Molecular Paleohydrology: Interpreting the Hydrogen-Isotopic Composition of Lipid Biomarkers from Photosynthesizing Organisms

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Abstract

Hydrogen-isotopic abundances of lipid biomarkers are emerging as important proxies in the study of ancient environments and ecosystems. A decade ago, pioneering studies made use of new analytical methods and demonstrated that the hydrogen-isotopic composition of individual lipids from aquatic and terrestrial organisms can be related to the composition of their growth (i.e., environmental) water. Subsequently, compound-specific deuterium/hydrogen (D/H) ratios of sedimentary biomarkers have been increasingly used as paleohydrological proxies over a range of geological timescales. Isotopic fractionation observed between hydrogen in environmental water and hydrogen in lipids, however, is sensitive to biochemical, physiological, and environmental influences on the composition of hydrogen available for biosynthesis in cells. Here we review the factors and processes that are known to influence the hydrogen-isotopic compositions of lipids—especially *n*-alkanes—from photosynthesizing organisms, and we provide a framework for interpreting their D/H ratios from ancient sediments and identify future research opportunities.

1. INTRODUCTION

The relative abundances of the stable isotopes of hydrogen [hydrogen (H) and deuterium (D); see sidebar, The Delta Notation and Enrichment Factors] as well as oxygen (^{16}O and ^{18}O) in precipitation are related to the fluxes of water in the hydrological cycle (Craig 1961, Craig & Gordon 1965, Dansgaard 1964, Gat 1996). Changes in precipitation δD and $\delta^{18}O$ values recorded in paleoarchives, such as continental ice cores (Thompson et al. 1985, 2003) or lake sediments (von Grafenstein et al. 1999), are critical tools for reconstructing the hydrological cycle over time. Suitable sites for ice-core drilling are, however, constrained to the high-latitude and high-altitude regions of Earth, and lake sediment records depend on the availability of ostracods or other suitable carbonate or silica producers, which are not ubiquitous. These preconditions hinder reconstructions of past changes in the hydrological cycle and limit our understanding of linkages between continental hydrology and both global paleoclimate and terrestrial paleoecology.

Organic matter from photosynthesizing organisms is an important component of most marine and lacustrine sediments. Water is the primary hydrogen source of photosynthesizing organisms and their biosynthetic products. Organic hydrogen preserved in sediments has thus been suggested to record the isotopic composition of water used during photosynthesis and could function as a paleohydrological proxy (Estep & Hoering 1980, Sternberg 1988). Organic matter in sediments is, however, a complex mixture of various organic compounds that can differ substantially in their isotopic compositions as a result of different biosynthetic pathways, different source organisms, and varying degrees of secondary exchange of bound hydrogen with environmental water (Schimmelmann et al. 2006). As a consequence, it is difficult to obtain robust paleohydrological proxy records using bulk sedimentary organic matter (Krishnamurthy et al. 1995).

THE DELTA NOTATION AND ENRICHMENT FACTORS

Isotope ratios R (R = D/H with ^{2}H or D for deuterium and ^{1}H or H for protium) are usually expressed as a δD value in per mil (‰) that represents the relative deviation of R in the sample from a standard [usually Vienna Standard Mean Ocean Water (VSMOW) with $\delta D = 0‰$]:

$$\delta D = \frac{R_{sample} - R_{standard}}{R_{standard}} \quad (1)$$

Enrichment factors (ε) are used to characterize the hydrogen-isotopic fractionation between source and product. The so-called net or apparent fractionation ($\varepsilon_{l/w}$) between source water (δD_w) and lipid (δD_l) product is one of the most commonly used parameters in the literature, where the equation

$$\varepsilon_{l/w} = \frac{(D/H)_l}{(D/H)_w} - 1 = \frac{\delta D_l + 1}{\delta D_w + 1} - 1 \quad (2)$$

represents the sum of many individual fractionations due to isotope effects on both physical and biochemical processes. Enrichment factors and delta values are commonly reported as per mil (‰) deviations, which implies multiplication by a factor of 1,000 (Cohen et al. 2007).

With analytical improvements in isotope-ratio mass spectrometry in the late 1990s, it is now possible to measure the stable hydrogen-isotopic composition of individual organic compounds (Burgoyne & Hayes 1998, Hilkert et al. 1999, Scrimgeour et al. 1999, Tobias & Brenna 1997). The direct analysis of individual compounds circumvents many of the problems described above. In particular, lipids are promising in this respect: Fatty acids, wax esters, ketones, hopanols, and sterols are present in the membranes of bacteria, algae, and higher plants, and some lipids are even specific to certain organisms. In addition, the cuticular wax layer of higher terrestrial plant leaves contains large amounts of long-chain *n*-alkanes, *n*-alcohols, *n*-alkanoic acids, and triterpenoid compounds (Eglinton & Hamilton 1967, Volkman et al. 1998). Lipids persist in the sedimentary record over geological timescales and are routinely used as biomarkers in paleoecosytem and paleoclimate reconstruction (Eglinton & Eglinton 2008). Furthermore, most lipid hydrogen atoms are covalently bound to carbon atoms and are not readily exchanged at temperatures below 100°C (Sessions et al. 2004).

Initial studies revealed that a variety of lipids from sedimentary terrestrial and aquatic lipid biomarkers have δD values that are offset from, but highly correlated with, that of the water source used by these organisms (**Figure 1**) (Chikaraishi & Naraoka 2003; Englebrecht & Sachs 2005; Huang et al. 2002, 2004; Sachse et al. 2004b; Sauer et al. 2001; Sessions et al. 1999). These studies generated a wave of excitement among paleoclimatologists, who look to reconstruct paleowater δD values from measurements of these individual lipids. Applications of lipid δD values for paleohydrological reconstruction now exist and show substantial promise (Pagani et al. 2006, Sachs et al. 2009, Schefuss et al. 2005, Tierney et al. 2008).

Subsequent studies investigating lipid δD values from living organisms and/or plants have revealed that additional environmental and physiological variables can influence isotopic fractionation between hydrogen in environmental water and in terrestrial and aquatic lipids. The relative effects of these processes are not completely understood, making it difficult to take them into account when interpreting δD values of lipid biomarkers in a paleohydrological context. Improved understanding of controls on fractionation not only will aid paleohydrology but also may eventually result in new applications, such as the use of lipid δD values as a paleosalinity

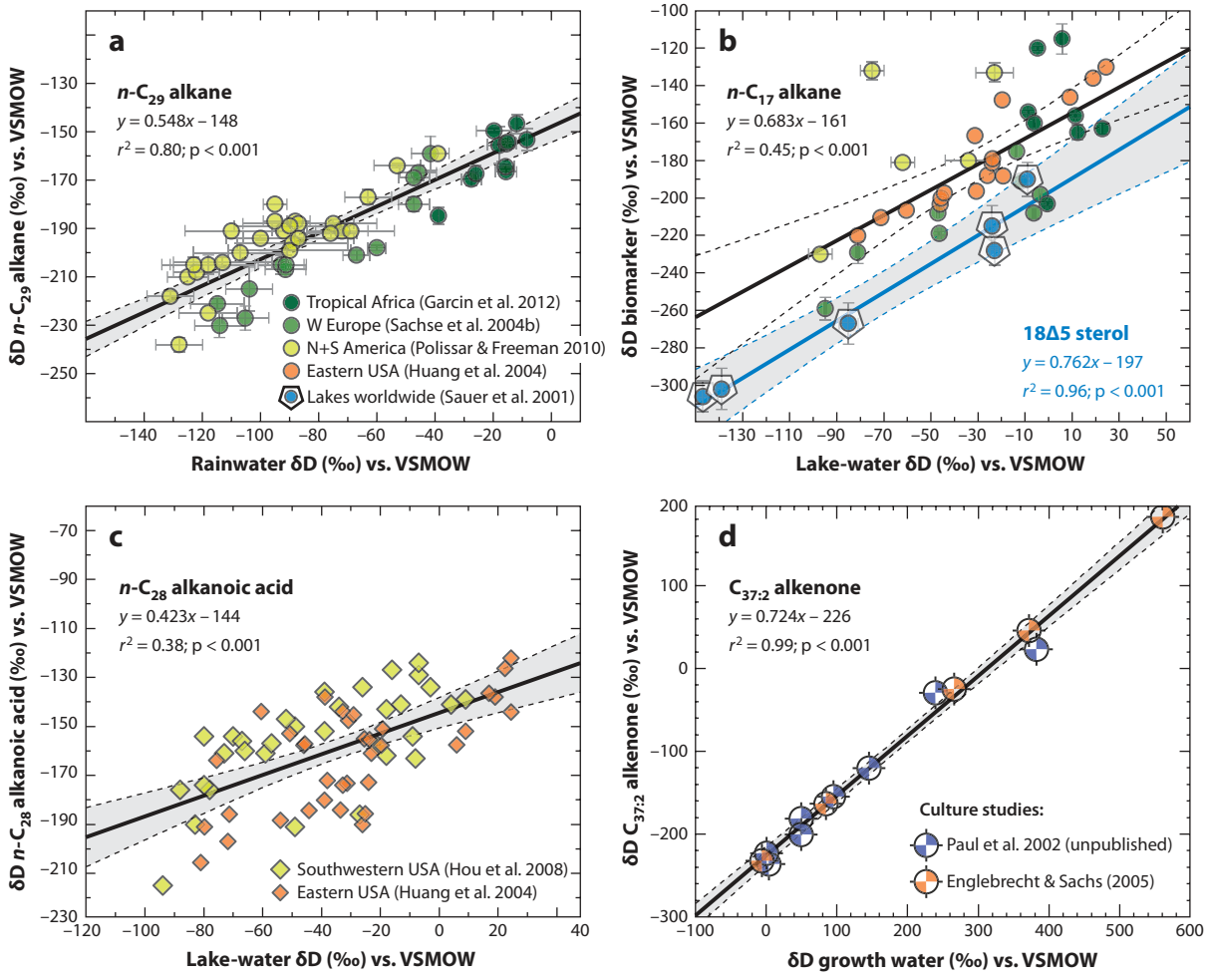

Figure 1

Relationships between source-water δD and lipid biomarker δD values from lake-surface sediment transects across (*a–c*) climatic gradients and (*d*) culture studies. Error bars are plotted if given in the original publications and are 1-sigma standard deviations of replicate measurements (for lipid analysis, lake water, growth water), and errors are estimated from precipitation or taken from the International Atomic Energy Agency GNIP database. Data from Hou et al. (2008) are plotted here against lake-water δD values to allow for better comparison with other *n*-alkanoic acid data sets (Huang et al. 2004), whereas in the original publication they are plotted against rainwater δD. Many of the lakes in arid areas from this study were dammed reservoirs fed by rivers draining snowmelt catchments. Abbreviation: VSMOW, Vienna Standard Mean Ocean Water.

proxy (Sachs et al. 2009, van der Meer et al. 2007) and as an ecohydrological tool (Krull et al. 2006).

The aim of this review is to summarize the variables that control the δD values of lipid biomarkers derived from aquatic and terrestrial photosynthesizing organisms. We do so by following hydrogen from the water source (precipitation, lake water, and seawater) into organic compounds during biosynthesis and through to the deposition of lipids in sediments (**Figure 2**). We conclude with recommendations for applying molecular δD values to paleohydrological questions and for further research.

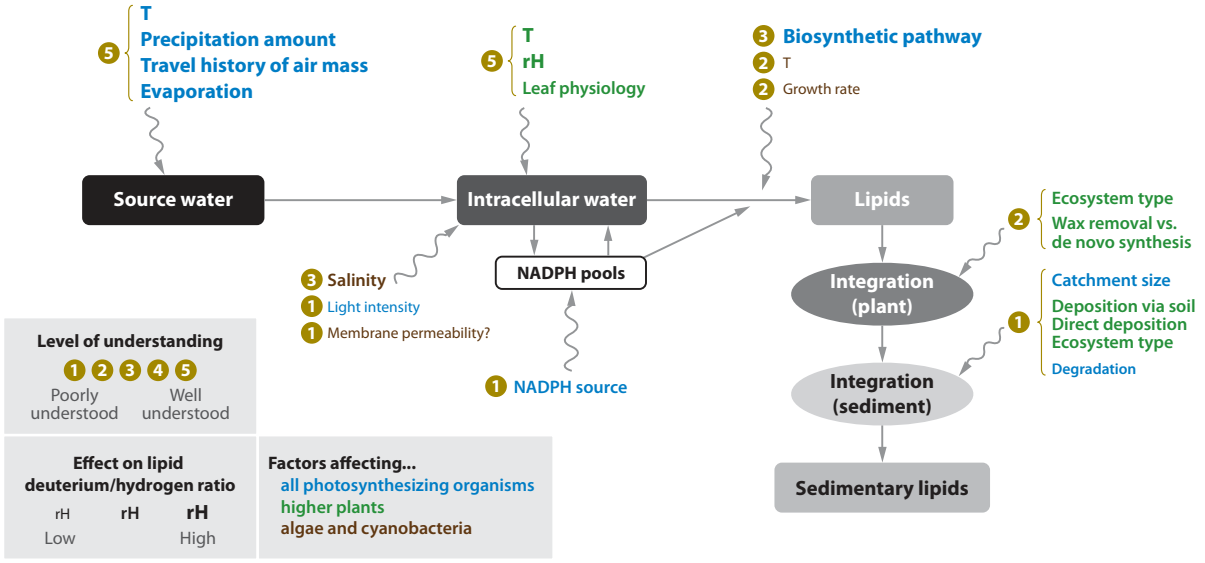

Figure 2

Overview of the processes affecting the hydrogen-isotopic composition of lipid biomarkers from phototrophic organisms. Abbreviations: NADPH, nicotinamide adenine dinucleotide phosphate (reduced); rH, relative humidity; T, temperature.

2. ISOTOPIC COMPOSITION OF WATER: THE PRIMARY HYDROGEN SOURCE FOR PHOTOSYNTHESIZING ORGANISMS

Environmental water is the primary source of hydrogen for biosynthesis in photoautotrophic organisms. Paleohydrological studies using lipid isotopic signatures aim to reconstruct the isotopic composition of environmental waters. If they are to do so, however, it is critical to understand the influences of environment and physiology on the isotopic composition of intracellular water and its use in subsequent biosynthetic processes by aquatic and terrestrial organisms.

2.1. Water Sources

Different organisms use different environmental water pools as their hydrogen sources. In the following, we discuss the major environmental variables affecting the δD values of these water sources; a more extensive discussion is found in the literature (e.g., Gat 1996).

2.1.1. Precipitation and atmospheric vapor. The hydrogen-isotopic composition of precipitation and that of atmospheric vapor vary substantially over space and time. Much of this variability can be explained by Rayleigh-type processes during evaporation and condensation (Craig 1961, Gat 1996). When seawater (δD = 0‰) evaporates, the corresponding vapor is depleted in the heavy isotope D because $^{1}H_2{}^{16}O$ has a higher vapor pressure and evaporates faster than $^{1}HD^{16}O$. When the water vapor condenses and eventually leaves the air mass in the form of precipitation, the resulting rain is enriched in D relative to the vapor, whereas the remaining vapor becomes depleted in D. Dansgaard (1964) identified several environmental factors that correlate with the resulting spatial and temporal patterns of precipitation δD:

1. Continental effect: As air masses progressively lose moisture over the continents, the preferential loss of D drives an evolution of subsequent precipitation to lower δD values further inland.
2. Temperature effect: Across regions characterized by strong temperature variability, the rainout process is strongly correlated with temperature. In addition, the equilibrium isotopic fractionation between vapor and condensate increases with temperature—e.g., at 25°C, liquid water is enriched in $^{1}HD^{16}O$ by approximately 74‰ relative to its vapor source, whereas at colder temperatures the fractionation is larger (101‰ at 0°C).
3. Amount effect: In tropical regions characterized by limited temperature variation but by strong seasonality in rainfall, the isotopic composition of precipitation is related to the amount of precipitation with stronger depletion in D at higher precipitation rates.

These factors combine with others influencing the source and transport of atmospheric moisture (e.g., atmospheric circulation, spatial patterns of evapotranspiration rates) to drive variation of isotopes in atmospheric precipitation over space and time (Bowen 2010, Liu et al. 2010). In particular, the temperature effect is prominent across many regions outside the tropics, whereas the amount effect is most prominent in tropical latitudes (Bowen 2008). In other cases, precipitation isotope ratios may serve as a more integrated proxy for atmospheric circulation changes or variability in climate modes (Baldini et al. 2008).

2.1.2. Water sources of aquatic organisms: lakes, bogs, and rivers. Most aquatic organisms (cyanobacteria, algae, aquatic plants) take up precipitation water that has accumulated in lakes, bogs, and rivers. These water reservoirs spatially and temporally integrate the effects discussed above as a function of their catchment size. In arid regions where evaporation locally exceeds precipitation, surface-water bodies become enriched in D. Lake-water δD values therefore additionally record the degree of evaporation experienced by the lake system.

2.1.3. Water sources of terrestrial plants: soil water. Soil moisture is the main water source for higher terrestrial plants, although in some ecosystems, plants may also use fog, dew, cloud water, or groundwater (Dawson 1998, Dawson & Ehleringer 1993). Precipitation is the ultimate source of soil water and groundwater; thus, the spatiotemporal variability of soil-water isotopic composition largely reflects an amount-weighted average of precipitation inputs. In the uppermost soil horizons, this general pattern can be altered by surface evaporation and D enrichment of water coupled with a small effect of exchange with atmospheric vapor (e.g., Riley et al. 2002). Although evaporative soil-water D enrichment is more common in arid climates, plants adapted to these environments often have deep roots, and many can "lift" and "redistribute" water in the soil profile from deeper soil horizons or groundwater and hence take up water not affected by surface evaporation (Dawson 1993, Dawson & Pate 1996). Therefore, at large spatial scales, plant source-water δD values generally follow those of precipitation (e.g., West et al. 2007).

2.2. The Isotopic Composition of Water in Leaves and Cells

During transport from the environment to the sites of lipid biosynthesis, the isotopic composition of water can undergo substantial changes. In the following, we discuss the major processes responsible for such changes in higher plants and unicellular organisms.

2.2.1. Leaf water in terrestrial plants. Although there is typically no isotopic fractionation during the uptake of source water via the root (but see Ellsworth & Williams 2007), the isotopic composition of leaf water can vary markedly from that of the plant's source water

MECHANISTIC LEAF-WATER ISOTOPE MODELS

Models describing the evaporative D enrichment of leaf water are based on the Craig-Gordon (CG) model for open water bodies, which has been adapted to describe evaporative enrichment of leaf water at the sites of evaporation in the leaf (ΔD_e):

$$\Delta D_e = \varepsilon^+ + \varepsilon_k + (\Delta D_v - \varepsilon_k)\frac{e_a}{e_i}. \quad (3)$$

ε^+ is the temperature-dependent equilibrium fractionation between liquid water and vapor at the air-water interfaces, ε_k is the kinetic fractionation during water vapor diffusion from the leaf intercellular air space to the atmosphere, ΔD_v is the isotopic enrichment or depletion of vapor in the atmosphere relative to source water, and e_a/e_i is the ratio of leaf vapor pressure to air vapor pressure, which is a product of atmospheric humidity, leaf temperature, and air temperature (Craig & Gordon 1965, Flanagan et al. 1991). To describe the evaporative enrichment of leaf water as a whole (ΔD_l or ΔD_{lw}), the original CG model (Equation 3) has been modified to account for the so-called Péclet effect—the diffusion of enriched water away from the sites of evaporation that is opposed by the transpirational advection of unenriched water to the site of evaporation. Summary and discussions of leaf-water models can be found in the recent literature (Barbour 2007, Farquhar et al. 2007, Ferrio et al. 2009, Kahmen et al. 2008).

(Barbour et al. 2004, Farquhar et al. 2007). This is the result of transpiration, or water loss from the leaf, where the lighter water isotopologs evaporate and diffuse in air faster than the heavier ones (e.g., $^1H_2{}^{16}O$ versus $^1HD^{16}O$). The isotopic deviation of leaf water relative to its xylem water (typically designated ΔD_l or ΔD_{lw}) is influenced by various environmental and physiological parameters, which have been integrated into mechanistic leaf-water models (see sidebar, Mechanistic Leaf-Water Isotope Models). The main drivers of this enrichment are relative humidity, temperature, and the isotopic composition of the water vapor surrounding the leaf (e.g., Kahmen et al. 2008).

Leaf-wax lipids are synthesized in plant leaves. Therefore, the isotopic composition of water available as a hydrogen source for biosynthesis of organic compounds within the plant leaves should integrate the processes discussed above. However, the relative importance of the potential water sources (leaf water, xylem water) for lipid synthesis is unknown. In Section 4.2, we discuss empirical evidence and current hypotheses pertaining to the effect of soil-water evaporation and leaf-water transpiration on leaf-wax δD values.

2.2.2. Intracellular water in unicellular, aquatic organisms. An implicit assumption in the use of lipid δD values for paleohydrology is that intracellular water used in biosynthetic reactions has the same isotopic composition as water external to the cell. There is increasing evidence to suggest that isotopic differences between environmental water and the intracellular environment may exist in aquatic organisms (e.g., bacteria, algae) that live submerged in water. For the heterotrophic bacterium *Escherichia coli*, Kreuzer-Martin et al. (2006) measured significant differences in δD values between intra- and extracellular water. These were interpreted as evidence for accumulation of metabolic water within the cell as a result of (relatively) slow diffusion of water across the cell membrane. In particular, the high proportion of metabolic water during log-phase growth was viewed as a consequence of high rates of respiration. The increased generation of metabolic water during log-phase growth was reflected in the isotopic composition of fatty acids from the *E. coli* cultures. If changes in the isotopic composition of intracellular water are mediated by the relative rates of photosynthetic H_2O consumption, respiratory H_2O production, and water

MECHANISMS FOR THE SALINITY DEPENDENCY ON D/H FRACTIONATION IN ALGAL AND CYANOBACTERIAL LIPIDS

The response of $\varepsilon_{l/w}$ to salinity was observed to be remarkably constant at 0.9‰ ± 0.2‰ in δD per salinity unit increase for lipids from two contrasting environmental settings (hypersaline lake cyanobacterial lipids and brackish estuary algal dinosterol) reported so far. A culture study by Schouten et al. (2006) on algal alkenones (**Figure 3**) showed a larger response of $\varepsilon_{l/w}$ to salinity (3–4‰ decrease per salinity unit increase). The different $\varepsilon_{l/w}$ to salinity sensitivities between the environmental lipids and alkenones from culture might be due to species-related differences, differences in isotopic fractionation during biosynthesis, and differences in growth rate between the environmental and culture samples.

The mechanism is hypothesized to be exercised via D enrichment of intracellular water: Restricted exchange with extracellular water at high salinity due to aquaporin downregulation (Sachse & Sachs 2008) or increased production of osmolytes (compatible solutes produced to maintain osmotic pressure) would preferentially remove light hydrogen from the intracellular water (Sachs & Schwab 2011). Alternatively, lower growth rates at higher salinity may reduce lipid-water fractionations. If the mechanism is further resolved, the relationship between $\varepsilon_{l/w}$ and salinity can provide a method to reconstruct paleosalinities (Sachs et al. 2009, van der Meer et al. 2007).

exchange across the cell membrane, then factors such as salinity, temperature, growth rate, and light intensity could also exert indirect control on the D/H ratios of lipids produced from this water. Although no isotopic data exist for intracellular water in photoautotrophs, several indirect observations suggest that these parameters affect the isotopic compositions of their lipids (see sidebars, Mechanisms for the Salinity Dependency on D/H Fractionation in Algal and Cyanobacterial Lipids; Effect of Temperature on D/H Fractionation?). Although such additional controls on the isotopic fractionation between lipids and source water ($\varepsilon_{l/w}$) may complicate paleoclimate interpretation in certain settings, a better understanding of these could result in new applications of the lipid δD proxy. For example, the salinity dependency of D/H fractionation in algal and cyanobacterial lipids has resulted in the application of lipid δD values as a paleosalinity proxy because $\varepsilon_{l/w}$ becomes smaller with increasing salinity in cyanobacteria (Sachse & Sachs 2008) and marine algae (Sachs & Schwab 2011, Schouten et al. 2006) (see **Figure 3** and sidebar, Mechanisms for the Salinity Dependency on D/H Fractionation in Algal and Cyanobacterial Lipids).

EFFECT OF TEMPERATURE ON D/H FRACTIONATION?

Whereas the temperature dependences of isotope effects in biosynthetic and $NADP^+$-reducing enzymes are unlikely to be large enough to have an effect on biosynthetic hydrogen-isotopic fractionation (ε_{bio}) (Kwart 1982, Siebrand & Smedarchina 2004), rates of respiration and photosynthesis are strongly temperature dependent. Therefore, the net fractionation ($\varepsilon_{l/w}$) may well be influenced by temperature changes. Indeed, Z. Zhang et al. (2009) showed that $\varepsilon_{l/w}$ increased at a rate of 2–4‰ per degree Celsius for algal lipids produced via different biosynthetic pathways (acetogenic pathway and mevalonic acid pathway). Wolhowe et al. (2009) observed a similar magnitude for alkenones in lab-grown haptophytes, although the relative abundance of the alkenones had also changed, and this may affect their isotopic compositions as well. Because different classes of lipids were influenced to a similar magnitude, the mechanism seems to affect their common hydrogen source—intracellular water. Thus, the observed effect of temperature on $\varepsilon_{l/w}$ may be due to changes of the isotopic composition of intracellular water itself, related to changes in physiology, metabolism, or membrane permeability.

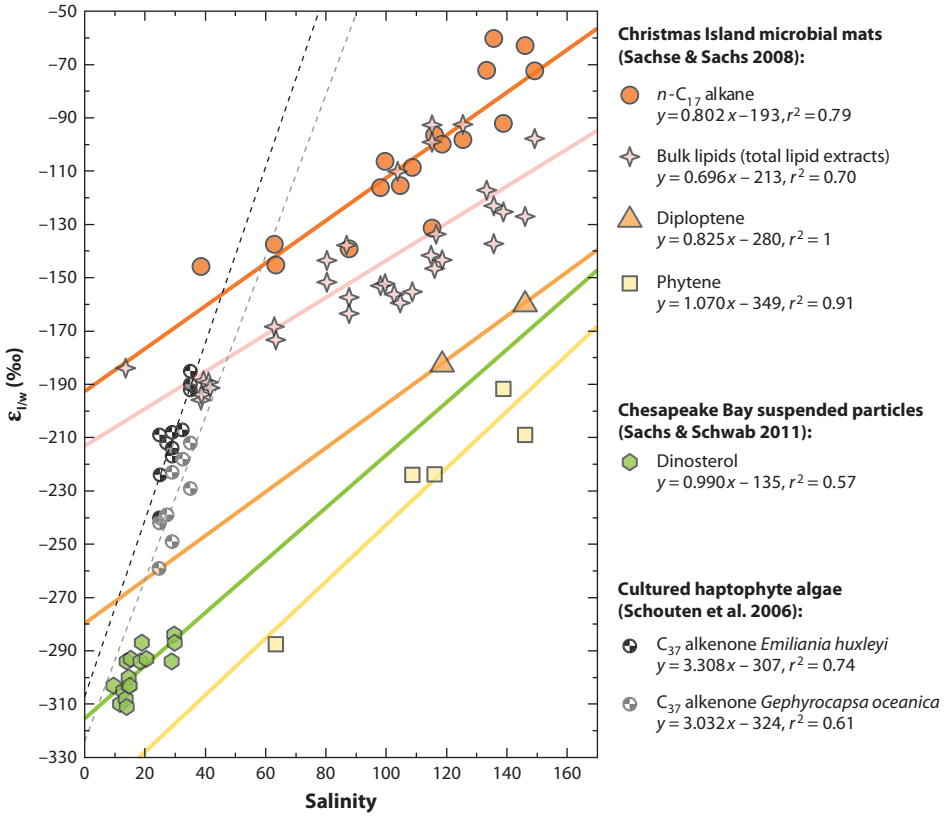

Figure 3

Relationships of the isotopic fractionation between lipids and source water ($\varepsilon_{l/w}$) and salinity (given in practical salinity units) for cyanobacterial and algal sediment samples, suspended particles, and culture studies.

3. BIOSYNTHESIS

Water is the ultimate source of hydrogen for all natural compounds produced by photosynthesizing organisms—specifically, leaf water/xylem water for terrestrial plants and intracellular water in aquatic algae or cyanobacteria. Organic molecules are usually depleted in D compared with the water source. Because biosynthetic hydrogen-transfer reactions express substantial isotopic fractionations, large ranges of δD values are observed for different organic compounds, with δD values between −400‰ and +200‰ for lipids commonly employed as biomarkers (Chikaraishi & Naraoka 2003; Chikaraishi et al. 2005, 2009; Sauer et al. 2001; Sessions et al. 1999; X. Zhang et al. 2009; Zhang & Sachs 2007). The observed variability in the isotopic compositions of these biomarkers within a single organism can be related to differences in biosynthesis and can mostly be explained by four factors: (*a*) isotopic fractionation associated with the different biosynthetic pathways; (*b*) secondary hydrogen exchange reactions, hydrogenations, and dehydrogenations; (*c*) differences in the isotopic composition of H^- (NADPH) originating from different pathways; and (*d*) influence of extrinsic secondary factors on isotopic fractionations. Each of these is discussed below.

3.1. Isotopic Fractionation Associated with the Different Biosynthetic Pathways of Individual Lipids

The biosynthesis of lipids in living organisms involves a complex array of enzymatic reactions. Such reactions, especially those in which hydrogen is added, removed, or exchanged, can lead to isotopic fractionations. As a result, the different pathways of lipid biosynthesis are characterized by different δD values in the resulting products. There are three major biosynthetic pathways for the synthesis of relevant lipid biomarkers (see **Figure 4**): (*a*) the acetogenic pathway for *n*-alkyl lipids; (*b*) the mevalonic acid (MVA) pathway for steroid, terpenoid, and hopanoid synthesis (which mostly operates in higher eukaryotes); and (*c*) the 1-deoxy-D-xylulose-5-phosphate (DOXP)/2-methyl-erythroyl-4-phosphate (MEP) pathway for isoprenoid lipids such as phytol, but also hopanoids (in cyanobacteria and plastids).

The common precursors of all three groups of lipids are descendants of carbohydrate metabolism [3-phosphoglyceric acid (3-PGA) and glyceraldehyde-3-phosphate (GA-3-P)], originating either directly from the Calvin cycle or from secondary carbohydrate metabolism. The acetogenic and the DOXP/MEP pathways are located in the plastids of photosynthesizing plants and algae, and in cyanobacteria. The MVA pathway, which operates only in higher eukaryotes and some heterotrophic bacteria, is found in the cytosol.

Lipids with the smallest D depletion relative to the water source are *n*-alkyl lipids, produced via the acetogenic pathway (Chikaraishi & Naraoka 2003, Chikaraishi et al. 2004a, Sessions et al. 1999). Acetogenic biosynthesis results in a butyryl chain containing seven hydrogen atoms from three different sources: three inherited from acetate [the original acetyl–coenzyme A (acetyl-CoA) methyl hydrogens], two derived from NADPH (the most depleted in D), and two directly transferred from water (the most enriched in D). Full correlation with these sources, however, is diminished if postmalonate exchange with water occurs (Sedgwick & Cornforth 1977, Sedgwick et al. 1977). The sequential addition of further acetyl-CoA units forms a typical C_{18} fatty acid, in which an alternating enriched/depleted pattern at the even/odd carbon positions is found (see Baillif et al. 2009 and references therein). In higher plants, fatty acids with 16 or 18 carbon atoms are exported from the chloroplasts for further elongation in the endoplasmic reticulum. In cyanobacteria, which have no subcellular organelles, fatty acid biosynthesis proceeds entirely within the cytoplasm (Lem & Stumpf 1984).

Isoprenoid lipids produced via the MVA pathway, such as sterols and terpenes, show a depletion in D by approximately 200–250‰ relative to source water (Chikaraishi et al. 2004a, Li et al. 2009, Sauer et al. 2001, Sessions et al. 1999, Zhang & Sachs 2007). The methyl groups of the terpene intermediates [such as farnesyl pyrophosphate (FPP)] contain hydrogen transferred from NADPH during the synthesis of MVA and are probably responsible for their additional D depletion relative to *n*-alkyl lipids.

Phytol and related compounds are generally observed to have the largest D depletions of any lipid (Li et al. 2009) and are produced via the DOXP/MEP pathway (Lichtenthaler 1999, Rohmer et al. 1993, Schwender et al. 1996). Although this pathway is located in the plastids, and is therefore spatially separated from the MVA pathway, exchange of intermediates such as dimethylallyl pyrophosphate (DMAPP) and isopentenyl diphosphate (IPP) takes place (Bartram et al. 2006, Hemmerlin et al. 2003, Z. Zhang et al. 2009). In contrast, cyanobacteria possess no MVA pathway; thus, they synthesize both hopanoids and isoprenoids via the DOXP/MEP pathway (Lange et al. 2000). Although not all fractionation steps at biosynthetic branching points and/or during enzymatic reactions are known, the different pathways explain the major isotopic differences between the lipid classes.

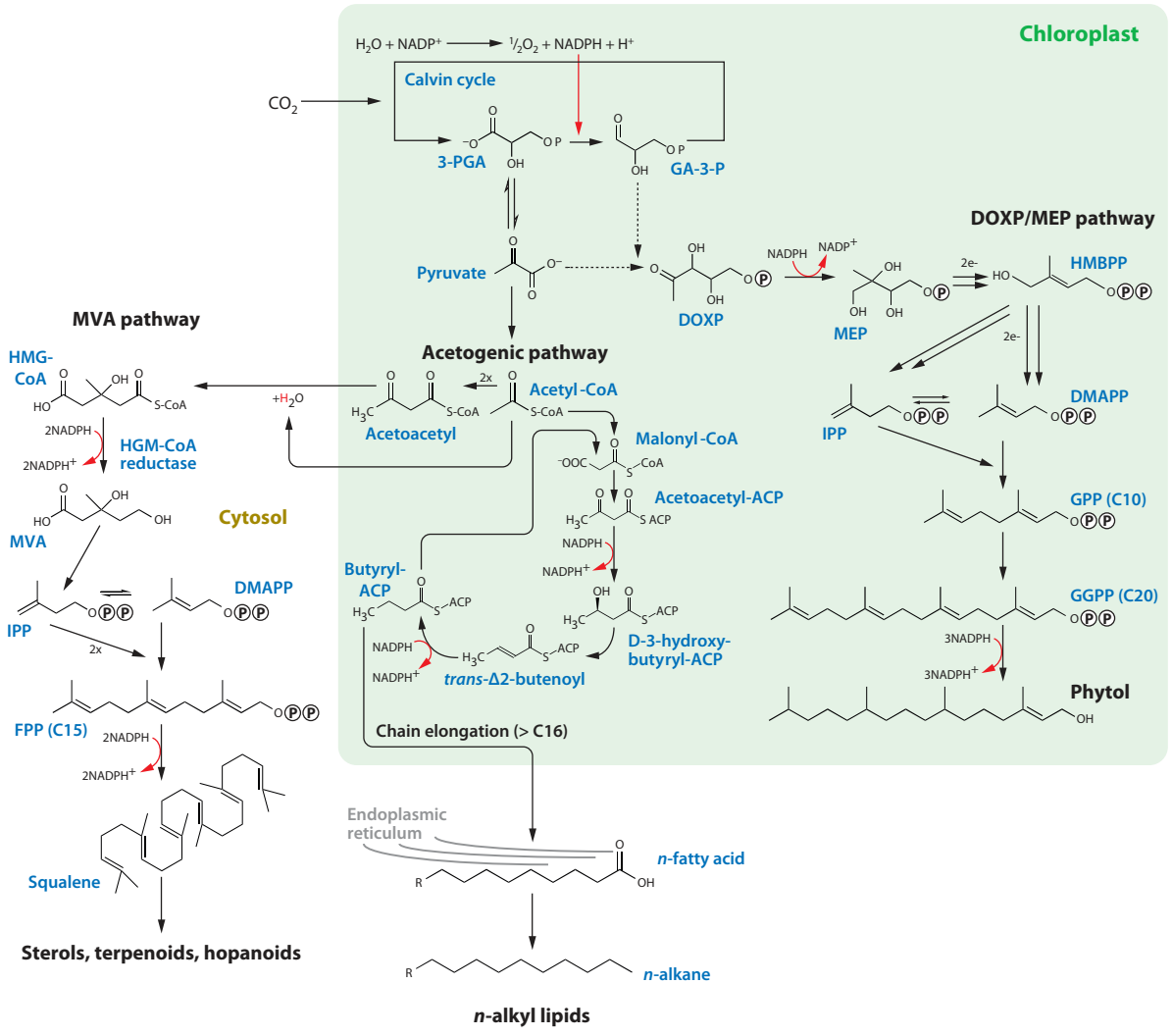

Figure 4

Overview of the three major biosynthetic pathways of lipid biosynthesis in photosynthesizing organisms. Red arrows indicate where H is transferred from reduced $NADP^+$ (NADPH), causing depletion in D of the product. Double arrows indicate that several transition steps are involved in these reactions. Abbreviations: 3-PGA, 3-phosphoglyceric acid; ACP, acyl carrier protein; CoA, coenzyme A; DMAPP, dimethylallyl pyrophosphate; DOXP, 1-deoxy-D-xylulose-5-phosphate; FPP, farnesyl pyrophosphate; GA-3-P, glyceraldehyde-3-phosphate; GGPP, geranylgeranyldiphosphate; GPP, geranyldiphosphate; HMG, 3-hydroxy-3-methylglutaryl; HMBPP, (*E*)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate; IPP, isopentenyl diphosphate; MEP, 2-methyl-erythritol-4-phosphate; MVA, mevalonic acid; NADPH, nicotinamide adenine dinucleotide phosphate (reduced). Modified from Chikaraishi et al. (2004a), Lichtenthaler (1999), Schmidt et al. (2003), and Zhang & Sachs (2007).

3.2. Influence of Secondary Hydrogen Exchange Reactions, Hydrogenations, and Dehydrogenations

Despite common biosynthetic pathways, substantial heterogeneity (up to 200‰) in the isotopic compositions of homologous molecules with different degrees of desaturation, such as fatty acids and alkenones, is observed (Chikaraishi et al. 2004b, D'Andrea et al. 2007, Schwab & Sachs

2009). Such variability can be attributed to large enzymatic isotope effects that occur during both hydrogenation (saturation) and dehydrogenation (desaturation) reactions (Behrouzian & Buist 2003, Chikaraishi et al. 2009). Another important secondary reaction is decarboxylation of *n*-alkanoic acids, leading to *n*-alkanes, which is associated with a D depletion of the *n*-alkane on the order of 25‰ ± 16‰ (Chikaraishi & Naraoka 2007).

3.3. Differences in the Isotopic Composition of H^- (NADPH) Originating from Different Pathways (Including the Role of Metabolism)

Hydrogen derived from NADPH, added to carbon skeletons during many hydrogenation reactions, seems to be strongly D depleted, on average, relative to water. This hydrogen can potentially come from several different metabolic sources. Experiments with heterotrophic bacteria suggest that different sources result in different isotopic compositions for NADPH (X. Zhang et al. 2009). Whereas NADPH produced during photosynthesis is likely to be strongly depleted in D by up to 600‰ (Luo et al. 1991, Schmidt et al. 2003), NADPH produced during sugar metabolism, including via the oxidative pentose phosphate pathway (PPP), is apparently less depleted in D (Schmidt et al. 2003, Yakir & Deniro 1990). The relative importance of these pathways for NADPH used in lipid biosynthesis therefore affects lipid δD values. In photosynthesizing organisms, however, the main NADPH source is photosynthesis, as evidenced by the observed strong linear relationships between water source and lipid δD. Modest increases in lipid δD values of higher plants can reflect increased reliance on stored carbohydrates and presumably a larger role for the PPP in generating NADPH during these conditions (Feakins & Sessions 2010b, Sessions 2006, Yakir 1992). In addition to these varying pathways for $NADP^+$ reduction, higher plants and algae may maintain isotopically distinct pools of NADPH in different subcellular compartments [e.g., chloroplast, mitochondria, and cytosol (Sessions et al. 1999)]. The combined effects of all these pools and pathways are currently impossible to predict, but measurements suggest that the net depletion of all NADPH hydrogen in the cell (compared to cell water) is typically close to 200‰.

3.4. Influence of Extrinsic Secondary (Environmental) Factors on Isotopic Fractionations

Mounting evidence suggests that several environmental factors, including salinity, temperature, growth rate, growth stage, and light intensity, can potentially affect the hydrogen-isotopic composition of lipids (see sidebars, Mechanisms for the Salinity Dependency on D/H Fractionation in Algal and Cyanobacterial Lipids; Effect of Temperature on D/H Fractionation?; Effect of Growth Rate and Growth Stage on D/H Fractionation). A key question is whether this influence is exerted directly (e.g., through changes in the biosynthetic fractionation) or indirectly (through its effects on the isotopic composition of cellular water) (see Section 2). Unfortunately, few controlled studies that would allow separation of these two mechanisms have been performed. A direct effect of growth rate on the biosynthetic fractionation is suggested by culture experiments with the marine diatom *Thalassiosira pseudonana* (Z. Zhang et al. 2009), in which $\varepsilon_{l/w}$ was greater at high growth rates for sterols but did not change significantly in fatty acids.

The influence of external factors on the biosynthetic isotopic fractionation is poorly understood and highlights the need for more systematic studies. Analyzing the intracellular water as well as different lipid classes from the same organism (Sachse & Sachs 2008) might help elucidate both the extrinsic effects that alter the isotopic composition of intracellular water and the direct biosynthetic effects.

EFFECT OF GROWTH RATE AND GROWTH STAGE ON D/H FRACTIONATION

Evidence is mounting that D/H fractionation in phytoplankton lipids is sensitive to growth rate. Schouten et al. (2006) observed increasing D depletion in C_{37} alkenones produced by batch-cultured *Emiliania huxleyi* and *Gephyrocapsa oceanica* at higher growth rates and proposed that a higher proportion of (D-depleted) metabolic water was responsible, although biosynthetic effects cannot be ruled out. Wolhowe et al. (2009) observed D enrichment of alkenones during the exponential phase (not directly comparable with growth rate) relative to the stationary phase in batch-cultured haptophytes. They explain this either by isolation of the intracellular water pool during exponential growth or by limited synthesis of isoprenoid lipids in the stationary phase, resulting in more D-depleted hydrogen being diverted to alkenone synthesis—which would mean a direct biosynthetic effect (see Section 3). This is supported by continuous-culture (chemostat) experiments with the marine diatom *Thalassiosira pseudonana* (Z. Zhang et al. 2009), in which $\varepsilon_{l/w}$ was greater at high than at low growth rates in a sterol but unchanged or slightly lower in fatty acids. The different effects of growth rate on $\varepsilon_{l/w}$ in acetogenic versus isoprenoid lipids may imply that metabolic water may not be the only or the primary control on D/H fractionation changes associated with growth rate.

4. OBSERVED PATTERNS IN δD VALUES OF LIPID BIOMARKERS ACROSS SPACE AND TIME

4.1. δD Values of Lipids Derived from Aquatic Organisms

Investigations of aquatic lipid biomarkers from lake-surface sediments along environmental gradients have yielded strong correlations between lake-water δD values and lipid δD values. This is true for compounds with well-constrained sources (phytoplanktonic sterols), compounds with several possible aquatic sources (C_{17} *n*-alkane), and even compounds that can be derived from aquatic and terrestrial sources (C_{16} *n*-alkanoic acid) (Huang et al. 2002, 2004; Sachse et al. 2004b; Sauer et al. 2001); see **Figure 1**. Similarly, for submerged wetland plants that predominantly produce C_{23} and C_{25} *n*-alkanes, relatively good correlations between source-water δD values and lipid δD values have been observed (Aichner et al. 2010; Nichols et al. 2010; Xie et al. 2000, 2004).

These observations point to the robustness of the signal, in which temporal and spatial (catchment-scale) integration processes appear to reduce the possible variability in individual lipid sources. Laboratory studies of marine and freshwater algae have largely resulted in similarly tight correlations between source-water δD values and lipid δD values for alkenones produced by marine haptophytes (Englebrecht & Sachs 2005), for freshwater algae–derived hydrocarbons (alkenes, long-chain alkadienes, and isoprenoids including botryococcene), and for *n*-alkanoic acids (Zhang & Sachs 2007). However, these batch-culture studies have revealed interspecies differences in the biosynthetic fractionation for ubiquitous compounds such as the C_{16} *n*-alkanoic acid of up to 90‰ (Zhang & Sachs 2007). The cause of species-specific variability of biosynthetic fractionation is not clear but may lie in differences in the exchange between intracellular and extracellular water (see Section 2) or differences in the metabolic networks feeding into biosynthesis (see Section 3.3). Further complications may arise if nonphotosynthesizing bacteria contribute *n*-alkanoic acids or other ubiquitous compounds to the sedimentary record because large differences in D/H fractionation have been observed for these compounds (Li et al. 2009, X. Zhang et al. 2009). These results stress the importance of constraining the biological sources of aquatic lipids. Ideally, this can be achieved by the application of specific lipid biomarkers that are produced only by a limited number of species, such as alkenones (marine haptophytes), 4-methyl dinosterols (freshwater and marine dinoflagellates), and botryococcenes (freshwater green algae *Botryococcus braunii*).

Because growth-rate-dependent and growth-stage-dependent differences in isotopic composition of aquatic lipids have been observed in culture studies (see sidebar, Effect of Growth Rate and Growth Stage on D/H Fractionation), it is likely that under certain circumstances (ecosystem perturbations, strong seasonality) these effects may be preserved in sediments. However, to date such effects have not been detected in sedimentary archives—or interpreted as such. In aqueous environments subject to seawater influence and/or strong evaporation, effects of salinity on lipid δD values have been observed (see sidebar, Mechanisms for the Salinity Dependency on D/H Fractionation in Algal and Cyanobacterial Lipids).

4.2. δD Values of Lipids Derived from Terrestrial Plants

δD values of lipids derived from terrestrial plants (long-chain *n*-alkanes, *n*-alcohols, and *n*-alkanoic acids with more than 24 carbon atoms) extracted from lake-surface sediments along climatic gradients have yielded strong linear relationships with mean precipitation δD values (Garcin et al. 2012, Hou et al. 2008, Huang et al. 2004, Polissar & Freeman 2010, Sachse et al. 2004b) (see **Figure 1**). These results imply relatively consistent offsets between source water and lipids, enabling qualitative paleohydrological reconstructions.

However, the slope and intercept values of the linear regressions obtained between source-water δD values and leaf-wax δD values suggest that a simple two-pool fractionation cannot explain the full variability observed in the data (Sessions & Hayes 2005). This is to be expected because numerous fractionation steps are involved in soil-water and leaf-water evapotranspiration and biochemical processes, all contributing to the overall net fractionation of the plant.

A process-based understanding of all potential drivers of the net or apparent fractionation ($\varepsilon_{l/w}$) is central to a quantitative application of molecular, organically bound hydrogen isotope data in the study of past climate and ecology. Numerous studies have tested the relationships between key hydrological variables and leaf-wax lipid δD values from living plants along environmental gradients (Bi et al. 2005; Chikaraishi & Naraoka 2003; Feakins & Sessions 2010a; Hou et al. 2007; Krull et al. 2006; Liu & Yang 2008; Liu et al. 2006; Pedentchouk et al. 2008; Sachse et al. 2006, 2009; Sessions et al. 1999; Smith & Freeman 2006; Yang & Huang 2003). These studies have identified climatic and/or plant physiological drivers affecting leaf-wax δD values in addition to the source-water isotopic composition. Here we compile and assess the data from diverse environmental studies to evaluate the role of precipitation δD, climate, and plant life-form in influencing δD values of C_{29} *n*-alkanes (δD_{C29}), which constitute the most commonly analyzed terrestrial biomarker (see **Supplemental Material** for data sources and treatment; follow the **Supplemental Materials link** from the Annual Reviews home page at **http://www.annualreviews.org**). We focus on *n*-alkanes, but data for other leaf-wax lipids (*n*-alcohols and *n*-alkanoic acids) exist, although in limited number. Results from these studies show correlations among compound classes that are considerably less strong than the within-class correlations (Hou et al. 2007), suggesting different controls on the isotopic compositions of different compound classes. Thus, a combination of *n*-alkane and *n*-alkanoic acid δD values may potentially record additional information.

4.2.1. Precipitation δD values as the primary control on leaf-wax *n*-alkane δD values.

Globally, site-averaged δD_{C29} and mean annual precipitation δD (δD_{MAP}) values are positively correlated (**Figure 5**), indicating that δD_{MAP} is the fundamental control on plant-wax δD values. However, among plant life-forms (trees, shrubs, forbs, and graminoids), there are differences in the slope, intercept, and significance of this relationship that are thought to result from multiple physical and biological controls on plant source-water, leaf-water, and biochemical fractionations, all of which are important determinants of the overall net fractionation and of plant-wax δD values.

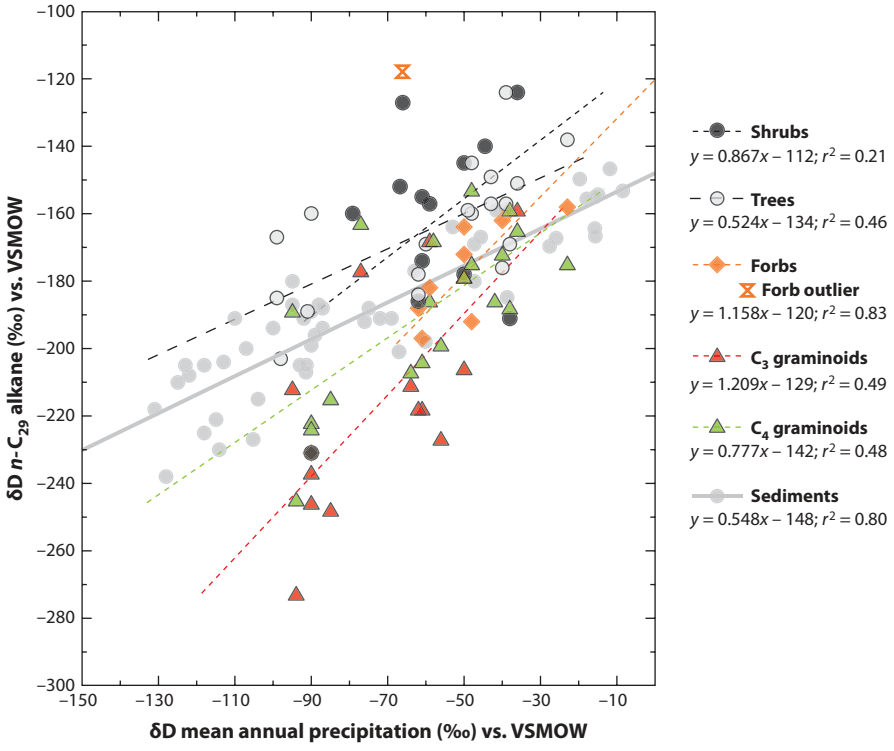

Figure 5

Site-averaged *n*-C_{29} alkane δD values plotted against site mean annual precipitation δD (δD_{MAP}) and grouped by growth form and comparison with sedimentary data (as in **Figure 1*a***). δD_{MAP} estimates are taken from the Online Isotopes in Precipitation Calculator version 2.2 (Bowen 2009, Bowen & Revenaugh 2003) or from on-site data if available (see **Supplemental Material**). Trees, forbs, and graminoids (C_3 as well as C_4) had significant, positive relationships with precipitation δD, whereas no significant relationship was observed for shrubs. One outlier was removed from the regression for forbs and is indicated. The regression parameters for C_3 graminoids and forbs were similar to each other and characterized by steeper slopes, although with considerable scatter. C_4 graminoids were characterized by a lower slope and a more negative intercept, whereas the regression for trees exhibited the lowest slope, possibly owing to stronger evapotranspirative enrichment of plant waters. The regression parameters for trees were similar to the parameters of the relationship between sedimentary *n*-C_{29} alkanes and δD_{MAP}, possibly indicating the importance of angiosperm tree-derived leaf-wax input into sedimentary archives (e.g., Diefendorf et al. 2011). Abbreviation: VSMOW, Vienna Standard Mean Ocean Water.

4.2.2. Physiological and climatic influences on leaf-wax *n*-alkane δD values. To account for variations in δD values caused by variables other than precipitation, hydrogen isotope data can be presented as apparent fractionations between lipid and precipitation water, or $\varepsilon_{l/w}$. Values for $\varepsilon_{l/w}$ in higher plants incorporate three potential sources of fractionation (**Figure 6**): soil-water evaporation (see Section 2.1), leaf-water transpiration (see Section 2.2), and biosynthetic fractionation (see Section 3). The biosynthetic fractionation is determined by the biosynthetic pathway, but the relative importance of soil-water evaporation and leaf-water transpiration on leaf-wax δD values is only poorly understood. This poor understanding is largely due to the limited availability of experimental studies under controlled environmental conditions and/or paired lipid δD and plant source-water (soil-water, xylem-water, and leaf-water) isotope data sets.

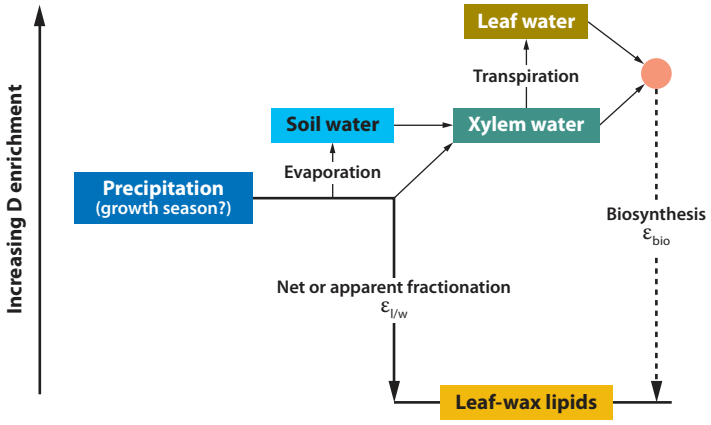

Figure 6

Conceptual diagram describing the hydrogen-isotopic relationships between precipitation and leaf-wax *n*-alkanes from terrestrial plants (not to scale). The red dot illustrates a hypothetical biosynthetic water pool, i.e., a potential mixture of different water pools within the leaf and the ultimate hydrogen source for lipid biosynthesis. Modified from Sachse et al. (2006) and Smith & Freeman (2006). Abbreviations: ε_{bio}, biosynthetic hydrogen-isotopic fractionation; $\varepsilon_{l/w}$, isotopic fractionation between lipids and source water.

We compiled a global data set of $\varepsilon_{C29/MAP}$ values from published data on living plants and estimates of mean annual precipitation (Bowen 2009, 2010) (see **Supplemental Material**). A broad trend to less negative values (yielding more D-enriched lipids) in drier regions becomes apparent. Less negative $\varepsilon_{C29/MAP}$ values at sites with relative humidity (rH) < 0.7 and evapotranspiration (E_t) < 1,000 mm year^{-1} may suggest a possible threshold for the effect of evaporation from soils and leaf water (Feakins & Sessions 2010a, Hou et al. 2008, Mügler et al. 2008, Pedentchouk et al. 2008, Sachse et al. 2006, Sauer et al. 2001, Smith & Freeman 2006, Yang et al. 2009) (**Figure 7**).

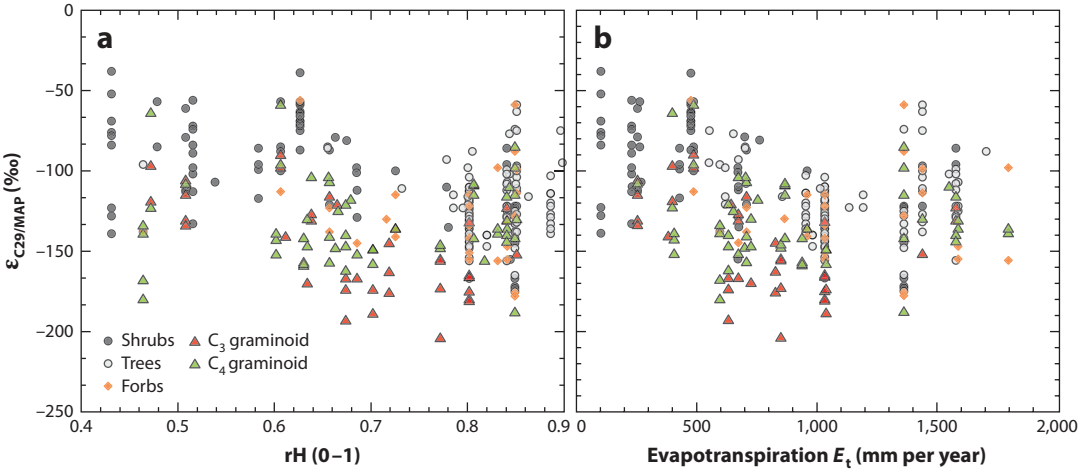

Figure 7

Apparent fractionations for individual species associated with (*a*) relative humidity (rH) and (*b*) evapotranspiration (E_t). Climate data are derived from the National Centers for Environmental Prediction (NCEP) Reanalysis data (1948–2009) provided by the National Oceanic and Atmospheric Administration/Earth System Research Laboratory (NOAA/ESRL) in Boulder, Colorado. δD_{MAP} was estimated as in **Figure 5**. Individual plant data are identified by major photosynthetic and life-form characteristics. See **Supplemental Material** for data sources and treatment.

Several lines of evidence indicate the importance of soil-water and leaf-water isotope enrichment: D enrichment in modern sedimentary leaf-wax *n*-alkanes relative to aquatic *n*-alkanes (Sachse et al. 2004b), smaller $\varepsilon_{l/w}$ values for lake sediments from arid regions compared with temperate regions (Hou et al. 2008, Polissar & Freeman 2010), and smaller $\varepsilon_{l/w}$ values for *n*-alkanes from living plants in drier compared with wetter sites (Feakins & Sessions 2010a, Smith & Freeman 2006). Feakins & Sessions (2010a) did find leaf-water evaporative enrichment to be reflected in leaf-wax δD values in woody plants, but other studies have not distinguished between leaf- and soil-water enrichment owing to the lack of isotope data for these water sources. A recent field study that compared the leaf-water δD values and soil-water δD values with leaf-wax lipid δD values of barley grasses concluded that leaf-water isotope enrichment, rather than soil-water isotope enrichment, was responsible for seasonal changes in leaf-wax δD—although the full amount of observed midday leaf-water enrichment did not become apparent in leaf-wax lipid δD values (Sachse et al. 2010). In contrast, a growth-chamber study found no difference in leaf-wax *n*-alkane δD values for grasses grown in high (96% and 80%) and low (37%) rH experiments. In that study, soil-water evaporative enrichment was prevented, and modeled leaf-water δD values showed no relationship with *n*-alkane δD values (McInerney et al. 2011). These contrasting results, and the lack of any greenhouse experiments including dicotyledonous plant species, point to the need for further experimental research that includes the careful assessment of the magnitude and variability of leaf-water evaporative enrichment in δD and its effects on leaf-wax *n*-alkane δD values.

In addition to soil-water and leaf-water evaporative enrichment, differences among species in rooting depth, thus in source water or microclimatic differences within the canopy, could explain some of the variation in leaf-wax *n*-alkane δD values shown in **Figures 5** and **7**. Few studies have reported on the effect of leaf shading or height in canopy, or other details of the growth microenvironment. Light intensity, through its influence on photosynthesis and transpiration, has the potential to affect δD values recorded in leaf-wax lipids. Recent studies have observed smaller $\varepsilon_{l/w}$ fractionations for leaf-wax lipids under continuous light conditions or from high latitudes (Liu & Yang 2008; Yang et al. 2009, 2011). Long-chain *n*-alkane δD values from high-altitude (>4,000-m) lake sediments (Polissar & Freeman 2010) are slightly but systematically enriched compared with records from lower-elevation lake sediments (see **Figure 1**). Interestingly, smaller $\varepsilon_{l/w}$ values have also been observed in algae for alkenones produced by *E. huxleyi* grown in culture at higher light intensities (Benthien et al. 2009), which are not subject to evapotranspirative D enrichment. Hence, the effect of light intensity on $\varepsilon_{l/w}$ may be attributable to a possible enrichment in D in the intracellular water owing to preferential H removal into metabolites at high rates of photosynthesis. To confirm these hypotheses, measurements of intracellular or leaf-water δD values in conjunction with lipid δD data are essential.

4.2.3. Influence of life-form and photosynthetic pathway on leaf-wax *n*-alkane δD values. Significant differences in $\varepsilon_{C29/MAP}$ values among plant life-forms were observed: Shrubs were the most D-enriched, with trees, forbs, and graminoids increasingly D-depleted (see **Figure 8** and **Supplemental Table X.2**).

We find the most positive $\varepsilon_{C29/MAP}$ values for long-lived shrubs and shrub-like trees (short statured). Shrubs are a common and widespread life-form/functional group in seasonally dry (clear mesic and dry periods), arid, and hyperarid environments. For this reason, the D-enriched nature of shrub waxes may partly be due to climatic factors that favor leaf-water evaporative enrichment signals.

The D-depleted $\varepsilon_{C29/MAP}$ values in graminoids (grasses), particularly C_3 graminoids, are likely related to physiological differences: Graminoids are monocotyledonous (monocot), whereas

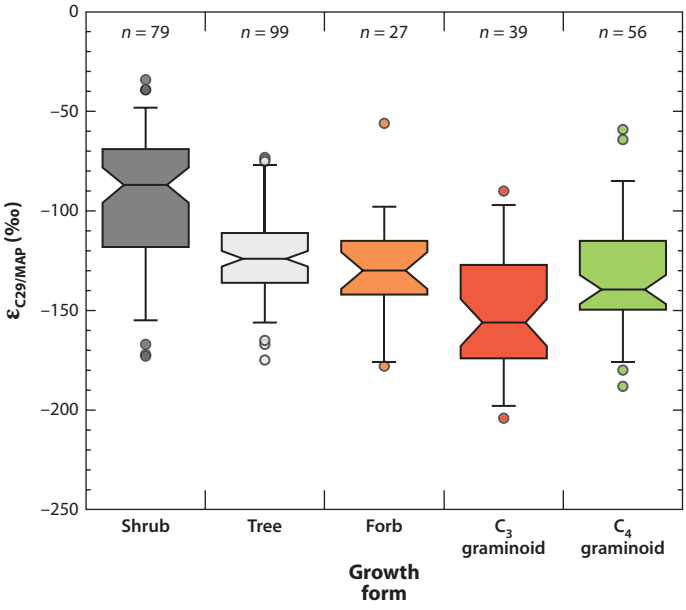

Figure 8

Apparent fractionation ($\varepsilon_{C29/MAP}$) by photosynthetic pathway divided by growth form. Notched box and whisker plots show median (*horizontal line*), upper and lower quartiles (*boxes*), and maximum and minimum values (*vertical lines*), in addition to any outliers, i.e., values that exceed the fifth or ninety-fifth percentile (*open circles*). Notch half-width is calculated according to Mcgill [half-width = (seventy-fifth percentile – twenty-fifth percentile) × $1.57/(\sqrt{N})$] and indicates confidence in differentiating the median values. All categories shown are also significantly different ($p < 0.05$) by heteroscedastic (two-sample, unequal variance) Student's t-tests, with a two-tailed distribution, except forbs, which are separated because of dissimilarity from trees in **Figure 5**. δD_{MAP} was estimated as in **Figure 5**.

shrubs, trees, and forbs are dicotyledonous (dicot). The C_3 monocot average $\varepsilon_{C29/MAP}$ is −149‰ ± 28‰ ($n = 47$), whereas C_3 dicots average −113‰ ± 31‰ ($n = 168$). Monocots and dicots differ in leaf architecture as well as in location and timing of wax synthesis. In monocot grasses, leaf water becomes progressively enriched in ^{18}O and D from base to tip (Helliker & Ehleringer 2002). Because growth occurs via the intercalary meristem at the base of the grass leaf blade, newly synthesized organic hydrogen could reflect the less enriched conditions at the base of the leaf (Helliker & Ehleringer 2002), consistent with seasonal data from barley grasses (Sachse et al. 2010).

Most C_4 plants belong to the angiosperm family Poaceae, which are graminoids that are common in subtropical grasslands (Still et al. 2003). C_4 monocots ($\varepsilon_{C29/MAP}$ −134‰ ± 27‰, $n = 53$) were 15‰ more D-enriched than were C_3 monocots (**Figure 8**). Initially, this offset was attributed to differences in interveinal distance and leaf-water enrichment between C_3 and C_4 grasses (Helliker & Ehleringer 2000, Smith & Freeman 2006). However, if leaf-water enrichment is translated into *n*-alkane δD values of grasses to a lesser extent (Sachse et al. 2010) or not at all (McInerney et al. 2011), biochemical differences related to different pathways of NADPH formation (see Section 3.3) may also be important. Similarly, biochemical influences have been tied to fractionation differences in another pathway, Crassulacean Acid Metabolism (CAM), that is used by succulent plants and epiphytes in tropical and subtropical regions (Feakins & Sessions 2010b).

The climatic and physiological differences expressed in $\varepsilon_{C29/MAP}$ among life-forms and photosynthetic pathways suggest that species changes have the potential to either reduce or exaggerate shifts in site-averaged $\varepsilon_{C29/MAP}$ across aridity gradients (Hou et al. 2008). For paleoenvironmental applications, the offsets among shrubs, C_3 trees, C_3 grasses, and C_4 grasses should be considered where these transitions are known to occur (e.g., via carbon isotope and pollen data). With the present state of knowledge, the observed larger net fractionations of grasses appear to be related primarily to physiological and/or biosynthetic differences, whereas the differences in other classes may be linked primarily to climatic and associated effects on soil-water and leaf-water evaporative enrichment. Thus, variations in net fractionations over time (i.e., from sediment cores) may carry valuable information that potentially could be separated with appropriate companion proxies.

4.2.4. Interspecies variability. Several studies have reported interspecies variability of $\varepsilon_{l/w}$ within growth forms of as much as 100‰ (Chikaraishi & Naraoka 2007, Feakins & Sessions 2010a, Hou et al. 2007, Pedentchouk et al. 2008). Across the data set compiled here, $\varepsilon_{C29/MAP}$ for individual species ranges from −204‰ to −34‰. Interspecies variability that cannot be explained by climate, photosynthetic pathway, life-form, or other gross categories must be related to more subtle aspects of plant physiology and biochemistry or undocumented differences in sampling protocol (e.g., sun versus shade leaves).

Another possible source of the large interspecies variability within growth forms may be related to differences in the timing of leaf-wax synthesis. Leaves of different plants, as well as different leaf generations from the same plant, form at different times of the growing season and therefore sample water with different isotopic compositions. In greenhouse-grown poplars watered with D-enriched water, only leaves that developed during tracer application recorded D enrichment in leaf waxes, whereas mature leaves were unaffected (Kahmen et al. 2011). Under field conditions, however, leaf-wax abrasion due to wind and rain may result in continued wax production. Nevertheless, Sachse et al. (2010) observed that *n*-alkane δD values of leaves from field-grown barley grass remain essentially unchanged after the short period of leaf emergence, and Feakins & Sessions (2010a) reported no seasonal variability in oak-leaf δD values. Other studies, however, indicate continued synthesis and rapid replacement of epicuticular waxes on mature leaves, especially during periods of stress, suggesting the potential for seasonally integrated isotopic signatures (Jetter et al. 2006, Pedentchouk et al. 2008, Sachse et al. 2009). As such, the temporal integration time of a given leaf-wax compound may vary widely among different plants.

4.3. Spatial and Temporal Integration of Sedimentary Records

The above discussions are based on samples from individual organisms. Studies of aquatic and terrestrial lipid biomarkers in soils and sediments offer a complementary approach to understanding δD variability in these compounds because of the large spatial and temporal integration times. Such data sets, spanning wide ranges of different climatic regimes, exhibit strongly reduced variability compared with data sets from studies of individual organisms (**Figure 1**). For example, sedimentary accumulations of plant leaf waxes cannot be attributed to individual species, as they integrate plant inputs over time and across spatial scales ranging from small catchments to river basins. The values observed in sedimentary archives are biased toward the most important plant sources and represent mixtures of different leaf generations developed during the growing season. Hence, sedimentary archives do not show the full range of values observed in modern environments. In addition, there are some special applications wherein the number of species is minimized, e.g., peat bogs (Nichols et al. 2010), or wherein species identifications can be preserved, e.g., leaf fossils (Yang & Huang 2003), middens (Carr et al. 2010), or sediment cores with associated preserved

pollen. Sites where species identifications and hydrogen-isotopic analysis can be combined provide ideal situations for maximum interpretive value in terms of both ecology and climate.

The extent of the temporal integration of sedimentary records is most likely related to catchment size, morphology, and catchment hydrology and therefore varies between different climate regimes and sedimentary contexts. Compound-specific ^{14}C analysis of plant biomarkers from large fluvial drainage areas has suggested residence times or "preaging" of these compounds before deposition in marine sediments on the order of centuries to millennia (Drenzek et al. 2007, Galy et al. 2011, Kusch et al. 2010). Residence times are likely to be significantly shorter in small lake catchments or eolian-dominated systems, allowing for much higher-resolution paleoclimate reconstructions. Further information is needed on the temporal and spatial integration of lipids preserved in different sedimentary archives: Catchment-scale studies combining stable carbon and hydrogen as well as ^{14}C measurements on sedimentary lipids hold great potential to elucidate these processes (e.g., Galy et al. 2011).

5. APPLICATIONS

Shortly after the first frameworks for interpreting this new proxy were articulated (Sauer et al. 2001, Sessions et al. 1999), paleohydrological reconstructions over various geological timescales were presented (Andersen et al. 2001, Huang et al. 2002, Sachse et al. 2004a). Over the past decade, the increased understanding of the proxy has netted a growing number of successful applications that are advancing the understanding of the timing and magnitude of changes in the hydrological cycle over geological timescales. In the scope of this review, we cannot present a comprehensive treatment of all current applications but choose to highlight three approaches to using compound-specific hydrogen isotope ratios that emphasize key areas of promise and focal points for future research.

5.1. Leaf-Wax δD Values as Recorders of Hydrological Changes on the Continents

The most commonly used application has been measurement of leaf-wax lipid (*n*-alkanes, *n*-alkanoic acids) δD values in marine or lake sediment cores. Owing to the still incomplete understanding of the relative importance of plant physiological versus climatic parameters in determining leaf-wax δD values (see Section 4), these applications are necessarily limited to qualitative interpretations. Nevertheless, they have resulted in important insights into changes in terrestrial hydrology across a range of catchments, exemplified by two leaf-wax δD records from the Congo River catchment, which drains much of central Africa: Schefuss et al. (2005) presented a record of *n*-alkane δD values from a marine sediment core from the Congo fan spanning 20,000 years. *n*-C_{29} alkane δD values were interpreted to indicate wetter (more negative δD values) or drier (more positive δD values) conditions associated with changes in Atlantic Ocean meridional temperature gradients over the last glacial cycle, reflecting the strength of the southerly trade winds counteracting monsoonal moisture inflow into central Africa. Tierney et al. (2008), in a 60,000-year record of *n*-C_{28} alkanoic acid δD values from Lake Tanganyika, found strikingly similar general patterns that indicated a consistent climate signal throughout the Congolese Basin (**Figure 9**) and also indicated a role for Indian Ocean sea-surface temperatures influencing the Eastern margin of the Congo catchment.

The absolute δD values were more enriched for the *n*-C_{28} acid; this can be explained partly by differences in biosynthesis (see Section 3) and partly by potential differences in water sources. Although the records are strikingly similar, the lacustrine record captures a late Holocene increase

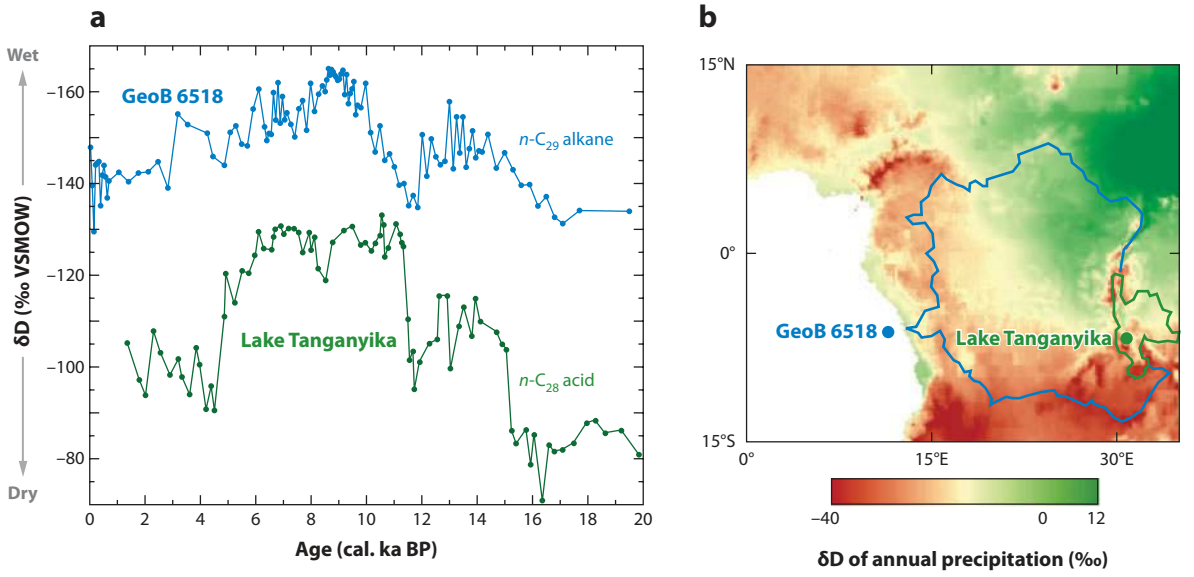

Figure 9

(*a*) Compound-specific δD records from lacustrine and marine sedimentary cores collected in tropical Africa, which span the past 20,000 years. The lacustrine core (*green line*) originates from Lake Tanganyika (Tierney et al. 2008), and the marine core (GeoB 6518, *blue line*) originates from the Congo River mouth (Schefuss et al. 2005). Note that the y-axis is reversed. (*b*) Spatial distribution of stable hydrogen isotope values in modern rainfall of central-western Africa; interpolated data from Bowen (2009) and Bowen & Revenaugh (2003). Catchment areas draining toward Lake Tanganyika and toward the Congo River are shown by green and blue lines, respectively. Abbreviations: cal. ka BP, calendar kiloyears before present; VSMOW, Vienna Standard Mean Ocean Water.

in *n*-C_{28} acid δD values, interpreted as increasingly dry conditions, not seen in the marine record and therefore perhaps indicative of regional changes affecting East Africa via the Indian Ocean influence. We also note that the amplitude of changes in the marine record is smaller, reflecting signal attenuation associated with greater spatial and temporal integration. This comparison between a large lake record and a marine record indicates that continental-scale as well as local-scale information is available from such nested analyses, emphasizing the value in obtaining leaf-wax δD records from a hierarchy of catchment sizes.

5.2. Leaf-Wax δD Values as Recorders of Paleoaltimetry

The observed decrease in the δD values of precipitation with altitude (mainly due to the temperature effect; see Section 2.1) has raised the possibility that sedimentary δD values of leaf-wax *n*-alkanes may be suitable as records of changes in mountain range uplift (Polissar et al. 2009). It is, however, often difficult to separate effects of changing climate and atmospheric circulation patterns from changes on δD precipitation due to uplift, especially over the multimillion-year timescales of mountain range uplift. A solution is to focus on the reconstruction of relative differences in the hydrology across mountain ranges, i.e., compare coeval records from the foreland to the mountain range. An example of this approach is given by Hren et al. (2010), who compared *n*-alkane δD values from fossil leaves across the Eocene Sierra Nevada mountain range in the western United States. Leaf-wax *n*-alkane δD values, as well as methylation index of branched tetraethers/cyclization ratio of branched tetraethers (MBT/CBT)-based temperature

reconstructions (i.e., Weijers et al. 2007) of the foreland-to-mountain-range gradient, suggest that a ~2 km high mountain range was present already during the Eocene.

5.3. Aquatic Lipid Biomarker δD Values as Recorders of Hydrological Shifts Across Different Temporal and Spatial Scales

Algal and cyanobacterial lipid δD values from lake and marine sediments are increasingly being used to infer changes in rainfall, runoff and salinity, and, by extension, climate (Pahnke et al. 2007; Sachs et al. 2009; Smittenberg et al. 2011; van der Meer et al. 2007, 2008). Although individual lake sedimentary sequences reflect, at most, regional climate changes, a combination of several sites can potentially elucidate even changes in large-scale, hemispheric phenomena. For instance, Sachs et al. (2009) used botryococcene δD values from a freshwater lake in the Galapagos Islands, dinosterol δD values from a brackish lake in Palau, and bulk cyanobacterial lipid δD values from a lake in the Northern Line Islands of Kiribati to infer that the Intertropical Convergence Zone was located approximately 500 km closer to the equator during the Little Ice Age (1400–1850 A.D.) than at present.

Alkenone δD values in sediment cores from the eastern Mediterranean Sea and the Black Sea were used by van der Meer et al. (2007, 2008) to infer the magnitude of surface-water freshening associated with the deposition of Mediterranean Sapropel S5 (variously dated between 116,000 and 127,000 years ago) and with salinity changes around the time of the invasion of the Black Sea by the coccolithophorid *E. huxleyi* (approximately 2,700 years ago).

SUMMARY POINTS

1. Know your proxy: It is now well established that source-water δD values are recorded in lipid biomarker δD values from photosynthesizing organisms. However, if sedimentary lipid δD values are to be interpreted in terms of changing paleohydrology and/or climate, the potential effects of other variables—including biological factors that modulate δD paleorecords—have to be taken into account. Conversely, when climate boundary conditions can be assessed, lipid δD values may even be useful in reconstructing ecophysiological changes.
2. Know your archive: Through the use of additional proxies—such as sedimentology, geochemistry, pollen records, biomarker presence and abundance, and biomarker indices—the environment can usually be reasonably well characterized, and the effects of certain biological processes or significant changes in vegetation can potentially be ruled out or accounted for. Pollen records can document possible vegetation shifts, allowing separation of climatic and physiological controls on leaf-wax δD values.
3. Know your molecule: The biosynthetic pathway of lipid synthesis can impact significantly the δD value of biomarkers; hence, it is prudent to be aware of the variation that can occur in different classes of lipids. Where biological effects are specific to certain organisms, biomarker sources can be constrained by the use of more specific molecules and by comparison with other lines of evidence, e.g., microfossils. Nonphotosynthesizing organisms using multiple hydrogen sources may synthesize lipids with widely variable δD values, and the relation to source-water δD values may be limited. Combination of compound-specific $\delta^{13}C$ measurements may help constrain the metabolic pathway of a given lipid biomarker.

4. Know your uncertainty: Biological variables can increase the uncertainty in reconstructing absolute source-water δD values. One solution is to interpret relative changes and to select records in which the signal change is anticipated to be bigger than the current uncertainty. Global or regional circulation models with a water isotope module may be helpful in predicting reasonable ranges for past changes in source-water δD values for different organisms; these ranges can be compared with reconstructed ranges in δD values. Possible discrepancies can then be better evaluated.

FUTURE ISSUES

1. Systematic greenhouse and field studies on higher plants investigating interspecies variability that include isotopic data for all possible hydrogen sources (source water, soil water, leaf water, water vapor) as well as meteorological and physiological observations over seasonal timescales are needed to characterize and quantify the sources of current uncertainty in the leaf-wax D/H proxy.
2. Controlled laboratory culture studies including the measurement of intra- and extracellular water are needed to understand and quantify the influence of salinity, temperature, growth rate, and light on D/H fractionation in a range of acetogenic and isoprenoid lipids in different species of phytoplankton.
3. Investigations estimating transport times of terrestrial lipids into sedimentary archives are essential to understand spatial and temporal integration of sediments. Compound-specific ^{14}C dating of lipid biomarkers over a range of different environments and catchments would help in understanding the temporal and spatial integration of the sedimentary leaf-wax δD signal.
4. Ultimately, empirical observations should be integrated into quantitative mechanistic models of the processes affecting lipid biomarker δD values. Eventually, such models will provide a basis for extracting quantitative paleohydrological reconstructions from the sedimentary record.

DISCLOSURE STATEMENT

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