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Geochimica et Cosmochimica Acta 74 (2010) 2128–2141
Geochimica et Cosmochimica Acta
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Controls on the D/H ratios of plant leaf waxes in an arid ecosystem

Sarah J. Feakins *, Alex L. Sessions

California Institute of Technology, Department of Geological and Planetary Sciences, Mail Stop 100-23, Pasadena, CA 91125, USA

Received 8 July 2009; accepted in revised form 5 January 2010; available online 15 January 2010

Abstract

The extent to which leaf water D-enrichment (transpiration) and soil water D-enrichment (evaporation) affect the D/H ratio of plant leaf waxes remains a contentious issue, with important implications for paleohydrologic reconstructions. In this study we measure δD values of precipitation (δD_p), groundwater (δD_{gw}), plant xylem water (δD_{xw}) and leaf water (δD_{lw}) to understand their impact on the δD values of plant leaf wax *n*-alkanes (δD_{wax}) in an arid ecosystem. Our survey includes multiple species at four sites across an aridity gradient (80–30% relative humidity) in southern California.

We find that many species take up groundwater or precipitation without significant fractionation. D-enriched soil water is a minor source even in species known to perform and utilize waters from hydraulic lift, such as *Larrea tridentata* (+10‰). Measurements of leaf water isotopic composition demonstrate that transpiration is an important mechanism for D-enrichment of leaf waters (+74 ± 20‰, 1σ), resulting in the smallest net fractionation yet reported between source water and leaf waxes (*L. tridentata* −41‰; multi-species mean value is −94 ± 21‰, 1σ). We find little change in leaf water D-enrichment or net fractionation across the climatic gradient sampled by our study, suggesting that a net fractionation of ca. −90‰ may be appropriate for paleohydrologic reconstructions in semi-arid to arid environments. Large interspecies offsets in net fractionations ($1\sigma = 21‰$) are potentially troublesome, given the observed floristic diversity and the likelihood of species assemblage changes with climate shifts.

1. INTRODUCTION

Fractionation of the stable isotopes of hydrogen and oxygen within the hydrologic cycle facilitates their use as tracers for climatic processes (see discussion in Craig, 1961). Hydrogen and oxygen isotope ratios in plant photosynthetic products have been found to reflect both the isotopic ratios of meteoric water and biochemical processes within the plant (Epstein et al., 1976; Sternberg, 1988). Of those plant photosynthetic products, certain long-chain lipids are well preserved in the sedimentary record, providing an archive for paleoclimatic and paleoecological processes (Eglinton and Hamilton, 1967; Huang et al., 1995). Here we consider the environmental and ecological origins of the D/H ratios of plant leaf waxes, which have recently emerged as a promising tool for investigating past changes in precipitation and evaporation regimes (Xie et al., 2000; Sauer et al., 2001; Huang et al., 2004; Sachse et al., 2004a; Schefuss et al., 2005; Smith and Freeman, 2006).

Evapotranspiration has long been known to drive D-enrichment of plant waters above that of source waters (e.g. Leaney et al., 1985; Yakir et al., 1990; Wang and Yakir, 1995) and it has been anticipated that this evapotranspiration signal would be recorded by elevated δD values of leaf wax lipids (Sauer et al., 2001; Sachse et al., 2004b). Here we combine the approaches of ecohydrology and organic geochemistry to measure the evapotranspiration signal in plant leaf waxes. Investigation of the magnitude of the evapotranspiration signal is needed, because the net fractionation between source water and leaf wax

* Corresponding author. Present address: Department of Earth Sciences, University of Southern California, Los Angeles, CA 90089-0740, USA. Tel.: +1 213 740 7168.
E-mail address: feakins@usc.edu (S.J. Feakins).

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doi:10.1016/j.gca.2010.01.016

n-alkanes integrates any D-enrichment due to evapotranspiration together with biosynthetic fractionations (Sachse et al., 2006; Smith and Freeman, 2006).

The theoretical basis for the influence of soil water evaporation on the δD values of plant leaf waxes comes from studies of plant xylem waters which have shown that species with different rooting depths may take up water of different isotopic composition, particularly in arid regions where there is a significant enrichment of soil water isotopic composition towards the surface (Ehleringer et al., 1991, 1998; Williams and Ehleringer, 2000). Several studies have predicted that such variations in plant water uptake may influence the hydrogen isotopic composition of lipid products (e.g., Krull et al., 2006; Smith and Freeman, 2006; Sachse et al., 2009), although no measurements of xylem water δD values (hereafter δD_{xw}) could directly confirm source water as the cause of shifts in leaf wax δD values (δD_{wax}) in those studies. In contrast, more positive δD_{wax} values have been found in deep-rooted trees relative to shallow-rooted grasses (Hou et al., 2007c) which points to confounding factors associated with transpiration or biosynthesis controlling δD_{wax} values.

Transpiration is known to drive D-enrichment in leaf water relative to xylem water, and several studies have suggested that this signal is recorded in δD_{wax} values. In the case of C_3 and C_4 grasses, model evidence suggests that the interveinal distance in plant leaves controls the degree of leaf water D-enrichment through transpiration and is recorded in elevated δD values of plant leaf waxes (Smith and Freeman, 2006). Observations of plant leaf water oxygen isotopic composition have further shown that unconstrained aspects of leaf physiology (the 'effective path length' fitting parameter in Péclet effect calculations) may be the primary control on leaf water isotopic differences even within C_3 plants of the same genus (Kahmen et al., 2008). Correlation of leaf wax δD values with plant life form (i.e. tree/shrub/grass) and carbon isotopic evidence for water use efficiency both suggest that interspecies variations in water budgets may be a significant source of variability in leaf wax δD values (Bi et al., 2005; Liu et al., 2006; Hou et al., 2007a). Enhanced transpiration has also been reported from a greenhouse experiment simulating the continuous light conditions in the Arctic summer (Yang et al., 2009). This study found a reduction in net fractionation of the C_{29} *n*-alkane relative to plants grown under normal diurnal light cycles, with some of the lowest published net fractionations $\varepsilon_{29/w} = -62‰$ (*Metasequoia*) and $-87‰$ (*Larix*). These studies demonstrate that leaf water D-enrichments may therefore be influenced by a variety of effects including leaf physiology and environmental conditions.

Prior studies provide constraints on biosynthesis from two sources. The first comes from the reported net fractionations for terrestrial plants (up to $-165‰$) (Sessions et al., 1999; Chikaraishi and Naraoka, 2003; Sessions, 2006; Smith and Freeman, 2006; Mügler et al., 2008), where the largest fractionations may include negligible effects of evapotranspiration. The second has been inferred (Sachse et al., 2004a; Mügler et al., 2008) from the net fractionations reported for *n*-alkane synthesis by aquatic plants (-90 to $-160‰$) which do not transpire (Sessions et al., 1999; Chikaraishi and Naraoka, 2003; Huang et al., 2004; Mügler et al., 2008; Li et al., 2009). However direct source water δD values have not always been obtained, such that there remains some uncertainty in reported net fractionations (see discussion in Yang et al., 2009). Additional complicating factors include variations in biosynthetic and metabolic pathways, chain length and compound type (Ziegler et al., 1976; Estep and Hoering, 1980; Sessions et al., 1999; Chikaraishi et al., 2004; Zhang et al., 2009).

Several studies provide insights into the magnitude and variability of the evapotranspiration signal in the δD values of plant leaf waxes. A study of grasses from natural and greenhouse plots across the central USA found a D-enrichment in plant leaf waxes associated with a decrease in relative humidity (Smith and Freeman, 2006). Their model predictions indicated that leaf water D-enrichment derived primarily from evaporation of soil water. In contrast, in a recent survey of catchment-integrated leaf waxes recorded in lake core tops, Hou et al. (2008) found that leaf wax δD values track shifts in precipitation δD values (δD_p) without the predicted influence of relative humidity. Hou et al. suggest that D-enrichment due to decreasing relative humidity is countered by changes in vegetation type along the climatic gradient, resulting in no net change in overall fractionation. Leaf wax δD values in the geological record have thus been variously interpreted in terms of changing evapotranspiration (Schefuss et al., 2005; Huang et al., 2007; Jacob et al., 2007) or δD_p values (Liu and Yang, 2008; Tierney et al., 2008). In a qualitative sense, a positive δD shift interpreted as an increase either in δD_p values or an increase in evapotranspiration would point to a drying trend in either case. Nevertheless, resolving these conflicting interpretations will be needed to support quantitative paleohydrologic reconstructions in arid regions.

Paired observations of δD values in plant waters and leaf waxes from plants growing in arid regions have the potential to clarify the extent to which evaporation and transpiration modulate plant waters and the D/H ratios recorded in leaf waxes. In this study, we measure the δD values of precipitation and groundwater, plant xylem waters, leaf waters and leaf wax *n*-alkanes across an aridity gradient in southern California. This approach enables us to distinguish and quantify contributions to δD_{wax} values from: (i) source water δD values, (ii) soil water evaporation, (iii) transpiration from the leaf and (iv) biosynthetic fractionations. Through this examination of hydrogen isotope ratios, from source water to leaf wax product, we document the range of variability at the plant scale and its implications for δD_{wax} as a paleohydrology proxy.

2. STUDY LOCATIONS AND SAMPLING STRATEGY

2.1. Study location and climate

Southern California (Fig. 1) has a strongly winter-dominated precipitation regime (Cayan and Rhoads, 1984), pronounced aridity gradients (Conil and Hall, 2006) and high floristic diversity (Rundel and Gustafson, 2005). We selected four sites with considerable differences in mean

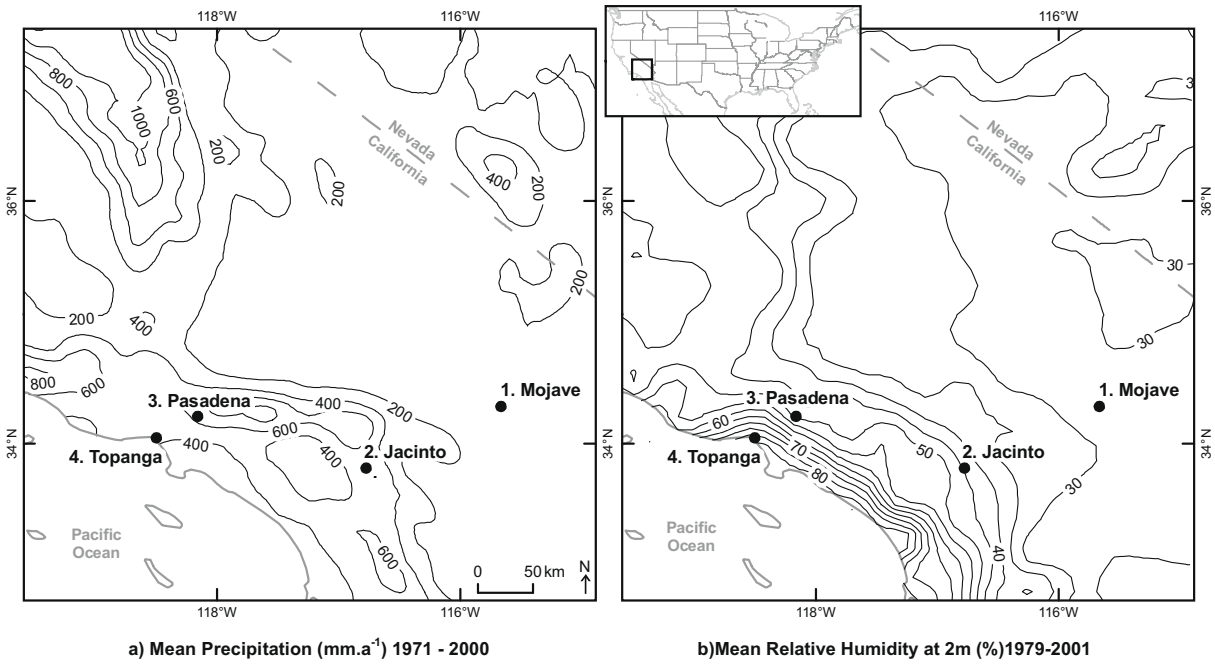

Fig. 1. Map of study locations in relation to a) mean annual precipitation and b) mean relative humidity at 2 m. California border shown in dashed line for reference. Climate data is derived from the North American Regional Reanalysis data provided by the NOAA/OAR/ESRL PSD, Boulder, Colorado, USA.

annual precipitation, relative humidity and δD values of precipitation (Table 1, Fig. 1), in order to assess the impact of variations in source water δD values and evapotranspiration on the δD values of plant leaf waxes.

The unimodal precipitation regime of this region provides a convenient setting in which to examine the linkages between δD_p and δD_{wax} values without the complication of large seasonal variations in δD_p values, as are typically found in regions with year-round or bimodal precipitation regimes (Ehleringer and Dawson, 1992; Friedman et al., 1992; Gat, 1996). There are, however, spatial gradients in δD_p values across southern California resulting from the progressive distillation of precipitation from water vapor along westerly storm tracks (Friedman et al., 1992). The result is precipitation with progressively more D-depleted isotopic compositions further inland. Given the limited spatial resolution of observational data for meteoric water isotopic compositions, and local deviations from global water isotope predictions (Williams and Rodoni, 1997; Poage and Chamberlain, 2001), we also monitored the δD values of precipitation and groundwater. Model estimates of δD_p values ($\delta D_{p\ (model)}$), obtained in 2009 from the Online Isotopes in Precipitation Calculator, version 2.2 (Bowen and Revenaugh, 2003), are given for comparison in Table 1.

2.2. Species diversity and implications for leaf wax isotopic composition

California, like other Mediterranean-type climates, is host to a high floristic diversity and plant community that is heterogeneous in space and time (Rundel and Gustafson, 2005). In this context, it is particularly important to examine the potential role of interspecies differences in isotope effects, which may complicate paleoclimate reconstructions from δD_{wax} values.

Modern plant samples were collected from each of the four study sites in order to quantify D/H fractionations in varied plant communities along the transect of increasing aridity. These sites capture some of the variety in southern Californian plant communities with assemblages characterized as mixed woody and succulent scrub, chaparral, oak and conifer woodland communities (dominated by shrubs and trees). Grasses were not included in this study as they are not a significant component of the year-round vegetation at any of the study sites, typically appearing only transiently following rains. Where possible we sampled at the University of California Natural Reserve System where additional climatic, ecological and historical data are available. We sampled a variety of plant species in each location in order to assess interspecies isotopic differences. Although the high floristic diversity of the region precludes a wide-ranging transect of one species, a genus-level comparison can be achieved for *Quercus* (oak).

2.3. Sampling methods

Leaf tissue samples were collected for extraction of leaf water and leaf waxes for D/H analysis (both analyses on the same sample). Leaf samples were collected from a single branch, if possible from locations where the leaves were in unshaded positions. Entire leaves were collected in order to ensure integration of the signal from the entire leaf, given likely isotopic gradients along the length of the leaf (Helliker and Ehleringer, 2000; Sessions, 2006). Leaves were cut at the junction of the leaf to the stalk using pruning shears. For pine and fir trees with needles, needles from

Table 1
Geographic and isotopic characteristics of site locations. $\delta D_{p\ (model)}$ values were obtained in 2009 from the Online Isotopes in Precipitation Calculator, version 2.2 (Bowen and Revenaugh, 2003).

Site	Location	Long. (W)	Lat. (N)	Elev. (m)	Dist. Inland (km)	Flora	Date sampled	δD_{gw} (‰)	δD_p (meas) (‰)	δD_p (model) (‰)	95% CI (‰)[a]	$\Delta\delta D_p$ (model-meas) (‰)
1. Mojave	UC Sweeney Granite Mountain Desert Research Center	115.664	34.806	1500	240	Mixed woody and succulent scrub	2.7.07	−79.1	na	−66	4	na
2. Jacinto	UC James Reserve, San Jacinto Mountain	116.778	33.808	1620	90	Chaparral, mixed woodland, riparian	9.14.06 and 5.5.07	−66.7	−46.7	−67	5	−20
3. Pasadena	Brown Mountain, San Gabriel Mountains	118.161	34.226	640	40	Oak woodland	4.2.07	−44.5	−40.0	−54	3	−14
4. Topanga	Topanga Canyon, Santa Monica Mountains	118. 589	34.09	400	4	Chaparral, coastal scrub, riparian	11.11.07	na	−37.3	−51	3	−14

[a] 95% confidence interval of model values.

multiple fasicles were sampled at random from each branch. Approximately 2 g of leaf tissue was collected per sample corresponding to two leaves in large-leaved plants to tens of leaves in the case of very small-leaved plants. Plant tissue samples for water extraction were immediately enclosed in screw-cap glass vials. All samples were collected between 12 and 5 pm in order to capture the maximum diurnal enrichment in leaf water isotopic composition (Li et al., 2006). Twig samples for xylem water extraction were cut from the same branch as the leaf samples and immediately enclosed in screw-cap glass vials. All samples were kept in a dry cooler in the field until transfer to the laboratory. Stem and leaf samples collected for water isotopic analyses were kept frozen until water was extracted using cryogenic vacuum distillation.

Precipitation, stream and ground water samples were collected for isotopic analysis in tightly sealed glass vials. The winter of 2006–2007 was anomalously dry, and there was no measurable precipitation at Site 1, the Mojave location. At Site 2, the Jacinto location, we captured rain and snow on a monthly basis for isotopic analysis in a polyethylene container with 5 mm of mineral oil to prevent evaporative losses. Precipitation from individual storms was collected from a Pasadena location close to Site 3 and a Topanga location close to study Site 4. Stream water was collected where available (at Sites 2 and 3). Groundwater samples were collected from tanks filled from existing wells at Sites 1 and 2. Water vapor isotopic composition is unconstrained.

3. ANALYTICAL METHODS

3.1. Xylem and leaf water extraction

Water was vacuum extracted from plant stem and leaf samples by heating (100 °C) the plant samples within an evacuated glass line and trapped in an adjacent cryogenic trap cooled with liquid N_2. Extraction was continued until visible condensation in the trap ceased or a minimum of 2 h (Ehleringer and Osmond, 1989; Ehleringer et al., 2000; West et al., 2006).

3.2. Lipid extraction

The same dried leaf samples were then cut into pieces with solvent-cleaned scissors and 1–2 g of each sample was used for extraction. *n*-Alkanes were extracted in 5 ml of hexane using a pumping action with a Pasteur pipette, repeated three times. Lipid extracts were transferred to a silica-gel column (column: 5 cm × 4 mm Pasteur pipette, 0.5 g 5% H_2O-deactivated silica-gel, 100–200 mesh) and the *n*-alkane fraction was collected by eluting with hexane.

3.3. Water isotopic analyses

Water samples, both from environmental waters and from plant tissues, were analyzed for D/H and $^{18}O/^{16}O$ ratios using a spectroscopic Liquid–Water Isotope Analyzer (Los Gatos Research, Inc.). Replicate measurements were made for each sample on 3–6 aliquots of water, each 0.8 μL in size. Samples are calibrated against three working standards of known isotopic composition which allow for normalization to the SMOW/SLAP isotopic scale. The precision of replicate injections during the period when these samples were analyzed averaged 0.7‰ and 0.2‰ for δD and $\delta^{18}O$, respectively (1σ, $n = 144$), with accuracy of replicate analyses determined for δD values as reproducible to within 0.7‰ (1σ, $n = 7$). Oxygen isotope data are reported in the Electronic Annex EA-2 and EA-4 to EA-7.

3.4. Bulk carbon isotopic analyses

Samples of leaf fragments of ca. 0.6 g dry weight were analyzed for $\delta^{13}C$ values of bulk plant leaf tissue. We used a Costech Elemental Analyzer (EA) coupled to a Finnigan Delta-S isotope-ratio mass spectrometer via a Conflo interface. All samples were blank-corrected, and $\delta^{13}C$ values were calculated by comparison to CO_2 reference gas with a $\delta^{13}C$ value of $-37.2‰$. Samples were analyzed as leaf fragments rather than homogenized powders. To estimate variability introduced by this approach, we repeatedly measured aliquots from a single specimen both as leaf fragments ($\sigma = 0.6‰$, $n = 7$) and as a homogenized powder ($\sigma = 0.3‰$, $n = 5$). Analytical precision for two working standards was 0.08‰ (urea, $n = 6$) and 0.05‰ (acetanilide, $n = 7$), with both yielding $\delta^{13}C$ values within 0.5‰ of known values.

3.5. Leaf wax isotopic analyses

The δD values of individual *n*-alkanes were measured using a Thermo-Finnigan Trace GC equipped with a DB-5 ms column (30 m × 0.25 mm × 0.25 μm) and a PTV injector operated in splitless mode, coupled to a Delta Plus XP mass spectrometer via a pyrolysis interface (Thermo GC/TC) operated at 1430 °C. Reference peaks of CH_4 or H_2 gas (Wang and Sessions, 2008) were co-injected between *n*-alkane peaks during the course of the GC-IRMS run. Two of these peaks were used for standardization of the isotopic analyses, while the remainder were treated as unknowns to assess accuracy. Data were then normalized to the SMOW/SLAP isotopic scale by comparison to an external standard. The results are reported using conventional delta notation (i.e. δD values) in permil (‰) units. The mean precision for replicate analyses of the external *n*-alkane standard compounds is typically better than 5‰ (1σ). For co-injected peaks of CH_4 or H_2 reference gas, precision was typically better than 2‰ (1σ). In addition to the δD values reported for individual *n*-alkanes we calculate an amount-weighted mean of the C_{27}, C_{29} and C_{31} *n*-alkanes (δD_{wax}) for comparison across all species and study sites.

We report isotopic fractionations between two measured substrates, δD_a and δD_b, as enrichment factors ($\varepsilon_{a/b}$) defined as:

$$\varepsilon_{a/b} = \alpha_{a/b} - 1 = \frac{\delta_a + 1}{\delta_b + 1} - 1 \quad (1)$$

Enrichment factors are reported in permil notation, which implies a factor of 1000 (Cohen et al., 2007). The "net (or apparent) fractionation" ($\varepsilon_{wax/w}$) is one of the most commonly used parameters in the plant leaf wax literature, where:

$$\varepsilon_{wax/w} = \alpha_{wax/w} - 1 = \frac{\delta_{wax} + 1}{\delta_w + 1} - 1 \quad (2)$$

Typically $\varepsilon_{wax/w}$ is calculated relative to D/H ratios of mean annual local precipitation, inherently assuming that the isotopic composition of w is identical to local precipitation. However, in this study we empirically determined the source of water taken up by the plant by measuring δD_{xw} directly and comparing this to potential sources (groundwater, precipitation or inferred D-enriched soil water). Measuring the δD values of precipitation, groundwater, xylem water and leaf water enables the net fractionation ($\varepsilon_{wax/w}$) to be parsed into individual components: soil evaporation ($\varepsilon_{xw/p}$), transpiration ($\varepsilon_{lw/xw}$) and biosynthesis ($\varepsilon_{wax/lw}$).

4. RESULTS

4.1. Environmental water isotopic analyses

Samples of environmental waters yielded δD values ranging from $-79.1‰$ (groundwater) at the most inland location (Site 1, Mojave) to $-37.3‰$ (mean precipitation) at the most coastal location (Site 4, Topanga; Table 1, Electronic Annex EA-4 to EA-6). Average δD_p values decrease with distance inland, and the values obtained here are in good agreement with previous studies of long-term precipitation averages (Friedman et al., 1992; Williams and Rodoni, 1997). For comparison, model estimates ($\delta D_{p\ (model)}$) obtained in 2009 from the Online Isotopes in Precipitation Calculator, version 2.2 (Bowen and Revenaugh, 2003) are given in Table 1. $\delta D_{p\ (model)}$ values are on average 16‰ more negative than measured δD_p values. Studies of storm-to-storm variability in precipitation amount and isotopic composition at Sites 3 and 4 demonstrated that individual precipitation events varied greatly in amount (0.3–84 mm) and isotopic composition (δD values from -2 to $-90‰$) (Electronic Annex EA-5 and EA-6). Groundwater δD values are consistent with the mean isotopic composition of *in situ* precipitation except for Site 2 (Jacinto). At this site, groundwater is more D-depleted ($\delta D_{gw} = -66.7‰$) than precipitation in any month (amount-weighted annual average $\delta D_p = -46.7‰$) (Fig. 2, Electronic Annex EA-4).

4.2. Plant water isotopic analyses

Plant xylem waters reflect the isotopic composition of precipitation (or groundwater, where these differ; Figs. 2 and 3; Table 2). The largest numbers of samples are reported for Sites 1 and 2; plant water samples were not collected at Site 4 (Topanga). At Site 1 (Mojave), δD_{xw} values average $-76.0 \pm 12.3‰$ ($n = 6$ specimens), consistent with uptake of groundwater ($\delta D_{gw} = -79.1‰$) which in turn presumably reflects mean precipitation, though the latter could not be measured. At Site 2 (Jacinto), δD_{xw} values average $-71.8 \pm 12.8‰$ ($n = 7$), indicating uptake of groundwater ($\delta D_{gw} = -66.7‰$) rather than *in situ* precipitation ($\delta D_p = -46.7‰$). At Site 3 (Pasadena), a δD_{xw} value of $-40.7‰$ for *Quercus agrifolia* (Table 3) is virtually identical to δD_p values of $-40.0‰$.

Plant leaf waters are significantly D-enriched relative to xylem water at all sites (Figs. 2 and 3; Table 2). δD_{lw} values average $-4.0 \pm 21.6‰$, ($n = 8$) at Site 1 (Mojave) and $-8.5 \pm 4.6‰$ ($n = 10$) at Site 2 (Jacinto). At Site 3 (Pasadena) we obtained δD_{lw} values of $+22.3 \pm 0.7‰$ ($n = 2$) for *Q. agrifolia* (where the stated uncertainty refers to leaf-to-leaf variability) and +23.1‰ for *C. leucodermis*.

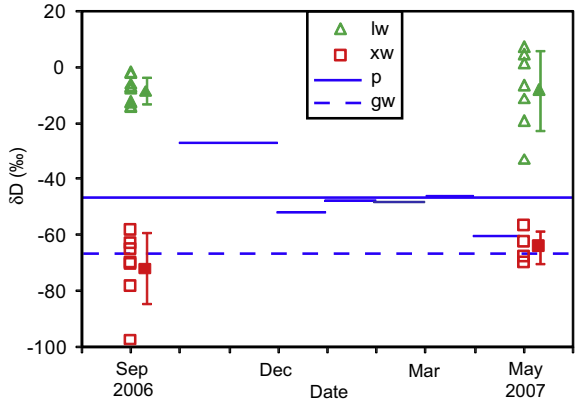

Fig. 2. Seasonal record of δD values for plant and environmental waters from Site 2, Jacinto. Open symbols represent plant water data for individual specimens, solid symbols represent site mean with 1 standard deviation (error bars). Isotopic values for precipitation (solid blue lines) integrate precipitation events over sampling intervals (~1 month) and the entire study interval, as indicated by the width of the symbol (–). Ground water (blue dashed line) was sampled on 9/14/2006 and is assumed to experience minimal variation during the year. (For interpretation of colours in this figure legend, the reader is referred to the web version of this article.)

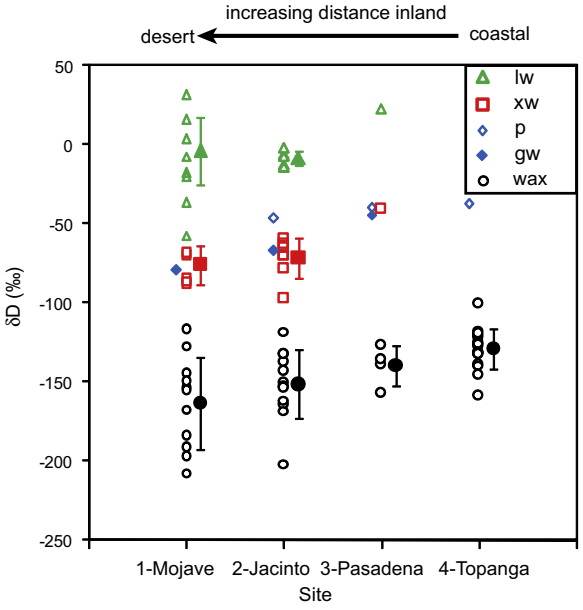

Fig. 3. Plant and environmental waters by site.

At Site 2 (Jacinto) we tested whether the isotopic composition of water taken up by plants (δD_{xw}) and present in plant leaves (δD_{lw}) records a seasonal shift in evapotranspiration. No seasonal difference was observed in the mean δD_{xw} values averaging $-71.8 \pm 12.8‰$ ($n = 7$) on 9/14/2006 and $-64.2 \pm 5.9‰$ ($n = 4$) on 5/5/2007 (Fig. 2 and Electronic Annex EA-7). Similarly, δD_{lw} values of $-8.5 \pm 4.6‰$ ($n = 10$) measured on 9/14/2006 and $-8.2 \pm 14.3‰$ ($n = 7$) on 5/5/2007, were not significantly different.

4.3. Bulk leaf carbon isotopic composition

Samples of bulk leaf tissue yielded $\delta^{13}C$ values of $-27 \pm 4‰$ ($n = 28$; Table 2). *Opuntia basilaris* (a CAM plant) yielded the lowest $\delta^{13}C$ value, $-13‰$. We note that this species produces only a very long-chain *n*-alkanoic acid (C_{33}), which was not included in calculated δD_{wax} values. We do not find a significant correlation between $\delta^{13}C$ values of bulk leaf tissue (often interpreted as a measure of plant water use efficiency) and δD_{wax} values (Fig. 4).

4.4. Leaf wax molecular abundance and hydrogen isotopic composition

Most species yielded long-chain C_{25} to C_{33} *n*-alkanes, with carbon chain length distribution varying between species (Table 2, Electronic Annex EA-2). Certain *Pinopsida*, *Liliopsida*, *Filicopsida* and *Pteridopsida* species that yielded insufficient *n*-alkane concentrations for isotopic analysis are omitted from consideration in this study. For all plants yielding measureable *n*-alkanes, δD values are reported in Table 2. We observe isotopic offsets between different chain lengths, as well as differences in *n*-alkane chain length abundance for different species and sites. Therefore, to provide a common basis for comparison we calculated an amount-weighted mean δD value for the C_{27}, C_{29} and C_{31} *n*-alkanes (δD_{wax}). These are the most common compounds as indicated by calculated values of average chain length ($ACL = \Sigma(C_n \cdot n)/\Sigma C_n$, where $n = 25, 27, 29, 31, 33$) and the modal chain length (C_{max}; Table 2). This approach enables comparison of the dominant wax compounds in various species regardless of chain length. There is a large spread in δD_{wax} values at all sites, averaging $-163 \pm 29‰$ ($n = 12$ species) at Site 1 (Mojave), $-151 \pm 22‰$ ($n = 12$) at Site 2 (Jacinto), $-140 \pm 13‰$ ($n = 4$) at Site 3 (Pasadena) and $-129 \pm 12‰$ ($n = 18$) at Site 4 (Topanga).

5. DISCUSSION

5.1. Plant water sources

The isotopic composition of plant xylem water (δD_{xw}) is a direct proxy for the plant water source (δD_w) where water uptake by roots does not involve significant fractionation (Ehleringer et al., 1991, 1998; Williams and Ehleringer, 2000). In general, the isotopic composition of sampled groundwater reflects that of mean annual precipitation, making them difficult to distinguish as potential water sources for plants. However, at the Jacinto site δD_{gw} values of $-66.7‰$ were D-depleted by $20‰$ relative to measured precipitation ($-46.7‰$, Fig. 2). We infer recharge from precipitation at higher elevations on San Jacinto Mountain, given the expected trend of $-10‰\ km^{-1}$ with elevation (Williams and Rodoni, 1997; Poage and Chamberlain, 2001). Stream water at this site yielded a δD value of $-59.8‰$, intermediate between those for ground water and precipitation, consistent with this explanation. At Jacinto, *Salix lasiolepsis* in a riparian setting appears to be sampling stream water, with an isotopic composition intermediate between *in situ* precipitation and groundwater

Table 2
δD values for xylem water, leaf water and leaf wax *n*-alkanes together with calculated fractionations and indices of molecular abundance.

Species	δD (‰)		δD of *n*-alkanes (‰)								Fractionation (‰)				δ^{13}C (‰)
	xw	lw	C_{25}	C_{27}	C_{29}	C_{31}	C_{33}	wax	C_{max}	ACL	xw/w	lw/xw	wax/lw	wax/w	bulk
1. Mojave															
Artemisia ludoviciana sp. *albula*	na	na			−127	−128		−127	31	30.1	na	na	na	−52	−25
Coleogyne ramosissima	−70	−7			−192	−150		−167	31	30.2	10	68	−161	−96	−26
Ephedra nevadensis	na	−20			−149	−157		−154	29	30.2	na	na	−136	−81	−23
Epilobium canum sp. *latifoiium*	na	na			−207	−221		−208	29	29.1	na	na	na	−140	−28
Ericameria cuneata var. *spathulata*	−85	31		−149	−143			−145	29	28.5	−6	127	−171	−71	−25
Larrea tridentata	−69	16			−114	−120		−117	31	30.1	11	91	−131	−41	−26
Opuntia basilaris var. *basilaris*	na	3					−124	na	33	33.0	na	na	na	na	−13
Purshia tridentata var. *gladulosa*	−88	−37		−191				−191	27	27.0	−10	56	−161	−122	−24
Quercus chrysolepsis	−86	−18		−147	−151	−153		−150	29	28.2	−8	75	−134	−77	−27
Rhamnus ilicifolia	na	0			−156			−156	29	29.0	na	na	−156	−83	−26
Salix exigua	−57	na			−197			−197	29	29.0	23	na	na	−128	−27
Yucca brevifolia	na	na				−184		−184	31	31.0	na	na	na	−114	−21
Mean	−76	−4		−163	−160	−159	−124	−163	29.8	29.6	3	83	−150	−91	−24
σ	12	22		25	32	34		29	1.6	1.5	13	27	16	32	4
2. Jacinto															
Alnus glutinosa	na	−13	na	−165	na			−165	27	27.0	na	na	−154	−105	−29
Arctostaphylos pringlei	−65	−2		na	−145	−163		−143	29	30.1	2	68	−141	−82	−30
Arctostaphylos pringlei	na	−14		−182	−191	−210		−202	31	30.0	na	na	−190	−145	−31
Erigonium wrightii	na	na	−126	−119	−119	−111		−118	27	27.8	na	na	na	−55	−26
Pinus lambertiana	−97	−6				−151		−151	29	29.0	−33	101	−146	−90	−25
Quercus chrysolepis	na	na	−168	−159	−150	−146		−153	27	27.4	na	na	na	−93	−30
Quercus chrysolepis	−63	−7	−137	−131	−134	−134		−132	27	27.8	4	60	−126	−70	−27
Quercus chrysolepis	−70	−7	−143	−143	−136	−129		−138	29	28.0	−3	67	−131	−76	−28
Quercus kelloggi	na	−13		−160	−162			−162	29	28.4	na	na	−151	−102	−30
Quercus kelloggi	−70	−14		−147	−172			−168	29	28.7	−4	60	−156	−109	−30
Quercus kelloggi	−78	−8		−137	−158			−154	29	28.6	−13	77	−148	−94	−27
Salix lasioiepsis	−58	−2	na	−145	na			−132	27	27.1	9	60	−130	−70	−31
Mean	−61	−6	−144	−149	−152	−149		−151	28.3	28.3	−5	70	−147	−91	−29
σ	13	5	18	18	22	31		22	1.3	1.0	14	15	18	23	2

3. Pasadena														
Ceanothus leucodermis	na	23		na	−156	−159		−157	31	29.7	na	na	−176	−118	−30
Quercus agrifolia – leaf sample 1	−41	22			−139			−139	29	29.0	na	65	−158	−99	−28
Quercus agrifolia – leaf sample 2		23			−127			−127	29	29.0	4	66	−146	−86	−29
Quercus agrifolia – leaf sample 3		na			−136			−136	29	29.0	na	na	na	−96	−29
Mean	−41	23			−139	−159		−140	29.5	29.2	4	66	−160	−100	−29
σ	na	±1			±12			±13	±1.0	±0.4	na	±1	±15	±13	±1
4. Topanga														
Adenostoma fasciculatum	na	na			−121	−102		−117	27	27.4	na	na	na	−83	na
Arctostaphyios glandulosa	na	na				−159	−158	−159	29	29.9	na	na	na	−126	na
Artemisia californica	na	na			−125	−127		−126	27	27.9	na	na	na	−92	na
Ceanothus intergerrimus	na	na			−126	−124		−125	27	27.6	na	na	na	−91	na
Ceanothus megacarpus	na	na			−136	−142		−139	29	28.1	na	na	na	−106	na
Ceanothus megacarpus	na	na			−130	−136		−133	27	28.0	na	na	na	−99	na
Ceanothus spinosus	na	na			−128	−137		−132	27	27.9	na	na	na	−98	na
Heteromeies arbutifolia	na	na			−132			−132	27	27.0	na	na	na	−98	na
Keckieiia cordifolia	na	na			−130	−121		−128	27	27.6	na	na	na	−94	na
Lonicera subspicata	na	na			−132			−132	27	27.0	na	na	na	−98	na
Maiacothamnus fasciculatus	na	na			−102	−90		−100	27	27.3	na	na	na	−66	na
Phacelia cicutaria	na	na			−118	−126		−122	29	28.1	na	na	na	−88	na
Platanus racemosa	na	na		−131		−148	−160	−140	25	28.1	na	na	na	−106	na
Quercus agrifolia	na	na			−126			−126	29	29.0	na	na	na	−92	na
Quercus agrifolia	na	na			−120			−120	29	29.0	na	na	na	−86	na
Quercus dumosa	na	na			−119			−119	29	29.0	na	na	na	−85	na
Rhus laurina	na	na		na	−147			−146	27	27.9	na	na	na	−112	na
Sambucus mexicana	na	na			−133			−133	29	29.0	na	na	na	−99	na
Mean	na	na			−126	−128	−159	−129	27.7	28.1	na	na	na	−95	na
σ	na	na			10	20	1	12	1.2	0.8	na	na	na	13	na
Mean overall	−71	−2	−144	−152	−142	−143	−147	−144	28.6	28.6	−1	74	−150	−94	−27
σ	15	17	18	20	24	29	20	24	1.6	1.2	13	20	17	21	4

na = not available/not determined.
wax = the abundance-weighted mean value as defined in the text.
C_{max} = modal chain length.
ACL = Average Chain Length as defined as in the text.

Table 3
Comparison of evergreen *Quercus* across all sites, showing δD values for source water and leaf wax *n*-alkanes, together with calculated net fractionations.

Site	Species	No. of trees	No. of samples	δD source water (‰)	δD_{wax} (‰)	wax/w (‰)
1. Mojave	*Q. chrysolepis*	1	1	−79	−150	−77
2. Jacinto	*Q. chrysolepis*	3	3	−67	−141	−80
3. Pasadena	*Q. agrifolia*	1	3	−45	−134	−94
4. Topanga	*Q. agrifolia*	2	2	−37	−123	−89
	Q. dumosa	1	1	−37	−119	−85
Mean		11	13	−53	−133	−85

(Table 2). All other sampled species have an isotopic composition equivalent to (or more depleted than) measured groundwater (Fig. 2). In areas were $\delta D_{gw} \neq \delta D_p$ this finding demonstrates the need to measure δD_{xw} values in order to assess water uptake. Reliance on published values of δD_p (or even measured δD values of soil waters) will introduce an additional source of uncertainty in leaf wax calibration studies (see discussion in Yang et al., 2009). Furthermore, measured precipitation δD values differed from modeled values by an average of -16‰ (Table 1), indicating the need for measurements of environmental waters where interpolated mean annual values are a poor match.

Measurements of δD_{xw} values also allow us to assess whether or not plants are taking up soil waters that have been substantially enriched in D by evaporation. Evaporative D-enrichment of soil waters had been predicted as an important component of the net fractionation between leaf waxes and precipitation (Krull et al., 2006; Sachse et al., 2006, 2009; Smith and Freeman, 2006). In the Mojave we found a slight (~10‰) D-enrichment of stem waters relative to groundwater in *Coleogyne ramosissima* and *Larrea tridentata*. *L. tridentata* has been shown to use waters released via hydraulic lift (Caldwell et al., 1998). Hydraulic lift is the process by which some deep-rooted plants take in water from lower soil layers and exude that water into upper, drier soil layers, which may be enriched via evaporation from the upper soil profile. The most D-enriched stem waters (offset by ~+25‰) were observed in *Salix exigua* which was dormant at the time of sampling (i.e. containing no leaf waters), and therefore has a very different seasonal strategy of photosynthesis and water use. However, in all other species sampled we find no evidence for significant uptake of D-enriched soil waters, with δD_{xw} values being equivalent to or more depleted than *in situ* precipitation (Figs. 2 and 3).

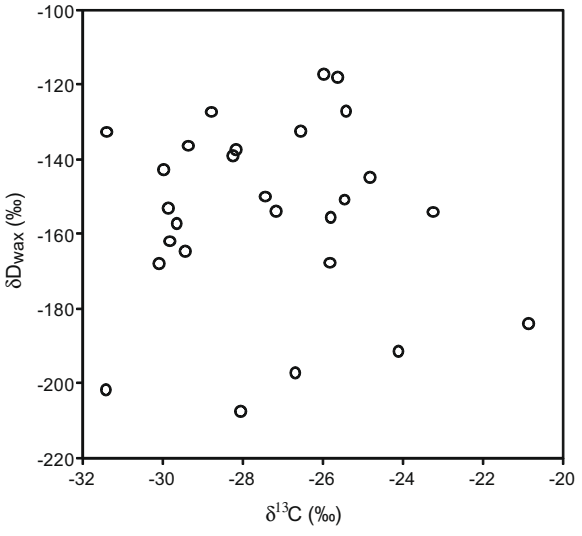

Fig. 4. Comparison of δ^{13}C bulk leaf tissue and δD_{wax}.

In some cases we observed δD_{xw} values that were more negative than any of the measured environmental waters (e.g. Fig. 3), suggesting that plants were accessing a different source of moisture than any of those sampled. Atmospheric water vapor is a possible source of relatively D-depleted water (Gat, 1996; Sachse et al., 2009). In coastal or montane arid regions, differences in water vapor uptake strategies (i.e. fog harvesting) may be important, particularly where there are significant differences in the isotopic compositions of fog and precipitation (Ingraham and Matthews, 1990; Dawson, 1998; Corbin et al., 2005) and where relative humidity is high (Farquhar and Cernusak, 2005). Fog has been shown to be an important source of summer moisture to vegetation along parts of the central and northern California coast, although we are aware of no studies of atmospheric water uptake in the coastal and inland mountains of southern California. Direct uptake of water vapor is reduced at lower relative humidity, however water vapor may also indirectly influence the isotopic composition of leaf water where water vapor and plant xylem water are out of equilibrium (Farquhar and Cernusak, 2005; Farquhar et al., 2007). Thus the isotopic composition of water vapor could be a significant influence on the δD value of plant leaf waters (Sachse et al., 2009), but we have no constraints on the δD values of water vapor in this study.

Alternatively, there is some evidence that certain xerophytic plants yield xylem water that is D-depleted relative to surrounding soil waters. This occurs when water taken up through the roots traverses cell membranes in the endodermis before entering the xylem (Ellsworth and Williams, 2007). In their study of 12 species of xerophytic and halophytic plants, the maximum relative depletion reported was 10‰ in old stems, and 5‰ in young stems of *Prosopis velutina*. Thus D-depletion of water during uptake remains a possible contributing factor for some species.

In summary, our studies of xylem water suggest that groundwater δD values may be considered as the source water for vegetation reflecting *in situ* or higher elevation precipitation in these mountainous sites in southern California. Locally-recharged groundwater integrates precipitation over many storm events, seasons, or years,

providing a more robust indicator of average precipitation isotopic composition than any short term sampling of precipitation could achieve. Given the multi-year averaging of groundwater, we should thus consider that plants may record heavily smoothed records of precipitation, and may incorporate a systematic bias towards precipitation from higher elevations. For paleoclimatic reconstructions this implies that small and well-constrained catchments will provide a more local record of paleohydrology.

5.2. Leaf water δD enrichment

Comparison of leaf water and xylem water δD values (Table 2) allows us to calculate the bulk leaf water D-enrichment associated with transpiration (Fig. 5). Averaging across all sites, this value is $\varepsilon_{lw/xw} = 74 \pm 20‰$. We find little evidence for any trend towards increasing $\varepsilon_{lw/xw}$ with increasing aridity, albeit based upon a relatively small dataset with large interspecies variability. Comparison between Sites 1 and 2 does suggest that the variability of $\varepsilon_{lw/xw}$ values may increase in more arid environments. We also find, from a limited study of seasonal variations, that any potential seasonal offset in plant waters appears to be smaller than the observed plant-to-plant variability (Fig. 2). This finding is consistent with a lack of seasonal variation in δD_{wax} values observed in an ongoing study of *Q. agrifolia* at Site 3 Pasadena (Electronic Annex EA-8). However, we note that significant seasonal variability in δD_{wax} values has been reported in a humid, temperate ecosystem (Sachse et al., 2009) and in a salt marsh grass (Sessions, 2006).

The considerable scatter in δD_{lw} values between samples (Table 2, Fig. 3) may reflect long-term differences in leaf water D-enrichment between plants, temporal differences in sampling, or both. Variations in δD_{lw} values between species are expected based on varied leaf morphology (Smith and Freeman, 2006; Kahmen et al., 2008). Large diurnal variations in leaf water δD and $\delta^{18}O$ values have previously been documented (Cernusak et al., 2002; Li et al., 2006; Kahmen et al., 2008), attributed to daytime transpiration with a concomitant enrichment of D or ^{18}O, followed by night-time recharge of leaf water with a D or ^{18}O content equivalent to that of xylem water. The magnitude of this cycle varies with incident sunlight intensity, temperature, relative humidity, and wind speed. Our sampling was necessarily completed over the course of several hours in the afternoon, perhaps leading to artificial variability between specimens. However, there is no correlation between isotopic enrichment and sampling time, suggesting that this is not a major source of bias.

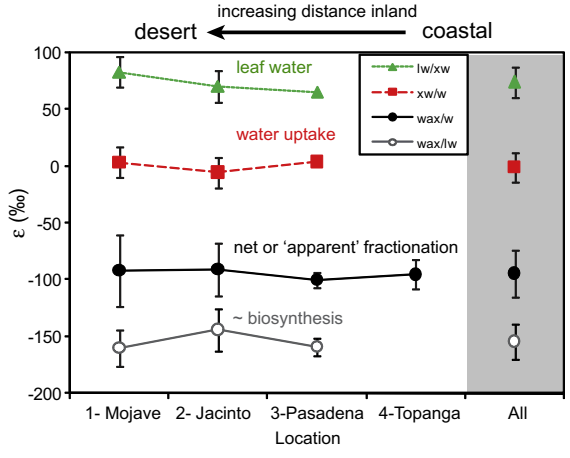

Fig. 5. Calculated fractionations between measured δD_{xw}, δD_{lw}, δD_{wax} and interpreted δD_w (see Eq. (2) and Section 3.5).

The timing of leaf water incorporation into plant leaf waxes, on both daily and seasonal scales, remains largely unknown. Thus collecting leaf waters that are representative of those used for biosynthesis is problematic. Indeed, leaf waxes are likely to be synthesized from discrete cellular pools of water, with isotopic compositions that differ from the empirically measured bulk leaf water (Sachse et al., 2009). In our sampling we sought to provide an upper limit on bulk leaf water isotopic enrichment by harvesting samples during the afternoon. Given uncertainty in the representative 'pool' of water used for biosynthesis, it is possible that this maximum enrichment of bulk leaf water overestimates the enrichment of leaf water used for biosynthesis. Values of $\varepsilon_{wax/lw}$ ($-149 \pm 16‰$) can therefore only provide an approximation of $\varepsilon_{biosynthesis}$ (Fig. 5). For this reason we do not seek to interpret our results in terms of variations in biosynthetic fractionations. Nevertheless, the mean value of $\varepsilon_{wax/lw}$ of $-150‰$ is consistent with estimates of biosynthetic fractionations reported elsewhere (Sessions et al., 1999; Sachse et al., 2004a).

5.3. Isotopic fractionations and the climatic signal preserved in plant leaf waxes

In this survey of southern Californian vegetation, δD_{wax} values respond to variations in source water isotopic composition (Fig. 6). There is a weak correlation ($R^2 = 0.338$, $n = 45$, $p < 0.001$) for data from individual plants, but a much stronger correlation when considering average δD values for each site ($R^2 = 0.972$, $n = 4$, $p < 0.015$). This difference suggests that the variability in fractionations between individuals is randomly distributed. Net fractionations ($\varepsilon_{wax/w}$) across the four study sites average $-94 \pm 21‰$. This value is similar to an average fractionation of $-99 \pm 8‰$ reported from a study of sedimentary C_{28} *n*-alkanoic acids across Texas to New Mexico, USA (Hou et al., 2008). However, it is considerably smaller than fractionations reported by various sedimentary and plant-based studies of *n*-alkanes and *n*-alkanoic acids from more humid climates in central and northeastern USA, southeastern Asia, and northern Europe, which range up to $-160‰$ (e.g. Chikaraishi and Naraoka, 2003; Sachse et al., 2006; Sessions, 2006; Smith and Freeman, 2006; Hou et al., 2007b). This result has important implications for quantitative reconstructions of paleoclimate in arid environments using the leaf wax δD proxy, where an appropriate $\varepsilon_{wax/w}$ value must be applied to calculate the δD value of source water (e.g. Sachse et al., 2004a).

The smaller fractionations between leaf wax and source water that we measure in southern California are consistent with previous predictions of D-enrichment in plant leaf waters – and thus in leaf waxes – in an arid climate (e.g.,

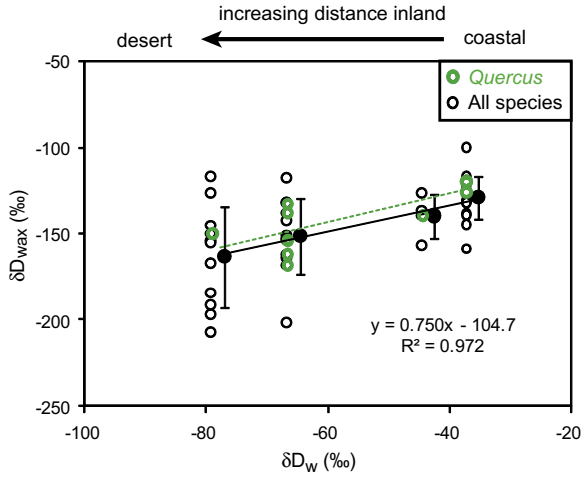

Fig. 6. Correlation of δD_w and δD_{wax} across all 4 sites for multiple species (black symbols) and a genus specific comparison of evergreen *Quercus* (highlighted in green). Black solid line represents correlation of the site mean values. (For interpretation of colours in this figure legend, the reader is referred to the web version of this article.)

Sauer et al., 2001; Sachse et al., 2004b). The prediction is supported by measured $\varepsilon_{lw/w}$ fractionations associated with transpiration processes that average +74‰, and which act to reduce the net fractionation ($\varepsilon_{wax/w}$) to an average of −94‰. This observation stands in contrast to the conclusions of Hou et al. (2008), who predicted on the basis of modeling and greenhouse experiments that most of the D-enrichment should occur through soil water evaporation. Measured δD_{lw} and δD_{xw} values demonstrate conclusively that most of the D-enrichment in our study area is due to leaf transpiration and not soil water evaporation.

Despite the large differences between $\varepsilon_{wax/w}$ values measured in humid versus arid climates, there is no significant shift in $\varepsilon_{wax/w}$ values across the climatic gradient that we sampled (Table 2, Fig. 5). $\varepsilon_{wax/w}$ values between sites are indistinguishable within the observed interspecies variability. Apparently, aridity has a significant impact on net D/H fractionations when comparing humid to arid regions, but only slight impact within arid regions. We speculate that the dependence of $\varepsilon_{wax/w}$ on aridity may exhibit a threshold effect. In arid regions, where conservation of tissue water is essential under all conditions, decreases in moisture availability may be countered by increases in plant water use efficiency and/or limitation of growth. However, our ^{13}C data do not support a simple construct of stomatal limitation to transpiration (water use efficiency) as being the major factor controlling $\varepsilon_{wax/w}$ variability. Other sources of interspecies variability (such as lifecycle and drought response) may be involved.

5.4. Interspecies isotopic differences and implications for the sedimentary record

Surveying the climatic response of δD_{wax} values at the individual plant scale reveals a large amount of scatter between individuals (Fig. 6), particularly at the most arid sites. We identify some of the smallest reported $\varepsilon_{wax/w}$ values for *L. tridentata* (−41‰) *Erigonium wrightii* (−55‰) and *Artemisia ludoviciana* (−55‰), alongside other species with $\varepsilon_{wax/w}$ values down to ~−140‰. The spread in δD values from our study is similar to that found by other studies where individual plants were sampled, thus significant scatter at the level of individual plants appears to be the norm across a wide range of climatic conditions (e.g., Chikaraishi and Naraoka, 2003; Liu and Huang, 2005; Sachse et al., 2006; Hou et al., 2007b). Several studies have linked variations in δD values to life form (e.g. tree, shrub and grass), with the largest offset observed for grasses (Liu et al., 2006; Hou et al., 2007c). We find no clear evidence for differences in D/H fractionations related to life form between the trees and shrubs sampled, but grasses could not be studied here because of their low frequency of occurrence. Expanded sampling programs are needed to confirm whether interspecies variations are related to abiotic conditions such as microsite or to biotic differences in water budget (e.g. hydraulic lift) or biosynthesis and metabolism.

A further question is whether interspecies variability has the potential to bias paleoclimatic reconstructions, given that ecosystem composition will inevitably adjust to climatic change. The inference that $\varepsilon_{wax/w}$ values are randomly distributed among individuals (see Section 5.3 above) is significant here. It is possible that catchment-scale average δD_{wax} values reliably record the climatic conditions of the region despite a large spread in values between individuals. Indeed, calibration studies using core-top sediments (e.g., Huang et al., 2004; Sachse et al., 2004b) have generally found much better correlations to climate than have studies of individual plants (Sachse et al., 2006). In our study there is very little change in average $\varepsilon_{wax/w}$ fractionations across the climatic gradient. We find a strong correlation between δD_w values and the site average δD_{wax} values, despite a large interspecies scatter. This is in rough agreement with the core-top sediment data reported by Hou et al. (2008) for the southern USA. Future studies should establish whether epsilon values are randomly distributed among species, in order to understand how isotopic signals in terms of plant-scale processes of water uptake, transpiration and biosynthesis are recorded at the catchment scale.

Although no one species can be sampled across all sites, several species of evergreen oaks (*Quercus*) can be compared across all sites (Table 3). *Q. agrifolia*, *Q. dumosa* and *Q. chrysolepis* are evergreen species with closely related morphologies, including small leaves with either smooth or saw-tooth edges and frequent hybrid forms. *n*-Alkane chain-length distribution differs between the *Quercus* species, with *Q. chrysolepis* producing C_{26}–C_{31} (with a strong odd chain length preference) whereas *Q. agrifolia* and *Q. dumosa* produce only C_{29} at detectable levels. We compare δD_{wax} values for *Quercus* species across the climatic gradient (Table 3 and Fig. 6). Values of $\varepsilon_{wax/w}$ for evergreen *Quercus* sampled in southern California range from −78 to −89‰. The modest decrease in $\varepsilon_{wax/w}$ fractionation between Sites 2 and 3 indicates that more leaf water D-enrichment is occurring at the more inland, arid sites resulting in a leaf wax D-enrichment of up to 10‰ in these closely related species (Table 3).

6. CONCLUSIONS

This survey of D/H ratios in water and leaf waxes from plants across southern California provides new insights into sources of leaf wax D/H variability in arid regions. Our approach, analyzing the isotopic compositions of both plant waters and leaf waxes, traces the origin of the leaf wax signal to variations in source water D/H and leaf water D-enrichment. Xylem water δD values indicate uptake of meteoric water, or in one case groundwater reflecting meteoric water from higher elevation. We note that where $\delta D_{gw} \neq \delta D_p$ it is therefore necessary to measure δD_{xw} values in order to assess water source for $\varepsilon_{wax/w}$ calibration studies. We do not find significant uptake of D-enriched soil waters in most of the trees and shrubs sampled ($\varepsilon_{xw/w} = -1 \pm 13‰$). Rather, plant water δD values provide direct evidence for substantial D-enrichment of leaf water ($\varepsilon_{lw/xw} = +74‰$) in this arid ecosystem, and importantly that this transpiration signal is recorded in plant leaf waxes. From our measurements of bulk leaf water we are able to provide an approximation of the total biosynthetic fractionation ($\varepsilon_{wax/lw} = -150‰$). This biosynthetic fractionation is opposed by leaf water D-enrichment associated with transpiration, yielding some of the smallest reported net fractionations ($\varepsilon_{wax/w} = -94 \pm 21‰$) – a clear signal of aridity. However, we observe no further discrimination across the aridity gradient in this survey within the limits of our dataset. Plant water regulation mechanisms must restrict transpiration which would affect the D-enrichment of leaf waters in the most arid climates. Thus, measurement of plant water D/H ratios, rather than prediction from abiotic conditions, are essential for the robust determination of net fractionation values for applications to leaf wax proxy archives of paleohydrology.

We conclude that while δD_{wax} values carry the signature of meteoric isotopic composition, appropriate $\varepsilon_{wax/w}$ values must be applied to accurately reconstruct absolute source water isotopic composition. The $\varepsilon_{wax/w}$ values display a lack of sensitivity to heightened aridity in the most arid sites, such that a constant $\varepsilon_{wax/w}$ value of $-90‰$ may be appropriate for similar sub-humid to arid environments. The largest complication may derive from interspecies variability in plant water and leaf wax δD values ($1\sigma \approx 20‰$). Site averages suggest that the effects may be mitigated in the catchment-integrated δD_{wax} value recorded by sediments.

ACKNOWLEDGMENTS

Part of this work was performed at the University of California (Riverside) Natural Reserve System (UCNRS) James Reserve and Sweeney Granite Mountain Desert Research Center. We are grateful to the assistance of Jim Andre, Jamie King and Michael Hamilton of the UCNRS for their assistance with plant identification and fieldwork support. Lauren Colloff assisted with fieldwork and sample preparation for the Topanga Canyon site. Michael Cheetham assisted with production of Fig. 1. Thanks to Darren Sandquist, Lisa Welp and Leonel Sternberg for helpful discussions and to the 6 anonymous reviewers. We gratefully acknowledge the support of the NOAA/UCAR Global and Climate Change postdoctoral fellowship to SF and NSF award EAR-0645502 to ALS.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.gca.2010.01.016. EA-1 to EA-3 contain the data included in Tables 1–3. EA-2 expands on data presented in Table 2, including *n*-alkane chain length abundance and $\delta^{18}O$ values for plant waters. We provide supplemental data in (EA-4 to EA-9). We report measured precipitation amounts and δD and $\delta^{18}O$ values for Jacinto (EA-4), Pasadena (EA-5) and Topanga (EA-6). We provide the primary data for a seasonal study of plant and environmental waters from Jacinto (Fig. 2, EA-7). We report data from an ongoing long-term study of δD_{wax} values collected from a single *Quercus agrifolia* tree at the Brown Mountain site (EA-8), which shows no clear seasonal variation in δD_{wax} values despite strong seasonality in precipitation amount and storm-to-storm variability in isotopic composition.

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Associate editor: Jaap S. Sinninghe Damste