in agreement with climatological precipitation data. In this case, we identified the same number of isotopic cycles as known years of growth, within age model error, illustrating the first successful test of the proposed isotopic chronometer in a tropical environment. Studies with known-age plantation samples from hyperarid northwestern coastal Peru revealed no clear annual cyclicity, likely due to the ability of the sampled tree to place a taproot into shallow, reliable ground-water.

Related work reported elsewhere supports the tropical isotopic chronometer hypothesis argued here. Samples collected from the Large-Scale Biosphere-Atmosphere Site near Santarem, Brazil (Evans et al. (2002)) show that the latest few years of growth rates, which are inferred from similar high-resolution isotopic measurements, are in agreement with growth rates measured directly using band dendrometers. Subsequent work on samples from the Thailand has shown that a specimen of *Podocarpus neriifolius,* which was manually scarred in 1992, shows 6 isotopic cycles between marking and sampling in early 1999 (Poussart et al., 2004). Poussart et al. (2004) also successfully confirmed the application of the stable isotopic chronometer in two replicate Suar wood data series using radiocarbon measurements. These results suggest cautious optimism for the application of the stable isotope chronometer, with some degree of species independence. However, local hydrology, tree physiology, sampling methods, and environmental seasonality are likely all important ingredients for the successful development of seasonal-to-annual chronometric data in tropical woods which are undatable by traditional dendrochronological means.

5.2. Paleoclimatic Calibration

Comparison of climatological regional rainfall data with age modeled isotope data suggests tropical isotope dendroclimatology is now feasible. We applied simple age modeling rules to develop replicated isotopic climatologies in qualitative agreement with RLE model predictions for the annual cycle in tropical dry and rain forest environments in Costa Rica (Figs. 6, 7). For the Costa Rican dry forest record, a tendency toward drought in ENSO years was not reflected in the isotopic data (Fig. 6b,c). For the Costa Rican rain forest record, differences between normal and enhanced ENSO-year rainfall were also not statistically significant, due to an irregular annual rainfall cycle, but were identified, on average, with a tendency toward lighter cellulose $\delta^{18}O$ values (Figs. 7b,c). In both records, growth rates were not simply related to variations in rainfall amount (Figs. 6d,e; 7d,e). These equivocal results are not entirely surprising: Waylen et al. (1996) analyzed the canonical rainfall response in northwest Costa Rica to ENSO warm phase activity between 1940 and 1992, and found that opposing seasonal rainfall anomalies through the course of an ENSO event may result in a rainfall anomaly integrated over the whole event that is close to zero. Both sites were useful for testing the stable isotope chronometer hypothesis, due to strong annual cycles in rainfall, but are not optimally located for recording large-scale climate variability such as the ENSO phenomenon.

Tantalizing data from an ENSO-sensitive region suggests that in the right environment, stable isotope dendroclimatology has great potential. The pilot data series from a northwestern coastal Peru plantation (Figure 8), although lacking replication and annual isotopic age control, does point to this location as a potentially useful addition to the extant high resolution paleoclimate data network used to study ENSO. Assuming a strong connection between rainfall and interannual growth rates for this sample suggests an 8–9‰ $\delta^{18}O$ anomaly during the very strong 1997–8 ENSO warm phase event rains experienced in the region. Further studies to replicate this result in trees of unknown total age, but which also record an annual cycle, are required to confirm this interpretation. Poussart et al. (2004) has subsequently developed two 20-year long, high-resolution $\delta^{18}O$ time series from Indonesia, with chronological control confirmed by multiple radiocarbon measurements. These records are significantly correlated ($r = 0.68$, $p < 0.0001$). In turn the data show elevated $\delta^{18}O$ values during known drought years 1983, 1987, 1991, and 1997, as predicted by the hypothesis proposed in Section 2. Given that no one location will unambiguously track a large-scale phenomenon such as ENSO, better observational coverage within the terrestrial tropics, in conjunction with other proxy data and modern data analysis techniques, will help to uncover the rich behavior of ENSO and other tropical phenomena over the past several centuries.

5.3. Uncertainties

The empirical application of RLE to the environmental interpretation of intra- and interannual $\delta^{18}O$ variation in tropical trees described here leaves open several important uncertainties. First, the fraction f of water O which does not undergo isotopic re-equilibration with leaf water prior to fixation as α-cellulose, has recently been observed, under temperate conditions, to vary in time and species (Roden et al., 2000; Anderson et al., 2002; Barbour et al., 2004, Section 2). Second, the extent to which precipitation, soil and ground waters are differentially used by trees cannot be determined using a single stable isotope measurement, although the construction of meteoric water lines for study sites could in principle be used to address this question in site calibration studies (Dawson et al., 2002). Third, many aspects of tree growth, structure and physiology, including aseasonal growth hiati, asymmetric radial structure, interseasonal photosynthate storage, and other adaptations of tropical trees may further complicate the environmental interpretation of the δ^{18}O of tropical wood. Further work to ground-truth the RLE model as applied to tropical environments is neccessary to verify the hypothesis which is supported here by empirical methods.

5.4. Summary

Current scientific understanding of the isotopic controls on the δ^{18}O of wood α-cellulose rapid α-cellulose extraction procedures, and continuous flow isotope ratio mass spectrometry technology now make tropical isotope dendroclimatology possible and relatively easy. We have found that the hypothesized isotopic clock functions in dry and moist tropical environments and seems to be species-independent, but is probably not environment-independent. Independent chronological evidence and data series replication are important proof-of-concepts for the approach. However, with demonstrable age control and series reproducibility, there is rich potential for tropical isotope dendroclimatology in the poorly-observed terrestrial tropics. Together with the mature ENSO proxies (corals, extratropical trees, ice cores, varved sediments, etc.) hydrological proxies from tropical trees may eventually be used to improve reconstructions of the past behavior of tropical climate phenomena over the past few hundred years.

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