

# Can sedimentary leaf waxes record $D/H$ ratios of continental precipitation? Field, model, and experimental assessments

Juzhi Hou, William J. D'Andrea, Yongsong Huang\*

*Department of Geological Sciences, Brown University, 324 Brook Street, Providence, RI 02912, USA*

Received 18 December 2007; accepted in revised form 23 April 2008; available online 11 May 2008

## Abstract

$D/H$  ratios of leaf waxes ( $\delta D_{\text{wax}}$ ) derived from terrestrial plants and preserved in lake sediments can provide important information on past continental hydrology. Ideally,  $\delta D_{\text{wax}}$  can be used to reconstruct precipitation  $D/H$  ratios ( $\delta D_P$ ) which is a well-established paleoclimate proxy. However, many other factors, such as vegetation and relative humidity (RH), also affect  $\delta D_{\text{wax}}$  variation. How the combination of these factors affects sedimentary  $\delta D_{\text{wax}}$  is unclear. Here, we use a transect of 32 lake surface sediments across large gradients of precipitation, relative humidity, and vegetation composition in the southwestern United States to study the natural factors affecting sedimentary  $\delta D_{\text{wax}}$ .  $\delta D$  values of  $C_{28}$   $n$ -alkanoic acids show significant correlation with  $\delta D_P$  values ( $R^2 = 0.76$ ) with an apparent isotopic enrichment of  $\sim 99 \pm 8\%$ , indicating that sedimentary  $\delta D_{\text{wax}}$  values track overall  $\delta D_P$  variation along the entire transect. Leaf waxes produced by plants grown under controlled conditions (RH = 80%, 60%, 40%) show a small increase in  $D/H$  ratios as RH decreases, consistent with prediction from the Craig-Gordon model. However, the isotopic effect of RH on  $\delta D_{\text{wax}}$  along the natural transect is partially countered by the opposing influence of vegetation changes. The correlation between  $\delta D_{\text{wax}}$  and  $\delta D_P$  values is significantly higher ( $R^2 = 0.84$ ) in the drier portions of the transect than in the wetter regions ( $R^2 = 0.64$ ). This study suggests that  $D/H$  ratios of sedimentary leaf waxes can be used as a proxy for precipitation  $\delta D$  variations, with particularly high fidelity in dry regions, although more studies in other regions will be important to further test this proxy.

© 2008 Elsevier Ltd. All rights reserved.

## 1. INTRODUCTION

Hydrogen and oxygen isotope ratios ( $D/H$  and  $^{18}O/^{16}O$ ) of precipitation are among the most effective and quantitative proxies for past continental climate variability. Ice cores (e.g., Grootes et al., 1993; Petit et al., 1999) and speleothems (e.g., Wang et al., 2001; Yuan et al., 2004) are the most common archives for reconstructing precipitation isotopic ratios. While the fidelity of such reconstructions is exceptional, the global distribution of ice cores and speleothems is very limited; speleothems to regions with carbonate bedrock and adequate precipitation; and ice cores to polar and/or high altitude locations. Continental climate is characterized by large regional variability, requiring re-

records from widely distributed sites to assess the underlying mechanisms of variation. The wide geographic distribution of lakes provides excellent opportunities to reconstruct the isotope ratios of past continental precipitation from sediment cores.

$D/H$  ratios of aquatic lipids in lake sediments have been shown to track  $D/H$  ratios of lake water and precipitation in regions with high precipitation/evaporation ( $P/E$ ) ratios (Sauer et al., 2001; Huang et al., 2002, 2004; Hou et al., 2006). However, in dry and warm regions,  $D/H$  ratios of lake water are strongly affected by evaporation, complicating climatic interpretation of  $\delta D$  records. Temporal variability in  $D/H$  ratios of leaf waxes ( $\delta D_{\text{wax}}$ ) derived from terrestrial plants and preserved in sediments has been attributed to past precipitation and hydrological changes (Liu and Huang, 2005; Schefuß et al., 2005; Pagani et al., 2006; Shuman et al., 2006; Huang et al., 2007). However, in addition to  $\delta D$  of precipitation ( $\delta D_P$ ), other factors, such

\* Corresponding author.

E-mail address: [yongsong\\_huang@brown.edu](mailto:yongsong_huang@brown.edu) (Y. Huang).

as relative humidity (RH) and vegetation composition, may also affect sedimentary  $\delta D_{\text{wax}}$ . Without a full evaluation of these factors, the reliability and limitations of leaf wax  $\delta D$  as a paleohydrological proxy remains uncertain.

Sachse et al. (2004) showed that leaf wax  $D/H$  ratios ( $\delta D_{\text{wax}}$ ) from lake surface sediments track  $\delta D_P$  variation along a 13-lake transect in Europe. However, the European sites used in the study have nearly constant relative humidity (RH = 75–85%), and relatively invariable, forest-dominated vegetation, and therefore cannot be used to assess whether  $\delta D_{\text{wax}}$  tracks  $\delta D_P$  across relative humidity gradients and/or different biomes. While we know that RH has a significant effect on the hydrogen isotopic fractionation of water (Gonfiantini, 1986; Gat, 1996), the effect of RH on the  $\delta D$  values of leaf waxes has not been studied directly. Previous studies reported that the  $D/H$  ratios of cellulose increase dramatically as RH decreases (Yapp and Epstein, 1982; Edwards and Fritz, 1986), but other studies (Gray and Song, 1984; White et al., 1994; Terwilliger and DeNiro, 1995) contradict the claim. Most published studies (e.g., Sauer et al., 2001; Liu and Huang, 2005; Sachse et al., 2006; Pagani et al., 2006) have assumed that  $\delta D_{\text{wax}}$  values are also affected by RH in addition to hydrological factors. Furthermore, recent studies have shown that hydrogen isotope enrichment between precipitation and leaf waxes ( $\epsilon_{\text{wax-p}}$ ) differs greatly among different types of higher plants (Liu and Huang, 2005; Liu et al., 2006; Hou et al., 2007a; Liu and Huang, 2008), with grasses displaying  $\sim 40$  to 50‰ lower  $\delta D$  values than trees. Therefore, in order to confidently reconstruct the variability of precipitation  $\delta D$  using leaf waxes, we must determine how RH and vegetation changes affect the relationship between  $\delta D_{\text{wax}}$  and  $\delta D_P$  values.

In this study, we aim to evaluate the degree to which  $\delta D_{\text{wax}}$  values in lake sediments reflect  $\delta D_P$  variation across large, natural environmental gradients of RH, precipitation and vegetation types. If it is to be used as a proxy for reconstructing past changes in  $\delta D_P$ ,  $\delta D_{\text{wax}}$  must first be shown to track modern precipitation  $\delta D$  values across sites with variable RH and vegetation composition. To achieve this goal, we examined lake surface sediments from a 32-lake transect across large gradients in precipitation, RH, and vegetation cover (from Phoenix, AZ to Houston, TX). We also grew plants under controlled conditions to accurately assess the effects of RH and modeled the isotopic effects under both experimental and field conditions. Our integrated field, experimental, and modeling approaches allow us to objectively assess the fidelity of leaf wax  $\delta D$  values as a proxy for  $\delta D$  variation of past continental precipitation.

## 2. SAMPLES AND METHODS

### 2.1. The southwestern transect

Surface sediment and lake water samples were collected from 32 lakes spanning large precipitation and relative humidity gradients (extending from Phoenix, Arizona to Houston, Texas) in May 2004 (Fig. 1; Table 1). For the purpose of this study (and based on our isotope data described below), the sampling sites along the transect are divided

into two general groups according to their geographic setting and mean annual precipitation: (1) the Interior Plains (Texas and northeastern New Mexico, referred to hereafter as “the Plains”), eastward of 104.5°W, with precipitation >400 mm, and (2) Southern Rocky Mountains and Basin and Range (Arizona and part of New Mexico, hereafter referred to as “Basin and Range”) westward of 104.5°W, with precipitation <400 mm. The lakes from the Plains are mostly naturally occurring, whereas the sampling sites from the Basin and Range represent reservoirs constructed by damming rivers and streams sourced from adjacent higher elevations.

There is a large mean annual precipitation gradient (120–1150 mm) and mean annual relative humidity gradient (36–82%) along the transect from Phoenix to Houston (Fig. 1A and B). Vegetation composition changes dramatically along the transect, from shrubland to grassland to savanna to subtropical forest (Fig. 1C). Mean annual temperature along the transect, excluding the highest elevation sites, ranges from 12 to 21 °C (Table 1). Three of the lakes (Upper Glacial Lake, Lake #1 and Mary’s Lake) are at much higher elevation and have lower mean annual temperature (Table 1) than the rest.

Surface sediments (0–2 cm below the sediment–water interface) were collected from the deepest part of each lake (in reservoirs, deepest sites are typically near the dam site, far from river inlets) using an Ekman Dredge, and were kept cold and in the dark until being frozen at Brown University. Duplicate lake water samples for isotopic analysis were collected using 4 ml glass vials. The vials were filled to capacity and sealed to prevent evaporation.

### 2.2. Growth chamber experiments

We grew 15 trees (representing 6 species) and 11 grasses (representing 4 species) in an EGC<sup>®</sup> GC series temperature and humidity-controlled growth chamber. *Fraxinus americana* L. (white ash), *Carya glabra* (pignut hickory), *Acer rubrum* L. (red maple) and *Quercus rubra* L. (red oak) were collected from the watershed of Blood Pond, Massachusetts. Two coniferous tree species, *Thuja Occidentalis* (eastern white cedar) and *Picea Glauca* (alberta spruce) were obtained from a local nursery. Tree seedlings were 30–50 cm in height before transplantation. The trees were transplanted into 8-inch pots using Canadian Growing Mix 2 (Fafard<sup>®</sup>). Grass seeds, including *Zea Mays* L. (corn), *Dactylis glomerata* L. (orchard grass), *Alopecurus* spp. (foxtail) and *Phleum pratense* L. (timothy weed), were obtained from a local grass seed distributor. The trees were kept in the greenhouse for 20 days and were irrigated with water of known isotopic composition twice daily before being moved into the growth chamber. The grasses were germinated and cultured in the greenhouse for 20 days before being moved to the growth chamber.

The temperature in the growth chamber was kept at 20 °C. RH was set to 80% during the first growing period (June 26–July 27, 2006), 60% for the second growing period (July 28–September 4, 2006), and 40% for the third growing period (September 5–October 11, 2006). A dehumidifier was placed in the growth chamber for experiments with 60%

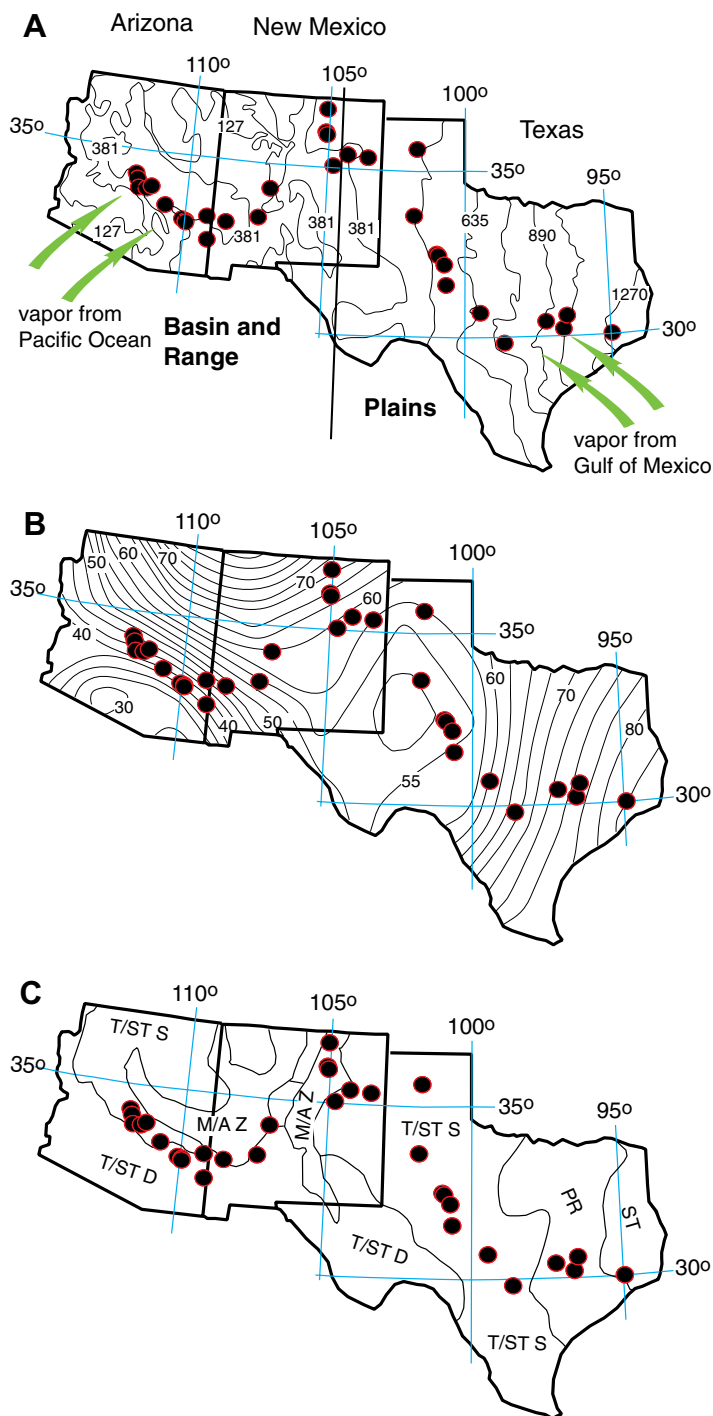


Fig. 1. The modern lake transect in the southwestern United States. (A) Precipitation gradient (mean annual precipitation (mm/yr) from 1961 to 1990; data from NOAA Cooperative Station Normals climate observations); (B) relative humidity (RH) gradient and mean annual relative humidity (%) (data from <http://www.cdc.noaa.gov>); (C) vegetation cover (data from <http://www.nationalatlas.gov>); ST, Subtropical forests; PR, Prairie; T/ST D, Tropical/subtropical Desert; T/ST S, Tropical/subtropical Steppe; M/A Z Mountain with Altitudinal Zonation. The arrows in (A) indicate the moisture source (Tropical Pacific Ocean from west, Gulf of Mexico from east) for precipitation in this region. The lakes are divided into two groups according to the geographic division and mean annual precipitation: Plains (including lakes from Texas and northeastern New Mexico); Basin and range (including lakes from Arizona and part of New Mexico).

and 40% RH settings, in order to help the system maintain stable RH levels. On the last day of each growing period, newly grown leaves from trees were collected for  $\delta D_{\text{wax}}$

analysis. There was no new leaf growth on the *Carya glabra* specimen during the experiment, so we collected preexisting leaves from *Carya glabra* on the last day of each growing

Table 1  
Geographical lake data and measured hydrogen isotopic ratios of lake water and sedimentary leaf waxes

Name	State	Lat (°)	Long (°)	Elv (m)	RH	P (mm)	E (mm)	T (°C)	$\delta D_p$	$\delta D_{\text{Lake}}$	$\delta D_{C_{26}}$	$\delta D_{C_{28}}$	$\delta D_{C_{30}}$
Horseshoe Lake <sup>a</sup>	AZ	33.99	-111.72	613	45.0	368	1615	16.2	-67	-73	-173	-161	-162
Bartlett Lake <sup>a</sup>	AZ	33.84	-111.64	527	44.0	500	1637	13.5	-65	-70	-164	-154	-155
Saguaro Lake <sup>a</sup>	AZ	33.57	-111.54	472	41.0	487	1717	13.2	-64	-49	-151	-150	-151
Apache Lake <sup>a</sup>	AZ	33.57	-111.26	567	41.0	304	1731	21.7	-65	-52	-154	-147	-147
Roosevelt Lake <sup>a</sup>	AZ	33.68	-111.10	655	43.0	234	1711	12.3	-67	-67	-164	-156	-157
San Carlos Reservoir <sup>a</sup>	AZ	33.17	-110.52	728	40.0	348	1665	20.4	-66	-59	-167	-161	-163
Cluff Pond <sup>a</sup>	AZ	32.81	-109.86	1006	37.0	463	1743	18.1	-69	-39	-150	-152	-153
Roper Lake <sup>a</sup>	AZ	32.76	-109.72	948	36.0	512	1734	11.3	-68	-57	-159	-157	-158
Dankworth Pond <sup>a</sup>	AZ	32.71	-109.71	978	36.0	503	1770	11.3	-68	-80	-166	-154	-155
Crescent Lake <sup>a</sup>	AZ	33.00	-109.00	1975	42.5	305	1650	15.1	-82	-88	-184	-176	-177
Animas Ditch <sup>a</sup>	AZ	32.28	-108.89	1268	43.0	305	1715	15.6	-70	4	-142	-141	-142
Caballo Lake <sup>a</sup>	NM	32.91	-108.31	1271	47.0	258	1572	15.7	-71	-66	-159	-160	-162
Elephant Butt Lake <sup>a</sup>	NM	33.17	-107.21	1335	54.0	215	1499	13.1	-71	-78	-183	-176	-178
Lake Escondida <sup>a</sup>	NM	34.11	-106.89	1423	60.0	127	1383	13.9	-74	-80	-174	-174	-175
Upper Glacier	NM	36.82	-105.05	2524	68.0	358	1246	9.7	-96	-94	-212	-215	-219
Lake #1	NM	36.08	-105.04	2420	65.0	396	1246	4.4	-92	-49	-177	-191	-196
Mary's Lake	NM	36.00	-105.00	2155	63.0	—	—	11.0	-88	-27	-191	-186	-188
Santa Rosa Lake <sup>a</sup>	NM	35.01	-104.69	1466	51.0	394	1537	14.4	-75	-83	-192	-190	-193
Conchas Lake <sup>a</sup>	NM	35.39	-104.19	1283	53.0	419	1547	11.9	-73	-21	-168	-158	-159
UTE Lake <sup>a</sup>	NM	35.33	-103.43	1155	53.0	194	1547	12.1	-69	-8	-165	-163	-165
Buffalo Spring Creek	TX	33.52	-101.71	919	51.0	583	1699	15.6	-56	-39	-131	-136	-136
Lake Meredith <sup>a</sup>	TX	35.65	-101.63	616	57.0	455	1654	12.9	-59	-9	-152	-154	-155
Lake Colorado City	TX	32.34	-100.92	631	53.0	338	1589	17.6	-48	-3	-106	-134	-134
Champion Creek	TX	32.29	-100.86	616	53.0	615	1589	16.7	-48	-18	-126	-143	-143
EV Spence Reservoir	TX	32.00	-100.67	607	54.0	483	1589	17.4	-47	-7	-105	-129	-128
Lake Nasworth	TX	31.37	-100.60	565	56.0	483	1663	17.4	-45	9	-108	-139	-140
Lake Junction	TX	30.49	-99.46	579	61.0	702	1461	18.2	-41	-34	-141	-142	-142
Lake Medina <sup>a</sup>	TX	29.57	-98.70	324	67.0	611	1375	18.5	-35	-18	-150	-162	-163
Lake Bastrop	TX	30.18	-97.29	137	73.0	850	1341	19.4	-31	16	-126	-127	-126
Cedar Creek Lake	TX	29.93	-96.73	98	76.0	995	1138	20.4	-30	13	-148	-141	-142
Somerville Lake	TX	30.33	-96.60	73	76.0	1108	1138	19.6	-29	-7	-116	-124	-124
Lake Houston	TX	29.70	-95.17	13	82.0	1150	1175	20.7	-27	-26	-135	-134	-134

<sup>a</sup> Dammed reservoir; Elv, elevation; RH, Mean annual relative humidity; P, mean annual precipitation; E, evaporation; T, temperature;  $\delta D_p$ , mean annual  $\delta D$  of precipitation;  $\delta D_{\text{lake}}$ ,  $\delta D$  of lake water;  $\delta D_{C_{26}}$ ,  $\delta D_{C_{28}}$ ,  $\delta D_{C_{30}}$ ,  $\delta D$  values of  $C_{26}$ ,  $C_{28}$ ,  $C_{30}$  *n*-acids.

period. For grass specimens, we collected whole leaves on the last day of each growing period. All plants were irrigated with 500 ml of water of known hydrogen isotopic ratio ( $\delta D = -49\%$ ) every two days. The irrigation water was stored in Reliance Fold-A-Carrier<sup>®</sup> collapsible containers at 4 °C prior to the experiment to ensure the isotope ratios remained constant.

### 2.3. Analyses of water $\delta D$ values

*D/H* ratios of lake water samples and irrigation water were measured using Thermal Conversion/Elemental Analyzer-Isotopic Ratio monitoring Mass Spectrometry (TC/EA-IRMS). Nine 0.2  $\mu$ l aliquots of each water sample were manually injected into the TC/EA. Standard Mean Ocean Water (SMOW,  $\delta D = 0\%$ ), Greenland Ice Sheet Precipitation (GISP,  $\delta D = -190\%$ ) and Standard Light Antarctic Precipitation (SLAP,  $\delta D = -428\%$ ) were measured between every 10 sample analyses for isotope calibration (Huang et al., 2002). A regression line ( $R^2 = 1$ ) was established between measured and actual  $\delta D$  values of the three isotope standards, and was used for instrument calibration. The standard deviation for water  $\delta D$  values was  $< \pm 1.5\%$ .

### 2.4. Analyses of sediment and plant samples

All sediment and leaf samples were freeze-dried. Sediment samples were extracted using an Accelerated Solvent Extractor (ASE200, Dionex) with dichloromethane:methanol (2:1 v/v) at 150 °C and 1200 psi for three 15-min cycles. Approximately 5 g of each dry sample were extracted. Leaves were soaked in dichloromethane:methanol (2:1 v/v) and ultrasonicated for 15 min to extract lipids. This extraction procedure was repeated three times. The total lipid extract obtained from sediment and plant leaves was separated into neutral and acid fractions using Supelco<sup>®</sup> Supelclean<sup>™</sup> LC-NH<sub>2</sub> SPE column and dichloromethane:isopropyl alcohol (2:1 v/v) and ether with 4% acetic acid (v/v), respectively, as eluents. Acid fractions were methylated with 5% anhydrous HCl in methanol at 60 °C for 12 h. Hydroxyl acids were removed by eluting the methylated samples through silica gel columns with hexane, and the fatty acid methyl esters were collected by elution with dichloromethane.

Quantification and identification of compounds was performed by Gas Chromatography-Flame Ionization Detection (GC-FID) and Gas Chromatography-Mass Spectrometry (GC-MS) (HP 6890, Agilent). An HP 6890

GC interfaced to a Finnigan DeltaPlus XL stable isotope spectrometer through a high-temperature pyrolysis reactor was used for hydrogen isotopic analysis (Huang et al., 2004). The compounds separated by the GC were pyrolyzed to  $H_2$  and  $CO$  at  $1445^\circ C$ . A tank of ultra high purity hydrogen gas with known  $\delta D$  values was used as the isotope standard during the measurements. The  $H_3^+$  factor was determined daily prior to sample analysis (average value was  $2.5 \pm 0.04$  (1 sigma) during the course of this study). The accuracy for the instrument was routinely checked by an injection of laboratory isotopic standards ( $C_{16}$ ,  $C_{18}$ ,  $C_{22}$ ,  $C_{24}$  *n*-alkanoic acid methyl esters) between every six measurements. The precision ( $1\sigma$ ) for the four laboratory standards was  $< \pm 2\text{‰}$  throughout the entire process. The precision ( $1\sigma$ ) for triplicate analyses of all samples was  $< \pm 2\text{‰}$ .  $\delta D$  values obtained from individual alkanolic acids (as methyl esters) were corrected by mathematically removing the isotopic contributions from added groups before reporting. The  $\delta D$  value of the added methyl group was determined by acidifying and then methylating (along with the samples) the disodium salt of succinic acid with a predetermined  $\delta D$  value (using TC/EA-IRMS) (Huang et al., 2002).

### 3. RESULTS

#### 3.1. Precipitation and lake water $\delta D$

The  $D/H$  ratio of precipitation ( $\delta D_P$ , weighted average values based on monthly precipitation amount) at the loca-

tion of each lake along the SW transect was calculated from the Online Isotopes in Precipitation Calculator (OIPC, Bowen and Revenaugh, 2003). The OIPC estimates the  $\delta D_P$  at a given site by combining an empirical model for isotopic trends related to latitude and altitude with detrended interpolation based on the isotope data from stations in Global Network of Isotopes in Precipitation (Bowen and Revenaugh, 2003).  $\delta D_P$  and lake water  $\delta D$  values ( $\delta D_{lake}$ ) show similar variation along the transect (Fig. 2; Table 1). Higher  $\delta D_P$  and  $\delta D_{lake}$  values occur in the eastern Plains and western Basin and Range. Lower  $\delta D_P$  and  $\delta D_{lake}$  values are found in the central part of the transect (Southern Rocky Mountains). Lake water samples from the Plains show greater deuterium enrichment relative to precipitation than the lakes from Basin and Range, which have  $\delta D_{lake}$  values that are very similar to that of precipitation (Fig. 2).

#### 3.2. $\delta D$ of sedimentary leaf waxes

We focus on  $C_{26}$ ,  $C_{28}$ , and  $C_{30}$  *n*-alkanoic acids, in the lake surface sediment samples as representative compounds for modern leaf waxes. We avoided use of long chain *n*-alkanes as terrestrial plant biomarkers to minimize the possibility of contamination from fossil fuel products (most lakes in the region are heavily used by recreational boaters and likely receive atmospheric input of combustion products). The hydrogen isotope ratios of the  $C_{26}$ ,  $C_{28}$ , and  $C_{30}$  *n*-alkanoic acids are strongly inter-correlated ( $R^2 = 1.0$  for  $C_{28}$  and  $C_{30}$ ,  $R^2 = 0.82$  for  $C_{28}$  and  $C_{26}$ ;  $n = 32$ ,  $p < 0.001$ ). Therefore, in the following discussion

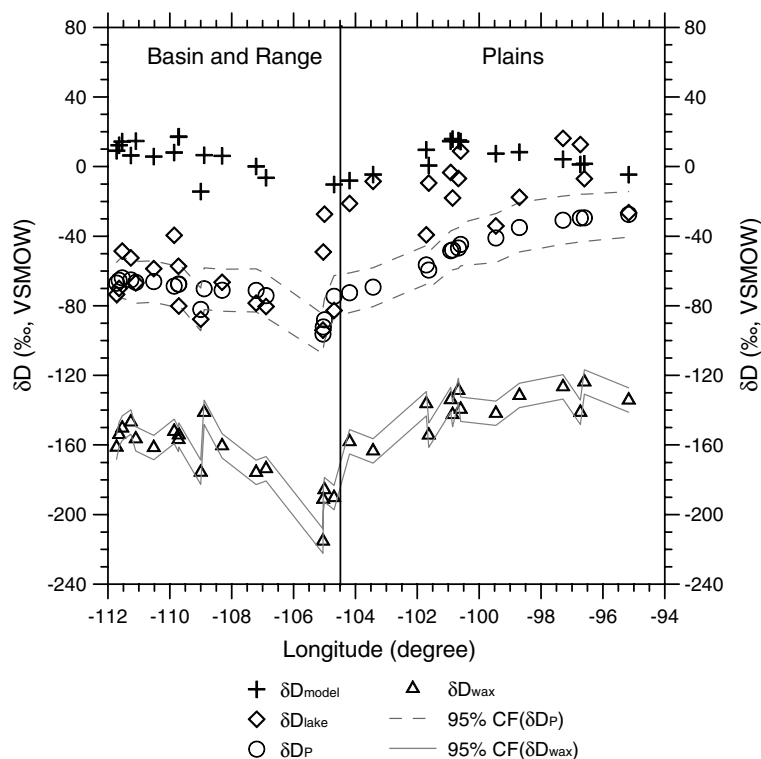


Fig. 2. Hydrogen isotope data for the SW transect:  $\delta D_{wax}$  = measured  $D/H$  ratios of sedimentary leaf waxes;  $\delta D_P$  =  $D/H$  ratios of precipitation calculated from OIPC;  $\delta D_{lake}$  = measured  $D/H$  ratios of lake water;  $\delta D_{model}$  = modeled  $D/H$  ratios of lake water; 95% CF ( $\delta D_P$ ) = 95% confidence interval for  $\delta D_P$ ; 95% CF ( $\delta D_{wax}$ ) = 95% confidence interval for  $\delta D_{wax}$ .

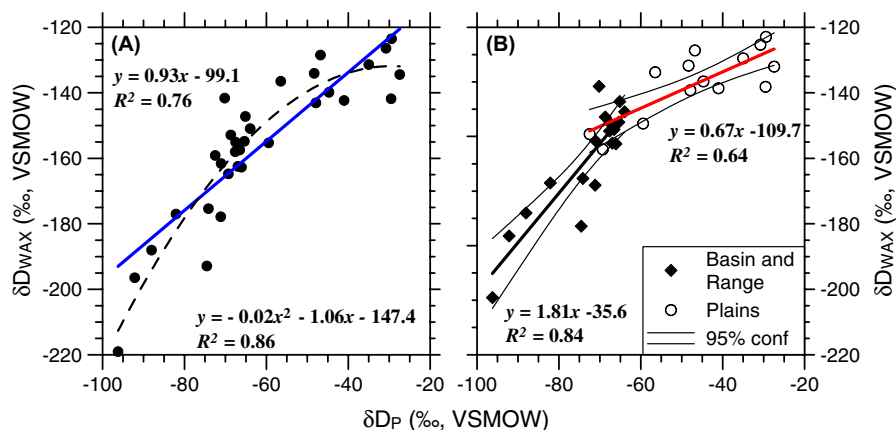


Fig. 3. Correlation between  $\delta D_{\text{wax}}$  and  $\delta D_P$  for the lake surface sediment samples along the SW transect. (A) Correlation for the entire data set showing both linear and polynomial regressions. (B) Correlation for data from distinct geographical provinces (Basin and Range, and Plains). 95% confidence intervals are shown.

$\delta D$  values of  $C_{28}$  *n*-alkanoic acid (hereafter referred to as  $\delta D_{\text{wax}}$ ) are taken as representative of integrated  $\delta D$  values of terrestrial leaf waxes from plants around the lakes.

Sedimentary  $\delta D_{\text{wax}}$  values show significant correlation with  $\delta D_P$  values along the entire transect ( $R^2 = 0.76$ ,  $y = 0.93x - 99.1$ ,  $n = 32$ ,  $p < 0.001$ , residual mean squares (RMS) = 122.3; Figs. 2 and 3A). However,  $\delta D_{\text{wax}}$  and  $\delta D_P$  correlate much more strongly within the Basin and Range ( $R^2 = 0.84$ ,  $y = 1.69x - 42$ ,  $n = 18$ ,  $p < 0.001$ , RMS = 70.2) than they do for the entire transect ( $R^2 = 0.76$ ) or the Great Plains lakes ( $R^2 = 0.64$ ,  $y = 0.67x - 109.7$ ,  $n = 14$ ,  $p < 0.001$ , RMS = 60.6; Fig. 3B). Furthermore, a second-order polynomial regression through the entire transect provides an overall better fit for the  $\delta D_{\text{wax}}$  and  $\delta D_P$  data ( $R^2 = 0.86$ ,  $y = -0.02x^2 - 1.06x - 147.4$ ,  $n = 32$ ,  $p < 0.001$ , RMS = 73.0, Fig. 3A) than does the linear regression. The correlation between  $\delta D_{\text{wax}}$  and  $\delta D_{\text{lake}}$  ( $R^2 = 0.46$ ,  $n = 32$ ,  $p < 0.001$ , RMS = 280.3) is not as strong as that between  $\delta D_{\text{wax}}$  and  $\delta D_P$ .

### 3.3. Leaf wax $\delta D$ for plants from growth chamber

$\delta D$  values of  $C_{28}$  *n*-alkanoic acids are strongly correlated with  $C_{30}$  *n*-alkanoic acids for the growth chamber samples ( $R^2 = 0.86$ ). As with the transect samples,  $\delta D$  values of  $C_{26}$  *n*-alkanoic acids show weaker correlation with  $C_{28}$  and  $C_{30}$  *n*-acids ( $R^2 = 0.61$  and  $0.44$ , respectively). This observation suggests that  $C_{28}$  or  $C_{30}$  *n*-alkanoic acids may be better choices for representative leaf wax compounds in sediment samples. It should also be noted that for certain growth chamber species,  $\delta D$  values of the three *n*-alkanoic acids show relatively large variation within and among specimens (Table 2), highlighting the natural variability of individual plants. The calculated leaf wax hydrogen isotopic enrichment values from source water ( $\epsilon_{\text{wax-water}}$ ) for the growth chamber samples show an approximately normal distribution (Fig. 4). The average  $\epsilon_{\text{wax-water}}$  values of all plants grown at 80%, 60% and 40% RH are  $-114\text{‰}$ ,  $-111.5\text{‰}$ , and  $-107\text{‰}$ , respectively, depicting minor deuterium enrichment as RH decreases.

## 4. DISCUSSION

### 4.1. Controls on $\delta D$ of precipitation and lake water

The patterns of  $\delta D_P$  variation along the transect in the southwestern United States result from differing water vapor sources and the continental isotope effect (Gat, 1996). The water vapor for precipitation in the Texas Plains mainly originates from the Gulf of Mexico, while the precipitation in the Basin and Range mainly derives from the eastern Pacific Ocean, off coastal Mexico (Fig. 1A; Adams and Comrie, 1997). Due to Rayleigh distillation, as water vapor moves inland the heavier isotopes are preferentially precipitated, resulting in isotopically lighter precipitation with increasing distance from the source. This helps explain the observation that higher  $\delta D_P$  values are found at either end of the transect, and lower  $\delta D_P$  values are found in the central portion of the transect. The lowest  $\delta D_P$  values are found at Mary's Lake,  $\sim 105^\circ\text{W}$  (Fig. 2). This site is the farthest from both vapor sources and is at high elevation (2155 m) (Table 1).

The  $\delta D_{\text{lake}}$  values along the transect are controlled not only by  $\delta D_P$ , but also by the degree of evaporation and local hydrological conditions. Evaporation causes lake water to become isotopically enriched in D and  $^{18}\text{O}$ , relative to precipitation (Gat, 1996). The evaporative effect explains the observation that  $\delta D_{\text{lake}}$  values are higher than  $\delta D_P$  values within the Great Plains. However, the lake waters from the Basin and Range do not show isotopic enrichment with respect to precipitation ( $\delta D$  values of lakes fall within 95% confidence intervals of estimated  $\delta D$  values for local precipitation; Fig. 2). This could be due to the fact that all Basin and Range lakes in this study are reservoirs, representing dammed rivers that originate in adjacent mountains (Table 1). The rivers are fed by precipitation within their catchments, including melt water from wintertime precipitation (e.g., snow) in the mountains. The water delivered to the reservoirs by river input is thus depleted in deuterium relative to precipitation falling directly onto the reservoirs (i.e., our estimated  $\delta D_P$ ). Subsequent evaporative enrichment in

Table 2

$\delta$ D values and mean values  $\pm$  standard deviation of long chain n-alkanoic acids from individual species cultured in growth chamber

Species	Life form	RH = 80%			RH = 60%			RH = 40%		
		C26	C28	C30	C26	C28	C30	C26	C28	C30
<i>Fraxinus americana</i> L.	tree	-156	-142	-136	-	-141	-129	-136	-139	-133
<i>Fraxinus americana</i> L.	tree	-160	-162	-160	-	-145	-152	-134	-156	-141
<i>Fraxinus americana</i> L.	tree	-163	-152	-155	-	-135	-136	-132	-139	-139
<i>Fraxinus americana</i> L.	tree	-143	-134	-132	-	-134	-121	-	-121	-125
<i>F. americana</i> mean		-156 $\pm$ 9	-147 $\pm$ 13	-146 $\pm$ 14	-	-139 $\pm$ 5	-135 $\pm$ 13	-134 $\pm$ 2	-139 $\pm$ 14	-134 $\pm$ 8
<i>Carya glabra</i>	tree	-157	-153	-142	-	-152	-144	-	-152	-140
<i>Acer rubrum</i> L.	tree	-171	-167	-160	-149	-157	-148	-138	-144	-133
<i>Acer rubrum</i> L.	tree	-169	-163	-151	-138	-143	-135	-141	-146	-135
<i>Acer rubrum</i> L.	tree	-162	-154	-147	-128	-136	-136	-139	-150	-144
<i>A. rubrum</i> mean		-168 $\pm$ 5	-161 $\pm$ 7	-153 $\pm$ 7	-138 $\pm$ 11	-145 $\pm$ 11	-140 $\pm$ 7	-139 $\pm$ 1	-146 $\pm$ 3	-137 $\pm$ 6
<i>Quercus rubra</i> L.	tree	-183	-171	-166	-169	-177	-169	-167	-173	-166
<i>Quercus rubra</i> L.	tree	-170	-163	-159	-146	-159	-159	-145	-153	-153
<i>Quercus rubra</i> L.	tree	-155	-165	-170	-150	-172	-178	-125	-167	-173
<i>Q. rubra</i> mean		-169 $\pm$ 14	-167 $\pm$ 4	-165 $\pm$ 5	-155 $\pm$ 12	-170 $\pm$ 9	-168 $\pm$ 9	-145 $\pm$ 21	-164 $\pm$ 11	-164 $\pm$ 10
<i>Thuja occidentalis</i>	tree	-154	-134	-128	-141	-136	-125	-140	-139	-133
<i>Thuja occidentalis</i>	tree	-138	-133	-134	-143	-140	-131	-140	-141	-136
<i>T. occidentalis</i> mean		-146 $\pm$ 11	-134 $\pm$ 1	-131 $\pm$ 4	-142 $\pm$ 1	-138 $\pm$ 3	-128 $\pm$ 4	-140 $\pm$ 0	-140 $\pm$ 1	-135 $\pm$ 2
<i>Picea glauca</i>	tree	-158	-141	-139	-152	-138	-138	-156	-147	-153
<i>Picea glauca</i>	tree	-161	-146	-145	-156	-141	-139	-151	-144	-144
<i>P. glauca</i> mean		-159 $\pm$ 2	-143 $\pm$ 4	-142 $\pm$ 4	-154 $\pm$ 3	-139 $\pm$ 2	-139 $\pm$ 1	-154 $\pm$ 4	-145 $\pm$ 3	-149 $\pm$ 6
<i>Zea mays</i> L.	grass	-165	-156	-160	-162	-154	-151	-158	-157	-166
<i>Zea mays</i> L.	grass	-164	-154	-146	-159	-159	-164	-155	-164	-163
<i>Z. Mays</i> mean		-164 $\pm$ 0	-155 $\pm$ 2	-153 $\pm$ 10	-161 $\pm$ 2	-156 $\pm$ 3	-157 $\pm$ 9	-157 $\pm$ 2	-160 $\pm$ 5	-165 $\pm$ 3
<i>Dactylis glomerata</i> L.	grass	-208	-190	-178	-	-192	-189	-	-188	-183
<i>Dactylis glomerata</i> L.	grass	-	-191	-189	-	-194	-183	-	-174	-170
<i>D. glomerata</i> mean		-208 $\pm$ 0	-190 $\pm$ 1	-184 $\pm$ 7	-	-193 $\pm$ 1	-186 $\pm$ 4	-	-181 $\pm$ 10	-177 $\pm$ 9
<i>Alopecurus</i> spp.	grass	-162	-158	-147	-170	-167	-160	-	-	-
<i>Alopecurus</i> spp.	grass	-169	-162	-149	-161	-164	-159	-	-	-
<i>Alopecurus</i> spp.	grass	-165	-160	-157	-157	-163	-159	-	-	-
<i>Alopecurus</i> mean		-166 $\pm$ 4	-160 $\pm$ 2	-151 $\pm$ 5	-163 $\pm$ 6	-165 $\pm$ 2	-159 $\pm$ 1	-	-	-
<i>Phleum pratense</i> L.	grass	-166	-160	-150	-166	-159	-143	-147	-144	-135
<i>Phleum pratense</i> L.	grass	-166	-152	-150	-159	-158	-158	-141	-138	-131
<i>Phleum pratense</i> L.	grass	-169	-160	-132	-158	-152	-130	-146	-144	-136
<i>P. pratense</i> mean		-167 $\pm$ 2	-157 $\pm$ 4	-144 $\pm$ 10	-161 $\pm$ 4	-156 $\pm$ 4	-144 $\pm$ 14	-145 $\pm$ 3	-142 $\pm$ 4	-134 $\pm$ 3

D/H ratios of leaf waxes and continental precipitation

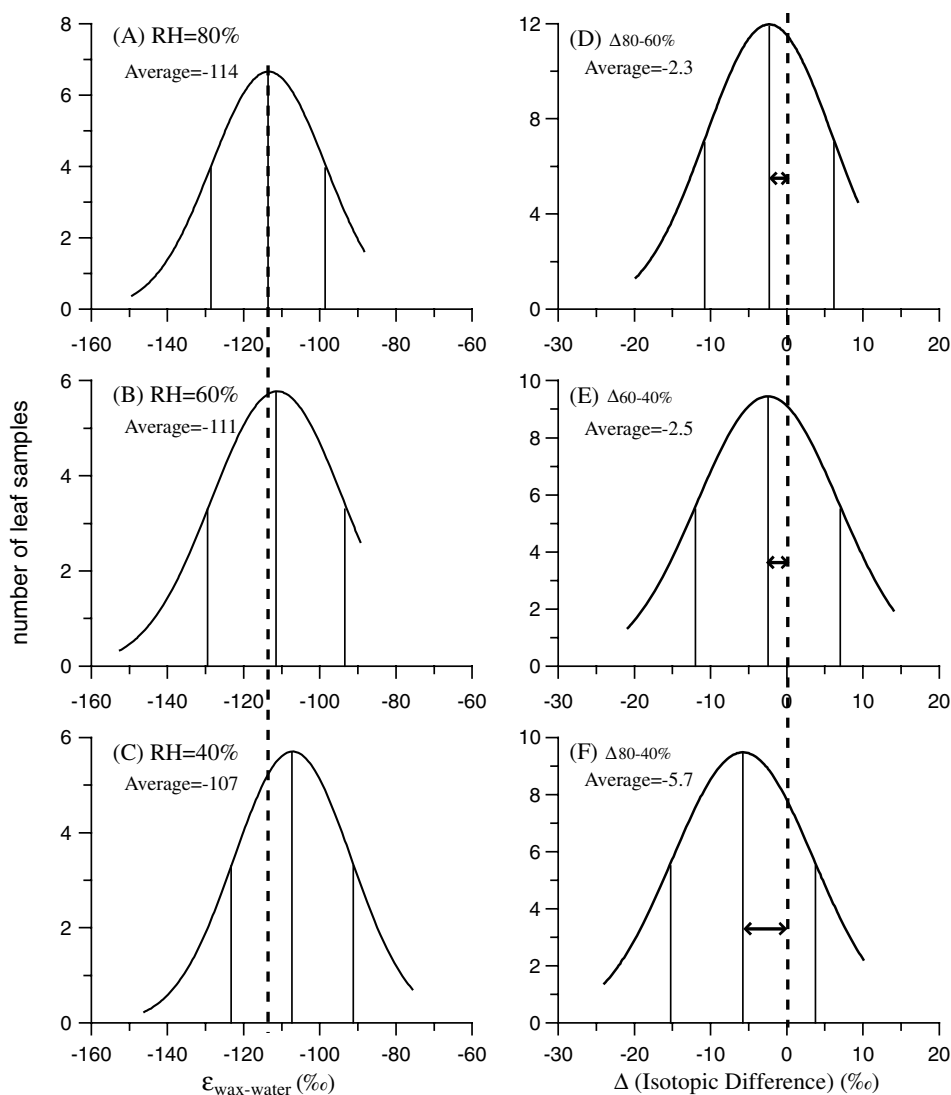


Fig. 4. Differences in the apparent isotopic enrichment of leaf waxes for plants grown in growth chamber at three humidity levels. The left-hand plots depict changes in enrichment factor ( $\epsilon_{\text{wax-water}}$ ) as RH changes from 80% to 40% (A: RH = 80%; B: RH = 60%; C: RH = 40%). The vertical dashed line represents the average  $\epsilon_{\text{wax-water}}$  value at RH = 80%. The righthand plots show hydrogen isotopic differences ( $\Delta$  values) between different RH levels. ( $D = \Delta D_{80-60\%}$ ;  $E = \Delta D_{60-40\%}$ ;  $F = \Delta D_{80-40\%}$ ). The vertical dashed line represents the  $\Delta = 0$ .

the reservoirs might counter the influence of lower  $\delta D$  values of the winter snow precipitation, and help explain the similarity between  $\delta D_{\text{lake}}$  and the estimated  $\delta D_P$  in the Basin and Range (Fig. 2).

As a further test of our explanation for lake water  $\delta D$  variations described above, we used the Craig-Gordon model to calculate the isotope ratios of lake water at steady state (Craig and Gordon, 1965; Gat, 1996), by assuming that all lake waters had the initial  $\delta D$  values of the precipitation prior to evaporative enrichment.

$$P = Q + E \quad (1)$$

$$P\delta_P = Q\delta_Q + E\delta_E = Q\delta_L + E\delta_E \quad (2)$$

where  $P$ ,  $Q$  and  $E$  represent the amount of precipitation, outflow, and evaporation, respectively, and  $\delta_P$ ,  $\delta_Q$ , and  $\delta_E$  are their associated hydrogen isotope ratios.  $\delta_L$  is the  $D/H$

ratio of lake water (note  $\delta_L = \delta_Q$ ). We adopted the modified Craig-Gordon model described by Gibson and Edwards (2002),

$$\delta_E = \frac{\alpha^* \delta_L - \text{RH} \times \delta_A - (\epsilon^* + \epsilon_K)}{1 - \text{RH} + 10^{-3} \epsilon_K} \quad (3)$$

where  $\epsilon^*$  is the equilibrium isotopic enrichment factor [ $\epsilon^* = 1158.8(T^3/10^9) - 1620.1(T^2/10^6) + 794.84(T/10^3) - 161.04 + 2.9992(10^9/T^3)$ ,  $T$  is temperature (K) (Gibson and Edwards, 2002)].  $\alpha^*$  is the equilibrium isotopic fractionation factor,  $\alpha^* = (1 + 10^{-3}\epsilon^*)$ .  $\epsilon_K$  is the kinetic isotopic enrichment factor [ $\epsilon_K = 12.5 \times (1 - \text{RH})$ ].  $\delta_A$  is the  $D/H$  ratio of atmospheric water vapor, estimated assuming equilibrium with flux-weighted precipitation ( $\delta_A = \alpha^* \delta_P - \epsilon^*$ ; Gibson and Edwards, 2002). RH is relative humidity, obtained from the NOAA Climate Diagnostic Center (<http://>

[www.cdc.noaa.gov/](http://www.cdc.noaa.gov/)) for individual study sites. Mean annual precipitation and pan evaporation data are from the State Climatological Summaries (<http://nndc.noaa.gov/ge-tsiteref>) for each site (Table 1). Pan evaporation data for lakes in Texas were also obtained from Texas Evaporation/Precipitation website (<http://hyper20.twdb.state.tx.us/Evaporation/evap.html>), and evaporation from lake surfaces has been estimated as 70% of that measured from evaporation pan (e.g., Gonfiantini, 1986; Gibson and Edwards, 1996; Gibson et al., 1998; Smith and Freeman, 2006).

Rearranging Eqs. (1)–(3) yields

$$\delta_L = \left( \frac{P}{E} \delta_P + \frac{\text{RH} \times \delta_A + \varepsilon^* + \varepsilon_K}{A^*} \right) / \left( \frac{\alpha^*}{A^*} + \frac{P}{E} - 1 \right) \quad (4)$$

where  $A^* = 1 - \text{RH} + 10^{-3} \varepsilon_K$ . We can obtain modeled  $D/H$  ratios of lake water ( $\delta D_{\text{model}} = \delta_L$ ) from Eq. (4).  $\delta D_{\text{model}}$  for all the lakes along the SW transect are plotted in Fig. 2. For lakes in the Plains, the  $\delta D_{\text{model}}$  values are similar to the measured  $\delta D_{\text{lake}}$  values, consistent with our explanation that these lakes are largely recharged by local precipitation. However, for most lakes/reservoirs in Basin and Range, the  $\delta D_{\text{model}}$  values are markedly higher than the measured  $\delta D_{\text{lake}}$  values. The data are again consistent with our explanation that the Basin and Range reservoirs are mainly recharged by rivers rather than by local precipitation. The  $\delta D$  values of river water (sourced at colder temperatures and higher elevations than the reservoirs they recharge) are expected to be lower than the estimated local  $\delta D_P$ , resulting in the correspondingly lower  $\delta D$  values for the observed lake waters than the modeled lake waters (the local  $\delta D_P$  is used to represent the initial or un-evaporated lake water in the model calculation) (Fig. 2).

#### 4.2. Sedimentary $\delta D_{\text{wax}}$ as a proxy for $\delta D_P$ : insights from the natural transect

Sedimentary  $\delta D_{\text{wax}}$  values are strongly correlated ( $R^2 = 0.76$ ) with mean annual  $\delta D_P$  values along the entire transect (Fig. 3A). Similar correlation ( $R^2 = 0.77$ ) is observed between  $\delta D_{\text{wax}}$  values and mean summer (April–October)  $\delta D_P$  values calculated from OIPC. Water is the

ultimate source for hydrogen in plant tissues, and organic matter derived from terrestrial plants should record the isotopic ratio of soil water, which is ultimately recharged by precipitation. The  $\delta D$  values of sedimentary leaf waxes could thus be expected to correlate with  $\delta D_P$  at a large scale. However, there are a number of other factors that could significantly impact the relative strength of this correlation. Two prominent factors are: (1) changes in evaporative isotopic enrichment at different RH due to evaporation from soil and leaf surfaces, and (2) variations in the apparent hydrogen fractionation among different vegetation types. Both factors could potentially impact  $\delta D_{\text{wax}}$  so strongly that reconstruction of  $\delta D_P$  from  $\delta D_{\text{wax}}$  would become too complicated and inaccurate for paleoclimate applications.

Surprisingly, however, even with the dramatic changes in RH and vegetation type along the lake surface sediment transect, the average apparent isotopic enrichments between local precipitation and sedimentary leaf waxes ( $\varepsilon_{\text{wax-p}}$ ) do not differ significantly over the entire transect ( $-98.8 \pm 7.8\text{‰}$ , Fig. 5). This finding would suggest that the combined impact of environmental factors (e.g. vegetation cover, RH) is relatively small. Moreover, the stronger correlation between  $\delta D_{\text{wax}}$  and  $\delta D_P$  in dry regions ( $R^2 = 0.84$ ) relative to the entire transect or the more humid regions (Fig. 3B) may imply that  $\delta D_{\text{wax}}$  tracks  $\delta D_P$  variation with greater fidelity in dry regions. We will provide a detailed interpretation for this observation in Sections 4.3 and 4.4 after presenting data from growth chamber and model calculations.

Three lakes of the transect (Animas Ditch (AD), Upper Glacial Lake (UGL), and Santa Rosa Lake (SRL)) stand out as having significantly different  $\varepsilon_{\text{wax-p}}$  (Fig. 5). These exceptions can be understood in the context of their local geographical and hydrological settings. Animas Ditch is located adjacent to a major highway, within an extensive playa (dry lake bed).  $\delta D$  values measured for Animas Ditch ( $\delta D_{\text{lake}} = 4\text{‰}$ ), a small remnant pond, are much higher than other lakes in Arizona ( $\delta D_{\text{lake}} \sim -40$  to  $110\text{‰}$ ), indicating the influence of very strong evaporation. Therefore, exceptionally strong evaporation of soil water at Animas Ditch could explain the reduced  $\varepsilon_{\text{wax-p}}$ . Upper Glacial Lake

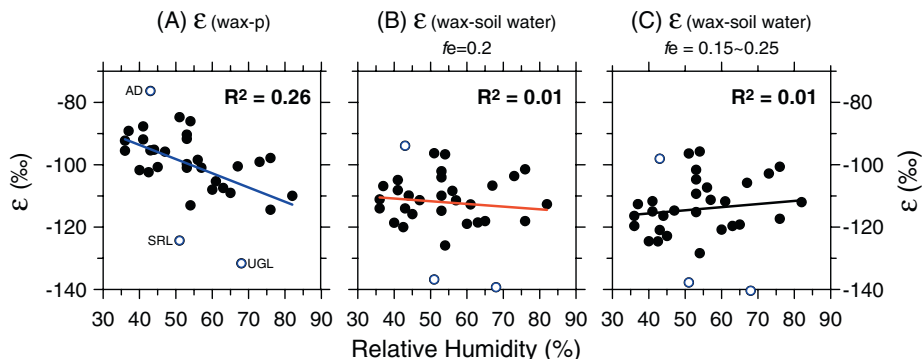


Fig. 5. The correlation between RH and apparent isotopic enrichment of sedimentary leaf waxes relative to (A) precipitation ( $\varepsilon_{\text{wax-p}}$ ), (B) modeled soil water ( $\varepsilon_{\text{wax-soil water}}$ ) assuming  $f_e = 15\%$ , (C) modeled soil water ( $\varepsilon_{\text{wax-soil water}}$ ) assuming a linear east to west gradient of  $f_e$  from 15% to 25% for our study sites.  $\varepsilon_{\text{wax-soil water}} = [(D/H)_{\text{wax}}/(D/H)_{\text{soil water}} - 1] \times 1000\text{‰}$ . Three of the lakes, AD (Animas Ditch), UGL (Upper Glacial Lake) and SRL (Santa Rosa Lake) show different  $\varepsilon_{\text{wax-p}}$  values (see text for discussion).

receives water from nearby glaciers, and both precipitation and lake water show low  $\delta D$  values ( $-96$  and  $-94\text{‰}$ , respectively). Glacial melt water and snowmelt in the catchment of this high-elevation lake may represent a large proportion of the soil water recharge. Plants utilizing this soil water would produce leaf waxes with correspondingly low  $\delta D$  values. Santa Rosa Lake is a recently constructed man-made reservoir, completed in 1981. Disturbance associated with construction activities may complicate the  $\delta D_{\text{wax}}$  signal of surface sediment. Reservoirs are dammed rivers. It is therefore possible that a small fraction of the leaf waxes in sediments might have been contributed from fluvial input. However, we collected all of our sediment samples from the deepest part of the reservoirs (dammed side), minimizing the fluvial components. Therefore, the leaf waxes extracted from the lake sediments in this study should reflect primarily the local vegetation inputs.

### 4.3. The effect of RH on $\varepsilon_{\text{wax-p}}$

#### 4.3.1. RH and $\varepsilon_{\text{wax-p}}$ variation along the transect

The apparent isotopic enrichment between local precipitation and sedimentary leaf waxes ( $\varepsilon_{\text{wax-p}}$ ) is plotted against RH in Fig. 5A. There appears to be a small decrease in absolute  $\varepsilon_{\text{wax-p}}$  values as RH decreases, although the correlation between RH and  $\varepsilon_{\text{wax-p}}$  is relatively weak ( $R^2 = 0.28$ ). To better understand whether soil evaporation or plant evapotranspiration causes the RH effect on  $\varepsilon_{\text{wax-p}}$ , we estimated the  $D/H$  ratios of soil water ( $\delta D_{\text{soil}}$ ) at all sites using the modified Craig-Gordon model. We then took the fraction of evaporative water into consideration and adopted a binary isotope model (Smith and Freeman, 2006):

$$\delta D_{\text{soil}} = (1 - f_e) \times \delta D_p + f_e \times \delta D_{\text{model}} \quad (5)$$

where  $\delta D_{\text{model}}$  (the modeled  $\delta D$  ratios of soil water subjected to evaporation) is calculated from Eq. (4);  $f_e$  is the fraction of soil water subjected to evaporation in bulk soil water. Because accurate measurements for  $f_e$  are not available for all sites along the SW transect,  $f_e$  was set to 20% according to Smith and Freeman (2006) who modeled soil evaporation over a similar relative humidity range. The estimated  $\varepsilon_{\text{wax-soil}}$  shows no correlation to RH ( $R^2 = 0.01$ , Fig. 5B). However, because the assumption of constant  $f_e$  along the SW transect might be too simplistic ( $f_e$  could be relatively higher in dry regions, such as in Basin and Range, than in wet regions, such as the Plains), we also modeled the soil water under the condition that  $f_e$  increases linearly from 15–25% for our sites from east to west along the transect. The new model results (shown in Fig. 5C) still show no significant correlation between RH and  $\varepsilon_{\text{wax-soil}}$  ( $R^2 = 0.01$ ), suggesting that the small effect of RH on  $\varepsilon_{\text{wax-p}}$  in our transect samples may be primarily due to soil evaporation rather than plant transpiration.

#### 4.3.2. RH and $\varepsilon_{\text{wax-p}}$ : growth chamber experiments

Natural samples are inevitably influenced by many complicated environmental factors that may affect  $\delta D_{\text{wax}}$ , and varying levels of inaccuracy exist in estimates of precipita-

tion  $\delta D$ . To examine the effect of RH on  $\delta D_{\text{wax}}$  while minimizing the complications inherent to natural systems, we conducted controlled plant growth experiments. The plants selected for the growth chamber experiments include broad leaf trees, conifers,  $C_3$  grasses and  $C_4$  grasses, and represent the typical plant types found in the catchments of lakes in the southwestern United States. The results of the growth experiments indicate that the effect of RH on the apparent hydrogen isotopic enrichment between irrigation water and leaf waxes ( $\varepsilon_{\text{wax-water}}$ ) is very small (Fig. 4). The average  $\varepsilon_{\text{wax-water}}$  values for plants grown at 80, 60 and 40% RH are  $-114 \pm 14$ ,  $-111.5 \pm 17$ , and  $-107 \pm 15\text{‰}$ , respectively. Therefore, the  $\varepsilon_{\text{wax-water}}$  values only increase by  $\sim 7\text{‰}$  over a 40% change in RH.

In order to quantify and to better understand the minor effect of RH on isotopic fractionation between plant leaf waxes and water, we modeled plant leaf water  $D/H$  ratios at different humidity levels using the modified Craig-Gordon model of Flanagan et al. (1991) and Roden et al. (2000), which considers the leaf boundary layer and diffusion through stomata (Eq. (6)). We also applied the same isotopic model to simulated plants around the lakes along the SW transect, to examine the RH effect on the isotopic composition of leaf waxes in the lake surface sediment.

$$R_{\text{leaf}} = \alpha^* \left[ \alpha_K R_{\text{soil}} \left( \frac{e_i - e_s}{e_i} \right) + \alpha_{KB} R_{\text{soil}} \left( \frac{e_s - e_a}{e_i} \right) + R_a \left( \frac{e_a}{e_i} \right) \right] \quad (6)$$

where  $R_{\text{leaf}}$ ,  $R_{\text{soil}}$ ,  $R_a$  refer to the  $D/H$  ratios of leaf water subject to evaporation, soil water, atmospheric water vapor, respectively.  $\alpha^*$  is the equilibrium fractionation factor;  $\alpha_K$  is kinetic fractionation associated with diffusion in air, and  $\alpha_{KB}$  is the kinetic fractionation associated with diffusion through the boundary layer ( $\alpha_{KB} = (\alpha_K)^{2/3}$ ; Roden et al., 2000).  $e_i$ ,  $e_s$ , and  $e_a$  represent the vapor pressures of intercellular air spaces in the leaf, leaf surface, and the atmosphere, respectively. These vapor pressures were estimated using stomatal conductance and transpiration rate (Ball, 1987; Roden et al., 2000). Eq. (6) estimates the leaf water  $\delta D$  values at the site of evaporation. Because not all of the water within the leaf is subjected to evaporation, a correction is needed for bulk leaf water isotopic ratios ( $\delta_{\text{bulk}}$ ).

$$\delta_{\text{bulk}} = \delta_{\text{leaf}} f_l + \delta_{\text{soil}} (1 - f_l) \quad (7)$$

where  $f_l$  is the proportion of the leaf water subjected to evaporative enrichment and ranges from 13% to 33% (Flanagan et al., 1991); because the accurate measurements of  $f_l$  are not available,  $f_l$  is set at 15% for all sites in this study in order to provide the most conservative estimate.

The model results for growth chamber plants and field plants are plotted in Fig. 6A and B, respectively. To facilitate comparison between the RH effect on the  $D/H$  ratios of soil water and leaf water, the precipitation for plants from the field is set to  $-49\text{‰}$ , the same as the irrigation water used in the growth chamber experiments. To be consistent with the plants in the growth chamber, the temperature in the field model was set to 20 °C. However, there are differences between the growth chamber model and the field model.  $\delta D_{\text{soil}}$  in the growth chamber experiments approxi-

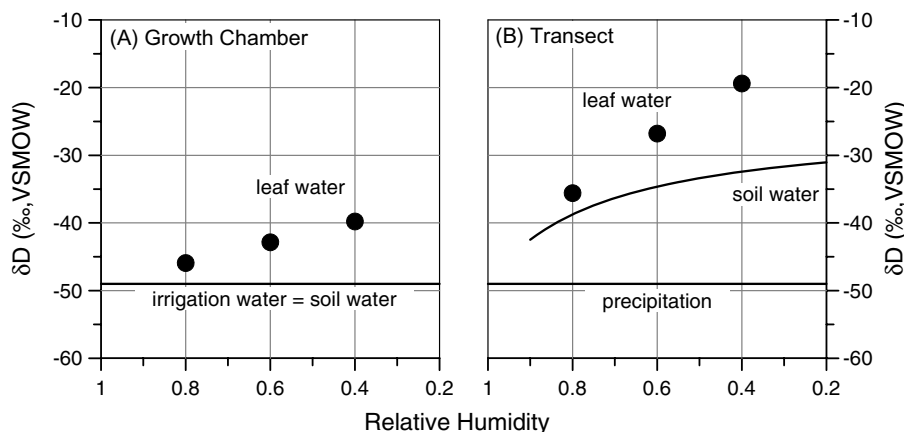


Fig. 6. Modeled  $D/H$  ratios of soil water and leaf water for plants (A) in the growth chamber and (B) in the field. To facilitate the comparison between the plants from the growth chamber and the field, the  $D/H$  ratio of precipitation is set to the  $\delta D$  value of irrigation water in the growth chamber ( $-49\text{‰}$ ).

mately equals  $\delta D$  of irrigation water because the plants were irrigated every two days, minimizing evaporative effects (note plant transpiration does not fractionate  $D/H$  ratios of the remaining water inside the growth chamber). Therefore, the RH effect on soil water in the growth chamber experiment is negligible. However, when modeling field conditions, soil evaporation must be considered.  $\delta D$  of soil water is estimated using Eq. (4), where  $f_e$  is set to 20% according to Smith and Freeman, 2006 and as discussed in Section 4.3.1. In other words, the field model takes into consideration both soil evaporation and plant transpiration, whereas the growth chamber model considers only plant transpiration. The modeled  $\delta D_{\text{leaf water}}$  value is  $-46\text{‰}$  at  $\text{RH} = 80\%$ ,  $\delta D_{\text{leaf water}} = -43\text{‰}$  at  $\text{RH} = 60\%$  and  $\delta D_{\text{leaf water}} = -40\text{‰}$  at  $\text{RH} = 40\%$  for the growth chamber plants. This  $6\text{‰}$  modeled increase in  $\delta D_{\text{leaf water}}$  as RH decreases from 80% to 40% agrees well with the observed increases in  $\delta D_{\text{wax}}$  of  $5.7\text{‰}$  as RH in the growth chambers was lowered from 80% to 40% (Fig. 4). The effect of RH on the  $D/H$  ratios of leaf water is small, as we observed in the transect samples (Fig. 5). For the simulated field plants, modeled  $\delta D_{\text{leaf water}}$  is slightly higher at corresponding humidity levels,  $-36$ ,  $-27$ ,  $-19\text{‰}$ , due to the additional isotope fractionation associated with soil water evaporation.  $\delta D_{\text{leaf water}}$  increases by  $17\text{‰}$  when RH decreases from 80% to 40%. This modeled RH effect must represent an average value, as natural desert plants show large variability in their leaf water isotopic ratios (e.g., Gat et al., 2007). Inevitably, growth chamber results can never fully represent all the complex variables of natural settings.

#### 4.4. The effect of vegetation types

Recent work has shown that there is significant difference in  $\delta D_{\text{wax}}$  among different plant types. Our recent study at Blood Pond, MA shows that  $\delta D_{\text{wax}}$  values from trees are  $\sim 40$  to  $50\text{‰}$  higher than those from grasses (Hou et al., 2007a). Because all plant samples at the Blood Pond site receive the same precipitation, our results suggest significantly larger apparent hydrogen isotopic fractionation during leaf wax synthesis in grasses relative to

trees. Similarly, Krull et al. (2006) found that leaf waxes from grasses and grassland soils are  $\sim 50\text{‰}$  lower than those from trees and woodland soils. Discrepancy between the  $\delta D_{\text{wax}}$  of grasses and trees was also reported by Liu et al. (2006), although the precipitation  $\delta D$  values in their study were not well constrained. The vegetation change along the SW transect is dramatic, shifting from shrubland to savanna to temperate grassland to subtropical forest (Fig. 1C). Relatively small  $\epsilon_{\text{wax-p}}$  would be expected in the eastern Texas Plains due to more extensive forest cover, while relatively large  $\epsilon_{\text{wax-p}}$  would be expected in the western part of the transect due to more extensive grassland, shrubland and CAM plants. However, these expectations are not observed and the large changes in vegetation cover are accompanied by a relatively small change in overall  $\epsilon_{\text{wax-p}}$ . We hypothesize that the opposing hydrogen isotope fractionation effects of RH (as expressed in the above model and our growth experiments) and vegetation type offset one another and result in relatively constant  $\epsilon_{\text{wax-p}}$  across the transect.

In order to test our hypothesis, we modeled separately the effects of RH and vegetation cover on  $\delta D_{\text{wax}}$  ratios along the southwestern US transect. First, we used the soil water model (Eq. (5)) and leaf water model (Eq. (7)) to estimate the effect of RH on  $\delta D_{\text{wax}}$  variation, while holding the vegetation constant (assuming 100% tree cover) (Fig. 7A). We then calculated the effect of vegetation types on  $\delta D_{\text{wax}}$  values by holding RH constant (assuming  $\text{RH} = 100\%$  for all sites). We used a binary isotopic model to estimate the vegetation effect. Because we were unable to find accurate productivity data for different plant types (e.g., trees, grasses, shrubs, CAM plants) along the transect, we assumed that the relative proportion of trees vs. grasses–shrubs is linearly correlated to RH. CAM plant leaf waxes have mean enrichment factor  $\epsilon_{\text{wax-p}}$  values  $\sim -145\text{‰}$  (Chikaraishi and Naraoka, 2003; Chikaraishi et al., 2004). Therefore, similar to an increase in grass–shrub cover, an increase in CAM plants relative to trees should also increase the  $\epsilon_{\text{wax-p}}$ , justifying our use of a simple two end member model to simulate the vegetation effect along the SW transect.

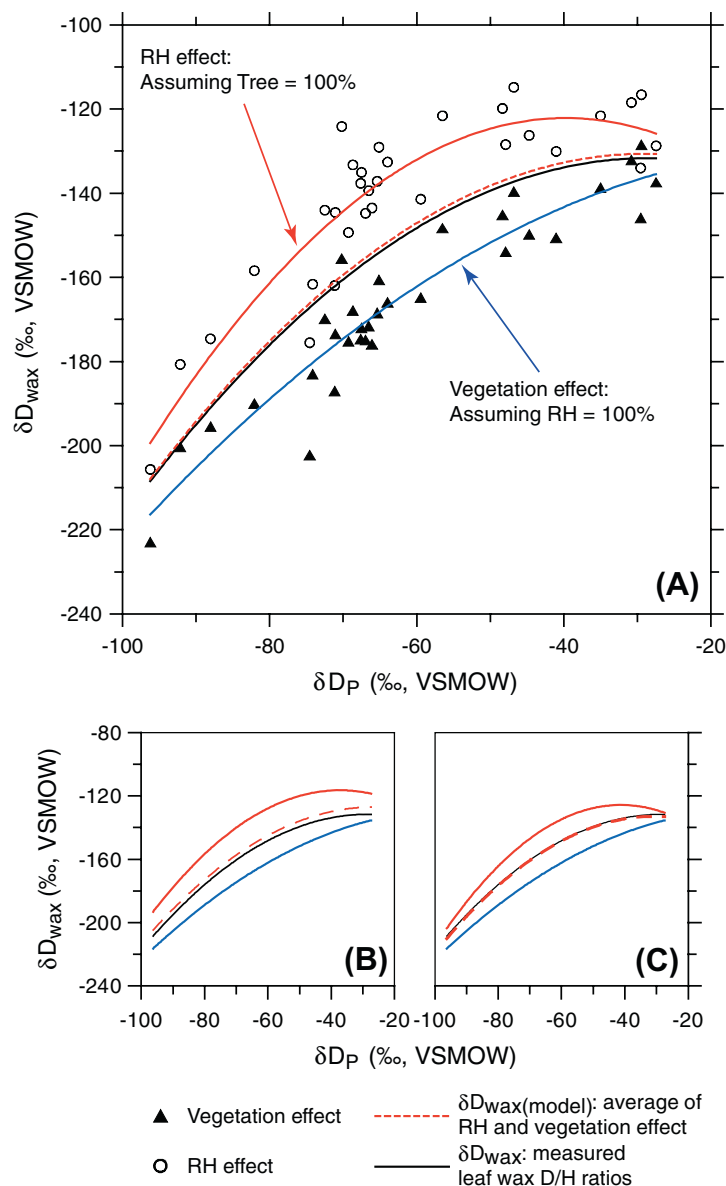


Fig. 7. (A) Models showing the opposing effects of RH and vegetation on the sedimentary  $\delta D_{wax}$ . The data for RH effect (circles) are obtained by assuming the production percentage of tree leaf waxes is 100%, whereas the data for vegetation effect (triangles) are obtained by assuming RH is 100% for all lakes. The average of the RH effect and vegetation effect for all sites ( $\delta D_{wax(\text{model})}$ , dashed line) is then compared with measured  $D/H$  ratios of leaf waxes from the lake sediments ( $\delta D_{wax}$ , black line). To facilitate comparison, second-order polynomial regressions are shown for all four datasets. (B) and (C) Sensitivity tests for the model combining RH and vegetation effects. As the percentage of tree cover increases from 0.7RH (B) to 1.2 RH (C), the modeled  $\delta D_{wax}$  vs.  $\delta D_p$  relationship does not change significantly.

The combined vegetation and RH effects are shown in Fig. 7A. To simplify the plots, we show the polynomial regression for the measured and modeled  $D/H$  ratios of leaf wax ( $\delta D_{wax(\text{model})}$ ) values and  $\delta D_p$  values along the transect. The  $\delta D_{wax(\text{model})} - \delta D_p$  curve matches the measured  $\delta D_{wax} - \delta D_p$  well (Fig. 7A), supporting our hypothesis that the opposing RH and vegetation effects could regulate  $\delta D_{wax}$  values. Altering the modeled relationship between percent tree cover and RH (by varying the coefficient of the linear relationship from 0.7 to 1.2) only results in minor changes in the modeled  $\delta D_{wax}$  (Fig. 7B and C). Of course, our model is a simplification of natural systems, and many other environmental factors may also play a part in deter-

mining the ultimate  $\epsilon_{wax-p}$  values at any given site. Moreover, there is very little published data showing that the vegetation effect extends to very arid regions. However, we are encouraged to see that this simple model experiment closely matches our observations. It is likely that the RH and vegetation effects are not always perfectly canceled. For example, we observe two distinct relationships between  $\delta D_p$  and  $\delta D_{wax}$  in the transect data (one for the dry portion and one for the wet portion) (Fig. 3B). In the Basin and Range, the dominance of grassland and shrubland may produce more leaf waxes with lower  $D/H$  ratios, resulting in a vegetation effect that overwhelms the RH effect. This would explain why sites from the Basin and Range show

a greater slope in the relationship between  $\delta D_P$  and  $\delta D_{wax}$  than those from Texas Plains (Fig. 3B).

Vegetation cover may also explain the much stronger correlation between  $\delta D_{wax}$  and  $\delta D_P$  observed in the dry portion of the transect (Fig. 3B,  $R^2 = 0.84$  for Basin and Range,  $R^2 = 0.64$  for the Plains). Diverse types of vegetation, more variable seasonal  $\delta D_P$  values, groundwater vs. summer precipitation input, and difference in plant growth seasons in the Plains may introduce greater variability to sedimentary  $\delta D_{wax}$  values, thereby introducing noise to the  $\delta D_{wax} - \delta D_P$  correlation over the more humid portion of the transect. In contrast, plants in dry regions might only grow in the rainy season, when soil water is available, reducing the variability in source water  $\delta D$  values used by plants during leaf wax biosynthesis.

## 5. IMPLICATIONS TO PALEOCLIMATE RECONSTRUCTIONS

### 5.1. Wet and/or cool regions with high PIE ratio

The  $\delta D$  values of aquatic lipids, biosynthesized using lake water, show strong correlation with  $\delta D$  values of lake water at a nearly constant isotopic fractionation (Sauer

et al., 2001; Huang et al., 2002, 2004; Hou et al., 2006; Hou et al., 2007b). In cool and wet regions, such as north-eastern North America (Huang et al., 2002) and western Europe (Sachse et al., 2004), lake water is mainly recharged by precipitation and/or ground water. Therefore,  $\delta D$  values of lake water more closely represent  $\delta D$  of mean annual precipitation (Fig. 8, right panel). However,  $\delta D_{wax}$  values, especially those from low lying areas, are affected by multiple factors, including multiple water sources (direct precipitation, groundwater), seasonality of precipitation, and diversity of vegetation cover. In addition to precipitation, plants can also use the shallow ground water for biosynthesis. The groundwater in north-eastern North America is mainly recharged in early spring, which adds isotopically-distinct (relative to direct summer precipitation) source water for plants. Diversity of plant types could also introduce significant uncertainties to the sedimentary  $\delta D_{wax}$  ratios, as different plant types at the same site have been shown to produce leaf waxes with quite different  $\delta D_{wax}$  values (Krull et al., 2006; Liu et al., 2006; Hou et al., 2007a; Liu and Huang, 2008). Therefore,  $\delta D_{wax}$  values from higher RH and/or cool regions could, generally, show larger variability relative to  $\delta D$  of precipitation (Fig. 8).

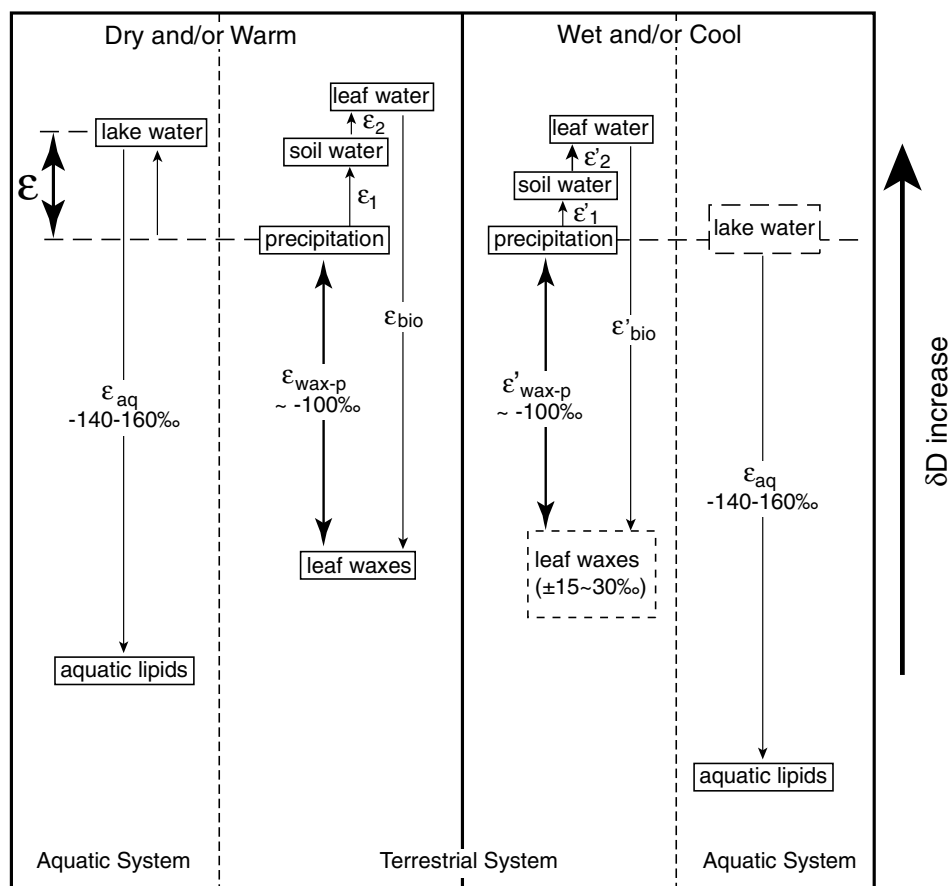


Fig. 8. The schematic variation of  $D/H$  ratios for low RH and/or warm regions (left panel), and high RH and/or cool regions (right panel; This panel is adapted from Sachse et al. (2006)).  $\epsilon_1$ ,  $\epsilon_2$ ,  $\epsilon'_1$ ,  $\epsilon'_2$  represent the evaporation effect from precipitation to soil water, then to leaf water;  $\epsilon_{bio}$  and  $\epsilon'_{bio}$  are the hydrogen isotopic enrichment during biosynthesis.  $\epsilon_{aq}$  is the biosynthesis effect for aquatic plants.  $\epsilon_{wax-p}$  and  $\epsilon'_{wax-p}$  are the apparent hydrogen isotopic enrichment from precipitation to leaf waxes.  $\epsilon$  is the isotopic enrichment between precipitation and lake water due to evaporation.

## 5.2. Dry and/or warm regions with low *PIE* ratios

In relatively dry and warm regions, such as the western part of the transect in this study, the evaporation effect on soil water and leaf water is enhanced due to the low RH ( $\epsilon_1$ ,  $\epsilon_2$  in Fig. 8). The net biosynthetic H isotopic fractionation ( $\epsilon_{\text{bio}}$  in Fig. 8) also increases relative to wet regions because of the reduced input from trees (Liu et al., 2006; Hou et al., 2007a). In such regions,  $\delta D$  values of aquatic lipids (which record the isotopic composition of lake water) are isotopically enriched relative to precipitation due to strong evaporation. Therefore, in dry regions the  $\delta D$  values of terrestrial leaf waxes may prove to be better proxies for  $\delta D$  of precipitation. Since speleothems tend not to form in areas of insufficient precipitation, leaf waxes extracted from lake sediments may provide the best opportunity for reconstructing the  $\delta D$  of precipitation in arid regions. Saline lakes in arid regions, recharged by mountain glaciers (e.g., Mono Lake in the Western U.S.; Lake Qinghai in China), could represent excellent candidates for paleohydrological reconstruction using the  $\delta D$  of leaf waxes.

## 6. CONCLUSIONS

The hydrogen isotopic composition of higher plant leaf waxes represents an effective proxy for  $\delta D$  of past continental precipitation. The proxy may prove particularly useful in dry regions, such as those with annual precipitation lower than 400 mm shown in this study. It appears that plants grown in dry regions use direct precipitation as source water for biosynthesis, as a means to maximize their water use efficiency. If this is indeed the case, leaf waxes from these plants are likely to track precipitation  $\delta D$  values with high fidelity. While the relationship between  $\delta D_{\text{wax}}$  and  $\delta D_P$  is also strong in wetter regions (such as those with annual precipitation exceeding 400 mm in this study), complicating factors such as multiple water sources (e.g., groundwater vs. summer precipitation), seasonality of precipitation and diversity of vegetation types could lead to reduced fidelity of  $\delta D_{\text{wax}}$  as a paleohydrologic proxy. However, because the sites in this study are restricted to the Southwestern United States, more research is needed to test whether our findings are also applicable to other regions around the world.

Our growth chamber experiments indicate that the effect of RH on  $\delta D_{\text{wax}}$  is small. Changing RH from 80% to 40% led to a mere  $\sim 7\text{‰}$  increase in hydrogen isotopic enrichment in  $\epsilon_{\text{wax-water}}$ . The experimental results are consistent with output from the latest leaf water model. Based on model results using the natural transect data, soil evaporation appears to make the largest contribution to the hydrogen isotopic variation induced by changes in relative humidity. However, the isotopic effect of relative humidity changes on plant leaf water and leaf waxes appears to be partially cancelled by the opposing isotopic effects of changing vegetation cover. We were able to reproduce the observed relationship between  $\delta D_{\text{wax}}$  and  $\delta D_P$  along our southwestern US transect using a simple model by combining the effects of RH with those of vegetation cover.

In arid settings, the combination of *D/H* ratios of terrestrial leaf waxes and aquatic lipids may provide important information concerning the past variability of the hydrologic cycle, including changes to both the isotopic composition of precipitation and lake water evaporation. In wet and/or cool regions, aquatic lipids from lakes in low lying areas are particularly useful for reconstructing past precipitation  $\delta D$  values. Leaf waxes in wet and/or cool regions may show greater variability relative to precipitation. However, if the effects of groundwater input could be minimized (e.g. by selecting lakes at topographic highs), leaf waxes could also provide valuable information on the  $\delta D$  values of growth season precipitation in wet and/or cool regions.

## ACKNOWLEDGMENTS

This work was supported by grants from the National Science Foundation (NSF 0318050, 0318123, 0402383) to Y. Huang. We thank the Koebeke Family (Dudley, MA) for access to Blood Pond. We thank Dana MacDonald for identifying and collecting the tree seedlings, Fred Jackson and Brian Leib for tending to plants in the greenhouse, David Murray and Joseph Orchardo for assistance during the growth chamber experiments, and Marcelo Alexandre for help during the sample analysis. We thank John Roden for providing the spreadsheet to model the leaf water *D/H* ratios. We thank Drs. Alex Sessions, Julian Sachs and Sarah Feakins for their thoughtful comments that helped improve the paper.

## REFERENCES

- Adams D. K. and Comrie A. C. (1997) The North American monsoon. *Bull. Am. Meteorolog. Soc.* **78**, 2197–2213.
- Ball J. T. (1987) Calculations related to leaf gas exchange. In *Stomatal Function* (eds. E. Zieger, G. D. Farquhar and I. R. Cowan). Stanford University Press, Stanford, California.
- Bowen G. J. and Revenaugh J. (2003) Interpolating the isotopic composition of modern meteoric precipitation. *Water Resources Res.* **39**, 1299, doi:10.1029/2003WR002086.
- Chikaraishi Y. and Naraoka H. (2003) Compound-specific  $\delta D$ - $\delta^{13}C$  analyses of *n*-alkanes extracted from terrestrial and aquatic plants. *Phytochemistry* **63**, 361–371.
- Chikaraishi Y., Naraoka H. and Poulson S. R. (2004) Hydrogen and carbon isotopic fractionations of lipid biosynthesis among terrestrial ( $C_3$ ,  $C_4$  and CAM) and aquatic plants. *Phytochemistry* **65**, 1369–1381.
- Craig H. and Gordon L. I. (1965) Deuterium and oxygen 18 variations in the ocean and marine atmosphere. In *Stable Isotopes in Oceanographic studies and Paleotemperatures* (ed. E. Tongiorgi), pp. 9–130. Lab. Geologia Nucleare.
- Edwards T. D. W. and Fritz P. (1986) Assessing meteoric water composition and relative from  $^{18}O$  and  $^2H$  in wood cellulose: paleoclimatic implications for southern Ontario, Canada. *Appl. Geochem.* **1**, 715–723.
- Flanagan L. B., Comstock J. P. and Ehleringer J. R. (1991) comparison of modeled and observed environmental-influences on the stable oxygen and hydrogen isotope composition of leaf water in *Phaseolus-Vulgaris* L.. *Plant Physiol.* **96**, 588–596.
- Gat J. R. (1996) Oxygen and hydrogen isotopes in the hydrologic cycle. *Annu. Rev. Earth Planet. Sci.* **24**, 225–262.
- Gat J. R., Yakir D., Goodfriend G., Fritz P., Trumborn P., Lipp J., Gev I., Adar E. and Waisel Y. (2007) Stable isotope composition of water in desert plants. *Plant Soil* **298**, 31–45.

- Gibson J. J. and Edwards W. T. (1996) Development and validation of an isotopic method for estimating lake evaporation. *Hydrol. Process.* **10**, 1369–1382.
- Gibson J. J., Reid R. and Spence C. (1998) A six-year isotopic record of lake evaporation at a mine site in the Canadian subarctic: results and validation. *Hydrol. Process.* **12**, 1779–1792.
- Gibson J. J. and Edwards T. W. D. (2002) Regional water balance trends and evaporation–transpiration partitioning from a stable isotope survey of lakes in northern Canada. *Global Biogeochem. Cycles* **16**. doi:10.1029/2001GB001839.
- Gonfiantini R. (1986) Environmental isotopes in lake studies. In *Handbook of Environmental Isotope Geochemistry* (eds. P. Fritz and J. C. Fontes). Elsevier, pp. 113–168.
- Gray J. and Song S. J. (1984) Climatic implications of the natural variations of D/H ratios in tree-ring cellulose. *Earth Planet. Sci. Lett.* **70**, 129–138.
- Groottes P. M., Stuiver M., White J. W. C., Johnsen S. and Jouzel J. (1993) Comparison of oxygen–isotope records from the GISP2 and GRIP Greenland Ice Cores. *Nature* **366**, 552–554.
- Hou J., Huang Y., Wang Y., Shuman B., Oswald W. W., Faison E. and Foster D. R. (2006) Postglacial climate reconstruction based on compound-specific D/H ratios of fatty acids from Blood Pond, New England. *Geochem. Geophys. Geosyst.* **7**. doi:10.1029/2005GC001076.
- Hou J., D'Andrea W. J., MacDonald D. and Huang Y. (2007a) Hydrogen isotopic variability in leaf waxes among terrestrial and aquatic plants around Blood Pond, Massachusetts (USA). *Org. Geochem.* **38**, 977–984. doi:10.1016/j.orggeochem.2006.12.009.
- Hou J., Huang Y., Oswald W. W., Foster D. R. and Shuman B. (2007b) Centennial-scale compound-specific hydrogen isotope record of Pleistocene–Holocene climate transition from southern New England. *Geophys. Res. Lett.* **34**, L19706. doi:10.1029/2007GL030303.
- Huang Y., Shuman B., Wang Y. and Webb, III., T. (2002) Hydrogen isotope ratios of palmitic acid in lacustrine sediments record late Quaternary climate variations. *Geology* **30**, 1103–1106.
- Huang Y., Shuman B., Wang Y. and Webb, III., T. (2004) Hydrogen isotope ratios of individual lipids in lake sediments as novel tracers of climatic and environmental change: a surface sediment test. *J. Paleolimnol.* **31**, 363–375.
- Huang Y., Clemens S. C., Liu W., Wang Y. and Prell W. L. (2007) Large scale hydrological change drove the late Miocene C<sub>4</sub> plant expansion in the Himalayan foreland and Arabian Peninsula. *Geology* **35**, 531–534.
- Krull E., Sachse D., Mugler I., Thiele A. and Gleixner G. (2006) Compound-specific  $\delta^{13}\text{C}$  and  $\delta^2\text{H}$  analyses of plant and soil organic matter: a preliminary assessment of the effects of vegetation change on ecosystem hydrology. *Soil Biol. Biochem.* **38**, 3211–3221.
- Liu W. and Huang Y. (2005) Compound specific D/H ratios and molecular distributions of higher plant leaf waxes as novel paleoenvironmental indicators in the Chinese Loess Plateau. *Org. Geochem.* **36**, 851–860.
- Liu Z. and Huang Y. (2008) Hydrogen isotopic compositions of plant leaf lipids are unaffected by a twofold pCO<sub>2</sub> change in growth chambers. *Org. Geochem.* doi:10.1016/j.orggeochem.2008.01.020.
- Liu W., Yang H. and Li L. W. (2006) Hydrogen isotopic compositions of n-alkanes from terrestrial plants correlate with their ecological life forms. *Oecologia* **150**, 330–338.
- Pagani M., Pedentchouk N., Huber M., Sluijs A., Schouten S., Brinkhuis H., Damste J. S. S., Dickens G. R. and Scientists E. (2006) Arctic hydrology during global warming at the Palaeocene/Eocene thermal maximum. *Nature* **442**, 671–675.
- Petit J. R., Jouzel J., Raynaud D., Barkov N. I., Barnola J. M., Basile I., Bender M., Chappellaz J., Davis M., Delaygue G., Delmotte M., Kotlyakov V. M., Legrand M., Lipenkov V. Y., Lorius C., Pepin L., Ritz C., Saltzman E. and Stievenard M. (1999) Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica. *Nature* **399**, 429–436.
- Roden J. S., Lin G. and Ehleringer J. R. (2000) A mechanistic model for interpretation of hydrogen and oxygen isotope ratios in tree-ring cellulose. *Geochim. Cosmochim. Acta* **64**, 21–35.
- Sachse D., Radke J. and Gleixner G. (2004) Hydrogen isotope ratios of recent lacustrine sedimentary n-alkanes record modern climate variability. *Geochim. Cosmochim. Acta* **68**, 4877–4889.
- Sachse D., Radke J. and Gleixner G. (2006)  $\delta D$  values of individual n-alkanes from terrestrial plants along a climatic gradient—implications for the sedimentary biomarker record. *Org. Geochem.* **37**, 469–483.
- Sauer P. E., Eglinton T. I., Hayes J. M., Schimmelmann A. and Sessions A. L. (2001) Compound-specific D/H ratios of lipid biomarkers from sediments as a proxy for environmental and climatic conditions. *Geochim. Cosmochim. Acta* **65**, 213–222.
- Scheffuß E., Schouten S. and Schneider R. R. (2005) Climatic controls on central African hydrology during the past 20,000 years. *Nature* **437**, 1003–1006.
- Shuman B., Huang Y., Newby P. and Wang Y. (2006) Compound-specific isotopic analyses track changes in the seasonality of precipitation in the Northeastern United States at ca 8200 cal yr BP. *Quater. Sci. Rev.* **25**, 2992–3002.
- Smith F. A. and Freeman K. H. (2006) Influence of physiology and climate on  $\delta D$  of leaf wax n-alkanes from C<sub>3</sub> and C<sub>4</sub> grasses. *Geochim. Cosmochim. Acta* **70**, 1172–1187.
- Terwilliger V. J. and DeNiro M. J. (1995) Hydrogen isotope fractionation in wood-producing avocado seedlings: biological constraints to paleoclimatic interpretations of delta D values in tree ring cellulose. *Geochim. Cosmochim. Acta* **59**, 5199–5207.
- Wang Y. J., Cheng H., Edwards R. L., An Z. S., Wu J. Y., Shen C. C. and Dorale J. A. (2001) A high-resolution absolute-dated late Pleistocene monsoon record from Hulu Cave, China. *Science* **294**, 2345–2348.
- White J. W. C., Lawrence J. R. and Broecker W. S. (1994) Modeling and interpreting D/H ratios in tree-rings—a test-case of white-pine in the Northeastern United-States. *Geochim. Cosmochim. Acta* **58**, 851–862.
- Yapp C. J. and Epstein S. (1982) A re-examination of cellulose carbon-bound hydrogen  $\delta D$  measurements and some factors affecting plant–water D/H relationships. *Geochim. Cosmochim. Acta* **46**, 955–965.
- Yuan D. X., Cheng H., Edwards R. L., Dykoski C. A., Kelly M. J., Zhang M., Qing J., Lin Y., Wang Y., Wu J., Dorale J. A., An Z. S. and Cai Y. (2004) Timing, duration, and transitions of last interglacial Asian monsoon. *Science* **304**, 575–578.