Aridity and vegetation composition are important determinants of leaf-wax δD values in southeastern Mexico and Central America

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Abstract

Leaf-wax hydrogen isotope composition (δD wax) is increasingly applied as a proxy for hydroclimate variability in tropical paleoclimate archives, but the factors controlling δD wax in the tropics remain poorly understood. We measured δD wax and the stable carbon isotope composition of leaf-waxes (δ13C wax), including both n-alkanes and n-alkanoic acids, from modern lake sediments and soils across a marked aridity gradient in southeastern Mexico and northern Central America to investigate the importance of aridity and vegetation composition on δD wax. In this region the estimated hydrogen isotope composition of meteoric water (δD w) varies by only 25‰, and variability in δD w does not explain the relatively large variance in δD wax (60‰). Instead, the aridity index, defined as the ratio of mean annual precipitation to mean annual potential evapotranspiration (MAP/PET), explains much of the variability in the hydrogen isotope fractionation between leaf-waxes and meteoric water (εwax/w). Aridity effects are more evident in lake sediments than in soils, possibly because integration of leaf-waxes across a broad catchment masks small-scale variability in εwax/w that is a consequence of differences in vegetation and microclimates.

In angiosperm-dominated environments, plant ecology, inferred from δ13C wax, provides a secondary control on εn-alkane/w for n-alkanoic acids (εn-alkanoic acid/w). Low δ13C n-alkane values are associated with high εn-alkane/w values, most likely reflecting differences in biosynthetic hydrogen isotope fractionation between C4 grasses and C3 trees and shrubs. A similar relationship between δ13C n-alkane and εn-alkane/w is not observed. These results indicate that changes in either aridity or vegetation can cause large variability in δD wax that is independent of the isotopic composition of precipitation, and these effects should be accounted for in paleoclimate studies.

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1. INTRODUCTION

A promising geochemical approach for detecting past hydrological change applies hydrogen isotope values of terrestrial leaf-waxes (δD wax) recovered from lake and marine sediments (Sauer et al., 2001; Sachse et al., 2004; Hou et al., 2008) as a proxy for the hydrogen isotopic composition (D/H) of precipitation. This proxy could prove particularly valuable for reconstructing hydroclimate changes in the tropics, where precipitation D/H is primarily controlled by rainfall amount (Rozanski et al., 1992; Risi et al., 2008), source effects and vapor transport pathways. δD wax records from lake and marine sediments have been used to reconstruct hydrological changes at a number of locations over various time periods (e.g. Pagani et al., 2006; Shuman et al., 2006; Hren et al., 2010) including numerous sites in the tropics (Schefuss et al., 2005, 2011; Jacob et al., 2007; Tierney et al., 2008; Jaramillo et al., 2010; Niedermeyer et al., 2010). However, there are very few data constraining the mechanisms controlling leaf-wax hydrogen isotopes in

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Dwax values are arguably controlled by three factors: (1) the isotopic composition of meteoric water; (2) D enrichment of soil and/or leaf-water due to evapotranspiration; and (3) differences in isotopic fractionation between source water and leaf-waxes in different plant groups (Sachse et al., 2004, 2012; Liu et al., 2006; Smith and Freeman, 2006; Hou et al., 2008; Liu and Yang, 2008; Feakins and Sessions, 2010; McNerney et al., 2011). Studies of Dwax variability in lacustrine sediments across mid-latitude transects indicate that the isotopic composition of meteoric waters is a primary determinant of Dwax (Sachse et al., 2004; Hou et al., 2008), with other environmental factors exerting a secondary influence. For example, a study of lakes in the southwestern United States showed that large changes in relative humidity did not strongly impact Dwax values (Hou et al., 2008). Conversely, grass extracts from the Great Plains show that Dwax values from drier settings are progressively D-enriched relative to meteoric water (Smith and Freeman, 2006), suggesting soil evaporation and transpiration enhance D enrichment in drier climates. Comparison of grasses grown in a growth chamber with field-grown grasses indicates that the D/H of grass leaf-waxes is not affected by transpiration, implicating soil evaporation as an important cause of grass leaf-wax D/H variability (McNerney et al., 2011). However, direct measurements of plant-water D values for trees and grasses indicate that isotopic enrichment of leaf-water due to transpiration does have a significant impact on Dwax values (Feakins and Sessions, 2010; Sachse et al., 2010).

There are differences in apparent hydrogen isotope fractionation between meteoric water and leaf-waxes (εwax/ν) for different plant groups grown under similar conditions. Research to date indicates that Dwax values are lower in C₄ grasses than in C₃ trees and shrubs by ~20% on average (Chikaraishi and Naraoka, 2003; Chikaraishi et al., 2004; Sachse et al., 2012). Furthermore, leaf-waxes in C₃ grasses are D-depleted by ~15% relative to C₄ grasses (Smith and Freeman, 2006; McNerney et al., 2011; Sachse et al., 2012). Dwax differences between gymnosperms and angiosperms can also be important (Chikaraishi and Naraoka, 2003; Chikaraishi et al., 2004; Pedentchouk et al., 2008; Polissar and Freeman, 2010), with most studies indicating lower Dwax values in gymnosperms. Evidence from plant extracts suggests that gymnosperms produce very few n-alkanes relative to angiosperms, although these two plant groups produce similar amounts of n-alkanoic acids (Diefendorf et al., 2011).

Most studies concerning modern variability of sedimentary Dwax values have focused on mid-latitude settings (Sachse et al., 2004; Hou et al., 2008). Polissar and Freeman (2010) analyzed Dwax values of sediments from high-elevation tropical lakes as part of a broad study that also included mid-latitude sites, but did not focus on low-elevation tropical environments. Rao et al. (2009) analyzed Dwax from tropical soils as part of a larger study across eastern China, but did not focus on Dwax variability between tropical sites. Garcin et al. (2012) examined δD variability in n-alkanes from lake sediments across a climatic gradient in Cameroon and found that the δD value of plant source water was the primary determinant of Dwax values in this tropical location, similar to results from mid-latitude settings.

For this study, we measured Dwax in lake sediments and soils across southeastern Mexico and northern Central America (Fig. 1) to evaluate the impact of aridity on Dwax in tropical environments. We also measured the carbon isotope composition of leaf-waxes (δ¹³Cwax) at each site to constrain the relative contribution of leaf-waxes from different plant groups. Large variance in aridity characterizes our sample localities. The aridity index (AI), defined as annual precipitation divided by annual potential evapotranspiration (MAP/PET), varies from 0.51 to 2.1, where lower values of AI indicate greater aridity. Meanwhile the estimated hydrogen isotopic composition of meteoric water (Dw) ranges from ~24‰ to ~49‰ with Dw at most sites...
varying by only 9%. The large aridity gradient across the study area, combined with little variability in the isotopic composition of precipitation, provides an ideal setting to study aridity effects on δDwax. Most of the sampling sites for this study are located within broadleaf forests (Fig. 1), and therefore changes in ecosystems and the dominant plant source of leaf-waxes related to climatic variability are reduced relative to previous studies (e.g. Hou et al., 2008; Garcin et al., 2012). However, local scale variation in vegetation cover due to anthropogenic deforestation and land-cover change (Wagner, 1964; Kok and Veldkamp, 2001), constrained by δ13Cwax values, provide an opportunity to assess the effects of differing vegetation on δDwax between sites with similar climates.

2. METHODOLOGY

2.1. Sediment, soil and water sampling

Surface sediment and water samples were collected from 20 lakes and cenotes (water-filled karst sinkholes) across southeastern Mexico (the Yucatan Peninsula), Guatemala, and Honduras (Fig. 1). Most of the lakes are located at low elevations, between 10 and 200 masl, although one lake, Yojoa, is located at 662 masl. Four of the sampled lakes (Cuerpo Uno, La Corona, Xoche and Yaalchak) have shorelines dominated by emergent aquatic plants (EAP lakes), which likely affects the δDwax signal at these sites (see Section 4.2.3.3). We collected soil samples from 23 sites across the region, including 9 sites near sampled lakes, and 14 sites in Honduras where there are few lakes. Soil localities span elevations from 5 to 1405 masl. Vegetation across the study region is dominated by moist and dry tropical broadleaf forests at low elevations (≪500 masl), and pine-oak forests at higher elevations (≫500 masl) (Fig. 1; Wagner, 1964; Olson et al., 2001). The location and environmental characteristics of all soil and sediment sampling sites are listed in Electronic Annex EA-1.

Lake sediments were collected using an Ekman dredge. Where possible, sediments were collected at water depths > 10 m. Soil samples were collected at a depth of 5 cm. Most soil samples were from forest or woodland environments. Stream, lake and groundwater samples were collected at 46 sites, including streams near the sampled lakes and soils. Replicate water samples were collected in August 2008 and May 2009 from three streams and two lakes to examine seasonal variability in surface-water isotopic values. Water sampling locations are listed in Electronic Annex EA-2.

In addition to sediment and soil samples, we collected leaf samples from grasses, angiosperm trees, and emergent aquatic plants from some of the lake and soil sampling sites. In this study we analyze the n-alkyl lipid distribution of these samples in order to constrain the source of leaf-waxes in lake sediment samples (see Section 4.2.3.3).

2.2. Leaf-wax isotope analyses

All lake sediment and soil samples were freeze-dried, sieved (400 μm), and extracted using an Accelerated Solvent Extractor (ASE3000, Dionex) with dichloromethane:methanol (9:1 v/v) at 150 °C and 1500 psi. Between 5 and 10 g dry weight of sediment/soil were extracted per sample. Leaf samples were freeze-dried, cut into pieces with solvent-cleaned scissors and ultrasonically extracted with dichloromethane:methanol (9:1 v/v). The total lipid extracts obtained from sediment, soil and leaf samples were separated into neutral and acid fractions using solid phase extraction with aminopropyl sorbent (Varian Bondesil). Neutral lipids were eluted first with 8 ml of 1:9 v/v acetone:dichloromethane, and acidic lipids were eluted in 8 ml of 2% formic acid in dichloromethane.

The neutral fraction was separated using silica gel (J.T. Baker) chromatography into three subfractions: aliphatic hydrocarbons, eluted with hexane; ketones, eluted with dichloromethane; and polar lipids, eluted with methanol. The ketone and polar subfractions were reserved for future analyses. Straight-chain aliphatic hydrocarbons were purified by urea adduction, and unsaturated hydrocarbons were removed using silver nitrate column chromatography.

The acid fraction was methylated using 14% boron trifluoride in methanol (70 °C for 20 min). The resulting fatty acid methyl esters (FAMEs) were extracted with hexane, and then purified via silica gel chromatography (eluted in 2:1 v/v hexane:dichloromethane). Unsaturated FAMEs were removed with silver nitrate column chromatography.

Normal alkane and FAME compound distributions were analyzed using a Thermo Trace 2000 GC equipped with an Rxi-1ms column (60 m × 0.25 mm × 0.25 μm), a pressure- and temperature-variable (PTV) injector and a flame ionization device (FID) with He as the carrier gas. δD and δ13C values for individual compounds were determined by gas chromatography-isotope ratio mass spectrometry (GC-IRMS). Measurements were performed using a Thermo Trace2000 GC equipped with an SGE Sol-Gel-1ms column (60 m × 0.25 mm × 0.25 μm) and a PTV injector coupled to a Finnigan MAT 253 stable isotope mass spectrometer via a Finnigan GC combustion III interface.

The H3+ factor for the GC-IRMS was measured daily prior to δD analysis, with a mean value for the measurement periods of 15.6 ± 0.3 (1σ). External n-alkane and FAME isotope standards (Mix B2 and Mix F8, Indiana University Biogeochemical Laboratories) were measured after every four to six sample analyses and used to standardize and normalize sample isotope values. The precision of the external standards was ±$\pm$0.5 for δD analyses and ±$\pm$0.5 for δ13C analyses. Most samples were run in duplicate for both hydrogen and carbon isotope analysis, and the reported isotope ratio values are averages of duplicate runs. All leaf-wax stable isotope data are reported in Electronic Annex EA-3.

We applied the precision of the external standards for δD (5%) and δ13C (0.5%) as the analytical error of these measurements in all statistical analyses because these values were consistently larger than the standard deviation of replicate analyses.

FAME δ13C and δD values were corrected for the isotopic composition of the methyl group added during transesterification. This was accomplished using a phthalic acid standard of known isotopic composition, acquired from
Indiana University Biogeochemical Laboratories, and calculating the change in isotopic composition due to methylation.

To investigate environmental controls on \( \delta D_{\text{wax}} \), we calculated the apparent hydrogen isotope fractionation \( (\epsilon_{\text{wax/w}}) \) between meteoric water, estimated from surface-water isotopic data (see Section 2.4) and leaf-waxes (Sauer et al., 2001):

\[
\epsilon_{\text{wax/w}} = \left( \frac{(\delta D_{\text{wax}} + 1000)}{(\delta D_{\text{w}} + 1000)} \right) - 1 \times 1000
\]

with \( \epsilon_{\text{wax/w}} \) expressed in per mil notation. Errors in \( \epsilon_{\text{wax/w}} \) were calculated by propagating errors in \( \delta D_{\text{wax}} \) measurements and \( \delta D_{\text{w}} \) estimates (Taylor, 1997; see Section 2.4 for a discussion of errors in \( \delta D_{\text{w}} \) estimates).

### Analysis of water samples

\( \delta D \) and \( \delta^{18}O \) values of lake, stream and groundwater samples were measured using thermal conversion/elemental analyzer-isotope ratio mass spectrometry (TC–EA-IRMS) and are represented as the average of five replicate analyses using 0.5-μl aliquots of water per measurement. Seven laboratory standards, with known isotopic values calibrated to the SMOW-SLAP scale, were measured after every five samples for instrument calibration. Precision for \( \delta D \) and \( \delta^{18}O \) standard measurements was \( \leq 2_{\text{oc}} \) and \( \leq 0.2_{\text{oc}} \), respectively, and these values were used as measurement error in subsequent statistical analyses. All water isotope data are reported in Electronic Annex EA-2.

### Estimating the isotopic composition of precipitation

Because of the scarcity of precipitation isotope measurements in southeastern Mexico and Central America, and the variable distribution and type of surface-water isotope measurements in our study area, we took a multi-pronged approach to estimating the isotopic composition of precipitation at our lake sediment and soil sampling sites. For each sediment and soil sampling site we compiled all surface-water isotopic data (Lachniet and Patterson, 2009; Hodell et al., 2012; this study), including lakes, streams and groundwater, within 90 km of the sampling site. This radius was determined by estimating the distance that would lead to a significant change in meteoric water isotopic composition (defined as 0.5%o for \( \delta^{18}O \) and 4%o for \( \delta D \) in the context of this study), using a multivariate model for streamwater isotopic composition in Guatemala and Belize (Lachniet and Patterson, 2009); a relatively large radius was selected to include sufficient data for sites in areas with sparse water isotope data, particularly in central Honduras. In the multivariate model of Lachniet and Patterson (2009), the distance variable is distance to the Pacific coast along a NE–SW heading. However, this direction has the greatest variability in isotopic composition of precipitation when elevation changes are accounted for, and isotopic variability in other directions is typically of a smaller magnitude. The compiled surface-water data were normalized to the elevation of the soil/sediment sample site using an empirical isotopic lapse rate determined for Belize and Guatemala (Lachniet and Patterson, 2009): -1.71%o/km for \( \delta^{18}O \) and -13.66%o/km for \( \delta D \). To normalize surface-water isotopic values we used the following equation:

\[
\delta_D = \delta_x + (z - z_{ref}) \Gamma_{d/dz}
\]

where \( \delta_x \) is the isotopic composition of the surface-water sample, \( z_{ref} \) is the elevation of the targeted soil/sediment sampling site, \( z \) is the elevation of the surface-water sample, and \( \Gamma_{d/dz} \) is the isotopic lapse rate (Polissar and Freeman, 2010). The surface-water locations compiled for each lake sediment and soil sampling site are catalogued in Electronic Annex EA-4. All surface-water isotope values are listed in Electronic Annex EA-5.

In areas where data are available for the isotopic composition of evaporative lake-waters, namely the northern Yucatan Peninsula and northern Guatemala, we constructed local evaporation lines for each area using compiled surface-water data within 90 km of sites and calculated the intersection of the local evaporation line and global meteoric water line in \( \delta D–\delta^{18}O \) space as the inferred precipitation isotopic composition for that site (Gonfiantini, 1986). Errors for estimates of meteoric \( \delta D \) and \( \delta^{18}O \) were calculated by propagating the standard error of the local evaporation line regression parameters through the calculation of local meteoric \( \delta D \) and \( \delta^{18}O \) using standard error propagation techniques (Taylor, 1997). The regression parameters and their standard errors for local evaporation lines are listed in Electronic Annex EA-6.

Surface-water isotope data from sites in central Honduras are much more sparse, consisting only of measurements on samples collected during this study. Many of these data come from stream and lake-waters that are enriched in \( ^{18}O \) and D due to evaporation. For these sites we also used local evaporation lines as described above to estimate the isotopic composition of precipitation, but the relative paucity of data leads to larger errors in the estimated isotopic composition of precipitation.

Numerous river water isotope data are available for southeastern Guatemala and western Honduras, but there are very few isotope measurements of evaporative surface-waters with which to construct a local evaporation line. Instead, we calculated the mean of elevation-normalized river water isotope data within 90 km of each site as the best estimate of the isotope composition of local precipitation. This is consistent with the analysis of Lachniet and Patterson (2009) who argued that river water isotope values are robust indicators of meteoric water isotopic values in Guatemala and Belize. Estimated \( \delta^{18}O_{\text{w}} \) and \( \delta D_{\text{w}} \) values from this region indicate deuterium excess (\( \delta \)) values between 8 and 10, suggesting that these values are not strongly influenced by evaporation (Electronic Annex EA-4). We consider the standard deviations (1σ) of the compiled water isotope data for each sampling site to be conservative (i.e. large) estimates of the error associated with these values. \( \delta D_{\text{w}} \) estimates for each sampling site and their corresponding errors are listed in Electronic Annex EA-4.

### Climate data

Estimates of mean annual precipitation amount (MAP) and mean annual relative humidity (MARH) used for
comparisons with isotopic data come from the Ten Minute Climatology dataset of the University of East Anglia Climatology Research Unit (New et al., 2002; data available online at http://www.cru.uea.ac.uk/cru/data/hrg/tmc/). Estimates of mean annual potential evapotranspiration (PET) come from the Consultative Group on International Agricultural Research Consortium for Spatial Information (CGIAR-CSI) Global Potential Evapo-Transpiration Geospatial Database (Trabucco et al., 2008; Trabucco and Zomer, 2009; data available online at http://www.cgiar.org/aridity). Climate variables for all sampling sites are listed in Electronic Annex EA-1.

### 2.6. Biome and ecoregion classification

All sites were assigned to a biome based on a global database of terrestrial biomes and ecoregions (Olson et al., 2001; data available online at http://www.worldwildlife.org/science/ecoregions). Biome boundaries are not precise, and ecotones typically occur in transitional areas between biomes (Olson et al., 2001). Because the presence of gymnosperms in ecotones between coniferous and broad-leaf biomes may affect leaf-wax isotope values, we classified sites within 10 km of a coniferous forest biome as belonging to that biome. This classification is supported by observations of coniferous trees at sites in this category during field campaigns. There is evidence that gymnosperms in temperate environments produce very few n-alkanes relative to angiosperms (Diefendorf et al., 2011). However, Diefendorf et al. (2011) also showed that gymnosperms and angiosperms produce similar amounts of n-alkanoic acids, so differentiating sites with abundant gymnosperms is relevant for this study. Similarly, we classified sites within 10 km of the dry broadleaf forest biome as belonging to that biome because the presence of more open canopies and deciduous trees in ecotones between moist and dry forests has the potential to affect leaf-wax isotope

All samples were assigned to an ecoregion to examine finer-scale differences in plant communities across the study area (Olson et al., 2001). The five eco-regions that occur in the study area (Yucatan dry forests, Peten moist forests, Central American Atlantic moist forests, Central American dry forests, and Central American pine-ack forests) correspond to five clusters of sampling sites, each with a relatively homogenous climate. Transition zones between ecoregions were addressed similarly to transition zones between biomes. Biome and ecoregion classifications are listed in Electronic Annex EA-1.

### 2.7. Linear regression analyses

To analyze relationships between $\delta_{D_{\text{wax}}}$ and $\epsilon_{\text{wax/w}}$ and potential controlling variables explored in this study ($\delta_{D_{\text{wax}}}$, MAP, MARH, AI, PET, $\delta^{13}C_{\text{wax}}$), we conducted a set of linear regression analyses for the dataset as a whole, as well as subsets of the data defined by sample type, biome, ecoregion and $\delta^{13}C_{\text{wax}}$ value. To assess whether lakes dominated by emergent aquatic plants (EAP lakes) influence regression results we conducted regression analyses both including and excluding these sites (see Section 4.2.3.3).

To account for variable and in some cases large errors associated with $\delta_{D_{\text{wax}}}$ and $\epsilon_{\text{wax/w}}$ values, we applied a weighted least squares regression in analyses involving these variables, with weights $W = 1/\sigma^2$ (Taylor, 1997). For $\delta_{D_{\text{wax}}}$ and $\delta^{13}C_{\text{wax}}$ for which there are more uniform measurement errors based on the precision of external isotope standards, we applied a simulation-extrapolation (SIMEX) method for fitting models with additive measurement error in both the independent and dependent variables (Cook and Stefanski, 1994). The SIMEX method adds additional measurement error to the data in known increments, computes regression estimates from the modified data, establishes a trend between these estimates and the variance of the added errors, and extrapolates this trend to compute regression estimates in the case of no measurement error. For the SIMEX regression models we applied a quadratic fitting method and jackknife variance estimation (Cook and Stefanski, 1994). Measurement error was added in the increments $\lambda = 0.5, 1, 1.5, 2$, where $\lambda$ is a set of multipliers of the actual measurement error variance. 100 bootstrap iterations were performed for each value of $\lambda$. For regression analyses that involved both weighted regression and SIMEX models (e.g. regressing $\epsilon_{\text{wax/w}}$ against $\delta^{13}C_{\text{wax}}$), we first produced a naïve model using weighted least squares, and then applied the SIMEX procedure to this naïve model to account for measurement error in both the dependent and independent variables. All regression analyses were performed in $R$.

### 3. RESULTS

#### 3.1. Water isotope measurements

Stream and groundwater $\delta^{18}O$ and $\delta D$ values range from $-2.5^{\circ}/$ and $-13^{\circ}/$, respectively, at a low-elevation coastal site (Rio Cienega) to $-6.5^{\circ}/$ and $-44^{\circ}/$, respectively, at a high-elevation inland site (Santa Lucia). Lake-water $\delta^{18}O$ and $\delta D$ values vary from $-4.3^{\circ}/$ and $-28^{\circ}/$, respectively, in a large, open-basin lake (L. Izabal Mina) to $4.6^{\circ}/$ and $18^{\circ}/$, respectively, in a highly evaporative, closed-basin lake (L. Salpeten). Generally, stream and groundwater $\delta D$ values decrease with increasing elevation (Fig. 2a) and with distance from the Caribbean Coast, in agreement with other stream-water datasets from the region (Lachniet and Patterson, 2009). Lake-water $\delta D$ values range widely and are controlled primarily by basin hydrology (Fig. 2b). Open-basin lakes and cenotes exhibit $\delta D$ values similar to stream/groundwater values, ranging from $-28^{\circ}/$ to $-22^{\circ}/$. More evaporative closed-basin lakes generally exhibit higher $\delta D$ values than open-basin lakes and range from $-17^{\circ}/$ to $18^{\circ}/$. There is a continuum between open and closed basin lakes related to the proportion of the water budget that is lost to evaporation.

Samples from three streams and two lakes collected in both August 2008 and May 2009 indicate that May surface-waters, for both lakes and two streams, were $\sim 5^{\circ}/$ more positive in $\delta D$ and $\sim 0.6^{\circ}/$ more positive in $\delta^{18}O$, relative to August surface-waters. For the third stream, seasonal differences in isotopic values were within analytical error.
3.2. Estimates of meteoric water isotopic composition

We estimated meteoric water isotopic compositions (δnw) for each lake sediment and soil sampling site using nearby surface-water isotopic measurements (see Section 2.4). δ18Ow values range from −4.1‰ to −7.4‰ and ΔDw ranges from −24‰ to −49‰ (Electronic Annex EA-4). The error associated with these estimates varies across the study area, primarily as a consequence of the variability in the frequency and type of surface-water measurements. Errors in the northern Yucatan Peninsula and northern Guatemala, where numerous surface-water data are available, average 0.4‰ for δ18O and 3‰ for ΔD. In southeastern Guatemala and northern Honduras, where estimates use averages of river water measurements, average errors are 1.6‰ for δ18O and 13‰ for ΔD. In central Honduras, where only sparse surface-water data were collected, average errors are 2.4‰ for δ18O and 19‰ for ΔD.

For all sites, we conducted a sensitivity test to determine whether changing the radius over which surface-water isotope values were integrated to 50 km affected the inferred isotopic composition of precipitation. Results of this test are shown in Electronic Annex EA-4. For most sites (27 out of 38), reducing the integration radius for surface-water data from 90 to 50 km did not change the inferred isotopic composition of precipitation by more than 0.5‰ for δ18O and 4‰ for ΔD. Most exceptions to this insensitivity to integration radius occurred in central Honduras, where scarcity of data led to large variance in estimates of the isotopic composition of precipitation derived from local evaporation lines calculated with a 50-km integration radius. There is substantial uncertainty regarding meteoric water isotopic compositions from central Honduras, but the large errors for these estimates reflect this uncertainty.

Comparison of our estimated meteoric water values with estimates from the Online Isotopes in Precipitation Calculator (OIPC) (Bowen and Revenaugh, 2003; Bowen, 2011) suggests broad agreement (Electronic Annex EA-4). Because there are few isotope monitoring stations to constrain model interpolations in southeast Mexico and northern Central America, we argue that meteoric water isotope values calculated from surface-water data measurements provide the best estimates of meteoric water isotope values in the context of this study.

3.3. Sediment and soil leaf-wax abundance and isotopic composition

All lake sediment and soil samples yielded n-C16 to n-C34 n-alkanoic acids. n-C27 to n-C33 n-alkanes were found in all samples, with some samples yielding shorter-chain and/or longer-chain compounds (n-C17 to n-C25, C35). n-Alkanoic acids exhibit strong even-over-odd peak preference, while almost all n-alkane samples exhibit strong odd-over-even peak preference, consistent with higher-plant sources. Three n-alkane samples had low carbon preference index (CPI) values (Lake Yojoa, Lake Izabal Mina, Ceibita), likely due to petroleum contamination, and were excluded from further analysis. We limit our isotopic analysis to long-chain n-alkanoic acids (n-C26 to n-C30) and n-alkanes (n-C29 to n-C33) to characterize the isotopic composition of vascular plant leaf-wax lipids. In some samples n-C24 is the dominant n-alkanoic acid homolog; however, because this homolog is not typically a target of modern or paleoclimate isotopic studies (e.g. Hou et al., 2008; Tierney et al., 2008), it is not a focus of our study. δD and δ13C values for n-C24 n-alkanoic acids co-vary positively with values for longer-chain length homologs, suggesting that this homolog follows a similar pattern of isotopic variability to the compounds analyzed in this study.

Long-chain n-alkanoic acid homologs (n-C26, n-C28, and n-C30) are positively correlated with one another both in δD (Fig. 3a) and δ13C values (Fig. 3b). We compared the mean isotopic signal of long-chain n-alkanoic acids between sites using abundance-weighted average ΔD and δ13C values for n-C26, n-C28, and n-C30 n-alkanoic acids for each sample. Abundance-weighted average leaf-wax isotope values have been applied in previous studies to compare the overall
leaf-wax isotopic signal between plant taxa or sediment samples, despite varying chain-length abundances and isotopic differences between leaf-wax homologs (Street-Perrott et al., 2004; Makou et al., 2007; Feakins and Sessions, 2010). This approach provides a clearer indication of overall isotopic variability across the study area than focusing on a single homolog, as has been done in some studies of modern sedimentary δD (Hou et al., 2008; Polissar and Freeman, 2010), given that the dominant homolog varies between samples in our study area. The weighted average δD of long-chain \( n \)-alkanoic acids (δD\(_{n-alkanoic\, acid} \)) varies between \(-111.9\%_{oo}\) (Chumayel-lake sediment) and \(-166.9\%_{oo}\) (Comayagua-soil). The weighted average δδ\(^{13}C\) value for long-chain \( n \)-alkanoic acids (δδ\(^{13}C\, acid\)) varies from \( -26.9\%_{oo} \) (Yojoa-soil) to \(-37.3\%_{oo}\) (Puente Cienega-soil). N-Alkane isotope and chain-length distribution data are reported in Electronic Annex EA-3.

Long-chain \( n \)-alkane homologs (\( n \)-C\(_{29}\), \( n \)-C\(_{31}\), \( n \)-C\(_{33}\)) are positively correlated with one another both in δD (Fig. 3c) and δδ\(^{13}C\) values (Fig. 3d). We calculated a weighted average δD (δD\(_{n-alkane} \)) and δδ\(^{13}C\) (δδ\(^{13}C_{n-alkane}\)) value for \( n \)-C\(_{29}\), \( n \)-C\(_{31}\), and \( n \)-C\(_{33}\) to compare the mean isotopic signal of long-chain \( n \)-alkanes between sites. Fig. 3d indicates differences in δδ\(^{13}C\) values among homologs, particularly between \( n \)-C\(_{29}\) and \( n \)-C\(_{31}\). Isotopic differences for specific compounds could be due to differences in plant sources for these homologs in sediment and soil samples and/or differences in biogenic isotopic fractionation between homologs. This variability supports the application of abundance-weighted mean average isotopic values to analyze the overall isotopic signature of leaf-waxes at a given site.

### 4. DISCUSSION

#### 4.1. Surface-water isotopic variability

Comparison of our surface-water measurements with other datasets (Lachniet and Patterson, 2009; Hodell et al., 2012) suggests broad agreement (Fig. 4). Our stream
and groundwater sample values, however, tend to be offset toward higher $\delta^{18}$O and higher deuterium excess ($d$) values than samples from the other datasets, suggesting that surface-waters sampled for this study generally show a stronger evaporative influence than samples from other studies. This difference could be due in part to the season when sampling was conducted. Sampling campaigns in Guatemala and Honduras in May and June 2009 occurred before the start of the wet season. The sampling campaign in Guatemala and Mexico during August 2008, however, occurred during the wet season. The Guatemalan samples of Lachniet and Patterson (2009) were collected in mid to late June 2007, after the onset of the wet season. The water data of Hodell et al. (2012), were primarily collected in August 2002, also during the wet season. Note that samples collected by Lachniet and Patterson (2009) from Belize in February 2008, during the dry season, generally plot closer to measurements from this study (Fig. 4), suggesting that seasonality affects the isotopic composition of surface-water samples. The suggested evaporative effects on our measured isotopic values support the use of additional datasets to derive accurate estimates of the isotopic composition of precipitation (Section 2.4).

The isotopic composition of lake-water in this region appears to be largely dependent on lake hydrology (Fig. 2b). The isotopic composition of hydrologically open lakes is generally similar to the isotopic composition of nearby streams or groundwater, whereas the isotopic composition of hydrologically closed lakes displays a range of values that are influenced by the relationship between water input, evaporation, and interactions with groundwater (Henderson and Shuman, 2009). Lake-water isotopic composition has been used as a proxy for aridity in other studies of $\delta D_{\text{wax}}$ variability (Polissar and Freeman, 2010), but because of the highly variable lake-water isotopic values in areas with similar climates (Fig. 2b), it is not a suitable proxy for spatial variability in aridity in southeastern Mexico and northern Central America. However, this does not negate the value of $\delta^{18}$O values from a single lake as an indicator of temporal climate variability.

4.2. Environmental controls on $\delta D_{\text{wax}}$

4.2.1. Relationship between $\delta D_{w}$ and $\delta D_{\text{wax}}$

Estimated $\delta D_{w}$ values range from $-24^{\circ}/o$ to $-49^{\circ}/o$, whereas $\delta D_{\text{wax}}$ values exhibit larger variability, from $-111^{\circ}/o$ to $-170^{\circ}/o$, suggesting that factors other than the isotopic composition of meteoric water exert important influences on $\delta D_{\text{wax}}$ in this region. Indeed, $\delta D_{w}$ and $\delta D_{\text{wax}}$ show no relationship across the study area (Fig. 5), unlike most previous studies of $\delta D_{\text{wax}}$ variability in sediments and plant extracts (Sachse et al., 2004, 2006; Smith and Freeman, 2006; Hou et al., 2008; Liu and Yang, 2008; Rao et al., 2009; Feakins and Sessions, 2010; Polissar and Freeman, 2010). This relationship is most likely not apparent in this study because $\delta D_{w}$ exhibits almost no variance (range = $25^{\circ}/o$, with most sites varying by only $9^{\circ}/o$) compared with a much wider range of $\delta D_{w}$ values in most other studies. For this reason this study provides a valuable opportunity to assess environmental variables other than $\delta D_{w}$, such as aridity and vegetation composition, that influence $\delta D_{\text{wax}}$ values.

4.2.2. $\varepsilon_{\text{wax/w}}$: Relationships with climate variables

$\varepsilon_{\text{wax/w}}$ for $n$-alkanoic acids ($\varepsilon_{n\text{-alkanoic/w}}$) ranges from $-71^{\circ}/o$ (Yojoa-soil) to $-131^{\circ}/o$ (Izabal Rio Oscuru-lake/Comayagua-soil), while $\varepsilon_{\text{wax/w}}$ for $n$-alkanes ($\varepsilon_{n\text{-alkane/w}}$) ranges from $-84^{\circ}/o$ (Chumayel-lake) to $-140^{\circ}/o$ (Peten Itza-lake). $\varepsilon_{\text{wax/w}}$ values for all sites are reported in Electronic Annex EA-7. This regional range in $\varepsilon_{\text{wax/w}}$ values ($56-60^{\circ}/o$) is larger than previously reported in most other studies, and reflects the broad range of $\delta D_{\text{wax}}$ values and limited variability in $\delta D_{w}$. However, the $\varepsilon_{\text{wax/w}}$ values reported here are well within the range of values reported from previous analyses of individual plant extracts, which vary from $-41^{\circ}/o$ for Larrea tridentata from arid regions of California (Feakins and Sessions, 2010) to $<-200^{\circ}/o$ for C3 grasses from the US Great Plains (Smith and Freeman, 2006; McInerney et al., 2011).

For the dataset as a whole there is a weak, but significant relationship between AI and both $\varepsilon_{n\text{-acid/w}}$ and $\varepsilon_{n\text{-alkane/w}}$ (Fig. 6). There is a similar negative relationship between MAP and both $\varepsilon_{n\text{-acid/w}}$ and $\varepsilon_{n\text{-alkane/w}}$, and a positive relationship between PET and $\varepsilon_{n\text{-acid/w}}$ and $\varepsilon_{n\text{-alkane/w}}$ (Electronic Annex EA-8). These relationships suggest that variability in aridity, due to both precipitation amount and potential evapotranspiration, partly explains the variability in $\varepsilon_{\text{wax/w}}$ across the study area. The large amount of scatter in these relationships, however, indicates that other variables also influence $\varepsilon_{\text{wax/w}}$.

In addition, we analyzed correlations between $\delta D_{\text{wax}}$ values and climate variables (Electronic Annex EA-8). In general, the same relationships described for $\varepsilon_{\text{wax/w}}$ values

![Fig. 4. Comparison of $\delta^{18}$O and $d$ for river waters from different studies in southeastern Mexico and northern Central America. Data from this study tend to be shifted towards higher $\delta^{18}$O relative to other studies, possibly due to seasonal influences on river water isotopic composition. Overall the data are relatively evenly distributed around the Global Meteoric Water Line (black line).]
occur with $\delta_{D_{\text{wax}}}$ values and the coefficient of determination is in many cases stronger. However, because variability in $\delta_{D_{\text{w}}}$ is not constrained when analyzing $\delta_{D_{\text{wax}}}$ values we focus our interpretation on comparisons with of climate variables with $\varepsilon_{\text{wax/w}}$.

We also analyzed a subset of the data, including only sites from the moist and dry tropical broadleaf forest biomes to evaluate how $\varepsilon_{\text{wax/w}}$ varies in tropical forests dominated by angiosperms. For these data, there is a significant correlation between climate variables (AI,
MAP, PET) and both $e_{\text{en}-\text{alkane/w}}$ and $e_{\text{en}-\text{acid/w}}$, that is similar but stronger than the relationships observed in the dataset as a whole (Electronic Annex EA-8). Polissar and Freeman (2010), using data from five sites, argued that aridity does not affect $e_{\text{en}-\text{alkane/w}}$ in tropical angiosperm forests. Our results, however, suggest that large variability in aridity can lead to sizeable variability in $e_{\text{wax/w}}$ (30–50%) in tropical angiosperm forest environments.

The observed correlation between aridity and $e_{\text{wax/w}}$ is likely caused by D-enrichment of plant-water relative to

![Fig. 6. AI vs. (a) $e_{\text{en}-\text{acid/w}}$; (b) $e_{\text{en}-\text{alkane/w}}$; (c) $e_{\text{en}-\text{C28/w}}$; (d) $e_{\text{en}-\text{C29/w}}$; (e) $e_{\text{en}-\text{C30/w}}$; and (f) $e_{\text{en}-\text{C31/w}}$ for all samples disaggregated by sample type. Black lines indicate regression lines for the entire dataset. Solid regression lines indicate correlation at the level of $p < 0.05$, while dashed regression lines indicate correlation at the level of $p < 0.1$. AI is correlated with $e_{\text{wax/w}}$ for the entire dataset and for lake sediment samples; in addition there is a significant correlation between AI and $e_{\text{en}-\text{alkane/w}}$, $e_{\text{en}-\text{C29/w}}$, and $e_{\text{en}-\text{C31/w}}$ for soil samples. Unfilled circles indicate lakes dominated by emergent aquatic plants (EAP lakes). EAP lakes are not included in plotted regression lines (see Section 4.2.3.3). $e_{\text{wax/w}}$ error bars indicate compounded errors for $\delta D_w$ and $\delta D_{\text{wax}}$ values.]
precipitation that is due to a combination of soil–water evaporation and plant-water transpiration (Smith and Freeman, 2006; Pedentchouk et al., 2008; Polissar and Freeman, 2010; Feakins and Sessions, 2010; McInerney et al., 2011; Sachse et al., 2012). A number of previous studies have observed variability in \( \Delta^{13}C/\Delta^{18}O \) related to climate, although there is contrasting evidence as to whether transpiration (Sachse et al., 2010; Feakins and Sessions, 2010) or soil–water evaporation (McInerney et al., 2011) are more important in determining \( \Delta^{13}C/\Delta^{18}O \) values. In this study we cannot resolve the mechanism causing the observed relationship between aridity and \( \Delta^{13}C/\Delta^{18}O \) since lake sediment and soil samples confute soil evaporation and transpiration processes.

Two aspects of this study, though, could prove important in understanding mechanisms for \( \Delta^{13}C/\Delta^{18}O \) variability. First, relative humidity and temperature are the primary climatic drivers for leaf-water enrichment in D and \( ^{18}O \) (Kahmen et al., 2008, 2011a). MARH is relatively invariant across the study area (72.9–82.1%; Electronic Annex EA-1), and is not significantly correlated with \( \Delta^{13}C/\Delta^{18}O \) (Electronic Annex EA-8). We have not explored temperature as an explanatory variable, but mean annual temperature (MAT) is also relatively invariant across the study area and is significantly correlated with \( \Delta^{13}C/\Delta^{18}O \) (Electronic Annex EA-8). We have not explored temperature as an explanatory variable, but mean annual temperature (MAT) is also relatively invariant across the study area, ranging from 21 to 27 °C (New et al., 2002). Most of the study sites are at low elevations (below 500 masl) where MAT varies between 24 and 27 °C (New et al., 2002). The large degree of variability in \( \Delta^{13}C/\Delta^{18}O \) despite minimal variability in MARH or MAT suggests that either (1) plant-water transpiration is not a strong driver of \( \Delta^{13}C/\Delta^{18}O \) variability in this region or (2) that seasonal, diurnal or microclimate scale variability in relative humidity or temperature that are not resolved in this study have a significant effect on leaf-water isotopic enrichment and \( \Delta^{13}C/\Delta^{18}O \). In contrast, MAP, and to a lesser extent PET, vary widely across the study area and are significantly correlated with \( \Delta^{13}C/\Delta^{18}O \) (Electronic Annex EA-8). These variables do not have a direct effect on leaf-water isotopic composition (Kahmen et al., 2008, 2011a), but can directly influence soil–water isotopic composition through soil–water recharge and evaporation (Hsieh et al., 1998; Smith and Freeman, 2006).

Second, much of the study area, particularly in Mexico and Guatemala, is a limestone platform overlying a large karstic aquifer (Bauer-Gottwein et al., 2011). In some areas the depth to the water table is over 100 m (Bauer-Gottwein et al., 2011). Existing ecohydrological data from the Yucatan Peninsula suggests that trees in this region do not typically access aquifer water, and instead rely on soil–water reservoirs that are enriched in \( ^{18}O \) and D due to evaporation, especially during the dry season (Querejeta et al., 2007). Plant reliance on soil water has also been observed in some other seasonally dry subtropical ecosystems (Ish-Shalom et al., 1992; Goldsmith et al., 2011). These studies suggest that soil water is a primary water source for many plants in the area, and that soil–water evaporation likely influences the isotopic composition of plant-water and leaf-waxes. This situation is in contrast with results from southern California, for instance, where desert-adapted plants often access groundwater, and therefore are not strongly influenced by soil–water evaporation (Feakins and Sessions, 2010). The correlation between \( \Delta^{13}C/\Delta^{18}O \) and climatic variables (AI, MAP, PET) is possibly consistent with a soil–water evaporation influence on \( \Delta^{13}C/\Delta^{18}O \). However, more research that directly compares plant-water and leaf-wax isotopic values in a range of environments is needed to better resolve the mechanisms that control \( \Delta^{13}C/\Delta^{18}O \) variability.

Some studies have suggested that the dependence of \( \Delta^{13}C/\Delta^{18}O \) on aridity could exhibit a threshold effect, with distinct differences between arid and humid climates, but with little variability across climatic gradients within arid and humid regions (Feakins and Sessions, 2010; Sachse et al., 2012). Our results suggest that the dependence of \( \Delta^{13}C/\Delta^{18}O \) on aridity is linear within our study area (Fig. 6). The range of \( \Delta^{13}C/\Delta^{18}O \) values we observe span the range of values reported from arid (\( \sim -100^{\circ}/_{\circ} \), Hou et al., 2008; Polissar and Freeman, 2010) and humid (\( \sim -130^{\circ}/_{\circ} \) to \( -140^{\circ}/_{\circ} \), Sachse et al., 2004; Polissar and Freeman, 2010; Garcin et al., 2012) environments, although some studies of plant extracts report \( \Delta^{13}C/\Delta^{18}O \) Values that are more positive or negative than observed in this study (Chikaraisi and Naraoka, 2003; Smith and Freeman, 2006; Feakins and Sessions, 2010; McInerney et al., 2011). Our results suggest that the dependence of \( \Delta^{13}C/\Delta^{18}O \) on aridity does not exhibit a threshold effect in climates with AI values between 0.5 and 2, but it is possible that aridity influenced variability in \( \Delta^{13}C/\Delta^{18}O \) is lessened in climates that are either more or less arid.

4.2.2.1. Seasonality of leaf-wax production. Southeastern Mexico and northern Central America have highly seasonal climates with pronounced wet and dry seasons, and it is likely that this seasonality has an effect on \( \Delta^{13}C/\Delta^{18}O \) values. Currently there is little consensus on the seasonality of leaf-wax production. Some studies have indicated leaf-wax synthesis predominantly occurs at the time of leaf formation (Sachse et al., 2010; Kahmen et al., 2011b), while others have shown evidence that leaf-wax isotopic compositions change throughout the growth season (Pedentchouk et al., 2008; Sachse et al., 2009). Further, no studies on the seasonality of leaf-wax production have been carried out in tropical deciduous or evergreen plants, which could differ markedly from the temperate taxa that have been studied.

Forests in our study area include both deciduous and evergreen tree species, with the proportion of deciduous taxa generally increasing with increasing aridity (Murphy and Lugo, 1986). There is a large amount of variability in the phenology of deciduous tree taxa in southern Mexico and Central America. Leaf flushing can occur both before and after onset of the wet season, while the timing of leaf fall varies widely across the dry season (Rivera et al., 2002; Valdez-Hernandez et al., 2010). In addition there is considerable interannual variability in phenology for a given taxa, driven in part by climatic variability (Murphy and Lugo, 1986; Valdez-Hernandez et al., 2010). It is possible that most leaf-waxes in this study area are produced during the wet season, but since some tree species form and/or retain their leaves during the dry season it is also possible that a significant proportion of leaf-waxes are
produced during the dry season. Due to this uncertainty we have focused on comparisons of δDwax with annual climatic data, but further study of the role of seasonality in leaf-wax isotopes in this and other tropical environments is warranted.

4.2.2.2. Comparison of Δ13Cwax and Δn13Cwax variability in lake sediment and soil samples. We disaggregated the data by sample type to more thoroughly examine relationships between climatic variables and Δ13Cwax. Lake sediments potentially provide a clearer signal of climate effects on Δ13Cwax than do soils, because they typically integrate leaf-waxes from a larger catchment area (Sachse et al., 2006; Feakins and Sessions, 2010), effectively averaging out variability in Δ13Cwax caused by microclimates or differences between plant communities. In addition, surficial lake sediment samples may represent a greater amount of time than soil samples and could average interannual climate variability to a greater degree. Indeed, the negative correlations between AI and Δ13Cwax are stronger for lake sediments than for the dataset as a whole (Fig. 6). Conversely, the correlation between AI and Δn13Cwax is weaker for soil samples relative to the whole dataset, and there is no significant correlation between AI and Δn13Cwax for soil samples (Fig. 6). The soil sample dataset includes sites from the tropical coniferous forest biome, whereas the lake sediment sample dataset does not. Because gymnosperms have been shown to typically have lower Δ13Cwax values than angiosperms (Chikaraishi and Naraoka, 2003; Chikaraishi et al., 2004; Pedentchouk et al., 2008) this difference could partly lead to the larger degree of scatter in the soil Δ13Cwax data. However, analysis of soil samples excluding the coniferous forest biome does not indicate an enhanced correlation between Δ13Cwax and climate variables relative to all soil samples (Electronic Annex EA-8). These results provide indirect evidence that there is larger variability in Δ13Cwax for soil samples relative to lake sediment samples because of the smaller catchment area and smaller amount of time averaging for soils.

4.2.3. Δ13Cwax: Influence of vegetation

4.2.3.1. Relationships between Δ13Cwax and Δn13Cwax. Although variability in AI accounts for some of the observed variability in Δn13Cwax, there is a notable amount of scatter in the relationships between AI and both Δn13Cwax and Δn13Calkane (Fig. 6), suggesting that other factors also influence Δn13Cwax. Plant physiology has been shown to significantly influence Δn13Cwax in angiosperms, with important differences between C3 grasses (lowest Δn13Cwax values), C4 grasses (intermediate Δn13Cwax values), and C3 angiosperm trees and shrubs (highest Δn13Cwax values) (Chikaraishi and Naraoka, 2003; Chikaraishi et al., 2004; Liu et al., 2006; Smith and Freeman, 2006; McInerney et al., 2011; Sachse et al., 2012). In lowland southeastern Mexico and northern Central America C3 angiosperm trees are typically dominant, but grasses are prevalent in many areas, and modern anthropogenic deforestation has greatly increased the proportion of grasses in some locations (Kok and Veldkamp, 2001). Gymnosperms are not typically present in lowland ecosystems, but are prevalent and can be dominant above 500 masl (Wagner, 1964; Farjon, 1996). Leaf-wax carbon isotope values (δ13Cwax) in part reflect the specific photosynthetic pathway of the source plant (Collister et al., 1994; Chikaraishi and Naraoka, 2003; Chikaraishi et al., 2004). In lowland ecosystems of southeastern Mexico and northern Central America, most grasses use the C4 photosynthetic pathway, while most shrubs and trees are C3 plants (Chazdon, 1978; Sage et al., 1999). In this study, δ13Cwax values from lake sediments and soils provide an approximate indication of the relative contribution of leaf-waxes from C4 grasses versus C3 trees and shrubs at a given site. Based on end-member values for C3 and C4 leaf-waxes (Collister et al., 1994; C3 = −36‰, C4 = −21‰, the range of δ13Cwax values from sediment and soil samples indicates that the proportion of leaf-waxes derived from C3 plants ranges between 100 ± 18% to 40 ± 24%. Caution is necessary in interpreting δ13Cwax as an indicator of plant type because there is a wide range of δ13Cwax values within both C3 and C4 plants, but soil and sediment samples at the upper end of the range of δ13Cwax values clearly include a C4 plant component.

Since C3 trees/shrubs and C4 grasses have different Δn13Cwax values under the same climate conditions (Chikaraishi and Naraoka, 2003; Chikaraishi et al., 2004), the wide range of observed δ13Cwax values is expected to correspond to variability in Δn13Cwax. For the purposes of analyzing vegetation effects on Δn13Cwax, we primarily focus on sites from biomes dominated by angiosperms (moist and dry tropical broadleaf forests). Gymnosperms can differ from angiosperms in both δ13Cwax and Δn13Cwax, with values that tend to be intermediate between angiosperm trees and shrubs (low δ13Cwax and high Δn13Cwax) and C4 grasses (high δ13Cwax and low Δn13Cwax) (Chikaraishi and Naraoka, 2003; Chikaraishi et al., 2004). Focusing our analysis on sites without significant gymnosperms simplifies our comparison of δ13Cwax and Δn13Cwax values. However, further study involving such a comparison in ecosystems with significant numbers of gymnosperms would be valuable.

δ13Cwax and Δn13Cwax values are not significantly correlated for the dataset as a whole or in broadleaf forests, suggesting that the composition of vegetation is not a primary control on Δn13Cwax in soils and lake sediments in this region. Because climate variability across the region could mask the effects of vegetation, we disaggregated the data to the level of eco-regions (Olson et al., 2001), which are relatively homogenous in terms of climate. Within three ecoregions (Central American Atlantic moist forests, Peten moist forests, Yucatan dry forests), we observe a negative correlation between δ13Cn13Cn13Cwax and Δn13Cwax (Fig. 7a), although this relationship is only statistically significant at the level of p ≤ 0.05 for the Peten moist forests and Yucatan dry forests. As a consequence of the small number of samples within specific ecoregions, determining an unequivocal relationship between these variables will require further testing. Samples from these three eco-regions fall into distinct δ13Cn13Cwax-Δn13Cwax fields, with the highest Δn13Cwax values for a given value of δ13Cn13Cwax in the Yucatan dry forests (most arid) and the lowest Δn13Cwax values for a given value of δ13Cn13Cwax in the Central American Atlantic moist forests (least arid) (Fig. 7a). This pattern suggests that for a given plant group, variability in Δn13Cwax is largely controlled by aridity, and by
Fig. 7. (a) $\delta^{13}$C$_{\text{n-accid}}$ vs. $\epsilon$$_{\text{n-accid/w}}$; (b) $\delta^{13}$C$_{\text{n-alkane}}$ vs. $\epsilon$$_{\text{n-alkane/w}}$; (c) $\delta^{13}$C$_{\text{n-C28}}$ vs. $\epsilon$$_{\text{n-C28/w}}$; (d) $\delta^{13}$C$_{\text{n-C29}}$ vs. $\epsilon$$_{\text{n-C29/w}}$; and (e) $\delta^{13}$C$_{\text{n-C30}}$ vs. $\epsilon$$_{\text{n-C30/w}}$ for samples from broadleaf forest ecosystems disaggregated by ecoregion. Solid regression lines indicate correlation at the level of $p < 0.05$, while dashed regression lines indicate correlation at the level of $p < 0.1$. A negative correlation between $\delta^{13}$C$_{\text{wax}}$ and $\epsilon$$_{\text{wax/w}}$ is apparent for n-alkanoic acids in some ecoregions, but is not apparent for n-alkanes. Black arrows indicate the general trend of increasing aridity. Unfilled markers indicate EAP lakes, which generally have low $\delta^{13}$C$_{\text{wax}}$ and $\epsilon$$_{\text{wax/w}}$ relative to other samples from their ecoregion, particularly in n-alkanoic acids. EAP lakes are not included in plotted regression lines (see Section 4.2.3.3). $\epsilon$$_{\text{wax/w}}$ error bars reflect compounded errors in $\delta$D$_{\text{wax}}$ and $\delta$D$_{\text{wax}}$. $\delta^{13}$C$_{\text{wax}}$ error bars indicate the pooled precision of external isotopic standards (0.5‰).
corollary, variability in $\varepsilon_{n\text{-acid}}$/w is largely controlled by differences between plant groups within a given climate. Samples from the Central American dry forests ecoregion exhibit greater scatter in $d_{13}C$/w-$n$-acid/w space, which could be partly due to greater uncertainty in $d_{Dw}$ estimates for these sites. Samples from this ecoregion have a similar range in $d_{13}C$/w-$n$-acid/w space to samples from the Yucatan dry forests ecoregion, suggesting similarity.
between dry forest environments. Samples from the tropical coniferous forest biome (not plotted) span a much larger range in $\delta^{13}C_{\text{n-alkane}}$–$\delta^{13}C_{\text{n-alkane/w}}$ space, possibly as a consequence of inclusion of gymnosperm derived $n$-alkanoic acids, which may complicate $\delta^{13}C_{\text{n-alkane}}$–$\delta^{13}C_{\text{n-alkane/w}}$ relationships.

For $n$-alkane samples, relationships between $\delta^{13}C_{\text{n-alkane}}$ and $\delta^{13}C_{\text{n-alkane/w}}$ are less apparent. In Fig. 7b, ecoregions are divided primarily by $\delta^{13}C_{\text{n-alkane/w}}$ values, with little corresponding differences in $\delta^{13}C_{\text{n-alkane}}$. Two sites with high $\delta^{13}C_{\text{n-alkane}}$ values have relatively low $\delta^{13}C_{\text{n-alkane/w}}$ values compared to other samples from their ecoregion (La Barca, Peten-Izta). $n$-Alkane samples in this study typically have lower $\delta^{13}C$ values than $n$-alkanoic acids (Fig. 7a and b), possibly reflecting different plant sources. Specifically, lower $\delta^{13}C$ values in $n$-alkanes suggest that a greater proportion of $n$-alkanes than $n$-alkanoic acids are derived from C$_3$ trees and shrubs, possibly influencing observed $\delta^{13}C_{\text{n-alkane}}$–$\delta^{13}C_{\text{n-alkane/w}}$ relationships.

Variability in $\delta^{13}C_{\text{wax/w}}$ between plant types (Chikaraishi and Naraoka, 2003; Chikaraishi et al., 2004; Smith and Freeman, 2006; Pedentchouk et al., 2008) and ecosystems (Krull et al., 2006; Polissar and Freeman, 2010) has been previously evaluated, but few studies have examined relationships between $\delta^{13}C_{\text{wax}}$ and $\delta^{13}C_{\text{wax/w}}$ in modern sediments, soils, or leaves. Hou et al. (2007) compared $\delta^{13}C$ and $\delta^{18}D$ values for individual $n$-alkanoic acid homologs from leaves at one site in Massachusetts and found a negative correlation. This mid-latitude setting, all samples derive from C$_3$ plants based on the end-members of Collister et al. (1994), and were chosen because they evenly divide the range of $\delta^{13}C_{\text{wax}}$ values. As discussed above (Section 4.2.3.1), identification of plant source using $\delta^{13}C_{\text{wax}}$ is associated with large uncertainty. A strong negative correlation between AI and $\delta^{13}C_{\text{n-alkane/w}}$ for samples with low $\delta^{13}C_{\text{n-alkane}}$ suggests that in ecosystems dominated by C$_3$ trees and shrubs $\delta^{13}C_{\text{n-alkane/w}}$ is largely controlled by aridity (Fig. 8a). The negative correlation between AI and $\delta^{13}C_{\text{n-alkane}}$ for samples with low $\delta^{13}C_{\text{n-alkane}}$ is somewhat weaker, but still significant (Fig. 8b). There is also a significant negative relationship between AI and $\delta^{13}C_{\text{n-alkane/w}}$ for samples with intermediate $\delta^{13}C_{\text{n-alkane}}$ with $\delta^{13}C_{\text{n-alkane/w}}$ values that are generally more negative than samples with low $\delta^{13}C_{\text{n-alkane}}$ (Fig. 8a). This suggests that for ecosystems with a greater proportion of C$_4$ grasses, aridity also influences $\delta^{13}C_{\text{n-alkane}}$, but that $\delta^{13}C_{\text{n-alkane/w}}$ values are lower relative to tree- and shrub-dominated ecosystems for a given climate. There is greater scatter for samples with intermediate $\delta^{13}C_{\text{n-alkane}}$, which most likely reflects that $\delta^{13}C_{\text{wax}}$ is an imperfect proxy for vegetation type since factors such as water-use efficiency and canopy effects can also influence plant $\delta^{13}C$ values (Diefendorf et al., 2010; Kohn, 2010).

For this reason, some samples with intermediate $\delta^{13}C_{\text{wax}}$ could be predominantly derived from C$_3$ trees and shrubs. The small number of samples with intermediate $\delta^{13}C_{\text{n-alkane}}$, high $\delta^{13}C_{\text{n-alkane}}$, and high $\delta^{13}C_{\text{n-alkane}}$ make it difficult to assess statistical relationships between aridity and $\delta^{13}C_{\text{wax/w}}$ within these groups. These samples tend to have relatively low $\delta^{13}C_{\text{wax/w}}$ values for a given level of aridity, but also exhibit a large degree of scatter. As with the dataset as a whole, MAP and PET are also significantly correlated with $\delta^{13}C_{\text{wax/w}}$ for the low $\delta^{13}C_{\text{n-alkane}}$, low $\delta^{13}C_{\text{n-alkane}}$, and intermediate $\delta^{13}C_{\text{n-alkane}}$ groups (Electronic Annex EA-8).

4.2.3.3. Leaf-wax distributions and isotopic signatures in lakes dominated by emergent aquatic plants. Emergent aquatic plants dominate the shoreline of four of the sampled lakes (EAP lakes: Xoché, Yaalchak, La Corona, Cuerpo Dos). Emergent aquatic plants often contain abundant long-chain $n$-alkyl lipids (Ficken et al., 2000), but, because of their aquatic habitat, controls on $\delta^{13}C_{\text{wax}}$ and $\delta^{13}C_{\text{wax}}$ values for these plants are likely different from those for terrestrial vegetation (Shuman et al., 2006; Mugler et al., 2008). This has been observed in a previous study from the midwestern USA, in which sediments from lakes with abundant emergent aquatic plants had lower $\delta^{13}C_{\text{wax}}$ values than other nearby lakes (Shuman et al., 2006).

One technique for identifying aquatic plant contributions to sedimentary leaf-waxes is to analyze their chain-length distributions. Although previous studies found distinct differences in leaf-wax chain-length distribution between submerged and floating aquatic plants and terrestrial vegetation (Ficken et al., 2000; Gao et al., 2011), differences between emergent aquatic and terrestrial plants are less clearly defined (Ficken et al., 2000). Our analyses of emergent aquatic plant extracts indicate some characteristic chain-length distributions that could potentially be useful in identifying leaf-wax input from these plants.

Overall, emergent aquatic plants exhibit widely varying $n$-alkanoic acid chain-length distributions (Fig. 9). Samples of Cladium jamaicense, an aquatic sedge that is dominant at three of the four EAP lakes, contain a greater proportion of the longest chain $n$-alkanoic acids (C$_{32}$ and C$_{34}$ homologs) than leaf samples from terrestrial angiosperms (Fig. 9). EAP lake sediments have a slightly greater abundance of longer-chain length $n$-alkanoic acids than other lakes (Fig. 9), with a modal chain-length of 28 as opposed to 26 carbon atoms.
Emergent aquatic plant samples have \( n \)-alkane distributions that are shifted to longer-chain length homologs relative to terrestrial angiosperms, with a greater proportion of \( n \)-C31 than \( n \)-C29 alkanes in most samples (Fig. 9). *Cladium* samples, in particular, contain a much greater amount of \( n \)-C33 alkanes than other plant extracts (Fig. 9). EAP lake sediments also have \( n \)-alkane distributions that are shifted to longer-chain length homologs relative to other lakes (Fig. 9), with a modal chain-length of 31 as opposed to 29 carbon atoms. All plant extract and lake sediment compound distribution data are shown in *Electronic Annex EA-9*.

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Lakes dominated by emergent aquatic plants (EAP) also exhibit unique patterns in terms of \( \delta \mathrm{D}_{\text{wax}} \) and \( \delta^{13} \mathrm{C}_{\text{wax}} \) values. \( \delta \mathrm{n}_{\text{acid/w}} \) values for three out of four EAP lakes are relatively low, although not outside the range of \( \delta \mathrm{n}_{\text{acid/w}} \) values for other lakes (Fig. 6a). EAP lakes also have relatively low \( \delta^{13} \mathrm{C}_{\text{n-alkane}} \) values (\(-32.9_{\%} \) to \(-35.2_{\%}\)). In \( \delta \mathrm{n}_{\text{acid/w}}-\delta^{13} \mathrm{C}_{\text{n-alkane}} \) space EAP lakes lie outside the range of other samples from their respective ecoregion, with lower
values for both variables (Fig. 7a). Similarly, when samples are subdivided by $\delta^{13}C_{\text{n-alkane}}$ values, three out of four EAP lakes have lower $\delta^{13}C_{\text{n-alkane}}$ than other samples with low $\delta^{13}C_{\text{wax/w}}$ values from the same climate (Fig. 8a).

EAP lakes from the Peten moist forests ecoregion have similar $\delta^{13}C_{\text{n-alkane}}$ values to other lakes from this region, while EAP lakes from the more arid Yucatan dry forests ecoregion have distinctively low $\delta^{13}C_{\text{n-alkane}}$ values (Figs. 6b and 7b). One of the EAP lakes from the Yucatan dry forests, L. Yaachalch, has the lowest $\delta^{13}C_{\text{n-alkane}}$ value of the dataset (−146‰). This lake also has a unique n-alkane chain-length distribution, with a modal chain length of 33 carbons (Electronic Annex EA-9). This suggests a large input of n-alkanes from Cladium, which is dominant along the shoreline of this lake. Three out of four EAP lakes have among the lowest $\delta^{13}C_{\text{n-alkane}}$ values (−35.8‰ to −37‰) for their respective ecoregion, while L. Yaachalch has a relatively high $\delta^{13}C_{\text{n-alkane}}$ value (−32.4‰) (Fig. 7b).

Broadly, EAP lakes tend to have low $\delta^{13}C_{\text{wax/w}}$ and $\delta^{13}C_{\text{wax}}$ values, although these patterns are not consistent across all EAP lakes. Low $\delta^{13}C_{\text{wax}}$ values are consistent with a growth environment with unlimited water, since aquatic plants would have less need to close their stomata to reduce water loss, and would tend to more strongly fractionate stomatal CO$_2$ during photosynthesis. Similarly, the absence of soil–water evaporation effects would potentially lead to low $\delta^{13}C_{\text{wax/w}}$ values. However, two of the four EAP lakes (Yaachalch and La Corona) have positive δD and δ18O values for lake-water (Electronic Annex EA-2), indicating major evaporative isotopic enrichment. The relatively low $\delta^{13}C_{\text{wax/w}}$ values for these lakes possibly suggest that the evaporation of lake-water does not strongly affect the isotopic composition of emergent aquatic plant leaf-waxes at these sites, perhaps because these plants are able to access less evaporated groundwater feeding the lakes.

The correlation between $\delta^{13}C_{\text{n-alkane}}$ and climatic variables (AI, MAP, PET) persists when EAP lakes are included in regression analyses, although the $R^2$ value is reduced (Electronic Annex EA-8). When EAP lakes are included there is no significant relationship between $\delta^{13}C_{\text{n-alkane}}$ and $\delta^{13}C_{\text{wax/w}}$ in the Peten Moist Forest and Yucatan Dry Forest ecoregions since the EAP lakes have lower values for $\delta^{13}C_{\text{n-alkane}}$ and $\delta^{13}C_{\text{wax/w}}$ than the other samples from these regions (Fig. 7a). Similarly, when EAP lakes are included there is no significant correlation between $\delta^{13}C_{\text{n-alkane}}$ and climatic variables (AI, MAP, PET) for samples with low $\delta^{13}C_{\text{wax/w}}$ values, since the EAP lake samples generally have lower $\delta^{13}C_{\text{n-alkane}}$ and plot more closely with samples with intermediate $\delta^{13}C_{\text{n-alkane}}$ values (Fig. 8a).

The correlation between $\delta^{13}C_{\text{n-alkane}}$ and climatic variables (AI, MAP, PET) also persists when EAP lakes are included in regression analyses, although the $R^2$ value is reduced to a greater extent than for $\delta^{13}C_{\text{n-alkane}}$ (Electronic Annex EA-8). There is no significant correlation between $\delta^{13}C_{\text{n-alkane}}$ and climatic variables for lake sediment samples when EAP lakes are included. The stronger influence of EAP lakes on regression statistics for $\delta^{13}C_{\text{n-alkane}}$ as opposed to $\delta^{13}C_{\text{wax/w}}$ is probably primarily caused by L. Yaachalch, which has a very low $\delta^{13}C_{\text{n-alkane}}$ value despite its relatively arid climate (Figs. 6b and 8b).

Overall, the inclusion of EAP lakes in regression analyses does not negate the indication of an aridity effect on $\delta^{13}C_{\text{wax/w}}$ values in this study area, although these lakes in many cases deviate from the relationship between $\delta^{13}C_{\text{wax/w}}$ and climatic variables. We argue that the negative correlations between $\delta^{13}C_{\text{n-alkane}}$ and $\delta^{13}C_{\text{wax/w}}$ for specific ecoregions that are observed when EAP lakes are excluded are robust, since the EAP lakes have unique $\delta^{13}C_{\text{n-alkane}}$ and $\delta^{13}C_{\text{wax/w}}$ values for their respective ecoregions that likely reflect the input of emergent aquatic plant material (Fig. 7a). However, more study is needed to ascertain whether (1) a negative correlation between $\delta^{13}C_{\text{n-alkane}}$ and $\delta^{13}C_{\text{wax/w}}$ is observed across a wide range of environments and (2) whether relatively low $\delta^{13}C_{\text{n-alkane}}$ and $\delta^{13}C_{\text{wax/w}}$, along with leaf-wax distributions shifted to higher-chain length homologs, are consistent features of lakes dominated by emergent aquatic plants.

4.2.4. Isotopic patterns in individual leaf-wax homologs

Many paleoclimate studies focus on isotopic variability in individual leaf-wax homologs (e.g. Schefuss et al., 2005, 2011; Tierney et al., 2008; Niedermeyer et al., 2010), and it is important to consider whether isotopic signatures of individual homologs differ from the patterns observed in abundance-weighted mean values discussed above. We focus our analysis on the n-C$_{28}$ and n-C$_{30}$ n-alkanoic acids and n-C$_{29}$ and n-C$_{31}$ n-alkanes since these homologs are most often applied in paleoclimate studies.

Patterns in $\delta^{13}C_{\text{n-C$_{28}$/w}}$ and $\delta^{13}C_{\text{n-C$_{30}$/w}}$ are similar to those found for $\delta^{13}C_{\text{n-alkane/w}}$. The correlation between $\delta^{13}C_{\text{n-C$_{28}$/w}}$ and climatic variables (AI, MAP, PET) is stronger than that for $\delta^{13}C_{\text{n-alkane/w}}$, while $\delta^{13}C_{\text{n-C$_{28}$/w}}$ is more weakly correlated with climatic variables (Fig. 6c and e, Electronic Annex EA-8). Negative correlations in $\delta^{13}C_{\text{n-C$_{28}$/w}}$ and $\delta^{13}C_{\text{n-C$_{30}$/w}}$ space for specific ecoregions are also apparent (Fig. 7c and e), although this relationship is not statistically significant at the level of $p > 0.1$ for n-C$_{28}$ in the Yucatan Dry Forests and for n-C$_{30}$ in the Central American Atlantic Moist Forests. There is a negative relationship between $\delta^{13}C_{\text{n-C$_{28}$/w}}$ and climatic variables for samples with intermediate $\delta^{13}C_{\text{n-C$_{28}$/w}}$ values, but not for samples with low $\delta^{13}C_{\text{n-C$_{28}$/w}}$ values (Fig. 8c). Conversely, there is a negative relationship between $\delta^{13}C_{\text{n-C$_{30}$/w}}$ and climatic variables for samples with low $\delta^{13}C_{\text{n-C$_{30}$/w}}$ values, but not for samples with low $\delta^{13}C_{\text{n-C$_{30}$/w}}$ values (Fig. 8e). $\delta^{13}C_{\text{n-C$_{28}$/w}}$ values for EAP lakes are clearly more negative than other lakes from similar environments, while $\delta^{13}C_{\text{n-C$_{28}$/w}}$ values for EAP lakes are more variable (Figs. 7c, e and 8c, e).

A stronger aridity effect for $\delta^{13}C_{\text{n-C$_{28}$/w}}$ could be related to different plant sources for n-C$_{28}$ and n-C$_{30}$ alkanoic acids, or to differences in the timing or location of biosynthesis of these two homologs within a given plant. In our study $\delta^{13}C_{\text{n-C$_{28}$/w}}$ values are generally higher than $\delta^{13}C_{\text{n-C$_{30}$/w}}$ values from the same sample, possibly suggesting greater C$_4$ grass input to n-C$_{28}$ alkanoic acids. Further studies of tropical plant extracts are needed to better understand isotopic differences between these two homologs.

$\delta^{13}C_{\text{n-C$_{28}$/w}}$ and $\delta^{13}C_{\text{n-C$_{30}$/w}}$ exhibit correlations with climate variables that are similar in strength and slope to those for $\delta^{13}C_{\text{n-alkane/w}}$ (Figs. 6d, f and 8d, f). As with $\delta^{13}C_{\text{n-alkane/w}}$ there
is no apparent relationship between $\delta n$-$C_{29}$/w and $\delta n$-$C_{31}$/w, and the respective $n$-alkane $\delta^{13}$C value in specific ecoregions, with the exception of $\delta n$-$C_{31}$/w in the Peten moist forests ecoregion (Fig. 7d and f). $\delta n$-$C_{29}$/w values in EAP lakes are less divergent from other sites than $\delta n$-$C_{31}$/w values. In particular, for L. Yaalchak $\delta n$-$C_{29}$/w is within the range of other samples from the Yucatan Dry Forests ecoregion, but $\delta n$-$C_{31}$/w for this lake is the lowest of the dataset. This is consistent with emergent aquatic plants, and in particular Cladium, producing greater amounts of $n$-$C_{31}$ alkanes than $n$-$C_{29}$ alkanes (Fig. 9).

While there are some minor differences between individual homologs and abundance-weighted mean values, particularly for $n$-alkanoic acids, the overall patterns of environmental controls for $\delta n$-acid/w and $\delta n$-alkane/w are also apparent for individual leaf-wax homologs. The findings of this study are therefore relevant for paleoclimate studies of individual leaf-wax homologs as well as abundance-weighted mean isotopic values.

### 4.3. Comparison with previous studies of sedimentary $\delta D_{WAX}$

$\delta D_{WAX}$ values in this study agree broadly with previous analyses of $\delta D_{WAX}$ in modern lake sediments across climatic gradients (Fig. 10; Sachse et al., 2004; Hou et al., 2008; Polissar and Freeman, 2010; Garcin et al., 2012). Compared with other datasets of lake sediment $\delta D_{WAX}$, our results support a global-scale positive correlation between $\delta D_w$ and $\delta D_{WAX}$ (Fig. 10). However, our results differ from previous regional studies of $\delta D_{WAX}$ in modern lake sediments and soils in two ways: (1) we observe a larger range of $\delta WAX/w$ ($\sim 60^\circ$) than most previous studies; and (2) we observe a significant correlation between aridity and $\delta WAX/w$. The first difference is largely explained by the relatively small range in $\delta D_w$ (25$^\circ$) in this study, which is less than that observed in other studies (Fig. 10), combined with relatively large variability in aridity and vegetation (see Section 4.2.2). In this sense our results provide important constraints on environmental effects on $\delta D_{WAX}$ when $\delta D_w$ is relatively constant.

The second difference is most salient in comparison with the results of Hou et al. (2008; Southwestern USA) and Garcin et al. (2012; Cameroon, West Africa), both of whom analyzed $\delta D_{WAX}$ across large gradients in precipitation and relative humidity, but did not observe corresponding variability in $\delta WAX/w$. We suggest that this difference can be primarily ascribed to changing ecosystems across the Southwestern USA and Cameroon transects. Namely, in both transects there is a pronounced shift to grass-dominated ecosystems with increasing aridity (Hou et al., 2008; Garcin et al., 2012). Since grasses have been shown to have lower $\delta WAX/w$ values than trees (Chikaraishi and Naraoka, 2003; Chikaraishi et al., 2004; Smith and Freeman, 2006; McInerney et al., 2011; Sachse et al., 2012), it is likely that the shifts to grass-dominated ecosystems in previous transect studies counteract increases in $\delta WAX/w$ related to aridity (Hou et al., 2008; Garcin et al., 2012). In this study, however, tree-dominated ecosystems are prevalent in spite of an aridity gradient, and our results indicate that in this more botanically invariant setting $\delta WAX/w$ values do become more positive with increasing aridity (Figs. 6 and 8).

Garcin et al. (2012) found divergent hydrogen isotope patterns between the $n$-$C_{29}$ and $n$-$C_{31}$ homologs, with $\delta D_{n-C_{29}}$ correlating strongly with the estimated $\delta D$ of precipitation and $\delta D_{n-C_{31}}$ correlating strongly with the $\delta D$ of lake-water. The authors suggest that this difference is due to different plant sources for these homologs, and that this difference could potentially form the basis of a higher-plant based evaporation proxy. We do not observe a similar phenomenon in our data; $\delta D_{n-C_{29}}$ and $\delta D_{n-C_{31}}$ are strongly correlated for our study (Fig. 3c), and neither $\delta D_{n-C_{29}}$ nor $\delta D_{n-C_{31}}$ is correlated with the estimated $\delta D$ of precipitation (Fig. 5e and f) or the $\delta D$ of lake-water (in the case of lake sediment samples). This would suggest that the differences between $\delta D_{n-C_{29}}$ and $\delta D_{n-C_{31}}$ observed by Garcin et al.

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Fig. 10. $\delta D_w$ vs. (a) $\delta D_{n-C_{28}}$ alkanolic acid and (b) $\delta D_{n-C_{29}}$ alkane for lake sediment samples from this study and other published datasets (Sachse et al., 2004, 2012; Hou et al., 2008; Polissar and Freeman, 2010; Garcin et al., 2012). Regression lines include all plotted data. $\delta D_{WAX}$ values from this study generally agree with other datasets, but also exhibit a relatively broad range of $\delta D_{WAX}$ for a given value of $\delta D_w$. Error bars for data from this study indicate errors associated with estimating the isotopic composition of precipitation ($\delta D_w$) and the pooled precision of external isotopic standards ($\delta D_{WAX}$). Error bars for other datasets are as published.
In general, changes in $\delta D_{\text{wax}}$ in paleoclimate records are interpreted to primarily reflect changes in the isotopic composition of precipitation (Schefuss et al., 2005, 2011; Tierney et al., 2008; Niedermeyer et al., 2010). Our results, however, indicate that aridity and vegetation composition can strongly influence $\delta D_{\text{wax}}$ values in the tropics (Figs. 6–8). The observed relationship between aridity and $e_{\text{wax/w}}$ in this study indicates that temporal shifts in $\delta D_{\text{wax}}$ could indicate changes in the isotopic composition of precipitation, and/or changes in aridity. In tropical settings where the isotopic composition of precipitation is primarily controlled by the amount effect (Risi et al., 2008), increasing aridity will be reflected in higher $\delta D_{\text{wax}}$ values, both due to D-enrichment of precipitation and increased evaporative enrichment of soil/leaf-water. It is important to clarify that although spatial variability in $\delta D_{\text{wax}}$ is not strongly controlled by local precipitation amount in Central America, temporal variability in the isotope composition of precipitation is significantly correlated with local precipitation amount in this region (Lachniet and Patterson, 2009), as it is in many other tropical environments. Due to their sensitivity to both changes in $\delta D_{\text{wax}}$ and aridity, $\delta D_{\text{wax}}$ measurements can provide a sensitive qualitative indicator of hydroclimate change in tropical precipitation amount. Aridity effects on $\delta D_{\text{wax}}$, however, complicate inference of past precipitation isotope values.

To address this complication, (Polissar and Freeman, 2010) suggested that paired analyses of $\delta D_{\text{wax}}$ and $\delta^{18}O$ in lacustrine carbonates be used to establish an isotopic evaporation line, which, when extrapolated to the global meteoric water line, provides an estimate of the isotopic composition of past precipitation. Our results support the premise of this approach. It is important to note, however, that this technique hinges on the establishment of a leaf-water/lake-water evaporation slope for a given ecosystem, which could prove problematic in some areas. In our study area, for instance, both $e_{\text{wax/w}}$ and the isotopic composition of lake-water vary widely, and are influenced by the evaporation of soil and lake-water, respectively (Figs. 2b and 6), but these variables are not correlated. The absence of a leaf-water/lake-water evaporation slope in this setting is likely due to factors other than evaporation that influence the isotopic composition of leaf-waxes (vegetation composition) and lake-waters (input hydrology and basin geometry; Henderson and Shuman, 2009).

Changes in vegetation may lead to large shifts in $\delta D_{\text{wax}}$ that are independent of climate. In this dataset, variability in $\delta^{13}C_{\text{n-alkane/w}}$ values corresponds to differences in $e_{\text{n-alkane/w}}$ of up to 35% in sites with similar climates (Fig. 7a, c, and e). This effect is less evident for n-alkanes in this dataset, but differences in $\delta^{13}C$ values suggest that in this environment a smaller proportion of n-alkanes are derived from C4 grasses relative to n-alkanoic acids. Previous studies of plant extracts indicate there are important differences in $e_{\text{n-alkane/w}}$ between different plant groups (Chikaraishi and Naraoka, 2003; Smith and Freeman, 2006; Fekins and Sessions, 2010; McInerney et al., 2011). If vegetation change is not constrained, this effect could lead to large errors when interpreting $\delta D_{\text{wax}}$ values in a paleoclimate context.

$\delta^{13}C_{\text{wax}}$ records offer one approach to reconstructing changes in vegetation composition and thereby constraining vegetation effects on $\delta D_{\text{wax}}$. Our results indicate that in angiosperm-dominated ecosystems in southeastern Mexico and northern Central America, which include varying proportions of C3 trees and shrubs and C4 grasses, $\delta^{13}C_{\text{wax}}$ values provide a predictor of vegetation-controlled variability in $e_{\text{n-alkane/w}}$ (Figs. 7 and 8). Vegetation reconstructions in areas with a large proportion of gymnosperms and C3 grasses will likely prove more complicated, because these groups can differ from angiosperm trees and shrubs in $e_{\text{n-alkane/w}}$ (McInerney et al., 2011), but are not as distinct in $\delta^{13}C_{\text{wax}}$ as C3 and C4 plants.

It is possible that analysis of leaf-wax chain-length distributions in sediment samples could also be used to constrain leaf-wax plant sources, but this will require more investigation of taxonomic differences in chain-length distributions. Pollen records could potentially help to constrain vegetation-related shifts in $\delta D_{\text{wax}}$, because pollen can provide a taxonomically detailed indication of vegetation change. Differences in sources and transport mechanisms for pollen and leaf-waxes, however, could complicate such comparisons, particularly for high-resolution paleoclimate records. Finally, another technique that may prove valuable is hydrogen isotope analysis of taxon-specific plant biomarkers, such as dehydroabietane and $\beta$-amyrin (Schouten et al., 2007), which could potentially avoid the complication of multiple plant sources of leaf-waxes with differing $e_{\text{wax/w}}$ values. Overall, our results highlight the importance of constraining vegetation change for interpreting climate signals from $\delta D_{\text{wax}}$ records in the tropics, and demonstrate the value of $\delta^{13}C_{\text{wax}}$ measurements for this purpose in some tropical environments.

5. CONCLUSIONS

Isotopic compositions of n-alkanes and n-alkanoic acids from lake sediments and soils were evaluated across a steep aridity gradient in southeastern Mexico and northern Central America. $\delta D_{\text{wax}}$ does not correlate with the estimated isotopic composition of precipitation, but $e_{\text{wax/w}}$ is significantly correlated with climate variables related to aridity, namely AI, MAP and PET. (Figs. 6 and 8, Electronic Annex EA-8). This relationship suggests that aridity significantly influences the expression of $e_{\text{wax/w}}$ in some tropical environments, leading to variability on the order of 30–50%o. Since there is little variability in relative humidity in the study area, it appears that the observed aridity effects are primarily related to precipitation amount and potential evapotranspiration as opposed to atmospheric relative humidity.

Aridity effects are more evident in lake sediments than in soils, possibly due to the integration of leaf-waxes across a broad catchment, which masks small-scale variability in $e_{\text{wax/w}}$. From differences in vegetation and microclimates...


