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 ($\delta^{13}C_{wax}$), 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 ($\varepsilon_{wax/w}$). 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 $\varepsilon_{wax/w}$ that is a consequence of differences in vegetation and microclimates. In angiosperm-dominated environments, plant ecology, inferred from $\delta^{13}C_{wax}$, provides a secondary control on $\varepsilon_{wax/w}$ for n-alkanoic acids ($\varepsilon_{n\text{-}acid/w}$). Low $\delta^{13}C_{n\text{-}acid}$ values are associated with high $\varepsilon_{n\text{-}acid/w}$ values, most likely reflecting differences in biosynthetic hydrogen isotope fractionation between C_4 grasses and C_3 trees and shrubs. A similar relationship between $\delta^{13}C_{n\text{-}alkane}$ and $\varepsilon_{n\text{-}alkane/w}$ 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.

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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the tropics (Polissar and Freeman, 2010; Garcin et al., 2012).

δD_{wax} 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; McInerney et al., 2011). Studies of δD_{wax} variability in lacustrine sediments across mid-latitude transects indicate that the isotopic composition of meteoric waters is a primary determinant of δD_{wax} (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 δD_{wax} values (Hou et al., 2008). Conversely, grass extracts from the Great Plains show that δD_{wax} 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 (McInerney et al., 2011). However, direct measurements of plant-water δD values for trees and grasses indicate that isotopic enrichment of leaf-water due to transpiration does have a significant impact on δD_{wax} values (Feakins and Sessions, 2010; Sachse et al., 2010).

There are differences in apparent hydrogen isotope fractionation between meteoric water and leaf-waxes ($\varepsilon_{wax/w}$) for different plant groups grown under similar conditions. Research to date indicates that δD_{wax} values are lower in C_4 grasses than in C_3 trees and shrubs by ~20‰ on average (Chikaraishi and Naraoka, 2003; Chikaraishi et al., 2004; Sachse et al., 2012). Furthermore, leaf-waxes in C_3 grasses

are D-depleted by ~15‰ relative to C_4 grasses (Smith and Freeman, 2006; McInerney et al., 2011; Sachse et al., 2012). δD_{wax} 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 δD_{wax} 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 δD_{wax} values have focused on mid-latitude settings (Sachse et al., 2004; Hou et al., 2008). Polissar and Freeman (2010) analyzed δD_{wax} 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 δD_{wax} from tropical soils as part of a larger study across eastern China, but did not focus on δD_{wax} 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 δD_{wax} values in this tropical location, similar to results from mid-latitude settings.

For this study, we measured δD_{wax} in lake sediments and soils across southeastern Mexico and northern Central America (Fig. 1) to evaluate the impact of aridity on δD_{wax} in tropical environments. We also measured the carbon isotope composition of leaf-waxes ($\delta^{13}C_{wax}$) 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 (δD_w) ranges from −24‰ to −49‰, with δD_w at most sites
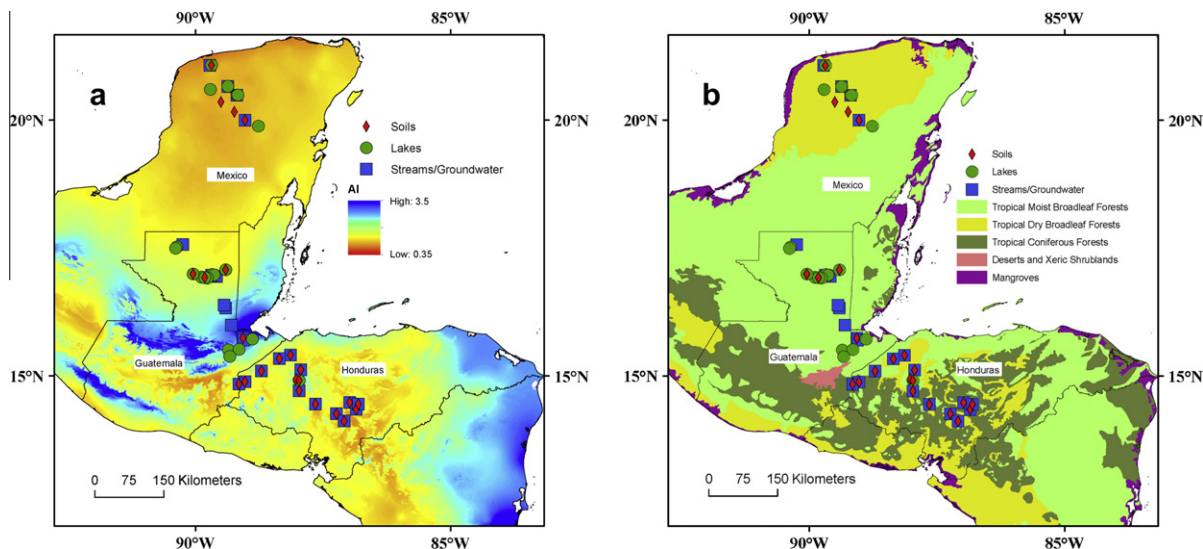

Fig. 1. Map of southeastern Mexico and northern Central America showing sampling locations with (a) aridity index values (data from New et al., 2002 and Trabucco and Zomer, 2009); and (b) biome distributions (from Olson et al., 2001).

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 δD_{wax}. 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 $\delta^{13}C_{wax}$ values, provide an opportunity to assess the effects of differing vegetation on δD_{wax} 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 δD_{wax} 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 forest 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 $\delta^{13}C$ 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 H_3^+ 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 ⩽±5‰ for δD analyses and ⩽±0.5‰ for $\delta^{13}C$ 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 $\delta^{13}C$ (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 $\delta^{13}C$ 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 δD_{wax}, we calculated the apparent hydrogen isotope fractionation ($\varepsilon_{wax/w}$) between meteoric water, estimated from surface-water isotopic data (see Section 2.4) and leaf-waxes (Sauer et al., 2001):

$$\varepsilon_{wax/w} = ([(\delta D_{wax} + 1000)/(\delta D_w + 1000)] - 1) * 1000 \quad (1)$$

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

2.3. Analysis of water samples

δD 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 δD and $\delta^{18}O$ standard measurements was $\leqslant 2\permil$ and $\leqslant 0.2\permil$ respectively, and these values were used as measurement error in subsequent statistical analyses. All water isotope data are reported in Electronic Annex EA-2.

2.4. 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 $0.5\permil$ for $\delta^{18}O$ and $4\permil$ for δD in the context of this study), using a multivariate model for stream-water 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): $-1.71\permil$/km for $\delta^{18}O$ and

$-13.66\permil$/km for δD. To normalize surface-water isotopic values we used the following equation:

$$\delta_{zref} = \delta_z + (z - z_{ref})\Gamma_{d\delta/dz} \quad (2)$$

where δ_z 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 $\Gamma_{d\delta/dz}$ 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 isotope 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 δD–$\delta^{18}O$ space as the inferred precipitation isotopic composition for that site (Gonfiantini, 1986). Errors for estimates of meteoric δD and $\delta^{18}O$ were calculated by propagating the standard error of the local evaporation line regression parameters through the calculation of local meteoric δD 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 $\delta^{18}O_w$ and δD_w values from this region indicate deuterium excess (d) 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. δD_w estimates for each sampling site and their corresponding errors are listed in Electronic Annex EA-4.

2.5. 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 Climate 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.csi.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 broadleaf 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 values.

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-oak 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 δD_{wax} and $\varepsilon_{wax/w}$ and potential controlling variables explored in this study (δD_w, MAP, MARH, AI, PET, $\delta^{13}C_{wax}$), 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 $\delta^{13}C_{wax}$ 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 δD_w and $\varepsilon_{wax/w}$ values, we applied a weighted least squares regression in analyses involving these variables, with weights $W = 1/\sigma^2$ (Taylor, 1997). For δD_{wax} and $\delta^{13}C_{wax}$, 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 λ is a set of multipliers of the actual measurement error variance. 100 bootstrap iterations were performed for each value of λ. For regression analyses that involved both weighted regression and SIMEX models (e.g. regressing $\varepsilon_{wax/w}$ against $\delta^{13}C_{wax}$), we first produced a naive model using weighted least squares, and then applied the SIMEX procedure to this naive 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 δD values range from $-2.5‰$ and $-13‰$, respectively, at a low-elevation coastal site (Rio Cienega) to $-6.5‰$ and $-44‰$, respectively, at a high-elevation inland site (Santa Lucia). Lake-water $\delta^{18}O$ and δD values vary from $-4.3‰$ and $-28‰$, respectively, in a large, open-basin lake (L. Izabal Mina) to $4.6‰$ and $18‰$, respectively, in a highly evaporative, closed-basin lake (L. Salpeten). Generally, stream and groundwater δD 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 δD values range widely and are controlled primarily by basin hydrology (Fig. 2b). Open-basin lakes and *cenotes* exhibit δD values similar to stream/groundwater values, ranging from $-28‰$ to $-22‰$. More evaporative closed-basin lakes generally exhibit higher δD values than open-basin lakes and range from $-17‰$ to $18‰$. 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 $\sim5‰$ more positive in δD and $\sim0.6‰$ more positive in $\delta^{18}O$, relative to August surface-waters. For the third stream, seasonal differences in isotopic values were within analytical error.
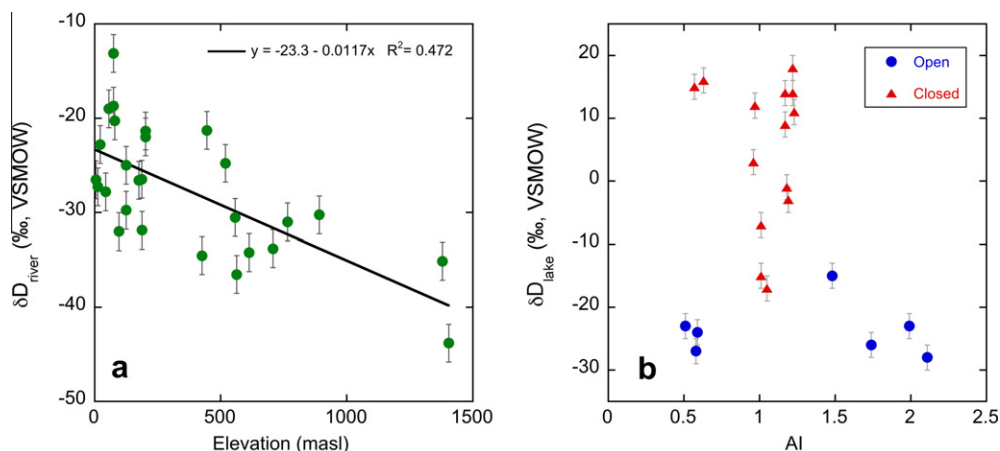
Fig. 2. (a) Elevation vs. river-water δD; the observed negative relationship is consistent with results from Lachniet and Patterson (2009); (b) AI vs. lake-water δD, divided into hydrologically open and closed systems. Open lake δD values are similar to stream-water values, while closed lake δD values show a wide range of enriched compositions that do not appear to be controlled by aridity. Error bars indicate analytical error of water δD measurements as described in the text.

3.2. Estimates of meteoric water isotopic composition

We estimated meteoric water isotopic compositions (δ_w) for each lake sediment and soil sampling site using nearby surface-water isotopic measurements (see Section 2.4). $\delta^{18}O_w$ values range from −4.1‰ to −7.4‰ and δD_w 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 $\delta^{18}O$ and 3‰ for δD. In southeastern Guatemala and northern Honduras, where estimates use averages of river water measurements, average errors are 1.6‰ for $\delta^{18}O$ and 13‰ for δD. In central Honduras, where only sparse surface-water data were collected, average errors are 2.4‰ for $\delta^{18}O$ 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 $\delta^{18}O$ 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 isotope 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-C_{16} to n-C_{34} n-alkanoic acids. n-C_{27} to n-C_{33} n-alkanes were found in all samples, with some samples yielding shorter-chain and/or longer-chain compounds (n-C_{17} to n-C_{25}, C_{35}). 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-C_{26} to n-C_{30}) and n-alkanes (n-C_{29} to n-C_{33}) to characterize the isotopic composition of vascular plant leaf-wax lipids. In some samples n-C_{24} 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 $\delta^{13}C$ values for n-C_{24} 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-C_{26}, n-C_{28}, and n-C_{30}) are positively correlated with one another both in δD (Fig. 3a) and $\delta^{13}C$ values (Fig. 3b). We compared the mean isotopic signal of long-chain n-alkanoic acids between sites using abundance-weighted average δD and $\delta^{13}C$ values for n-C_{26}, n-C_{28}, and n-C_{30} n-alkanoic acids for each sample. Abundance-weighted average leaf-wax isotope values have been applied in previous studies to compare the overall
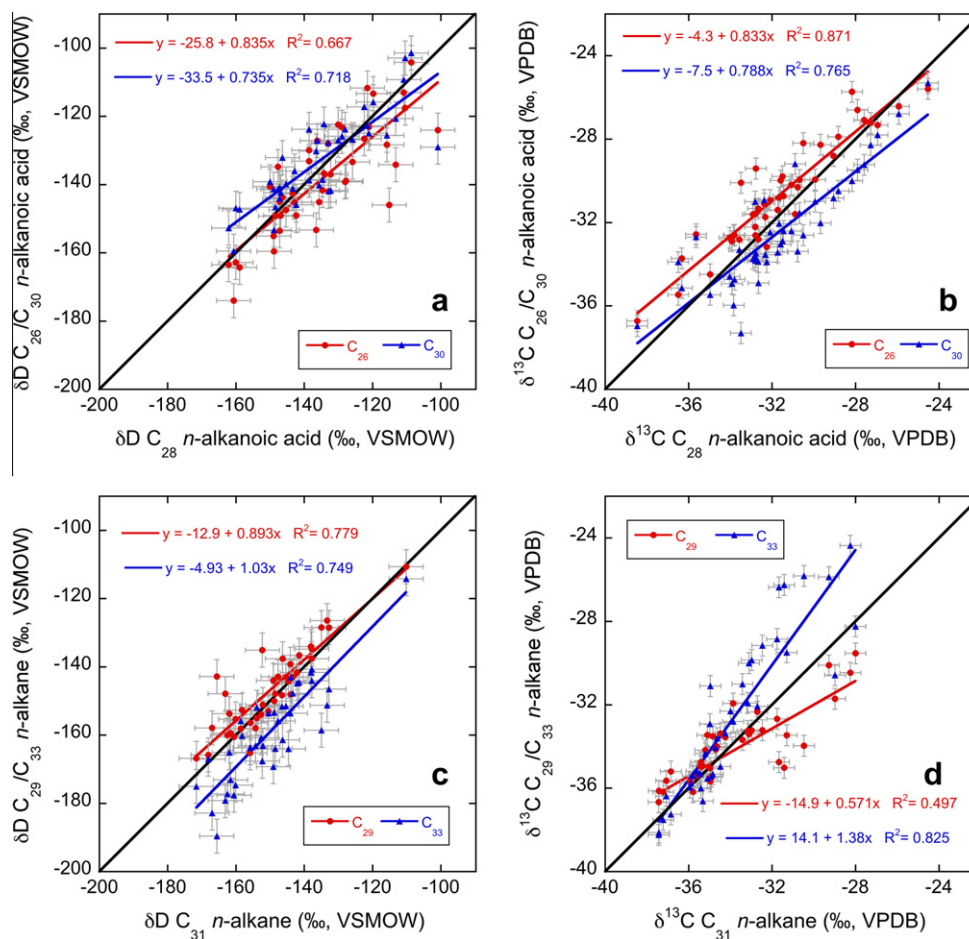
Fig. 3. Comparison of stable isotope values between long-chain n-alkyl lipid homologs: (a) δD values for long-chain n-alkanoic acids; (b) δ^{13}C values for long-chain n-alkanoic acids; (c) δD values for long-chain n-alkanes; and (d) δ^{13}C values for long-chain n-alkanes. All homologs exhibit positive correlations, although the distribution of δ^{13}C values differs markedly between n-alkane homologs. Black lines indicate 1:1 relationships. Error bars indicate pooled precision of external isotope standards (5‰ for δD and 0.5‰ for δ^{13}C).

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$_{wax}$ (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$_{n\text{-acid}}$) varies between -111‰ (Chumayel-lake sediment) and -166‰ (Comayagua-soil). The weighted average δ^{13}C value for long-chain n-alkanoic acids (δ^{13}C$_{n\text{-acid}}$) varies from -26.2‰ (Yojoa-soil) to -37.6‰ (Izabal Centro-lake sediment). n-Alkanoic acid 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 δ^{13}C values (Fig. 3d). We calculated a weighted average δD (δD$_{n\text{-alkane}}$) and δ^{13}C (δ^{13}C$_{n\text{-alkane}}$) 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 δ^{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 biosynthetic 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. δD$_{n\text{-alkane}}$ ranges from -111‰ (Chumayel-lake sediment) and -170‰ (Comayagua-soil), while δ^{13}C$_{n\text{-alkane}}$ ranges from -26.9‰ (Yojoa-soil) to -37.3‰ (Puente Cienega-soil). n-Alkane isotope and chain-length distribution data are reported in Electronic Annex EA-3.

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 δD_{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 δD_{wax}

4.2.1. Relationship between δD_w and δD_{wax}

Estimated δD_w values range from $-24‰$ to $-49‰$, whereas δD_{wax} values exhibit larger variability, from $-111‰$ to $-170‰$, suggesting that factors other than the isotopic composition of meteoric water exert important influences on δD_{wax} in this region. Indeed, δD_w and δD_{wax} show no relationship across the study area (Fig. 5), unlike most previous studies of δD_{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 δD_w exhibits almost no variance (range $= 25‰$, with most sites varying by only $9‰$) compared with a much wider range of δD_w values in most other studies. For this reason this study provides a valuable opportunity to assess environmental variables other than δD_w, such as aridity and vegetation composition, that influence δD_{wax} values.

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

$\varepsilon_{wax/w}$ for n-alkanoic acids ($\varepsilon_{n\text{-acid}/w}$) ranges from $-71‰$ (Yojoa-soil) to $-131‰$ (Izabal Rio Oscuro-lake/Comayagua-soil), while $\varepsilon_{wax/w}$ for n-alkanes ($\varepsilon_{n\text{-alkane}/w}$) ranges from $-84‰$ (Chumayel-lake) to $-140‰$ (Peten Itza-lake). $\varepsilon_{wax/w}$ values for all sites are reported in Electronic Annex EA-7. This regional range in $\varepsilon_{wax/w}$ values (56–$60‰$) is larger than previously reported in most other studies, and reflects the broad range of δD_{wax} values and limited variability in δD_w. However, the $\varepsilon_{wax/w}$ values reported here are well within the range of values reported from previous analyses of individual plant extracts, which vary from $-41‰$ for *Larrea tridentata* from arid regions of California (Feakins and Sessions, 2010) to $<-200‰$ for C_3 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 $\varepsilon_{wax/w}$ across the study area. The large amount of scatter in these relationships, however, indicates that other variables also influence $\varepsilon_{wax/w}$.

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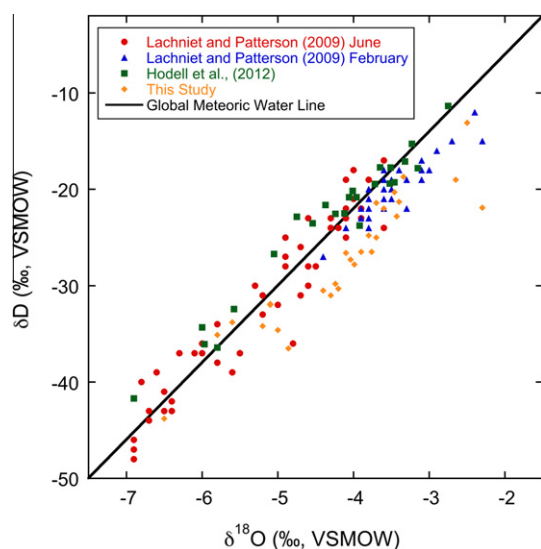

Fig. 4. Comparison of $\delta^{18}O$ and δD for river waters from different studies in southeastern Mexico and northern Central America. Data from this study tend to be shifted towards higher $\delta^{18}O$ relative to other studies, possibly due to seasonal influences on river water isotopic composition. Overall the data are relatively evenly distributed around the Global Meteoric Water Line (black line).
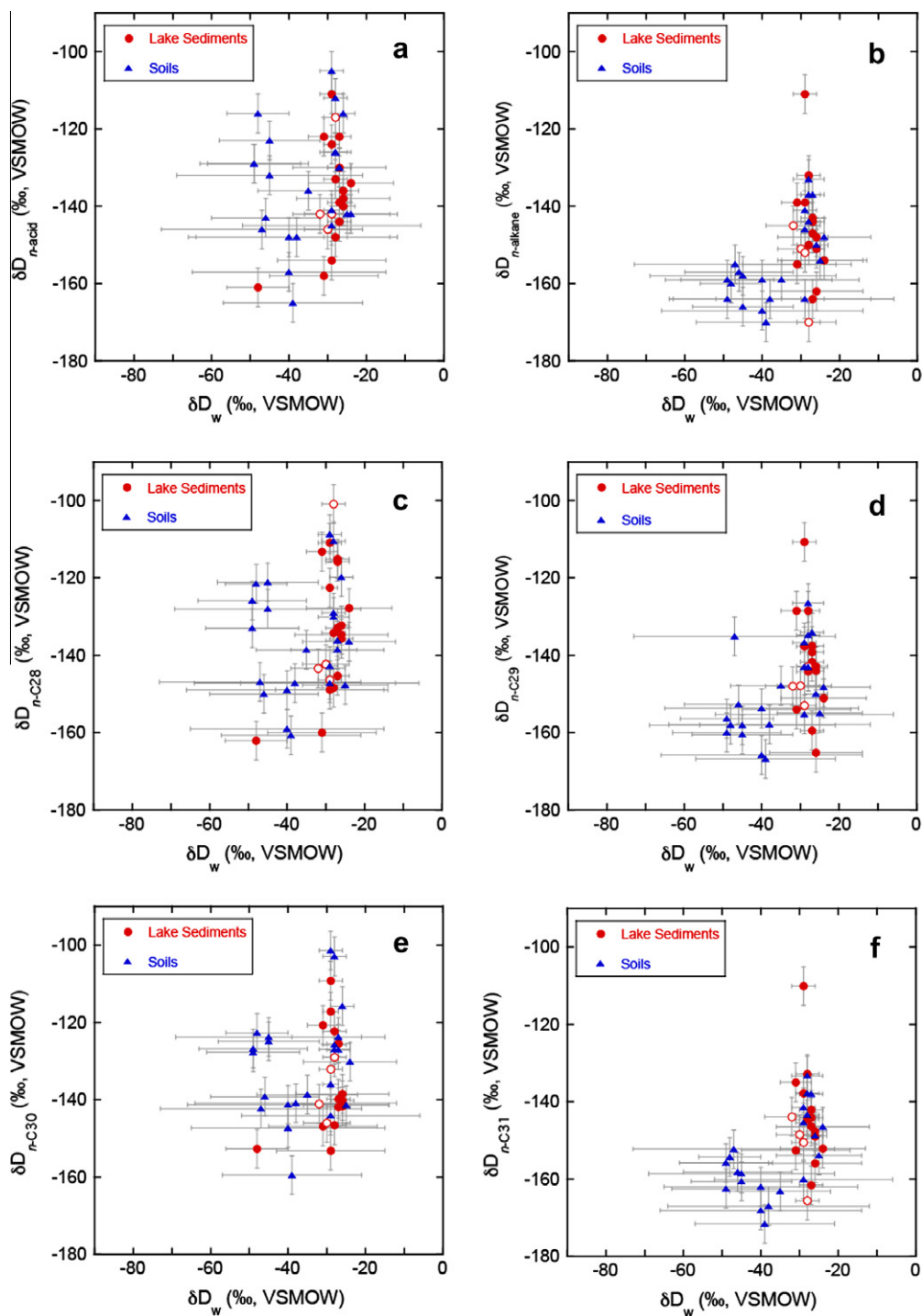
Fig. 5. δD_w vs. (a) $\delta D_{n\text{-acid}}$; (b) $\delta D_{n\text{-alkane}}$; (c) $\delta D_{n\text{-C28}}$ alkanoic acid; (d) $\delta D_{n\text{-C29}}$ alkane; (e) $\delta D_{n\text{-C30}}$ alkanoic acid; and (f) $\delta D_{n\text{-C31}}$ alkane for all samples subdivided by sample type. Lake sediment samples show a wide range of δD_{wax} values despite minimal variability in estimated δD_w. Soil samples have greater variability in estimated δD_w. Unfilled circles indicate lakes dominated by emergent aquatic plants (EAP lakes). Error bars for δD_{wax} indicate the pooled precision of external isotopic standards (5‰). Error bars for δD_w indicate errors associated with estimating the isotopic composition of precipitation.

occur with δD_{wax} values and the coefficient of determination is in many cases stronger. However, because variability in δD_w is not constrained when analyzing δD_{wax} values we focus our interpretation on comparisons with of climate variables with $\varepsilon_{wax/w}$.

We also analyzed a subset of the data, including only sites from the moist and dry tropical broadleaf forest biomes to evaluate how $\varepsilon_{wax/w}$ varies in tropical forests dominated by angiosperms. For these data, there is a significant correlation between climate variables (AI,
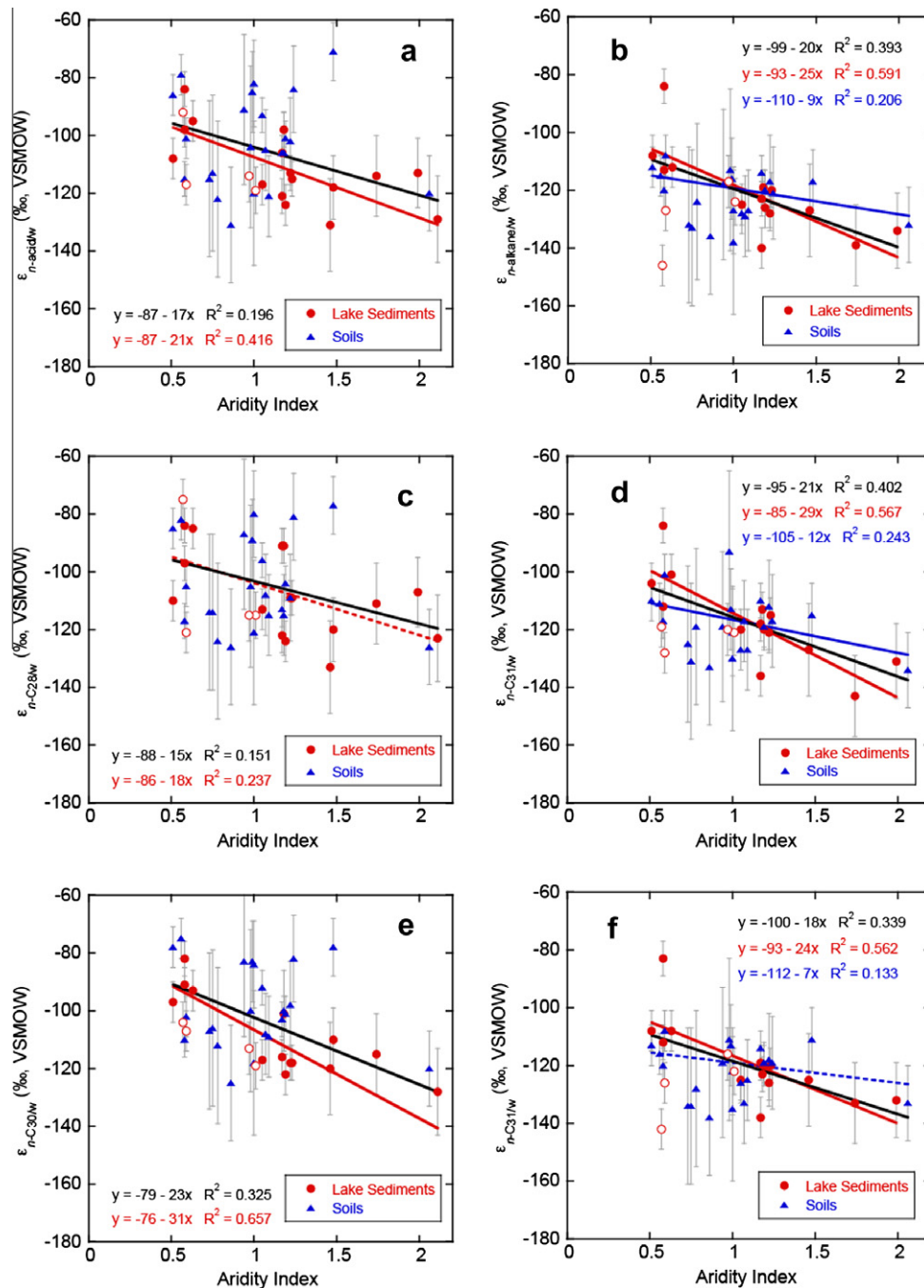
Fig. 6. AI vs. (a) $\varepsilon_{n\text{-acid/w}}$; (b) $\varepsilon_{n\text{-alkane/w}}$; (c) $\varepsilon_{n\text{-C28/w}}$; (d) $\varepsilon_{n\text{-C29/w}}$; (e) $\varepsilon_{n\text{-C30/w}}$; and (f) $\varepsilon_{n\text{-C31/w}}$ for all samples disaggregated by sample type. Black lines indicate 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 $\varepsilon_{\text{wax/w}}$ for the entire dataset and for lake sediment samples; in addition there is a significant correlation between AI and $\varepsilon_{n\text{-alkane/w}}$, $\varepsilon_{n\text{-C29/w}}$, and $\varepsilon_{n\text{-C31/w}}$ 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). $\varepsilon_{\text{wax/w}}$ error bars indicat compounded errors for δD_w and δD_{wax} values.

MAP, PET) and both $\varepsilon_{n\text{-acid/w}}$ and $\varepsilon_{n\text{-alkane/w}}$, 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 $\varepsilon_{n\text{-alkane/w}}$ in tropical angiosperm forests. Our

results, however, suggest that large variability in aridity can lead to sizeable variability in $\varepsilon_{\text{wax/w}}$ (30–50‰) in tropical angiosperm forest environments.

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

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 $\varepsilon_{wax/w}$ 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 $\varepsilon_{wax/w}$ values. In this study we cannot resolve the mechanism causing the observed relationship between aridity and $\varepsilon_{wax/w}$ since lake sediment and soil samples conflate soil evaporation and transpiration processes.

Two aspects of this study, though, could prove important in understanding mechanisms for $\varepsilon_{wax/w}$ 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 $\varepsilon_{wax/w}$ (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 $\varepsilon_{wax/w}$ despite minimal variability in MARH or MAT suggests that either (1) plant-water transpiration is not a strong driver of $\varepsilon_{wax/w}$ 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 $\varepsilon_{wax/w}$. In contrast, MAP, and to a lesser extent PET, vary widely across the study area and are significantly correlated with $\varepsilon_{wax/w}$ (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 $\varepsilon_{wax/w}$ and climate variables (AI, MAP, PET) is possibly consistent with a soil–water evaporation influence on $\varepsilon_{wax/w}$. 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 $\varepsilon_{wax/w}$ variability.

Some studies have suggested that the dependence of δD_{wax} 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 $\varepsilon_{wax/w}$ on aridity is linear within our study area (Fig. 6). The range of $\varepsilon_{wax/w}$ values we observe span the range of values reported from arid ($\sim$−100‰; Hou et al., 2008; Polissar and Freeman, 2010) and humid ($\sim$−130‰ to −140‰; Sachse et al., 2004; Polissar and Freeman, 2010; Garcin et al., 2012) environment lake sediments, although some studies of plant extracts report $\varepsilon_{wax/w}$ values that are more positive or negative than observed in this study (Chikaraishi and Naraoka, 2003; Smith and Freeman, 2006; Feakins and Sessions, 2010; McInerney et al., 2011). Our results suggest that the dependence of $\varepsilon_{wax/w}$ 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 $\varepsilon_{wax/w}$ 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 δD_{wax} 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 δD_{wax} 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 $\varepsilon_{wax/w}$ variability in lake sediment and soil samples. We disaggregated the data by sample type to more thoroughly examine relationships between climatic variables and $\varepsilon_{wax/w}$. Lake sediments potentially provide a clearer signal of climate effects on $\varepsilon_{wax/w}$ 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 $\varepsilon_{wax/w}$ 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 $\varepsilon_{wax/w}$ are stronger for lake sediments than for the dataset as a whole (Fig. 6). Conversely, the correlation between AI and $\varepsilon_{n\text{-alkane}/w}$ is weaker for soil samples relative to the whole dataset, and there is no significant correlation between AI and $\varepsilon_{n\text{-acid}/w}$ 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 $\varepsilon_{wax/w}$ 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 $\varepsilon_{wax/w}$ data. However, analysis of soil samples excluding the coniferous forest biome does not indicate an enhanced correlation between $\varepsilon_{wax/w}$ and climate variables relative to all soil samples (Electronic Annex EA-8). These results provide indirect evidence that there is larger variability in $\varepsilon_{wax/w}$ 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. $\varepsilon_{wax/w}$: Influence of vegetation
4.2.3.1. Relationships between $\delta^{13}C_{wax}$ and $\varepsilon_{w/wax}$.
Although variability in AI accounts for some of the observed variability in $\varepsilon_{wax/w}$, there is a notable amount of scatter in the relationships between AI and both $\varepsilon_{n\text{-acid}/w}$ and $\varepsilon_{n\text{-alkane}/w}$ (Fig. 6), suggesting that other factors also influence $\varepsilon_{wax/w}$. Plant physiology has been shown to significantly influence $\varepsilon_{wax/w}$ in angiosperms, with important differences between C_3 grasses (lowest $\varepsilon_{wax/w}$ values), C_4 grasses (intermediate $\varepsilon_{wax/w}$ values), and C_3 angiosperm trees and shrubs (highest $\varepsilon_{wax/w}$ 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 C_3 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 ($\delta^{13}C_{wax}$) 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 C_4 photosynthetic pathway, while most shrubs and trees are C_3 plants (Chazdon, 1978; Sage et al., 1999). In this study, $\delta^{13}C_{wax}$ values from lake sediments and soils provide an approximate indication of the relative contribution of leaf-waxes from C_4 grasses versus C_3 trees and shrubs at a given site. Based on end-member values for C_3 and C_4 leaf-waxes (Collister et al., 1994; $C_3 = -36‰$, $C_4 = -21‰$), the range of $\delta^{13}C_{wax}$ values from sediment and soil samples indicates that the proportion of leaf-waxes derived from C_3 plants ranges between $100 \pm 18\%$ to $40 \pm 24\%$. Caution is necessary in interpreting $\delta^{13}C_{wax}$ as an indicator of plant type because there is a wide range of $\delta^{13}C_{wax}$ values within both C_3 and C_4 plants, but soil and sediment samples at the upper end of the range of $\delta^{13}C_{wax}$ values clearly include a C_4 plant component.

Since C_3 trees/shrubs and C_4 grasses have different $\varepsilon_{wax/w}$ values under the same climate conditions (Chikaraishi and Naraoka, 2003; Chikaraishi et al., 2004), the wide range of observed $\delta^{13}C_{wax}$ values is expected to correspond to variability in $\varepsilon_{wax/w}$. For the purposes of analyzing vegetation effects on $\varepsilon_{wax/w}$, we primarily focus on sites from biomes dominated by angiosperms (moist and dry tropical broadleaf forests). Gymnosperms can differ from angiosperms in both $\delta^{13}C_{wax}$ and $\varepsilon_{wax/w}$, with values that tend to be intermediate between angiosperm trees and shrubs (low $\delta^{13}C_{wax}$ and high $\varepsilon_{wax/w}$) and C_4 grasses (high $\delta^{13}C_{wax}$ and low $\varepsilon_{wax/w}$) (Chikaraishi and Naraoka, 2003; Chikaraishi et al., 2004). Focusing our analysis on sites without significant gymnosperms simplifies our comparison of $\delta^{13}C_{wax}$ and $\varepsilon_{wax/w}$ values. However, further study involving such a comparison in ecosystems with significant numbers of gymnosperms would be valuable.

$\delta^{13}C_{wax}$ and $\varepsilon_{wax/w}$ 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 $\varepsilon_{wax/w}$ 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 ecoregions (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 $\delta^{13}C_{n\text{-acid}}$ and $\varepsilon_{n\text{-acid}/w}$ (Fig. 7a), although this relationship is only statistically significant at the level of $p \leqslant 0.05$ 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 $\delta^{13}C_{n\text{-acid}} - \varepsilon_{n\text{-acid}/w}$ fields, with the highest $\varepsilon_{n\text{-acid}/w}$ values for a given value of $\delta^{13}C_{n\text{-acid}}$ in the Yucatan dry forests (most arid) and the lowest $\varepsilon_{n\text{-acid}/w}$ values for a given value of $\delta^{13}C_{n\text{-acid}}$ in the Central American Atlantic moist forests (least arid) (Fig. 7a). This pattern suggests that for a given plant group, variability in $\varepsilon_{n\text{-acid}/w}$ is largely controlled by aridity, and by
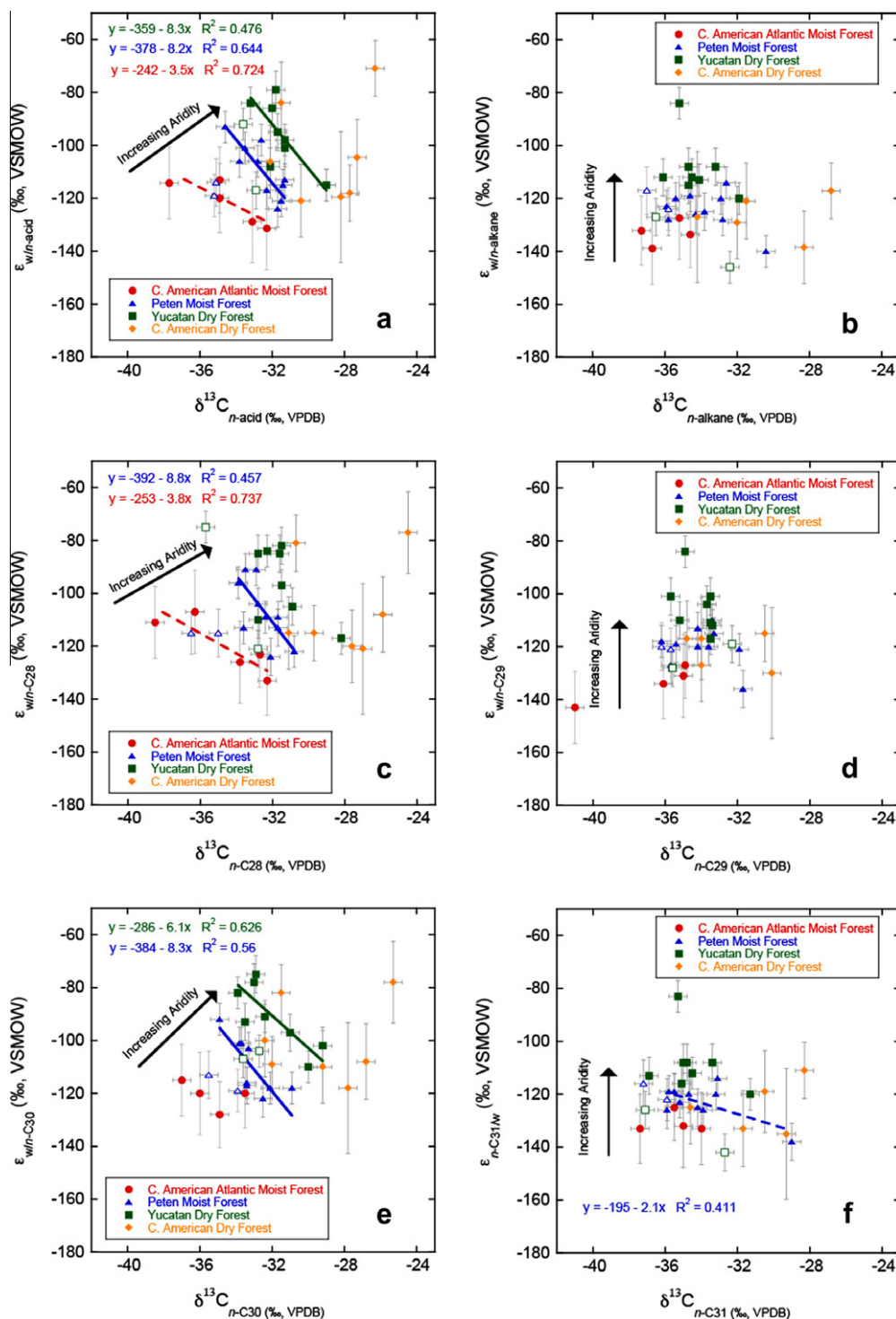
Fig. 7. (a) $\delta^{13}C_{n\text{-acid}}$ vs. $\varepsilon_{n\text{-acid/w}}$; (b) $\delta^{13}C_{n\text{-alkane}}$ vs. $\varepsilon_{n\text{-alkane/w}}$; (c) $\delta^{13}C_{n\text{-C28}}$ vs. $\varepsilon_{n\text{-C28/w}}$; (d) $\delta^{13}C_{n\text{-C29}}$ vs. $\varepsilon_{n\text{-C29/w}}$; (e) $\delta^{13}C_{n\text{-C30}}$ vs. $\varepsilon_{n\text{-C30/w}}$; and (f) $\delta^{13}C_{n\text{-C29}}$ vs. $\varepsilon_{n\text{-C29/w}}$ 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 $\delta^{13}C_{wax}$ and $\varepsilon_{wax/w}$ 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 $\delta^{13}C_{wax}$ and $\varepsilon_{wax/w}$ 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). $\varepsilon_{wax/w}$ error bars reflect compounded errors in δD_w and δD_{wax}. $\delta^{13}C_{wax}$ error bars indicate the pooled precision of external isotopic standards (0.5‰).
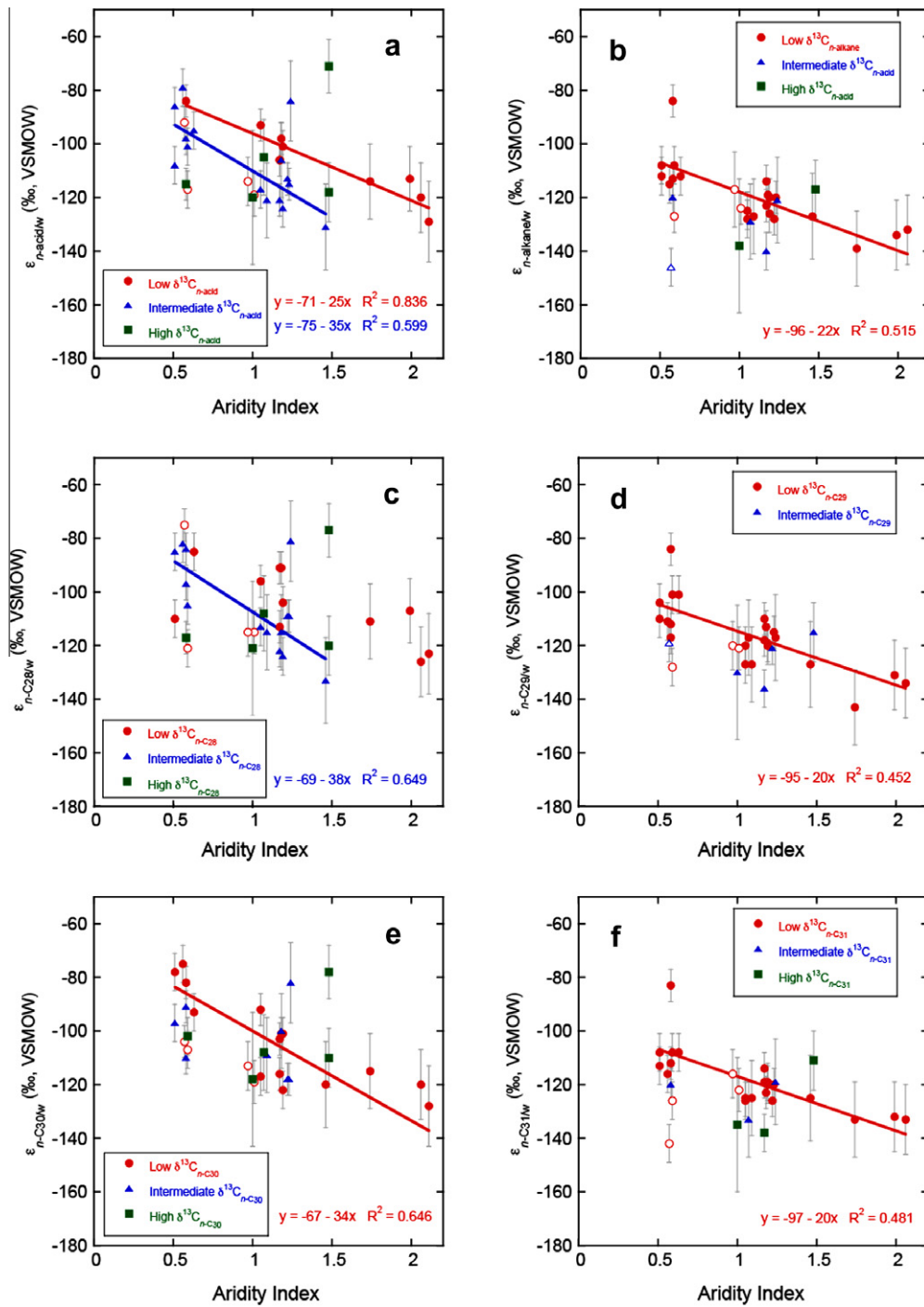
Fig. 8. AI vs. (a) $\varepsilon_{w/n\text{-}acid}$; (b) $\varepsilon_{w/n\text{-}alkane}$; (c) $\varepsilon_{n\text{-}C28/w}$; (d) $\varepsilon_{n\text{-}C29/w}$; (e) $\varepsilon_{n\text{-}C30/w}$; and (f) $\varepsilon_{n\text{-}C31/w}$ for samples from broadleaf forest ecosystems disaggregated by $\delta^{13}C_{wax}$ values (Low = <−32.4‰, Intermediate = −32.4‰ to −29.6‰, High = >−29.6‰). Generally sites with higher $\delta^{13}C_{wax}$ have lower $\varepsilon_{wax/w}$ values for a given level of aridity. There are significant negative correlations between AI and $\varepsilon_{wax/w}$ for samples with low $\delta^{13}C_{wax}$, with the exception of $\varepsilon_{n\text{-}C28/w}$. There is also a negative relationship between AI and $\varepsilon_{n\text{-}acid/w}$ and $\varepsilon_{n\text{-}C28/w}$ for samples with intermediate $\delta^{13}C_{n\text{-}acid}$. Unfilled markers indicate EAP lakes, which generally have low $\varepsilon_{wax/w}$ relative to samples with similar $\delta^{13}C_{wax}$ values. EAP lakes are not included in plotted regression lines (see Section 4.2.3.3). $\varepsilon_{wax/w}$ error bars reflect compounded errors in δD_w and δD_{wax}.

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 $\delta^{13}C_{n\text{-}acid}$–$\varepsilon_{n\text{-}acid/w}$ space,

which could be partly due to greater uncertainty in δD_w estimates for these sites. Samples from this ecoregion have a similar range in $\delta^{13}C_{n\text{-}acid}$–$\varepsilon_{n\text{-}acid/w}$ 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 $\delta^{13}C_{n\text{-acid}}$–$\varepsilon_{n\text{-acid/w}}$ space, possibly as a consequence of inclusion of gymnosperm derived n-alkanoic acids, which may complicate $\delta^{13}C_{n\text{-acid}}$–$\varepsilon_{n\text{-acid/w}}$ relationships.

For n-alkane samples, relationships between $\delta^{13}C_{n\text{-alkane}}$ and $\varepsilon_{n\text{-alkane/w}}$ are less apparent. In Fig. 7b, ecoregions are divided primarily by $\varepsilon_{n\text{-alkane/w}}$ values, with little corresponding differences in $\delta^{13}C_{n\text{-alkane}}$. Two sites with high $\delta^{13}C_{n\text{-alkane}}$ values have relatively low $\varepsilon_{n\text{-alkane/w}}$ values compared to other samples from their ecoregion (La Barca, Peten-Itza). 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 $\delta^{13}C_{n\text{-alkane}}$–$\varepsilon_{n\text{-alkane/w}}$ relationships.

Variability in $\varepsilon_{wax/w}$ 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 $\delta^{13}C_{wax}$ and $\varepsilon_{wax/w}$ in modern sediments, soils, or leaves. Hou et al. (2007) compared $\delta^{13}C$ and δD values for individual n-alkanoic acid homologs from leaves at one site in Massachusetts and found a negative correlation. In this mid-latitude setting, all samples derive from C_3 plants, and Hou et al. (2007) interpreted the relationship as an expression of plant-water-use efficiency, which partly controls $\delta^{13}C_{wax}$ values. Our results for n-alkanoic acids in sediments and soils are broadly similar to those of Hou et al. (2007). Because the most positive $\delta^{13}C_{wax}$ values in our study are outside the most positive values typically found in C_3 angiosperm plants (Chikaraishi and Naraoka, 2003; Chikaraishi et al., 2004), we suggest that the observed $\delta^{13}C_{n\text{-acid}}$–$\varepsilon_{n\text{-acid/w}}$ relationships are, at least in part, due to differences in $\varepsilon_{n\text{-acid/w}}$ between C_3 trees and shrubs and C_4 grasses.

4.2.3.2. The role of $\delta^{13}C_{wax}$ in understanding aridity effects on $\varepsilon_{wax/w}$. Because there is evidence that different plant groups differ widely in $\varepsilon_{wax/w}$, it would be valuable to compare how $\varepsilon_{wax/w}$ within different plant groups varies across climate gradients. To approximate such an analysis, we disaggregated the sediment and soil samples from the broadleaf forest biomes into three classes using $\delta^{13}C_{wax}$: low $\delta^{13}C_{wax}$ ($<-32.4‰$), intermediate $\delta^{13}C_{wax}$ ($-32.4‰$ to $-29.6‰$), and high $\delta^{13}C_{wax}$ ($>-29.6‰$). These groups correspond to $>80\%$, 80–60%, and $<60\%$ of leaf-waxes derived from C_3 plants based on the end-members of Collister et al. (1994), and were chosen because they evenly divide the range of $\delta^{13}C_{wax}$ values. As discussed above (Section 4.2.3.1), identification of plant source using $\delta^{13}C_{wax}$ is associated with large uncertainty. A strong negative correlation between AI and $\varepsilon_{n\text{-acid/w}}$ for samples with low $\delta^{13}C_{n\text{-acid}}$ suggests that in ecosystems dominated by C_3 trees and shrubs $\varepsilon_{n\text{-acid/w}}$ is largely controlled by aridity (Fig. 8a). The negative correlation between AI and $\varepsilon_{n\text{-alkane/w}}$ for samples with low $\delta^{13}C_{n\text{-alkane}}$ is somewhat

weaker, but still significant (Fig. 8b). There is also a significant negative relationship between AI and $\varepsilon_{n\text{-acid/w}}$ for samples with intermediate $\delta^{13}C_{n\text{-acid}}$, with $\varepsilon_{n\text{-acid/w}}$ values that are generally more negative than samples with low $\delta^{13}C_{n\text{-acid}}$ (Fig. 8a). This suggests that for ecosystems with a greater proportion of C_4 grasses, aridity also influences $\varepsilon_{n\text{-acid/w}}$, but that $\varepsilon_{n\text{-acid/w}}$ values are lower relative to tree- and shrub-dominated ecosystems for a given climate. There is greater scatter for samples with intermediate $\delta^{13}C_{n\text{-acid}}$, which most likely reflects that $\delta^{13}C_{wax}$ 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 $\delta^{13}C_{wax}$ could be predominantly derived from C_3 trees and shrubs. The small number of samples with intermediate $\delta^{13}C_{n\text{-alkane}}$, high $\delta^{13}C_{n\text{-alkane}}$, and high $\delta^{13}C_{n\text{-acid}}$ make it difficult to assess statistical relationships between aridity and $\varepsilon_{wax/w}$ within these groups. These samples tend to have relatively low $\varepsilon_{wax/w}$ 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 $\varepsilon_{wax/w}$ for the low $\delta^{13}C_{n\text{-acid}}$, low $\delta^{13}C_{n\text{-alkane}}$, and intermediate $\delta^{13}C_{n\text{-acid}}$ 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: Xoche, 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 δD_{wax} and $\delta^{13}C_{wax}$ 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 δD_{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 (n-C_{32} and n-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.
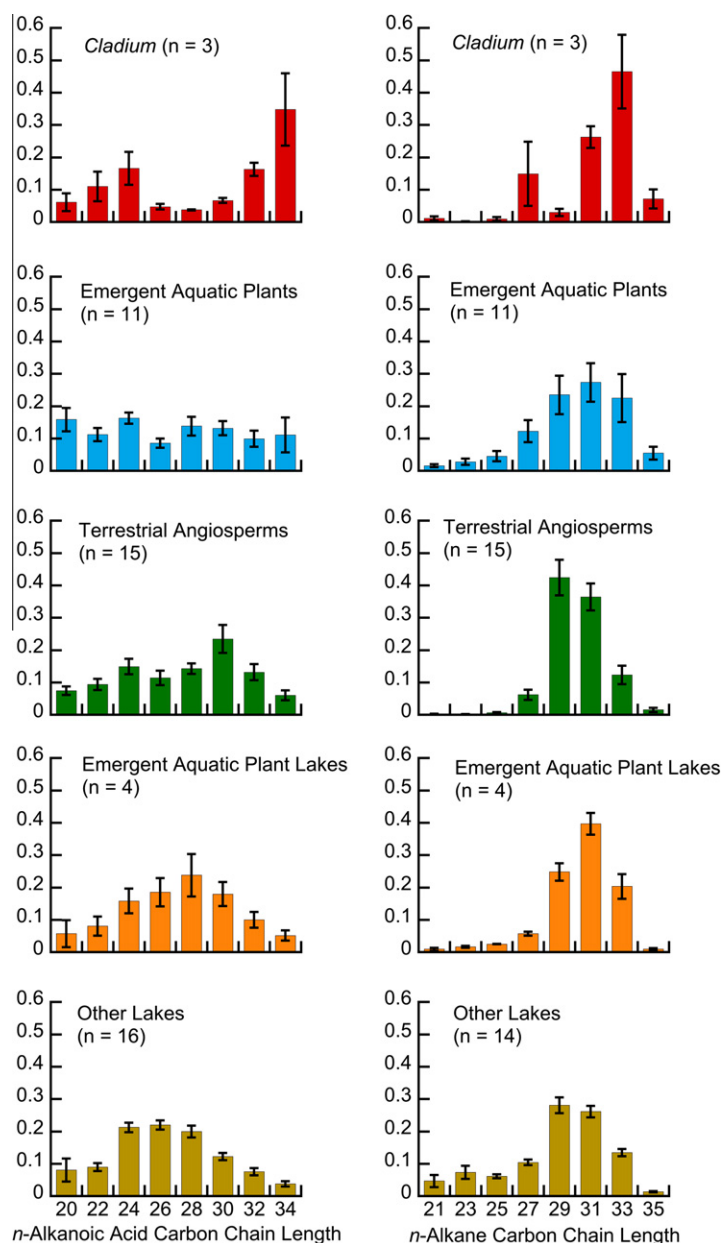
Fig. 9. Distribution of n-alkyl lipid homologs in groups of plant extracts and lake sediment samples. All y-axes indicate the mean relative abundance of even (n-alkanoic acids) or odd (n-alkanes) carbon chain-length homologs. Error bars indicate standard error ($\sigma/\sqrt{n}^2$). Chain-length distribution data are available in *Electronic* Annex EA-9. *Cladium* samples have n-alkyl lipid distributions that are strongly shifted to longer-chain length homologs. Emergent aquatic plants have n-alkane distributions that are shifted to longer-chain length homologs relative to terrestrial plants. EAP lakes have n-alkyl lipid distributions that are shifted to longer-chain length homologs relative to other lakes, particularly for n-alkanes.

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-C_{31} than n-C_{29} alkanes in most samples (Fig. 9). *Cladium* samples, in particular, contain a much greater amount of n-C_{33} 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.

Lakes dominated by emergent aquatic plants (EAP) also exhibit unique patterns in terms of δD_{wax} and $\delta^{13}C_{wax}$ values. $\varepsilon_{n\text{-acid/w}}$ values for three out of four EAP lakes are relatively low, although not outside the range of $\varepsilon_{n\text{-acid/w}}$ values for other lakes (Fig. 6a). EAP lakes also have relatively low $\delta^{13}C_{n\text{-acid}}$ values ($-32.9‰$ to $-35.2‰$). In $\varepsilon_{n\text{-acid/w}}$–$\delta^{13}C_{n\text{-acid}}$ 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 $\delta^{13}C_{n\text{-acid}}$ values, three out of four EAP lakes have lower $\varepsilon_{n\text{-acid/w}}$ than other samples with low $\delta^{13}C_{n\text{-acid}}$ values from the same climate (Fig. 8a).

EAP lakes from the Peten moist forests ecoregion have similar $\varepsilon_{n\text{-alkane/w}}$ values to other lakes from this region, while EAP lakes from the more arid Yucatan dry forests ecoregion have distinctively low $\varepsilon_{n\text{-alkane/w}}$ values (Figs. 6b and 7b). One of the EAP lakes from the Yucatan dry forests, L. Yaalchak, has the lowest $\varepsilon_{n\text{-alkane/w}}$ 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 $\delta^{13}C_{n\text{-alkane}}$ values ($-35.8‰$ to $-37‰$) for their respective ecoregion, while L. Yaalchak has a relatively high $\delta^{13}C_{n\text{-alkane}}$ value ($-32.4‰$) (Fig. 7b).

Broadly, EAP lakes tend to have low $\varepsilon_{wax/w}$ and $\delta^{13}C_{wax}$ values, although these patterns are not consistent across all EAP lakes. Low $\delta^{13}C_{wax}$ values are consistent with a growth environment with unlimited water, since aquatic plants would have less need to close their stoma 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 $\varepsilon_{wax/w}$ values. However, two of the four EAP lakes (Yaalchak and La Corona) have positive δD and $\delta^{18}O$ values for lake-water (Electronic Annex EA-2), indicating major evaporative isotopic enrichment. The relatively low $\varepsilon_{wax/w}$ 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 $\varepsilon_{n\text{-acid/w}}$ 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 $\delta^{13}C_{n\text{-acid}}$ and $\varepsilon_{n\text{-acid/w}}$ in the Peten Moist Forest and Yucatan Dry Forest ecoregions since the EAP lakes have lower values for $\delta^{13}C_{n\text{-acid}}$ and $\varepsilon_{n\text{-acid/w}}$ than the other samples from these regions (Fig. 7a). Similarly, when EAP lakes are included there is no significant correlation between $\varepsilon_{n\text{-acid/w}}$ and climatic variables (AI, MAP, PET) for samples with low $\delta^{13}C_{n\text{-acid}}$ values, since the EAP lake samples generally have low $\varepsilon_{n\text{-acid/w}}$ values and plot more closely with samples with intermediate $\delta^{13}C_{n\text{-acid}}$ values (Fig. 8a).

The correlation between $\varepsilon_{n\text{-alkane/w}}$ 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 $\varepsilon_{n\text{-acid/w}}$ (Electronic Annex EA-8). There is no significant correlation between $\varepsilon_{n\text{-alkane/w}}$ and climatic variables for lake sediment samples when EAP lakes are included. The stronger influence of EAP lakes on regression statistics for $\varepsilon_{n\text{-alkane/w}}$ as opposed to $\varepsilon_{n\text{-acid/w}}$ is probably primarily caused by L. Yaalchak, which has a very low $\varepsilon_{n\text{-alkane/w}}$ 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 $\varepsilon_{wax/w}$ values in this study area, although these lakes in many cases deviate from the relationship between $\varepsilon_{wax/w}$ and climatic variables. We argue that the negative correlations between $\delta^{13}C_{n\text{-acid}}$ and $\varepsilon_{n\text{-acid/w}}$ for specific ecoregions that are observed when EAP lakes are excluded are robust, since the EAP lakes have unique $\delta^{13}C_{n\text{-acid}}$ and $\varepsilon_{n\text{-acid/w}}$ 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 $\delta^{13}C_{n\text{-acid}}$ and $\varepsilon_{n\text{-acid/w}}$ is observed across a wide range of environments and (2) whether relatively low $\delta^{13}C_{wax}$ and $\varepsilon_{wax/w}$, 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 $n\text{-}C_{28}$ and $n\text{-}C_{30}$ n-alkanoic acids and $n\text{-}C_{29}$ and $n\text{-}C_{31}$ n-alkanes since these homologs are most often applied in paleoclimate studies.

Patterns in $\varepsilon_{n\text{-}C28/w}$ and $\varepsilon_{n\text{-}C30/w}$ are similar to those found for $\varepsilon_{n\text{-acid/w}}$. The correlation between $\varepsilon_{n\text{-}C30/w}$ and climatic variables (AI, MAP, PET) is stronger than that for $\varepsilon_{n\text{-acid/w}}$, while $\varepsilon_{n\text{-}C28/w}$ is more weakly correlated with climatic variables (Fig. 6c and e, Electronic Annex EA-8). Negative correlations in $\varepsilon_{n\text{-}C28/w}$–$\delta^{13}C_{n\text{-}C28}$ space and $\varepsilon_{n\text{-}C30/w}$–$\delta^{13}C_{n\text{-}C30}$ 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 $n\text{-}C_{28}$ in the Yucatan Dry Forests and for $n\text{-}C_{30}$ in the Central American Atlantic Moist Forests. There is a negative relationship between $\varepsilon_{n\text{-}C28/w}$ and climatic variables for samples with intermediate $\delta^{13}C_{n\text{-}C28}$ values, but not for samples with low $\delta^{13}C_{n\text{-}C28}$ values (Fig. 8c). Conversely, there is a negative relationship between $\varepsilon_{n\text{-}C30/w}$ and climatic variables for samples with low $\delta^{13}C_{n\text{-}C30}$ values, but not for samples with low $\delta^{13}C_{n\text{-}C30}$ values (Fig. 8e). $\varepsilon_{n\text{-}C30/w}$ values for EAP lakes are clearly more negative than other lakes from similar environments, while $\varepsilon_{n\text{-}C28/w}$ values for EAP lakes are more variable (Figs. 7c, e and 8c, e).

A stronger aridity effect for $\varepsilon_{n\text{-}C30/w}$ could be related to different plant sources for $n\text{-}C_{28}$ and $n\text{-}C_{30}$ alkanoic acids, or to differences in the timing or location of biosynthesis of these two homologs within a given plant. In our study $\delta^{13}C_{n\text{-}C28}$ values are generally higher than $\delta^{13}C_{n\text{-}C30}$ values from the same sample, possibly suggesting greater C_4 grass input to $n\text{-}C_{28}$ alkanoic acids. Further studies of tropical plant extracts are needed to better understand isotopic differences between these two homologs.

$\varepsilon_{n\text{-}C29/w}$ and $\varepsilon_{n\text{-}C31/w}$ exhibit correlations with climate variables that are similar in strength and slope to those for $\varepsilon_{n\text{-alkane/w}}$ (Figs. 6d, f and 8d, f). As with $\varepsilon_{n\text{-alkane/w}}$ there

is no apparent relationship between $\varepsilon_{n\text{-}C29/w}$ and $\varepsilon_{n\text{-}C31/w}$ and the respective n-alkane $\delta^{13}C$ value in specific ecoregions, with the exception of $\varepsilon_{n\text{-}C31/w}$ in the Peten moist forests ecoregion (Fig. 7d and f). $\varepsilon_{n\text{-}C29/w}$ values in EAP lakes are less divergent from other sites than $\varepsilon_{n\text{-}C31/w}$ values. In particular, for L. Yaalchak $\varepsilon_{n\text{-}C29/w}$ is within the range of other samples from the Yucatan Dry Forests ecoregion, but $\varepsilon_{n\text{-}C31/w}$ 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 $\varepsilon_{n\text{-}acid/w}$ and $\varepsilon_{n\text{-}alkane/w}$ 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 δD_{wax}

δD_{wax} values in this study agree broadly with previous analyses of δD_{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 δD_{wax}, our results support a global-scale positive correlation between δD_{w} and δD_{wax} (Fig. 10). However, our results differ from previous regional studies of δD_{wax} in modern lake sediments and soils in two ways: (1) we observe a larger range of $\varepsilon_{wax/w}$ (~60‰) than most previous studies; and (2) we observe a significant correlation between aridity and $\varepsilon_{wax/w}$. The first difference is largely explained by the relatively small range in δD_{w} (25‰) 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 δD_{wax} when δD_{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 δD_{wax} across large gradients in precipitation and relative humidity, but did not observe corresponding variability in $\varepsilon_{wax/w}$. 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 $\varepsilon_{wax/w}$ 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 $\varepsilon_{wax/w}$ 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 $\varepsilon_{wax/w}$ 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 $\delta D_{n\text{-}C29}$ correlating strongly with the estimated δD of precipitation and $\delta D_{n\text{-}C31}$ correlating strongly with the δD 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; $\delta D_{n\text{-}C29}$ and $\delta D_{n\text{-}C31}$ are strongly correlated for our study (Fig. 3c), and neither $\delta D_{n\text{-}C29}$ nor $\delta D_{n\text{-}C31}$ is correlated with the estimated δD of precipitation (Fig. 5e and f) or the δD of lake-water (in the case of lake sediment samples). This would suggest that the differences between $\delta D_{n\text{-}C29}$ and $\delta D_{n\text{-}C31}$ observed by Garcin et al.
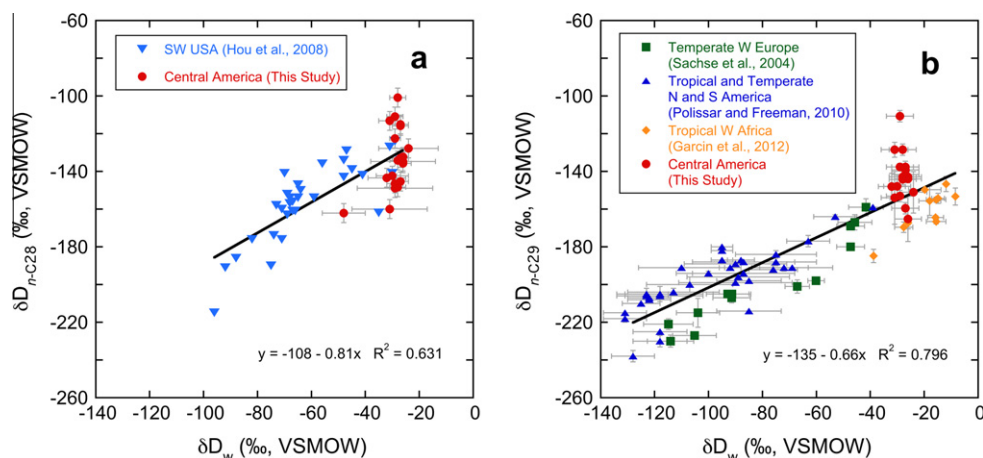
Fig. 10. δD_{w} vs. (a) $\delta D_{n\text{-}C28}$ alkanoic acid and (b) $\delta D_{n\text{-}C29}$ alkane for lake sediment samples from this study and other published datasets (Sachse et al., 2004, 2012; Hou et al., 2008; Polissar and Freeman, 2010; Garcin et al., 2012). Regression lines include all plotted data. δD_{wax} values from this study generally agree with other datasets, but also exhibit a relatively broad range of δD_{wax} for a given value of δD_{w}. Error bars for data from this study indicate errors associated with estimating the isotopic composition of precipitation (δD_{w}) and the pooled precision of external isotopic standards (δD_{wax}). Error bars for other datasets are as published.

(2012) are a regional feature of Western African lakes, or, at least, are not a global feature of tropical environments.

4.4. Implications for paleoclimate studies

In general, changes in δD_{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 δD_{wax} values in the tropics (Figs. 6–8). The observed relationship between aridity and $\varepsilon_{wax/w}$ in this study indicates that temporal shifts in δD_{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 δD_{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 δD_w is not strongly controlled by local precipitation amount in Central America, temporal variability in the isotopic 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 δD_w and aridity, δD_{wax} measurements can provide a sensitive qualitative indicator of hydroclimate change in tropical precipitation amount. Aridity effects on δD_{wax}, however, complicate inference of past precipitation isotope values.

To address this complication, (Polissar and Freeman, 2010) suggested that paired analyses of δD_{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 $\varepsilon_{wax/w}$ 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 δD_{wax} that are independent of climate. In this dataset, variability in $\delta^{13}C_{n-acid}$ values corresponds to differences in $\varepsilon_{n-acid/w}$ 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 C_4 grasses relative to n-alkanoic acids. Previous studies of plant extracts indicate there are important differences in $\varepsilon_{n-alkane/w}$ between different plant groups (Chikaraishi and

Naraoka, 2003; Smith and Freeman, 2006; Feakins and Sessions, 2010; McInerney et al., 2011). If vegetation change is not constrained, this effect could lead to large errors when interpreting δD_{wax} values in a paleoclimate context.

$\delta^{13}C_{wax}$ records offer one approach to reconstructing changes in vegetation composition and thereby constraining vegetation effects on δD_{wax}. Our results indicate that in angiosperm-dominated ecosystems in southeastern Mexico and northern Central America, which include varying proportions of C_3 trees and shrubs and C_4 grasses, $\delta^{13}C_{wax}$ values provide a predictor of vegetation-controlled variability in $\varepsilon_{n-acid/w}$ (Figs. 7 and 8). Vegetation reconstructions in areas with a large proportion of gymnosperms and C_3 grasses will likely prove more complicated, because these groups can differ from angiosperm trees and shrubs in $\varepsilon_{wax/w}$ (McInerney et al., 2011), but are not as distinct in $\delta^{13}C_{wax}$ as C_3 and C_4 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 δD_{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 β-amyrin (Schouten et al., 2007), which could potentially avoid the complication of multiple plant sources of leaf-waxes with differing $\varepsilon_{wax/w}$ values. Overall, our results highlight the importance of constraining vegetation change for interpreting climate signals from δD_{wax} records in the tropics, and demonstrate the value of $\delta^{13}C_{wax}$ 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. δD_{wax} does not correlate with the estimated isotopic composition of precipitation, but $\varepsilon_{wax/w}$ 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 $\varepsilon_{wax/w}$ in some tropical environments, leading to variability on the order of 30–50‰. 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 $\varepsilon_{wax/w}$ from differences in vegetation and microclimates

(Fig. 6). In addition, aridity effects on $\varepsilon_{wax/w}$ in angiosperm-dominated biomes appear to be modulated by vegetation composition, as inferred from $\delta^{13}C_{wax}$ values, with higher $\varepsilon_{wax/w}$ values for a given climate in samples with low $\delta^{13}C_{wax}$ (Fig. 8). Within some ecoregions (Yucatan dry forests, Peten moist forests, Central American Atlantic moist forests), our results suggest a negative correlation between $\delta^{13}C_{n\text{-acid}}$ and $\varepsilon_{n\text{-acid/w}}$ (Fig. 7a). These results suggest that changes in vegetation composition can lead to important differences in $\varepsilon_{n\text{-acid/w}}$, on the order of 30‰, that are independent of climate. Our results do not indicate a clear relationship between $\delta^{13}C_{n\text{-alkane}}$ and $\varepsilon_{n\text{-alkane/w}}$ (Fig. 7b). Aridity and vegetation effects on δD_{wax} should be taken into account for paleoclimate studies that interpret tropical leaf-waxes. In tropical regions, aridity effects generally complement changes in the isotopic composition of meteoric water associated with precipitation amount, leading to increased sensitivity of δD_{wax} to changes in hydroclimate. At the same time aridity effects complicate the reconstruction of the isotopic composition of precipitation. Vegetation effects on δD_{wax} can be partly accounted for with $\delta^{13}C_{wax}$ measurements in tropical environments that are dominated by C_3 angiosperm trees and shrubs and C_4 grasses, but such an approach could prove more complicated in environments with large populations of gymnosperms and/or C_3 grasses.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.gca.2012.09.005.

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