

Life form-specific gradients in compound-specific hydrogen isotope ratios of modern leaf waxes along a North American Monsoonal transect

Melissa A. Berke^{1,2} · Brett J. Tipple^{1,3} · Bastian Hambach¹ · James R. Ehleringer^{1,3}

Received: 11 November 2013 / Accepted: 15 August 2015 / Published online: 27 August 2015
© Springer-Verlag Berlin Heidelberg 2015

Abstract The use of hydrogen isotope ratios ($\delta^2\text{H}$) of sedimentary *n*-alkanes from leaf waxes has become an important tool for reconstructing paleoenvironmental and ancient hydrologic conditions. Studies of modern plant waxes can elucidate driving ecological mechanisms behind geologic deposits. Here, we used a transect across the North American Monsoon region of the western USA from Tucson, Arizona to Salt Lake City, Utah to study variations in leaf wax $\delta^2\text{H}$ among co-occurring plants. Three co-occurring life forms were selected: perennial shrub (rabbit brush, *Chrysothamnus nauseosus*; sagebrush, *Artemisia tridentata*); tree (Gambel's oak tree, *Quercus gambelii*); and annual (sunflower, *Helianthus annuus*). Our results showed that the distributions and abundances of *n*-alkanes in perennial plants were similar across all sites and generally did not vary with environmental conditions (e.g., precipitation and temperature). In contrast, variations in *n*-alkane $\delta^2\text{H}$ were significantly correlated with the fraction of the annual precipitation coming during the summer monsoon period. We use a modified Craig–Gordon model to speculate on the possible drivers of the $\delta^2\text{H}$ values of leaf wax *n*-alkanes of plants across the region. The model results suggest that the most likely explanation for variation in wax $\delta^2\text{H}$ values was

a combination of seasonal source water usage and subsequent environmental conditions.

Keywords *n*-Alkanes · Craig–Gordon model · Paleoenvironment · Source water · Fractionation

Introduction

For paleoclimatologists seeking to reconstruct ancient hydroclimates using sedimentary records, the stable isotopes of water are an important interpretative tool, since the isotopic composition of water informs us of past precipitation (Dansgaard 1964; Rozanski et al. 1993). Both hydrogen and oxygen isotopes from water are incorporated into organic matter and can thereby record climate patterns (Epstein and Yapp 1976; Roden et al. 2000; Bowen 2010; Sachse et al. 2012). Our understanding and ability to quantify the varying processes and sources in sediment archives are far from complete (Sachse et al. 2012) and there is an opportunity to bridge ecological and geological fields by understanding the ecological drivers influencing biologic materials recovered from ancient sediments.

For ancient systems, hydrogen isotopes from compounds in leaf waxes have been applied as a proxy for past hydrologic changes that alleviates many of the associated difficulties with source characterization based on bulk sediment isotope analyses (Sauer et al. 2001; Huang et al. 2004; Schefuß et al. 2005; Smith and Freeman 2006). *n*-Alkanes are long lived and are particularly abundant in modern and ancient angiosperm species, providing a strong link between ecology, geology, and climate (Diefendorf et al. 2011; Bush and McInerney 2013). In geologic environments that have not received excessive pressure or temperature with burial, epicuticular leaf wax compounds,

Communicated by Nina Buchmann.

✉ Melissa A. Berke
mberke@nd.edu

¹ Global Change and Sustainability Center, University of Utah, Salt Lake City, UT 84112, USA

² Present Address: Department of Civil and Environmental Engineering and Earth Sciences, University of Notre Dame, Notre Dame, IN 46556, USA

³ Department of Biology, University of Utah, Salt Lake City, UT 84112, USA

particularly *n*-alkanes, have been shown to exhibit little exchange of hydrogen on the C–H bonds (Schimmelmann et al. 1999).

Studies of modern leaf waxes and of wax distributions in plants that contribute to the sedimentary record enhance our understanding of ecosystem-level reconstruction through knowledge of the controls on the hydrogen isotope values and degree to which these control ancient leaf wax $\delta^2\text{H}$ values. Recent field observation and controlled environment studies have examined controls on isotope composition of modern plant leaf waxes (Smith and Freeman 2006; Feakins and Sessions 2010; Kahmen et al. 2011; Tipple et al. 2013; Tipple and Pagani 2013). These studies point to environmental water, biosynthetic fractionation, and plant phenology as factors critical to understanding the nature of isotope composition of plant *n*-alkanes.

Transect studies have become an increasingly useful method to address the controls on the $\delta^2\text{H}$ of lipids along climate gradients (Sachse et al. 2006; Feakins and Sessions 2010; Guenther et al. 2013; Tipple and Pagani 2013). In addition to constraining vegetation type along a gradient, we must also consider the source of water used by plants along a given transect. Water is a major limitation to growth of arid land plants (Ehleringer and Mooney 1983), and can also be the primary determinant in the structure of a plant community (Fonteyn and Mahall 1978), most notably in semi-arid and arid environments. The North American Monsoon (NAM) region in the southwestern US is one climate gradient where understanding the interactions among plant water availability, seasonality of moisture source, and the resulting response in leaf wax $\delta^2\text{H}$ could be critical to interpreting *n*-alkane isotope ratio data.

The ability to use $\delta^2\text{H}$ values from plant leaf waxes to reconstruct hydrologic conditions is primarily governed by the sum of processes that occur before wax formation (Sachse et al. 2012, 2015; Tipple et al. 2013). These include climate-driven differences in source water isotopes, alteration of the plant water environment through leaf-level biophysical processes, and lastly, biosynthesis of plant lipids in the leaf water environment. Arguably, the most important control on the hydrogen composition of leaf waxes for the purposes of climatic reconstruction is the $\delta^2\text{H}$ of local precipitation (Sauer et al. 2001; Sessions 2006; Hou et al. 2007, 2008). The isotopic composition of this local environmental water varies based on regional factors such as altitude, temperature, dominant wind patterns influencing moisture sourcing, intensity of storms, distance from coastal regions, and humidity (Dansgaard 1964; Craig and Gordon 1965; Rozanski et al. 1993; Bowen 2010). The continental effect of Rayleigh distillation helps explain the observation of progressive ${}^2\text{H}$ depletion with increasing latitude (Dansgaard 1964). The ${}^2\text{H}$ depletion with latitude and with associated rain-out effects within a single storm

in global monsoonal regions (so-called amount effect) have been documented in instrumental records and models as well as theorized for proxy records (Rozanski et al. 1982; Bowen and Revenaugh 2003; Hou et al. 2008; Schefuß et al. 2011). We suggest that precipitation will become ${}^2\text{H}$ enriched with both progressive movement southward along the NAM transect from decreased rain-out distance and an increased supply of warm, summer (monsoonal) fraction of annual precipitation.

Evapotranspiration is also known to control ${}^2\text{H}$ enrichment of plant leaf waters following precipitation (Leaney et al. 1985; Flanagan and Ehleringer 1991; Ehleringer and Dawson 1992). For instance, though little to no fractionation is thought to occur in plants during initial uptake of water by plant roots and its transport to leaves (White et al. 1985; Ehleringer and Dawson 1992), evaporative enrichment occurs in leaves associated with transpiration, whereby water entering and leaving the leaves is isotopically the same, but the pool within the leaves becomes ${}^2\text{H}$ enriched through evaporation and condensation fractionations (Flanagan and Ehleringer 1991; Roden and Ehleringer 1999). It is from this enriched leaf water environment that epicuticular waxes are synthesized (Sachse et al. 2012; Kahmen et al. 2013a, b; Tipple et al. 2013). The $\delta^2\text{H}$ values of leaf water can be altered either via direct transpiration by the leaves or more indirectly from soil moisture evaporation (Flanagan and Ehleringer 1991). The relative contribution of this enrichment to leaf wax $\delta^2\text{H}$ values is often debated, focusing on the importance of the drivers of relative humidity, temperature, and water vapor surrounding the leaf (Kahmen et al. 2008; Sachse et al. 2012). We suggest that in the relatively arid environment along this NAM transect, evapotranspiration will play a significant role in shaping the $\delta^2\text{H}$ values of leaf waxes observed.

The NAM is a large-scale atmospheric circulation system characterized by a pronounced increase in moisture, primarily between July and early September in southwestern North America (Barron et al. 2012). The NAM creates a seasonally, interannually, and geographically variable precipitation regime across this region. The winds shift from westerly in winter to southerly in summer, delivering moisture for this summer rainfall primarily from the subtropical eastern Pacific, Gulf of California, and minimally from the Gulf of Mexico. The movement of this precipitation is primarily driven by a movement of the Intertropical Convergence Zone northward (Adams and Comrie 1997; Higgins and Shi 2001; Barron et al. 2012). This supplies the generally dry region from northern Mexico through parts of Utah with an average of 35 to 60 % of their annual moisture (Mitchell 1976; Carleton et al. 1990; Mitchell et al. 2002). Timing of the NAM is controlled by both sea surface temperatures off the west coast of North America and thermal contrast between marine and continental regions,

as precipitation migrates from south to north (Higgins et al. 1999). There has been much work characterizing the modern signature of the NAM with modeling efforts, climatological measurements, and observations (Maddox et al. 1995; Adams and Comrie 1997; Barron et al. 2012). Efforts to reconstruct the paleo-monsoon have also been extensive, particularly those studies using tree ring records (Griffin et al. 2013). The effort to reconstruct past precipitation in the desert southwest over a large range of timescales (~10 to >100,000 years) has successfully used a wide variety of proxies (Wurster et al. 2008; English et al. 2010; Fawcett et al. 2011).

While seasonality governs delivery of precipitation to the NAM region, plant rooting distributions influence the availability of this moisture for plant growth. Moisture from intense summer monsoonal storms is found only in the upper soil profile and does not remain for long due to high amounts of evaporation (Schwinnning et al. 2005a). Evaporation of this uppermost soil moisture occurs within 5–10 days of rainfall and plant uptake of this water begins within 1–3 days (Lin et al. 1996; Schwinnning et al. 2005b, a). Winter storms penetrate deeper into the soil than summer storm events and may contribute more to total annual precipitation than summer rainfall events (Ehleringer and Dawson 1992; Reynolds et al. 2004; Schwinnning et al. 2005a, b). Winter or spring storms are gentler and occur during times of overall lower evaporation, allowing a greater percentage of this moisture to recharge deep soil layers than in summer (Caldwell 1985). Additionally, cooler temperatures in winter limit plant growth, allowing this water to penetrate deeper into long-term soil storage (Caldwell 1985; Ehleringer et al. 1998). Winter recharge events or multi-year averages are more likely to be reflected in moisture at greater depth, whereas summer monsoonal events are more likely to be reflected in moisture in shallower soil layers (Schwinnning and Sala 2004).

Plants found in arid regions have rooting structures to access multiple levels of soil water, exploiting the variable timing of precipitation delivery and storage in the desert (Ehleringer et al. 1991; Williams and Ehleringer 2000; Chesson et al. 2004; Reynolds et al. 2004; Ryel et al. 2004). Perennials have been noted to have dimorphic rooting structure, a rooting architecture that allows them to extract moisture from both shallow and deep positions in the soil (Forseth et al. 1984; Manning and Barbour 1988; Ehleringer et al. 1991; Schwinnning and Sala 2004; Schwinnning et al. 2005a). Those perennials at the northern end of the NAM and with only deep roots are thought to be less likely to utilize monsoonal precipitation, suggesting surface water in these arid regions may be of less importance to long-term water balance (Ehleringer and Dawson 1992; Williams and Ehleringer 2000). However, perennials with shallow roots (<1 m) may have a competitive advantage

because of their ability to use the most abundant precipitation source to the region, monsoonal rain (Lin et al. 1996), as well as winter precipitation. The relative usage of these sources by perennials with multiple rooting depths is likely to vary year to year depending on seasonal storms (Flanagan et al. 1992). Ehleringer et al. (1991) found that perennial species utilize surface or deep soil moisture sources for growth, often switching sources depending on moisture availability during the growing season. Winter recharge events are more likely to be reflected in moisture at greater depth, whereas summer monsoonal events are more likely to be reflected in moisture in shallower soil layers (Schwinnning and Sala 2004).

Recent observations have found that the leaf wax $\delta^2\text{H}$ of broadleaf angiosperms records only a narrow timeframe surrounding leaf flush and does not further integrate climate or water conditions in the remainder of the growing season (Kahmen et al. 2011; Tipple et al. 2013). We can exploit this limited timing of wax formation to look for signals of precipitation dynamics associated with the NAM, a seasonally restricted precipitation regime, in leaf wax *n*-alkanes of angiosperms. In this study, we measured the $\delta^2\text{H}$ values of *n*-alkanes and relative abundances of *n*-alkane chain lengths from four common plants found along the length of a south-to-north US monsoonal transect. This survey allowed us to assess the possibility that different phenologies and precipitation seasonality affect the $\delta^2\text{H}$ values of leaf waxes.

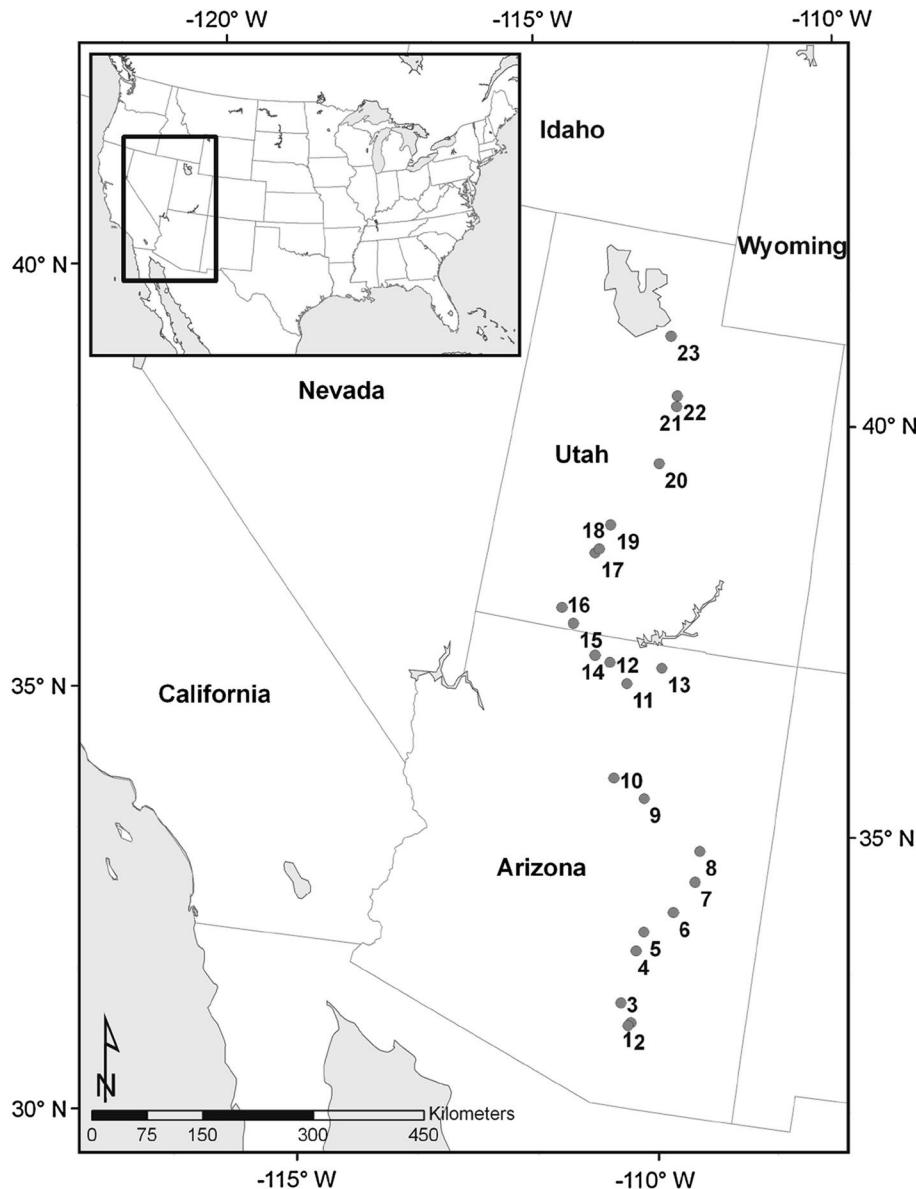
While leaf wax $\delta^2\text{H}$ values are a complex mixture of environmental, physiological, and biochemical variables, modern plant studies can provide a framework with which to interpret future sedimentary data sets, providing the ability to interpret $\delta^2\text{H}$ in light of these complications. Thus, strengthening the utility of $\delta^2\text{H}$ in leaf waxes for reconstructions of past hydrologic variability necessarily revolves around looking at both environmental and physiological controls of modern leaf waxes.

Materials and methods

Sampling locations and climate

We sampled four species spanning a 960-km (direct distance) precipitation transect between Tucson, Arizona and Salt Lake City, Utah (USA) from 22 to 26 September 2012 (Fig. 1; Table 1): sunflower (*Helianthus annus*), rabbit brush (*Chrysothamnus nauseosus*), sagebrush (*Artemesia tridentata*), and Gambel's oak tree (*Quercus gambelii*). These species were selected because of their wide geographic distribution, contrasting phenology, differential rooting structure (Fig. 2), and generally ubiquitous co-occurrence. Figure 2 shows rooting structures and their

Fig. 1 Map of North American Monsoon transect showing locations of the 23 unique sample sites from Tucson, Arizona to Salt Lake City, Utah



typical season of water uptake used in leaf wax development. Namely, *Q. gambelii* are often deeply rooted perennials, with winter deciduous leaves and active summer growth. Both perennials *A. tridentata* and *C. nauseosus* have dimorphic rooting structure, with drought deciduous leaves and active spring/summer growth. Lastly, *H. annus* is a shallowly rooted annual, with active growth in the summer. The distribution of sampled species spanned the entire transect, though not every species was available for collection at each site. This allowed for comparison of leaf waxes within and between species found at the northern and southern extent of the transect. We also compare deep- and shallow-rooted species in order to evaluate the relative importance of seasonal soil water on the $\delta^2\text{H}$ signal of these semi-arid plants, particularly between short-term

storm surface water and accumulated moisture deeper in the soil profile (Ehleringer et al. 1991, 1998; Williams and Ehleringer 2000).

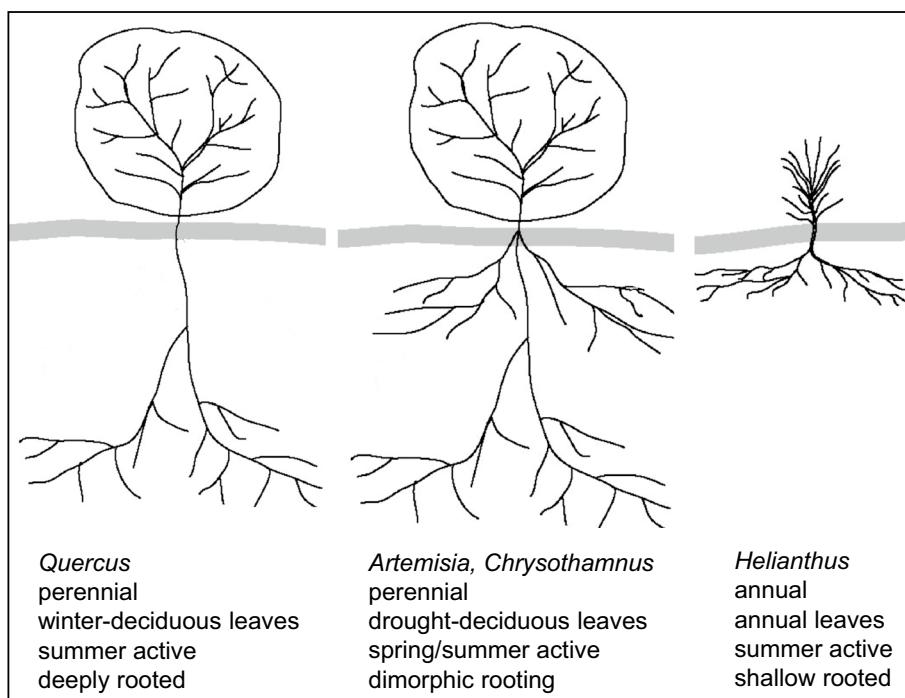
The sampling strategy for this study was based on evenly spaced collections of leaves from plants along the transect length, resulting in 23 sample sites (Fig. 1) and 43 different plant acquisitions (Table 1). Sites were chosen to provide coverage along the transect and to maximize the number of sites with as complete a complement of plant types as possible. Precipitation at sites ranged from 130 to 550 mm annual precipitation. July–August–September (JAS) precipitation at these sites varied from 43 to 212 mm, which accounted for 10–70 % of the annual precipitation in 2012, generally from north to south, respectively (Fig. 3). We examine the relationship of leaf wax $\delta^2\text{H}$ to the fraction of

Table 1 Sampled sites in Arizona and Utah

ID	Location	Latitude (°N)	Longitude (°W)	Elevation (m)	Plant types
NAM Stop 1	Mt Lemmon, AZ	32.38	-110.68	1868	QG
NAM Stop 2	Mt Lemmon, AZ	32.34	-110.72	1481	QG
NAM Stop 3	Catalina, AZ	32.60	-110.87	1150	H
NAM Stop 4	Tonto Natl Forest, AZ	33.26	-110.78	1486	QG
NAM Stop 5	Globe, AZ	33.50	-110.71	1287	QG
NAM Stop 6	Show Low, AZ	33.79	-110.33	1630	H, QG
NAM Stop 7	Show Low, AZ	34.20	-110.08	1986	QG
NAM Stop 8	Snowflake, AZ	34.59	-110.09	1691	H
NAM Stop 9	Coconino, AZ	35.11	-111.03	1643	H, RB
NAM Stop 10	N Flagstaff, AZ	35.30	-111.52	1675	RB
NAM Stop 11	N of Tuba City, AZ	36.47	-111.57	1715	RB
NAM Stop 12	N Grand Canyon, AZ	36.69	-111.88	1493	S
NAM Stop 13	Kaibito, AZ	36.73	-111.09	1708	RB, S
NAM Stop 14	Jacob Lake, AZ	36.74	-112.12	2062	QG, RB, S, H
NAM Stop 15	Kanab, UT	37.08	-112.54	1518	QG, RB, S, H
NAM Stop 16	Zion Park, UT	37.25	-112.76	1784	QG, RB, S, H
NAM Stop 17	Panguitch, UT	37.98	-112.40	1965	S
NAM Stop 18	N Panguitch, UT	38.04	-112.36	1920	H
NAM Stop 19	Marysvale, UT	38.35	-112.24	2095	QG, S
NAM Stop 20	Sterling, UT	39.20	-111.66	1786	QG, S, RB, H
NAM Stop 21	Birdseye, UT	39.93	-111.54	1666	RB, S, QG
NAM Stop 22	Spanish Fork, UT	40.06	-111.56	1477	H
NAM Stop 23	SLC, UT	40.77	-111.82	1510	RB, H, QG, S

Description of 23 sampled sites in Arizona and Utah, including latitude, longitude, elevation (m), and plant types sampled at each stop

Natl National, SLC Salt lake City, QG *Quercus gambelii*, H *Helianthus annuus*, S sagebrush (*Artemisia tridentata*), RB rabbit brush (*Chrysothamnus nauseosus*)

Fig. 2 Schematic showing general rooting structures and typical seasonal preference of water uptake for leaf wax development

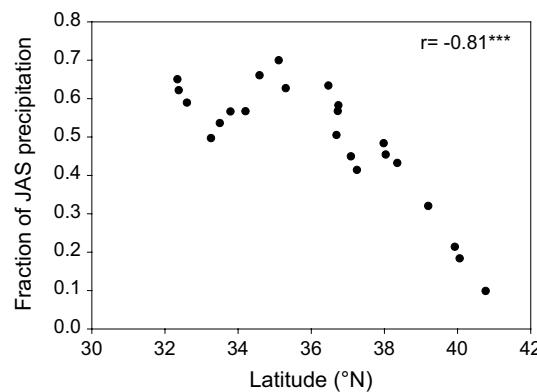


Fig. 3 Latitudinal distribution of the fraction of total, annual precipitation that occurs in the summer months of July–August–September (JAS)

precipitation arriving in certain seasons as a proxy for the influence of the NAM by looking at the fraction of precipitation to arrive in the summer or winter months relative to the total amount received in the year prior to this sampling.

All precipitation, temperature, and relative humidity data were determined using the parameter-elevation regressions on independent slopes model (PRISM) (PRISM Climate Group, Oregon State University, <http://prism.oregonstate.edu>), using discrete measurements of climate factors to interpolate continuous, digital grid estimations, providing high spatial resolution over the geographic area and time period of this study. PRISM uses point measurements of temperature, precipitation, and dew point to create a monthly 2.5×2.5 -mile grid across the US with data originating from, but not limited to, the National Weather Service, Natural Resources Conservation Service, US Forest Service and Bureau of Land Management stations, Bureau of Reclamation stations, other state and local station networks and National Weather Service/Federal Aviation Administration Automated surface observation stations. Relative humidity estimates were determined using monthly dewpoint (T_d) and monthly air temperatures (T) from PRISM using the equation:

$$RH = \frac{e^{\left(\frac{17.269 \times T_d}{273.3 + T_d}\right)}}{e^{\left(\frac{17.269 \times T_{\text{mean}}}{273.3 + T_{\text{mean}}}\right)}} \times 100\%$$

All $\delta^2\text{H}$ values of precipitation were determined using long-term averages of monthly precipitation at specified location using the online isotopes in precipitation calculator (OIPC) (<http://waterisotopes.org>). The OIPC uses geo-statistical modeling and spatial interpolation of isotope precipitation data from 1960 to 2004 from 348 global network for isotopes in precipitation instrumental record locations to yield site-specific monthly $\delta^2\text{H}$ values and amount-weighted annual $\delta^2\text{H}$, based on $\delta^2\text{H}$ values for each month

weighted to the amount of precipitation that fell in each month (Bowen and Wilkinson 2002; Bowen and Revenaugh 2003). OIPC provides a quantitative and statistically robust estimation of average precipitation isotope values along a spatial gradient (Bowen and Wilkinson 2002; Bowen and Revenaugh 2003).

Sampling methods

Leaf collections for $\delta^2\text{H}$ analysis of epicuticular wax *n*-alkanes began with collections of exposed sun leaves from the south side of the plants to minimize potential isotopic effects associated with shading. Collections focused on whole leaves of the newest, active growth in order to study the most recent growth. Leaves were stored in paper bags, allowed to air dry, and then moved to a 50 °C oven to finish drying upon return to the laboratory.

Analytical methods

Lipid extraction

Leaves were ground with a solvent-rinsed mortar and pestle. Ground samples (between 0.3 and 2 g) were then sonically extracted using ~10 mL of 2:1 dichloromethane:methanol (v:v) for 20 min, repeated three times in a manner similar to Feakins and Sessions (2010). This lipid extract was then dried under a stream of gaseous dinitrogen before being transferred to a silica-gel column (5 $\frac{3}{4}$ -inch Pasteur pipette with solvent extracted and water-deactivated silica gel, 70–230 mesh) for *n*-alkane fraction elution using hexane.

Leaf wax analyses

n-Alkanes were identified in select samples using a gas chromatograph–mass spectrometer (GC–MS) with a split-splitless injector at 300 °C with a fused silica, DB-5 phase column and helium carrier gas at a flow of 1.5 mL/min and comparison of elution times of *n*-alkane reference standards. The GC oven temperature program utilized was 60–320 °C at 15 °C/min with an isothermal for 30 min. *n*-Alkanes were quantified using a GC-flame ionization detector (FID) with comparison to a known amount of internal standard, C₃₆ alkane, which was not naturally occurring in any of the samples analyzed. The GC-FID temperature program began at 80 °C and increased at a rate of 6 °C/min to 320 °C and held for 20 min.

Compound-specific isotope analyses were performed using a Thermo Trace 2000 GC coupled to a Finnigan Delta V isotope ratio mass spectrometer interfaced with a high-temperature conversion system. The H₃⁺ factor was determined daily prior to standard calibration and sample analysis. All compound-specific $\delta^2\text{H}$ values are expressed

relative to Vienna Standard Mean Ocean Water (VSMOW). Individual *n*-alkane isotope ratios were corrected to primary in-house *n*-alkane reference materials that are run daily, and previously calibrated to the VSMOW scale (Tipple et al. 2013). The results are described using standard delta notation [$\delta^2\text{H}$ values in per mil (\textperthousand)]. Accuracy of the measurement of compound-specific $\delta^2\text{H}$ was $\pm 3\text{ \textperthousand}$ as determined from a secondary quality control *n*-alkane standard reference material, C₃₆ alkane. All samples were run in duplicate and the average value was used for this study, with a mean error of $\pm 4\text{ \textperthousand}$ or better. The dominant chain lengths of *n*-alkanes in all species were determined by normalizing abundance of chain lengths in micrograms of *n*-alkane to dry weight of leaves extracted in grams.

Statistics

Statistical analysis was done using PRISM and InStat (Graphpad Software) and Microsoft Excel for Windows. The correlation coefficient (r) was calculated to determine the measure of linear dependence between variables, with the sign defining the direction of the relationship. The associated p -value is used to assess the significance of this correlation.

Leaf water model

Craig and Gordon (1965) originally created a model to describe the evaporative isotope fractionation from an open water source. Modifications to this model through the addition of relevant parameters for transpiration from a leaf (Flanagan and Ehleringer 1991) allow us to describe water fractionation associated with transpiration through stomata:

$$R_{lw} = \alpha^* \left[\alpha_k R_{xw} \left(\frac{e_i - e_a}{e_i} \right) + \alpha_{kb} R_{xw} \left(\frac{e_s - e_i}{e_i} \right) + R_a \left(\frac{e_a}{e_i} \right) \right]$$

where R_{lw} (leaf water), R_{xw} (xylem water), and a (bulk air) are described. The partial pressures of water vapor of the leaf intercellular spaces (e_i), the leaf surface (e_s), and the air (e_a) are also present in this equation. Leaf surface pressures were determined from equations in Ball (1987), which needs stomatal conductance and leaf transpiration estimations (Roden and Ehleringer 1999). In the above equation, α^* is the equilibrium fractionation factor with movement from the liquid to vapor state, dependent on temperature and determined by the equations of Majoube (1971). The term α_k refers to the kinetic fractionation factor related to diffusion in air from the site of evaporation, 1.0164 for $\delta^2\text{H}$ (Cappa et al. 2003). Lastly, α_{kb} describes

the kinetic fractionation factor associated with diffusion across the boundary layer, $\delta^2\text{H} = 1.0110$ (Flanagan 1993).

Measured leaf wax $\delta^2\text{H}$ were compared to model leaf wax, determined using the modified Craig–Gordon model described above with a few assumptions. For the purposes of this model, the fractionation (hereafter referred to as ε , in keeping with more recent modeling efforts) between leaf wax compounds and plant source water was set at $-123\text{ \textperthousand}$ (reported in \textperthousand notation), consistent with the offset found in previous studies (Chikaraishi et al. 2004; Tipple et al. 2013). This fractionation is defined as

$$\varepsilon = [R_{\text{lipid}}/R_{\text{water}} - 1]$$

where R represents the $\delta^2\text{H}$ abundance ratio, and R_{lipid} and R_{water} are the ratios in the leaf wax *n*-alkanes and source growth water, respectively. The $\delta^2\text{H}$ value of leaf water was modeled using average conditions along the transect, related to the source water, equilibrium isotope effect, and kinetic isotope effect. The equilibrium fractionation factor was modeled in isotopic equilibrium with source water (Craig and Gordon 1965), barometric pressure and leaf temperature offset (2 °C) held constant (Flanagan 1993) and with an offset of atmospheric water vapor dependent on temperature related to modeled season of precipitation temperature (10 °C = 1.09788, 20 °C = 1.0840, 30 °C = 1.0740) (Majoube 1971). The modeled leaf water isotopic composition at the site of evaporation (Yakir 1998) also accounted for relative humidity estimates based on reasonable humidity estimates for JAS (35 %) and December (2011)–January–February (2012) (DJF) (50 %) using PRISM as described above.

Results and discussion

Environmental control on *n*-alkane distribution

All samples yielded sufficient *n*-alkanes for $\delta^2\text{H}$ analysis of at least the dominant chain length, although the distribution varied among species. The chain lengths C₂₇, C₂₉, and C₃₁ were the three most abundant in all species (Fig. 4). C₂₉ was the dominant *n*-alkane found in all species sampled, with the exception of *H. annuus*, where C₂₇ was always the most abundant. There was a strong odd-over-even predominance in *n*-alkane carbon chain lengths in all species, as has previously been described (Eglinton and Hamilton 1967).

Measurements of absolute *n*-alkane abundances, normalized to dry leaf weight of extracted leaf material, show wide variability between species. *Q. gambelii* showed the lowest cumulative *n*-alkane abundance with 122 µg/g dry leaf weight. Conversely, *A. tridentata* had the most *n*-alkane-rich leaf waxes with 716 µg/g dry leaf weight. *H.*

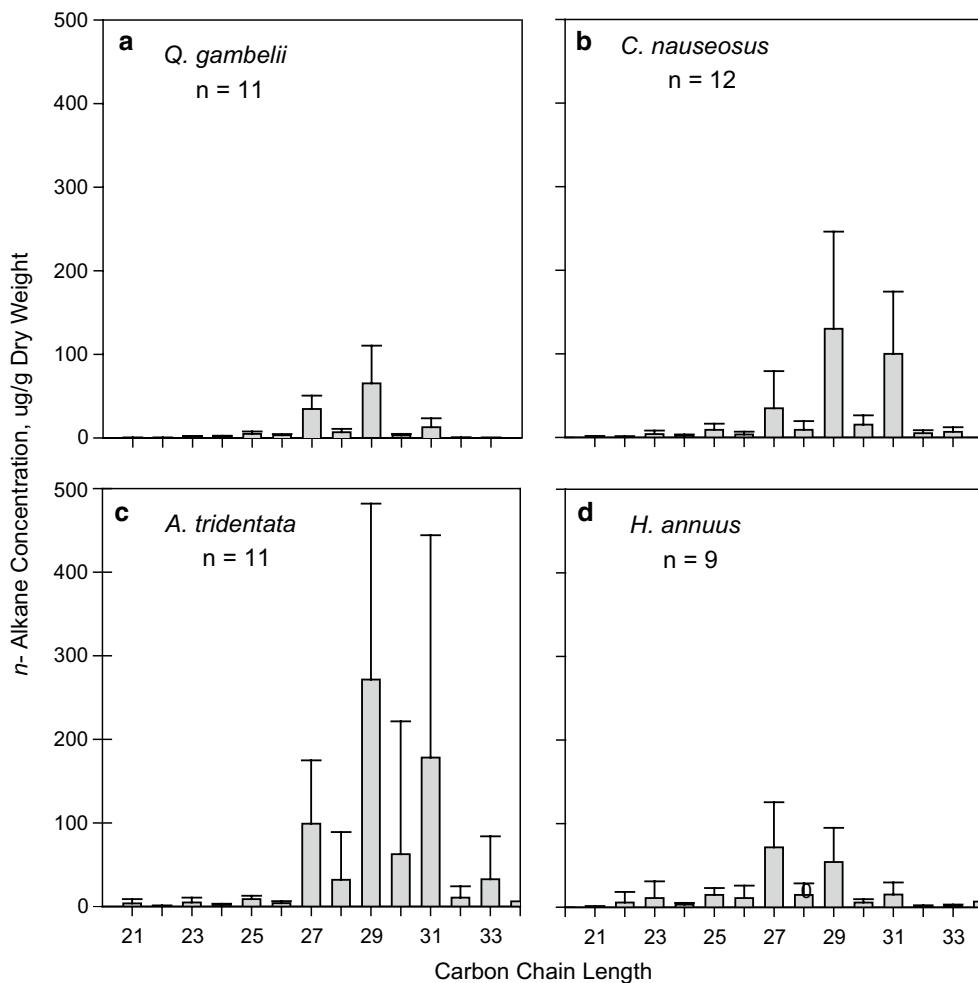


Fig. 4 Histogram of mean concentration of individual *n*-alkanes from sampled leaf waxes. Concentrations are reported in micrograms *n*-alkane per mass dry leaf extracted in grams ($\mu\text{g/g}$). Error bars depict 1 SD, indicating the variability around the mean for chain

lengths of these four species. These means and SDs are based on the following number of individuals: **a** *Quercus gambelii*, $n = 11$; **b** *Chrysothamnus nauseosus*, $n = 12$; **c** *Artemisia tridentata*, $n = 11$; **d** *Helianthus annuus*, $n = 9$

annuus and *C. nauseosus* contained 217 and 322 $\mu\text{g/g}$ dry leaf weight, respectively.

Average chain lengths (ACL) were calculated for each individual of each species using the equation $\text{ACL} = \Sigma(C_n \cdot n) / \Sigma C_n$, where $n = 25, 27, 29, 31$, and 33 (Poynter and Eglinton 1990). The mean ACL of each species were distributed between 27 and 30. *H. annuus* had the lowest ACL of these species (27.9) and *C. nauseosus* had the highest (29.5). *A. tridentata* had an ACL of 29.3 and *Q. gambelii* is somewhat lower at 28.3. Mean ACL is representative of all individuals, with no SD greater than 0.65 (observed for *A. tridentata*).

While each species produced a different abundance of *n*-alkanes, there did not appear to be any consistent trend with any environmental variable in general with the sampled species or for a given species. *Q. gambelii* and *H. annuus* were the only species with marginally

significant correlations between *n*-alkane concentration and the environmental parameters tested (Table 2). *Q. gambelii* *n*-alkane concentration ($n = 11$) is marginally significant with mean annual precipitation ($r = 0.72$, $p = 0.03$). Though absolute abundances are often less cited in the literature, studies suggest that *n*-alkane concentration data may serve as estimates of past environmental change with associated uncertainty (Bush and McInerney 2013; Hoffmann et al. 2013). The absence of a strong correlation for these four species and four environmental variables suggested that while there may be a discernable relationship between amount of *n*-alkanes and local conditions, caution was necessary when using concentration data along a transect for environmental interpretation.

With one exception, we found no significant relationship between ACL and environmental parameters mean

Table 2 Matrix of correlation coefficients for environmental variables and leaf wax $\delta^2\text{H}$ values

Correlation coefficients	ACL		$\delta^2\text{H}$ wax				
	Mean annual pptn	Mean annual temperature	Annual $\delta^2\text{H}$ pptn ^a	JAS $\delta^2\text{H}$ pptn ^a	DJF $\delta^2\text{H}$ pptn ^a	Fraction pptn, JAS ^b	Fraction pptn, DJF ^b
<i>C. nauseosus</i>	0.44	0.15	0.83***	0.83***	0.87***	0.73**	-0.78**
<i>A. tridentata</i>	0.00	-0.15	0.80**	0.76**	0.83***	0.30	-0.39
<i>Q. gambelii</i>	0.34	0.05	0.90***	0.91***	0.88***	0.73**	0.30
<i>H. annuus</i>	0.23	-0.13	0.21	0.22	-0.32	0.57	-0.66*

Correlation coefficients of average chain length (ACL) with mean annual precipitation (pptn) amount (as determined by PRISM) and ACL with mean annual temperature (as determined by PRISM) to investigate possible environmental influences on ACL

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

^a Correlation coefficients between the $\delta^2\text{H}$ values of leaf wax *n*-alkanes and annual pptn, July–August–September (JAS) 2012, and December (2011)–January–February (2012) (DJF) pptn $\delta^2\text{H}$ values (as determined by the online isotopes in precipitation calculator)

^b Correlation coefficients of $\delta^2\text{H}$ values of leaf wax *n*-alkanes and the fraction of annual pptn that falls in JAS and DJF

annual precipitation and mean annual temperature of the sampled sites (Table 2). While selected studies have observed significant changes of ACL along climate gradients for a large variety of species and environmental conditions, in modern plants and fossil plant waxes in sediments and soils these results are not always consistent (Dodd and Afzal-Rafii 2000; Dodd and Poveda 2003; Rommerskirchen et al. 2003; Sachse et al. 2006; Bush and McInerney 2013). *n*-Alkane chain length distribution is often linked to environmental changes such as temperature or aridity, thought to be due to a plant's physiological and structural response of the crystalline component of epicuticular leaf waxes to these conditions (Schönherr 1976; Riederer and Schneider 1990; Schreiber and Riederer 1996). An increased growing season, preferential evaporative loss of shorter chain lengths with increased evaporation, selective increase in formation of longer chain lengths to prohibit evaporative water loss, or increased solar radiation have all been theorized as possible explanations for the observations of more abundant long chain lengths at higher temperature, low latitudes, or more arid sites (Rommerskirchen et al. 2003; Sachse et al. 2006). Theorized relationships of chain lengths to environmental conditions are then applied to sediment and soil reconstructions in order to describe shifts in dominant vegetation or other environmental parameters. As such, some sedimentary *n*-alkanes have shown similar relationships, with increased chain length corresponding to increased temperature (Hughen et al. 2004; Castañeda et al. 2009) while other sedimentary reconstructions of ACL have shown no shift along a significant climatic gradient (Ernst et al. 2013). We found no consistent trend in ACL data, which suggests that there is little environmental control on *n*-alkane chain length and that the use of ACL for paleoenvironmental reconstructions of environmental conditions in arid ecosystems may not be successful.

Leaf wax *n*-alkane $\delta^2\text{H}$ values along a transect

As predicted, the summer fraction of annual precipitation correlated significantly with latitude (Fig. 3). The decreasing influence of the NAM on summer precipitation was clearly evident progressing from south to north, with only ~10 % of annual precipitation falling in the summer months in the northern end of this transect (Salt Lake City, UT). Notably, all but the four northernmost sites (north of ~38–39°N) received more than 40 % of their annual precipitation in the summer (Fig. 3). This suggests a significant summer monsoonal precipitation contribution for the sampled year (2012) for all sites south of 39°N.

We note large variations in $\delta^2\text{H}$ values of leaf wax *n*-alkanes among individuals of each species, between species, as well as across the NAM transect (Fig. 5). For comparative purposes, the C_{29} *n*-alkane $\delta^2\text{H}$ values were used for all species, including *H. annuus*, because chromatographic separation of C_{27} in some *H. annuus* individuals did not allow for isotopic determination. The average $\delta^2\text{H}$ value of all individuals of the five species was $-162 \pm 31 \text{‰}$ (1σ), $n = 43$ individuals. *n*-Alkane $\delta^2\text{H}$ values of *C. nauseosus* ranged from -146 to -239‰ (mean $-195 \pm 31 \text{‰}$, 1σ , $n = 12$), *H. annuus* ranged from -124 to -190‰ (mean $-151 \pm 27 \text{‰}$, 1σ , $n = 9$), *A. tridentata* ranged from -122 to -195‰ (mean $-159 \pm 23 \text{‰}$, 1σ , $n = 11$), and *Q. gambelii* ranged from -110 to -195‰ (mean $-171 \pm 24 \text{‰}$, 1σ , $n = 11$). There is a progressive depletion in ^2H wax values moving northward away from the regions most impacted by monsoonal moisture (Fig. 5).

At all sites with at least one representative of each species sampled (Table 1), *C. nauseosus* always exhibited the most ^2H -depleted leaf wax *n*-alkanes, while *Q. gambelii* was most often the most ^2H enriched at these sites. The southernmost collocated site had the lowest range among species, ~18 % between *Q. gambelii* and *C. nauseosus* at

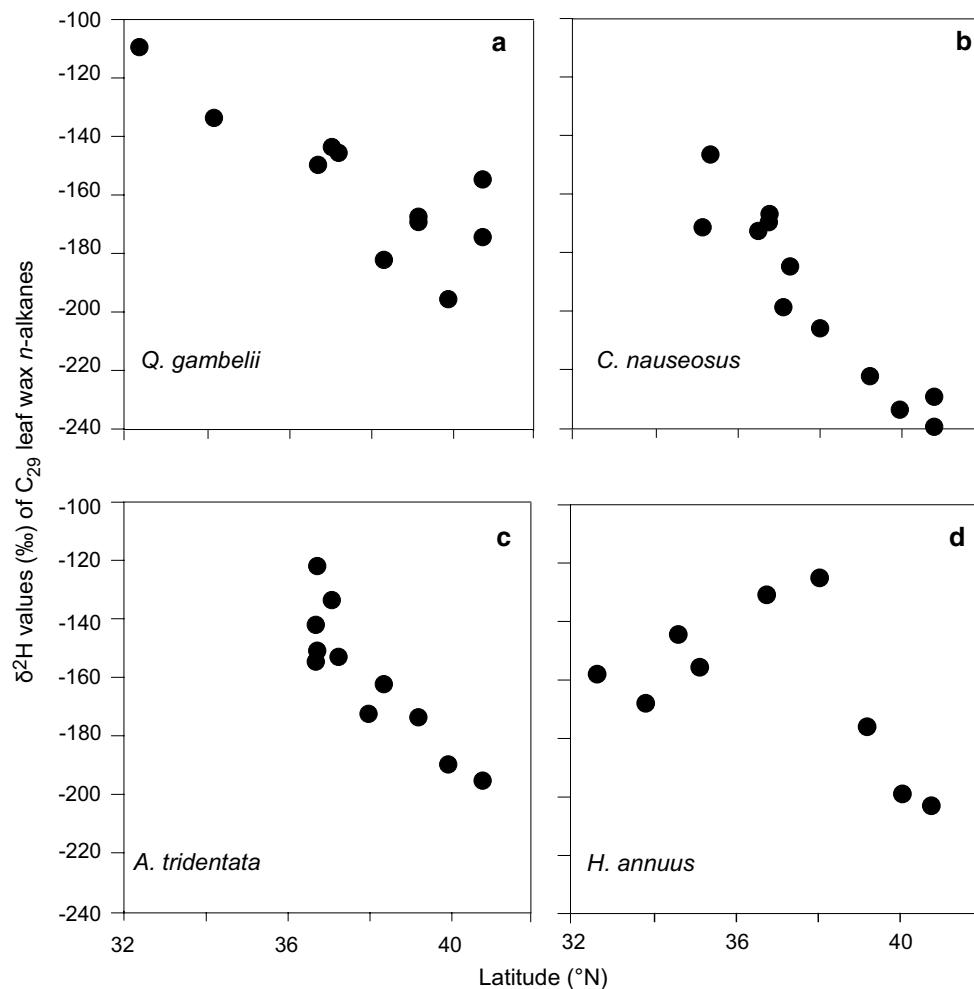


Fig. 5 Hydrogen isotope ratio ($\delta^2\text{H}$) values of C_{29} *n*-alkanes from leaf waxes of individual plant species **a** *Q. gambelii*, **b** *C. nauseosus*, **c** *A. tridentata*, **d** *H. annuus* versus latitude ($^{\circ}\text{N}$)

36.74°N. The largest range in *n*-alkane $\delta^2\text{H}$ values among species occurred at the most northerly site (40.77°N), with *C. nauseosus* ~55–85 ‰ ^2H depleted relative to *Q. gambelii* based on multiple co-located individuals from each species. *H. annuus* is more ^2H enriched at equivalent latitudes than *C. nauseosus*, but not than *Q. gambelii* or *A. tridentata*. *Q. gambelii* is often more ^2H enriched at equivalent sites than the other species sampled.

Seasonal precipitation influence on leaf wax $\delta^2\text{H}$ values

Prior research suggests that not all vegetation types on this transect would equally utilize summer moisture in a “pulse-driven ecosystem,” where brief, intense rains are unpredictable with regards to timing, spatial coverage, and contribution of moisture towards annual total moisture amount (Ehleringer et al. 1998; Chesson et al. 2004; Schwinnning and Sala 2004). Significant correlations between $\delta^2\text{H}$ and MAP in previous transect studies have been attributed

to less seasonally biased precipitation input than in this monsoon-influenced region (Sachse et al. 2006; Tipple and Pagani 2013). There was no correlation to MAP for *Q. gambelii*, *H. annuus*, and *C. nauseosus* ($r = -0.04, p = 0.9070$; $r = -0.67, p = 0.0483$; $r = -0.24, p = 0.4772$, respectively); however, *A. tridentata* showed weak but significant correlation with MAP ($r = -0.68, p = 0.0213$).

Thus, we compared measured leaf wax *n*-alkanes to PRISM-estimated precipitation amounts from different seasons along this transect to look at possible seasonal bias in source water usage influencing $\delta^2\text{H}$ values. These comparisons allow us to speculate on some of the potential drivers of observed $\delta^2\text{H}$ value variation, but cannot eliminate the influence of multiple variables, as discussed below, particularly since xylem water is not available for comparison to measured leaf wax *n*-alkanes in this study.

Leaf wax *n*-alkane $\delta^2\text{H}$ values of *C. nauseosus* ($r = 0.73, p = 0.0046$) and *Q. gambelii* ($r = 0.73, p = 0.0007$) exhibited positive significant correlations with the amount of

precipitation arriving in JAS at each site across the entire transect (Table 2). Leaf wax *n*-alkane $\delta^2\text{H}$ values of *A. tridentata* were almost statistically significant with JAS precipitation amount ($r = 0.57, p = 0.053$) (Table 2). Further, the only perennial to exhibit a statistically significant correlation with winter DJF precipitation amount was *C. nauseosus* ($r = -0.78, p = 0.0077$). This negative correlation suggests that as the amount of precipitation arriving in DJF decreases, the $\delta^2\text{H}$ values become more ^2H enriched. Based on prior studies we might expect that the $\delta^2\text{H}$ values of leaf waxes would correlate to seasonality of precipitation, and might expect to observe these relationships with amount of precipitation that arrives at each location. However, there is inconsistent sensitivity of leaf wax $\delta^2\text{H}$ values to seasonal amount of precipitation in perennials, suggesting that seasonal precipitation influence on ^2H values of leaf waxes might not be best tracked by amount of precipitation along the transect.

Comparison of leaf wax *n*-alkane $\delta^2\text{H}$ values and $\delta^2\text{H}$ values of seasonal precipitation indicates significant correlations for all perennial plants (Table 2), *C. nauseosus* ($r = 0.83, p = 0.0004$), *A. tridentata* ($r = 0.76, p = 0.0041$), and *Q. gambelii* ($r = 0.91, p = 0.0001$), with wax $\delta^2\text{H}$ values and $\delta^2\text{H}$ values of JAS precipitation. However, we also found statistically significant correlations with $\delta^2\text{H}$ values of DJF precipitation for *C. nauseosus* ($r = 0.87, p = 0.0001$), *A. tridentata* ($r = 0.83, p = 0.0008$), and *Q. gambelii* ($r = 0.88, p = 0.0002$). Perennials *C. nauseosus*, *A. tridentata*, and *Q. gambelii* showed a strong positive correlation to the arrival of both summer and winter precipitation, perhaps due to a tiered rooting structure and access to both long-term stored water, more similar in abundance and isotopic value to DJF precipitation, and soil water within the upper 1 m supplied by intense summer precipitation. *Q. gambelii*, often represented as predominantly deeply rooted (Fig. 2), appears along this transect to behave more like the other perennials that have tiered rooting, perhaps to take advantage of monsoon precipitation. Similarly, Feakins and Sessions (2010) observe that *Q. agrifolia* leaf waxes exhibit no seasonal variability in $\delta^2\text{H}$ values, though the isotopic composition of precipitation at the site is dominated by clear seasonal precipitation amount and isotopic variability. However, Hou et al. (2007) hypothesized that trees in their data set did not appear to reflect the $\delta^2\text{H}$ values of deeper groundwater, since trees appeared to have more ^2H -enriched leaf wax *n*-alkanes (~60–70 %) than herbs and grasses, which are thought to rely on shallow, more evaporated, surface waters. *H. annuus*, the only annual plant sampled on this transect, germinates in late spring and grows throughout the summer (Everett et al. 1980; Caldwell 1985). This window of leaf development and expansion would likely encapsulate the source water isotopic signature as well as the leaf-water enrichment effects (Sachse

et al. 2010; Kahmen et al. 2011; Tipple et al. 2013), ensuring that the isotopic composition of *n*-alkanes reflects leaf water at the time of leaf expansion. However, the $\delta^2\text{H}$ of *H. annuus* leaf waxes did not show significant correlations with either the amount or $\delta^2\text{H}$ of summertime precipitation. This finding is inconsistent with past studies of plant water sources, where desert annuals exhibited the strongest utilization of summer rain (Ehleringer et al. 1991).

Additional drivers of leaf wax *n*-alkane $\delta^2\text{H}$ values

Observations of precipitation and leaf wax $\delta^2\text{H}$ values point towards added complexity in these relationships, likely in the fractionation of source water or leaf water, ultimately observed in leaf wax $\delta^2\text{H}$ values (Sachse et al. 2012). There are several possible explanations for the observed relationships between source water and leaf wax $\delta^2\text{H}$ values. We note a particularly large range of $\delta^2\text{H}$ values (~100 ‰) for all species, compared to a rather limited range of $\delta^2\text{H}$ values of precipitation (~20–30 ‰), regardless of the season. Further fractionation of source water must have additional control on $\delta^2\text{H}$ values of leaf wax lipids to account for this variability. Net fractionation, or the tracing of source water to lipid synthesis, is rarely straightforward, and more often involves a series of steps, driven by multiple physiological and environmental factors (Sachse et al. 2012). One such influence is varying rates of evapotranspiration, which has long been known to contribute to ^2H enrichment in lipid isotope values (Sauer et al. 2001; Sachse et al. 2004). We observe similar trends of *n*-alkane $\delta^2\text{H}$ values with latitude within plant life forms (i.e., shrubs) (Fig. 5). Interestingly, Sachse et al. (2012) noted pronounced scatter in the relationship of shrub C_{29} alkane $\delta^2\text{H}$ values and precipitation (mean annual), with no significant, positive relationship, suggesting an often complicated fractionation between source water and shrub wax $\delta^2\text{H}$ values. In most cases, the slopes of these data are less steep than those of global data in Sachse et al. (2012) and this may indicate a stronger evapotranspirative ^2H enrichment of source waters.

It is likely that evapotranspiration acted on the plants in this study, though we do not distinguish between the more direct ^2H enrichment of transpiration from the more indirect evaporative processes involved in soil evaporation. Previous studies have hypothesized that soil evaporation may play a significant role in ^2H enrichment of plant lipids (e.g., Smith and Freeman 2006; Sachse et al. 2009). A transect of Californian desert plants showed $\delta^2\text{H}$ values of leaf lipids and plant waters with little influence from soil evaporative conditions (Feakins and Sessions 2010). The plants on this arid transect were likely deeply rooted and not utilizing the significantly ^2H enriched surface soil waters (Ehleringer et al. 1991, 1998). Conversely, less arid locations with shallow rooted grasses indicate the importance of soil water

evaporation in ^2H enrichment seen in grass wax lipids (Smith and Freeman 2006; Hou et al. 2007). Plant water isotopic values, sources of wax isotopic values, have also long been known to be affected by transpiration (Flanagan and Ehleringer 1991). With measured plant water, Feakins and Sessions (2010) distinguished between soil evaporation and leaf transpiration, a distinction made in another field study that was looking at changing seasonal $\delta^2\text{H}$ values in barley (Sachse et al. 2010). The perennial plants on this transect are likely accessing ^2H -enriched surface waters as well as deeper stored soil water that has little significant ^2H enrichment from soil evaporation. These plants may potentially switch water sources depending on seasonal availability (Ehleringer et al. 1991, 1998; Williams and Ehleringer 2000). Monsoonal precipitation delivery in the region adds to this complexity, because warmer monsoonal precipitation is more ^2H enriched than winter precipitation and more likely to reside in surface soils to be further ^2H enriched.

In order to further explore what is driving the patterns of leaf wax $\delta^2\text{H}$ values along this transect we use a mechanistic model to look at plant source water and lipid $\delta^2\text{H}$ values. We examined two possible scenarios using a modified Craig–Gordon model (1965), initially put forth by Flanagan and Ehleringer (1991), to evaluate controls on observed $\delta^2\text{H}$ values. In the first model scenario, we assume a constant biosynthetic fractionation (ε_{bio}) between leaf water and *n*-alkane $\delta^2\text{H}$ values across the transect and for all species in order to predict leaf water and leaf wax *n*-alkane hydrogen isotope ratios for a given set of environmental conditions. In the second model scenario, we determine the apparent fractionation (ε_{app}) that provides the best agreement between measured leaf wax and modeled $\delta^2\text{H}$ lipid values using a given set of environmental conditions.

Determining the relative constancy of ε_{bio} , the fractionation between leaf water and lipid $\delta^2\text{H}$ values, has proven elusive due to the difficulty in gathering this data (Sachse et al. 2009). Studies have tried to estimate ε_{bio} by controlling influences of environmental variables including source water isotopic values, temperature, and light intensity. The $\delta^2\text{H}$ values of leaf waxes are most directly determined by $\delta^2\text{H}$ values of leaf water (Sachse et al. 2012; Tipple et al. 2014), which is made up of source (precipitation) water that can be significantly altered by subsequent evaporation, varying biosynthetic pathways among plant types (Sessions et al. 1999; Diefendorf et al. 2011), and metabolic pathways among plant types (Zhang et al. 2009; Kahmen et al. 2013b). Biosynthetic pathways among plant groups are thought to be the same (Schmidt et al. 2003), though ε_{bio} specific to plant species has been suggested (Kahmen et al. 2013b; Tipple et al. 2014). Previous field studies have suggested ε_{bio} may be relatively stable, with approximations ranging between -123 and -170‰ (Sessions et al. 1999; Chikaraishi et al. 2004; Feakins and Sessions 2010; Tipple

et al. 2013). However, others have shown that the ε_{bio} may not be constant in plants (Zhou et al. 2011; Sachse et al. 2012; Kahmen et al. 2013a).

We look for the set of conditions that best explain the measured *n*-alkane $\delta^2\text{H}$ values using OIPC interpolated seasonal precipitation (Fig. 6). For the purposes of the first modeling exercise described here, ε_{bio} was held at -123‰ across the transect for all species, an estimate chosen due to stable ε_{bio} determined and limited sampling location overlap with Tipple et al. (2013). Further, the $\delta^2\text{H}$ value of atmospheric water vapor is modeled in equilibrium with source water for these plants (Craig and Gordon 1965) with barometric pressure and leaf temperature offset (2 °C) held constant (Flanagan 1993). The remaining input parameters of temperature and relative humidity are calculated averages across the transect for JAS (average conditions across the transect are 35 % relative humidity, 30 °C) and DJF (average conditions across the transect are 50 % relative humidity, 10 °C).

The model with constant ε_{bio} , relative humidity, and temperature does not capture the overall variation of $\delta^2\text{H}$ values along the transect (Fig. 6). However, the best approximation of measured leaf wax *n*-alkane $\delta^2\text{H}$ values, where data align well along the 1:1 line, can be seen for different portions of the transect during different seasons (Fig. 6). In general, modeled $\delta^2\text{H}$ values are most similar to measured $\delta^2\text{H}$ values using DJF precipitation in the northern end of the transect and JAS precipitation in the southern portion of the transect. The intermediate $\delta^2\text{H}$ values are best modeled using spring (March–April–May) precipitation. The model suggests that we can only capture measured $\delta^2\text{H}$ values along the transect by starting with different seasonal source water (or incorporating humidity and temperature estimates), and that not all plants along this transect are using the same source water. Southern sites receive the largest fraction of their total precipitation in summer (Fig. 2), which tied to this modeling, suggests that these plants may be using shallow roots and short-lived monsoonal precipitation at this end of the transect. Northern sampled sites, which often have the most ^2H depleted *n*-alkane values in this study, which receive very little summer precipitation, seem to indicate a reliance on winter precipitation and thus perhaps deeper rooting structures.

However, the model does not fully capture measured leaf wax *n*-alkane $\delta^2\text{H}$ values, which stresses the importance of complicating evaporative factors beyond source water in determining leaf wax *n*-alkane $\delta^2\text{H}$ values. The modeling described is an oversimplification because it uses a fixed ε_{bio} and one set of average input temperature and humidity along the entire transect.

Apparent (net) fractionation (ε_{app}) between measured leaf wax and source precipitation water has been documented in the range between -34 and -204‰ , depending

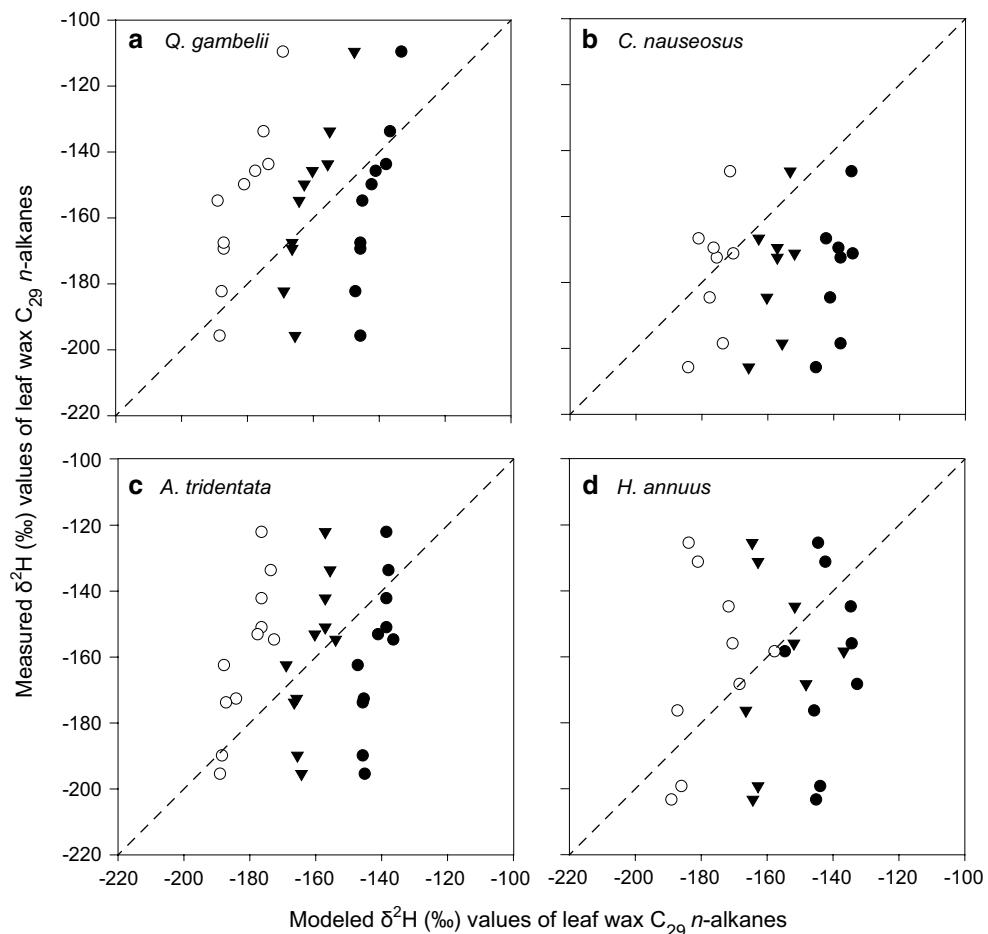


Fig. 6 $\delta^{2\text{H}}$ values of leaf wax C_{29} n -alkanes from individual species **a** *Q. gambelii*, **b** *C. nauseosus*, **c** *A. tridentata*, **d** *H. annuus* versus Craig–Gordon (1965) model-simulated leaf wax n -alkanes $\delta^{2\text{H}}$ values. Biosynthetic fractionation (ε_{bio}), defined as the fractionation between leaf water and n -alkane $\delta^{2\text{H}}$ values, was held constant (−123 ‰). Relative humidity (35 %) and temperature (30 °C) were

held constant, used as average of conditions along the transect [coinciding with averages for JAS across this region]. Precipitation (initial source water) is plotted for JAS (solid circle), December–January–February (open circle), and March–April–May (triangle), with vapor $\delta^{2\text{H}}$ in equilibrium with these values. The dashed line represents a 1:1 relationship

on influencing environmental and climate conditions (Sachse et al. 2012). The second model approach described here looks at the ε_{app} that matches measured and modeled leaf wax $\delta^{2\text{H}}$ lipid values. ε_{app} across all sites, species, and possible seasons of source water was -128 ± 32 , which falls within the published range of Sachse et al. (2012). Calculating the ε_{app} using JAS precipitation yields the most ^2H -depleted values (average of all sites—*Q. gambelii*, -137 ± 21 ; *A. tridentata*, -141 ± 20 ; *C. nauseosus*, 178 ± 28 ; *H. annuus*, -144 ± 28), with DJF precipitation showing the most ^2H -enriched (average of all sites—*Q. gambelii*, -95 ± 20 ; *A. tridentata*, -100 ± 19 ; *C. nauseosus*, 139 ± 27 ; *H. annuus*, -107 ± 28) ε_{app} . The difference in ε_{app} between JAS and DJF season precipitation and leaf wax $\delta^{2\text{H}}$ values remains relatively constant across all individuals and species (40 ± 2). However, individuals of a species show a range $>70 \%$ ε_{app} ,

indicating a significant environmental influence on ε_{app} across the transect. On average, *Q. gambelii* had the smallest ε_{app} and *C. nauseosus* often the largest. We noted that the calculated ε_{app} appears to correlate to environmental variables (Fig. 7). This strong relationship between ε_{app} and precipitation amount has been observed before (Smith and Freeman 2006). As predicted, the ε_{app} appears to vary as a function of climate in addition to plant species described above.

Conclusion

We collected two perennial shrubs (rabbit brush, *Chrysothamnus nauseosus*; sagebrush, *Artemisia tridentata*), a tree (Gambel's oak tree, *Quercus gambelii*), and an annual forb (sunflower, *Helianthus annuus*) along a transect from Tucson, Arizona to Salt Lake City, Utah to investigate the

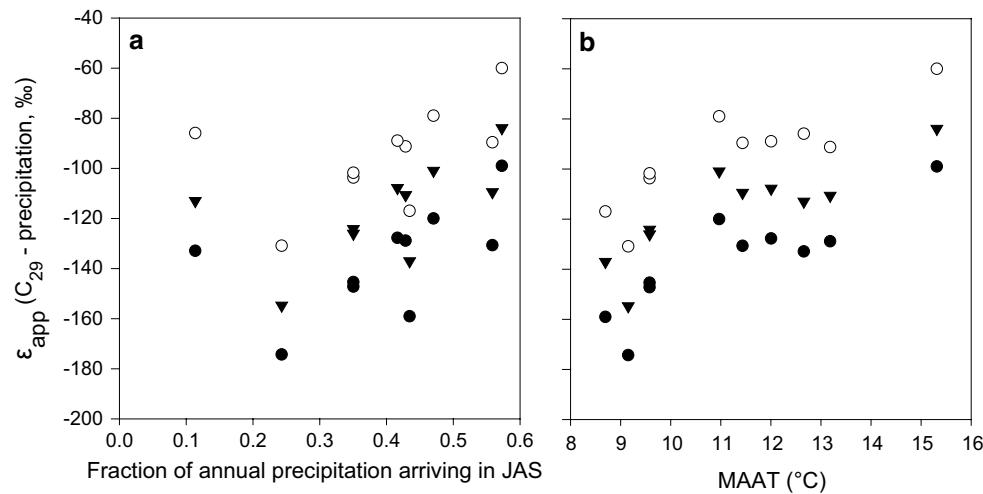


Fig. 7 Average apparent enrichment (ε_{app}) between leaf wax C_{29} *n*-alkane $\delta^2\text{H}$ values and **a** the fraction of total, annual precipitation that arrives in JAS and **b** mean annual air temperature (MAAT; in $^{\circ}\text{C}$)

potential use of NAM precipitation by plants for leaf wax lipid formation. We analyzed the distributions and abundances of *n*-alkanes for all species and observed that there was a poor correlation to environmental variables such as precipitation amount and temperature. However, we analyzed the C_{29} *n*-alkane $\delta^2\text{H}$ values and found significant correlations with estimated source water $\delta^2\text{H}$ values. Using a modified Craig–Gordon model we see that a constant biosynthetic fractionation (ε_{bio}) and seasonal source water usage do not fully capture the variability of $\delta^2\text{H}$ values along the transect. The relationships observed do suggest that the northern and southern end of the transect are relying on different source waters, perhaps due to different rooting strategies. These ends of the transect would also likely be experiencing different average relative humidity and temperatures, which are likely to account for the differences observed. Calculated ε_{app} between measured leaf wax and source precipitation water falls within the range previously documented. The relationships observed, with large variability of apparent fractionation within a species across this transect, indicate that in order for modeled $\delta^2\text{H}$ values to replicate measured leaf wax *n*-alkane $\delta^2\text{H}$ values, there must be a significant environmental influence on apparent fractionation. These results all point towards strong climate controls on wax $\delta^2\text{H}$ values and not just source water from a combination of seasonal source water usage. They also highlight the need for additional studies of coupled climate variables, leaf wax, and plant water $\delta^2\text{H}$ values to better constrain this variability. Leaf water $\delta^2\text{H}$ enrichments, and ultimately leaf wax $\delta^2\text{H}$ values, are likely being driven by changes in relative humidity, temperature, and source water $\delta^2\text{H}$ values. Within the range of likely relative humidity and

temperatures experienced along this transect, the modeled relationships show some sensitivity to these parameters, leading to leaf water $\delta^2\text{H}$ enrichment with increased temperatures and decreased humidity as expected.

The observed leaf wax *n*-alkane $\delta^2\text{H}$ values suggest an influence of seasonally derived precipitation. The NAM supplies the southern end of the transect with the majority of the total precipitation for the year. These plants are all significantly more ^2H enriched than the northern end of the transect, where a much smaller fraction of precipitation arrives from the monsoon. Data suggest that perennial species with dimorphic rooting structure are able to use both surface roots for monsoonal moisture, which does not penetrate the deeper soil, and deeper roots, which are able to obtain groundwater from winter precipitation and longer-term accumulation. Our observations suggest that these leaf wax isotope ratios show a combination of source moisture that reflects monsoonal delivery and subsequent environmental ^2H enrichment from climate conditions on this arid gradient.

Acknowledgments The authors wish to thank Susan Bush for invaluable help in the field and Brad Erkkila in the laboratory. Additionally the manuscript was improved with helpful comments from three anonymous reviewers as well as discussions with Rosemary Bush. All analyses were performed at the University of Utah. This research was supported by NSF IOS grant 1052551 (to J. E. and B. T.).

Author contribution statement M. A. B., B. J. T., and J. R. E. originally formulated the research idea; M. A. B. conducted the fieldwork; M. A. B. and B. H. generated the isotopic data; M. A. B. performed the statistical analyses; M. A. B. wrote the manuscript with input from B. J. T., B. H., and J. R. E. All authors agree to this submission and vouch that this work has not been submitted in full or in part to any other journal.

References

- Adams DK, Comrie AC (1997) The North American Monsoon. *Bull Am Meteorol Soc* 78:2197–2213
- Ball JT (1987) Calculations related to leaf gas exchange. In: Zieger E, Farquhar GD, Cowen IR (eds) *Stomatal function*. Stanford University Press, Stanford, pp 455–476
- Barron JA, Metcalfe SE, Addison JA (2012) Response of the North American monsoon to regional changes in ocean surface temperature. *Paleoceanography* 27:PA3206
- Bowen GJ (2010) Isoscapes: spatial pattern in isotopic biogeochemistry. *Annu Rev Earth Planet Sci* 38:161–187
- Bowen GJ, Revenaugh J (2003) Interpolating the isotopic composition of modern meteoric precipitation. *Water Resour Res* 39:1299
- Bowen GJ, Wilkinson B (2002) Spatial distribution of $\delta^{18}\text{O}$ in meteoric precipitation. *Geology* 30:315–318
- Bush RT, McInerney FA (2013) Leaf wax n-alkane distributions in and across modern plants: implications for paleoecology and chemotaxonomy. *Geochim Cosmochim Acta* 117:161–179
- Caldwell MM (1985) Cold desert. In: Mooney HA, Chabot BF (eds) *Physiological ecology of North American plant communities*. Chapman and Hall, New York
- Cappa CD, Hendricks MB, DePaolo DJ, Cohen RC (2003) Isotopic fractionation of water during evaporation. *J Geophys Res* 108:4525–4534
- Carleton AM, Carpenter DA, Wese PJ (1990) Mechanisms of interannual variability of southwest United States summer precipitation maximum. *J Clim* 3:999–1015
- Castañeda IS, Werne JP, Johnson TC, Filley TR (2009) Late Quaternary vegetation history of southeast Africa: the molecular isotopic record from Lake Malawi. *Palaeogeogr Palaeoclimatol Palaeoecol* 275:100–112
- Chesson P, Gebauer RLE, Schwinnig S, Huntly N, Wiegand K, Ernest MSK, Sher A, Novoplansky A, Weltzin JF (2004) Resource pulses, species interactions, and diversity maintenance in arid and semi-arid environments. *Oecologia* 141:236–253
- Chikaraishi Y, Naraoka H, Poulson SR (2004) Hydrogen and carbon isotopic fractionations of lipid biosynthesis among terrestrial (C₃, C₄ and CAM) and aquatic plants. *Phytochemistry* 65:1369–1381
- Craig H, Gordon LI (1965) Deuterium and oxygen 18 variations in the ocean and marine atmosphere. In: Tongiogi E (ed) *Proceedings of a conference on stable isotopes in oceanographic studies and paleotemperatures*. Lishi, Spoleto, Italy, pp 9–130
- Dansgaard W (1964) Stable isotopes in precipitation. *Tellus* 16:436–468
- Diefendorf AF, Freeman KH, Wing SL, Graham HV (2011) Production of n-alkyl lipids in living plants and implications for the geologic past. *Geochim Cosmochim Acta* 75:7472–7485
- Dodd RS, Afzal-Rafii Z (2000) Habitat-related adaptive properties of plant cuticular lipids. *Evolution* 54:1438–1444
- Dodd RS, Poveda MM (2003) Environmental gradients and population divergence contribute to variation in cuticular wax composition in *Juniperus communis*. *Biochem Syst Ecol* 31:1257–1270
- Eglinton G, Hamilton RJ (1967) Leaf epicuticular waxes. *Science* 156:1322–1335
- Ehleringer JR, Dawson TE (1992) Water uptake by plants: perspectives from stable isotopes. *Plant Cell Environ* 15:1073–1082
- Ehleringer J, Mooney HA (1983) Productivity of desert and Mediterranean-climate plants. In: Lange OL, Nobel PS, Osmond CB, Ziegler H (eds) *Physiological plant ecology IV*. Springer, Berlin Heidelberg New York, pp 205–231
- Ehleringer JR, Phillips SL, Schuster WSF, Sandquist DR (1991) Differential utilization of summer rains by desert plants. *Oecologia* 88:430–434
- Ehleringer JR, Evans RD, Williams D (1998) Assessing sensitivity to change in desert ecosystems—a stable isotope approach. In: Griffiths H (ed) *Stable isotopes integration of biological, ecological, and geochemical processes*. BIOS Scientific, Oxford, pp 223–237
- English NB, Dettman DL, Williams DG (2010) A 26-year stable isotope record of humidity and El Niño-enhanced precipitation in the spines of saguaro cactus, *Carnegiea gigantea*. *Palaeogeogr Palaeoclimatol Palaeoecol* 293:108–119
- Epstein S, Yapp CJ (1976) Climatic implications of the D/H ratio of hydrogen in C-H groups in tree cellulose. *Earth Planet Sci Lett* 30:252–261
- Ernst N, Peterse F, Breitenbach SFM, Syiemlieh HJ, Eglinton TI (2013) Biomarkers record environmental changes along an altitudinal transect in the wettest place on Earth. *Org Geochem* 60:93–99
- Everett RL, Tueller PT, Davis JB, Brunner AD (1980) Plant phenology in galleta-shadscale and galleta-sagebrush associations. *J Range Manage* 33:446–450
- Fawcett PJ, Werne JP, Anderson RS, Heikoop JM, Brown ET, Berke MA, Smith SJ, Goff F, Donohoo-Hurley L, Cisneros-Doval LM, Schouten S, Sinninghe Damsté JS, Huang Y, Toney J, Fessenden J, WoldeGabriel G, Atudorei V, Geissman JW, Allen CD (2011) Extended megadroughts in the southwestern United States during Pleistocene interglacials. *Nature* 470:518–521
- Feehins SJ, Sessions AL (2010) Controls on the D/H ratios of plant leaf waxes in an arid ecosystem. *Geochim Cosmochim Acta* 74:2128–2141
- Flanagan LB (1993) Environmental and biological influences on the stable oxygen and hydrogen isotopic composition of leaf water. In: Ehleringer JR, Hall AE, Farquhar GD (eds) *Stable isotopes and plant carbon-water relations*. Academic Press, San Diego, pp 71–90
- Flanagan LB, Ehleringer JR (1991) Stable isotope composition of stem and leaf water: applications to the study of plant water use. *Funct Ecol* 5:270–277
- Flanagan LB, Ehleringer JR, Marshall JD (1992) Differential uptake of summer precipitation among co-occurring trees and shrubs in a pinyon-juniper woodland. *Plant Cell Environ* 15:831–836
- Fonteyn PJ, Mahall BE (1978) Competition among desert perennials. *Nature* 275:544–545
- Forseth IN, Ehleringer JR, Werk KS, Cook CS (1984) Field water relations of Sonoran Desert annuals. *Ecology* 65:1436–1444
- Griffin D, Woodhouse CA, Meko DM, Stahle DW, Faulstich HL, Carrillo CL, Touchan R, Castro CL, Leavitt SW (2013) North American monsoon precipitation reconstructed from tree-ring latewood. *Geophys Res Lett.* doi:10.1002/grl.50184
- Guenther F, Aichner B, Siegwolf R, Xu B, Yao T, Gleixner G (2013) A synthesis of hydrogen isotope variability and its hydrological significance at the Qinghai-Tibetan Plateau. *Quatern Int* 313–314:3–16
- Higgins RW, Shi W (2001) Intercomparison of the principal modes of interannual and intraseasonal variability of the North American monsoon system. *J Clim* 14:403–417
- Higgins RW, Chen Y, Douglas AV (1999) Interannual variability of the North American warm season precipitation regime. *J Clim* 12:653–680
- Hoffmann B, Kahmen A, Cernusak LA, Arndt SK, Sachse D (2013) Abundance and distribution of leaf wax n-alkanes in leaves of *Acacia* and *Eucalyptus* trees along a strong humidity gradient in northern Australia. *Org Geochem* 62:62–67
- Hou J, D'Andrea WJ, MacDonald D, Huang Y (2007) Hydrogen isotopic variability in leaf waxes among terrestrial and aquatic plants around Blood Pond, Massachusetts (USA). *Org Geochem* 38:977–984

- Hou J, D'Andrea WJ, Huang Y (2008) Can sedimentary leaf waxes record D/H ratios of continental precipitation? Field, model, and experimental assessments. *Geochim Cosmochim Acta* 72:3503–3517
- Huang Y, Shuman B, Wang Y, Webb T III (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
- Hughen KA, Eglinton TI, Xu L, Makou M (2004) Abrupt tropical vegetation response to rapid climate changes. *Science* 304:1955–1959
- Kahmen A, Simonin K, Tu KP, Merchant A, Callister A, Siegwolf R, Dawson TE, Arndt SK (2008) Effects of environmental parameters, leaf physiological properties and leaf water relations on leaf water $\delta^{18}\text{O}$ enrichment in different *Eucalyptus* species. *Plant Cell Environ* 31:738–751
- Kahmen A, Dawson TE, Vieth A, Sachse D (2011) Leaf wax *n*-alkane δD values are determined early in the ontogeny of *Populus trichocarpa* leaves when grown under controlled environmental conditions. *Plant Cell Environ* 34:1639–1651
- Kahmen A, Hoffmann B, Schefuß E, Arndt SK, Cernusak LA, West JB, Sachse D (2013a) Leaf water deuterium enrichment shapes leaf wax *n*-alkane δD values of angiosperm plants. II. Observational evidence and global implications. *Geochim Cosmochim Acta* 111:50–63
- Kahmen A, Schefuß E, Sachse D (2013b) Leaf water deuterium enrichment shapes leaf wax *n*-alkane δD values of angiosperm plants. I. Experimental evidence and mechanistic insights. *Geochim Cosmochim Acta* 111:39–49
- Leaney FW, Osmond CB, Allison GB, Ziegler H (1985) Hydrogen-isotope composition of leaf water in C3 and C4 plants: its relationship to the hydrogen-isotope composition of dry matter. *Planta* 164:215–220
- Lin G, Phillips SL, Ehleringer JR (1996) Monsoonal precipitation responses of shrubs in a cold desert community on the Colorado plateau. *Oecologia* 106:8–17
- Maddox RA, McCollum DM, Howard KW (1995) Large-scale patterns associated with severe summertime thunderstorms over central Arizona. *Weather Forecast* 10:763–778
- Majoube M (1971) Fractionnement en oxygène-18 et en deutérium entré l'eau au sa vapeur. *J Chim Phys* 10 68:1423–1436
- Manning SJ, Barbour MG (1988) Root systems, spatial patterns, and competition for soil moisture between two desert subshrubs. *Am J Bot* 75:885–893
- Mitchell VL (1976) The regionalization of climate in the western United States. *J Appl Meteorol* 15:920–927
- Mitchell DL, Ivanova D, Rabin R, Brown TJ, Redmond K (2002) Gulf of California sea surface temperature and the North American monsoon: mechanistic implication from observation. *J Clim* 15:2261–2281
- Poynter JG, Eglinton G (1990) Molecular composition of three sediments from hole 717C: the Bengal fan. In: Cochran JR, Stow DAV et al (eds) Proceedings of the Ocean Drilling Program scientific results, pp 155–161
- Reynolds J, Kemp P, Ogle K, Fernández R (2004) Modifying the ‘pulse-reserve’ paradigm for deserts of North America: precipitation pulses, soil water, and plant responses. *Oecologia* 141:194–210
- Riederer M, Schneider G (1990) The effect of the environment on the permeability and composition of *Citrus* leaf cuticles. *Planta* 180:154–165
- Roden JS, Ehleringer JR (1999) Observations of hydrogen and oxygen isotopes in leaf water confirm the Craig–Gordon model under wide-ranging environmental conditions. *Plant Physiol* 120:1165–1174
- Roden JS, Lin G, Ehleringer JR (2000) A mechanistic model for interpretation of hydrogen and oxygen isotope ratios in tree-ring cellulose. *Geochim Cosmochim Acta* 64:21–35
- Rommerskirchen F, Eglinton G, Dupont L, Güntner U, Wenzel C, Rullkötter J (2003) A north to south transect of Holocene southeast Atlantic continental margin sediments: relationship between aerosol transport and compound-specific $\delta^{13}\text{C}$ land plant biomarker and pollen records. *Geochem Geophys Geosyst* 4:1101
- Rozanski K, Sonntag C, Münnich KO (1982) Factors controlling stable isotope composition of European precipitation. *Tellus* 34:142–150
- Rozanski K, Araguas-Araguas L, Gonfiantini R (1993) Isotopic patterns in modern global precipitation. In: Swart PK, Lohmann KC, McKenzie JA, Savin S (eds) Climate change in continental isotopic records. AGU monograph, Washington, pp 1–36
- Ryel RJ, Leffler AJ, Peek MS, Ivans CY, Caldwell MM (2004) Water conservation in *Artemisia tridentata* through redistribution of precipitation. *Oecologia* 141:335–345
- Sachse D, Radke J, 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, Gleixner G (2006) δD values of individual *n*-alkanes from terrestrial plants along a climatic gradient—implications for the sedimentary biomarker record. *Org Geochem* 37:469–483
- Sachse D, Kahmen A, Gleixner G (2009) Significant seasonal variation in the hydrogen isotopic composition of leaf-wax lipids for two deciduous tree ecosystems (*Fagus sylvatica* and *Acer pseudoplatanus*). *Org Geochem* 40:732–742
- Sachse D, Gleixner G, Wilkes H, Kahmen A (2010) Leaf wax *n*-alkane δD values of field-grown barley reflect leaf water δD values at the time of leaf formation. *Geochim Cosmochim Acta* 74:6741–6750
- Sachse D, Billault I, Bowen GJ, Chikaraishi Y, Dawson TE, Feakins SJ, Freeman KH, Magill CR, McInerney FA, van der Meer MTJ, Polissar P, Robins RJ, Sachs JP, Schmidt H-L, Sessions AL, White JWC, West JB, Kahmen A (2012) Molecular paleohydrology: interpreting the hydrogen-isotopic composition of lipid biomarkers from photosynthesizing organisms. *Annu Rev Earth Planet Sci* 40:221–249
- Sachse D, Dawson TE, Kahmen A (2015) Seasonal variation of leaf wax *n*-alkane production and $\delta^2\text{H}$ values from the evergreen oak tree, *Quercus agrifolia*. *Isotop Environ Health Stud* 51:1–19
- Sauer PE, Eglinton TI, Hayes JM, Schimmelmann A, Sessions AL (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
- Schefuß E, Schouten S, Schneider RR (2005) Central African hydrologic changes during the past 20,000 years. *Nature* 437:1003–1006
- Schefuß E, Kuhlmann H, Mollenhauer G, Prange M, Patzold J (2011) Forcing of wet phases in southeast Africa over the past 17,000 years. *Nature* 480:509–512
- Schimmelmann A, Lewan MD, Wintsch RP (1999) D/H isotope ratios of kerogen, bitumen, oil, and water in hydrous pyrolysis of source rocks containing kerogen types I, II, IIS, and III. *Geochim Cosmochim Acta* 63:3751–3766
- Schmidt H-L, Werner R, Eisenreich W (2003) Systematics of ^2H patterns in natural compounds and its importance for the elucidation of biosynthetic pathways. *Phytochem Rev* 2:61–85
- Schönherr J (1976) Water permeability of isolated cuticular membranes: the effect of pH and cations on diffusion, hydrodynamic permeability and size of polar pores in the cutin matrix. *Planta* 128:113–126
- Schreiber L, Riederer M (1996) Ecophysiology of cuticular transpiration: comparative investigation of cuticular water permeability of plant species from different habitats. *Oecologia* 107:426–432
- Schwinning S, Sala OE (2004) Hierarchy of responses to resource pulses in arid and semi-arid ecosystems. *Oecologia* 141:211–220

- Schwinning S, Starr BI, Ehleringer JR (2005a) Summer and winter drought in a cold desert ecosystem (Colorado Plateau). Part I. Effects on soil water and plant water uptake. *J Arid Environ* 60:547–566
- Schwinning S, Starr BI, Ehleringer JR (2005b) Summer and winter drought in a cold desert ecosystem (Colorado Plateau). Part II. Effects on plant carbon assimilation and growth. *J Arid Environ* 61:61–78
- Sessions AL, Burgoyne TW, Schimmelmann A, Hayes JM (1999) Fractionation of hydrogen isotopes in lipid biosynthesis. *Org Geochem* 30:1193–1200
- Sessions AL (2006) Seasonal changes in D/H fractionation accompanying lipid biosynthesis in *Spartina alterniflora*. *Geochim Cosmochim Acta* 70:2153–2162
- Smith FA, Freeman KH (2006) Influence of physiology and climate on δD of leaf wax *n*-alkanes from C_3 and C_4 grasses. *Geochim Cosmochim Acta* 70:1172–1187
- Tipple BJ, Pagani M (2013) Environmental control on eastern broadleaf forest species' leaf wax distributions and D/H ratios. *Geochim Cosmochim Acta* 111:64–77
- Tipple BJ, Berke MA, Doman CE, Khachaturyan S, Ehleringer JR (2013) Leaf-wax *n*-alkanes record the plant–water environment at leaf flush. In: *Proc Natl Acad Sci USA* 110(7):2659–2664
- Tipple BM, Berke MA, Hambach B, Roden J, Ehleringer JR (2014) Predicting leaf wax *n*-alkane $^{2}H/^{1}H$ ratios: controlled water source and humidity experiments with hydroponically grown trees confirm predictions of Craig–Gordon model. *Plant Cell Environ* 38:1035–1047
- White JWC, Cook ER, Lawrence JR, Wallace SB (1985) The D/H ratios of sap in trees: implications for water sources and tree ring D/H ratios. *Geochim Cosmochim Acta* 49:237–246
- Williams DG, Ehleringer JR (2000) Intra- and interspecific variation for summer precipitation use in pinyon-juniper woodlands. *Ecol Monogr* 70:517–537
- Wurster CM, Patterson WP, McFarlane DA, Wassenaar LI, Hobson KA, Athfield NB, Bird MI (2008) Stable carbon and hydrogen isotopes from bat guano in the Grand Canyon, USA, reveal Younger Dryas and 8.2 ka events. *Geology* 36:683–686
- Yakir D (1998) Oxygen-18 of leaf water: a crossroad for plant-associated isotopic signals. In: In: Griffiths H (ed) *Stable isotopes: integration of biological and geochemical processes*. BIOS Scientific, Oxford, pp 147–168
- Zhang X, Gillespie AL, Sessions AL (2009) Large D/H variations in bacterial lipids reflect central metabolic pathways. *Proc Natl Acad Sci USA* 106:12580–12586
- Zhou Y, Grice K, Chikaraishi Y, Stuart-Williams H, Farquhar GD, Ohkouchi N (2011) Temperature effect on leaf water deuterium enrichment and isotopic fractionation during leaf lipid biosynthesis: results from controlled growth of C_3 and C_4 land plants. *Phytochemistry* 72:207–213