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Invited Review

# Extracting the most from terrestrial plant-derived *n*-alkyl lipids and their carbon isotopes from the sedimentary record: A review

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ABSTRACT

Terrestrial plant biomarkers and their carbon isotopes provide insights into carbon cycling, paleovegetation and paleoclimate, ranging in scale from local to global. Over the past decade, considerable efforts have been made to constrain the factors that influence plant biomarkers and their carbon isotope composition to improve their utility for paleo applications. Global and regional replication of time intervals of great interest, such as during carbon cycle perturbations, has increased the need to compare among sites, but doing so has also complicated interpretation of carbon cycle perturbations due to the differences among records. This has led to questions regarding the fidelity of isotope records, the sensitivity of the isotope record to climate, and the best practices for reconciling records. But, at the same time, it has led to new exciting information on ecosystem responses to climate change. By removing competing influences of climate, ecosystem and biology, modern biomarker and isotope calibrations provide a means of reconciling and improving paleorecords and placing quantitative constraints on their interpretation. Here, we review the factors that influence the concentration of plant biomarkers and their carbon isotope composition and provide best practices for reconciling biomarker carbon isotope records for interpreting climate, ecosystem, and carbon cycling in the geologic past.



## 1. Introduction

Plant biomarkers have been recognized for decades in the sedimentary record. Since their discovery, they have developed into an important tool for organic geochemists to reconstruct paleoclimate and paleovegetation change, especially when other lines of evidence, such as pollen or macrofossils, are poorly preserved. Of these biomarkers, long chain *n*-alkyl plant wax compounds are the most commonly used. Their utility is strengthened both by excellent preservation potential in lacustrine, terrestrial and nearshore marine sediments and rocks, and by relatively easy extraction and analysis. Their carbon isotopic composition provides additional information as it passes evidence about the carbon cycle, vegetation, and climate to the geologic record.

Much of what we know about the carbon isotopic signals preserved in sedimentary plant wax are inferred from studies made on individual living species. However, sediments accumulate mixtures of wax from individual plants that are integrated at scales ranging from small ecosystems to large basins. In large spatial scale studies, plant wax may represent integrated signals of one to many biomes. This poses challenges when interpreting plant wax with species-level information (the focus of almost all calibration studies) because scaling relationships have not been developed between species, ecosystems and biomes. The role that vegetation, climate, taphonomy and transport have on plant wax concentration and carbon isotopic composition complicates interpretation of sedimentary plant wax. The evolution of plants and plant biomarkers over the past several hundred million years can also not be overlooked, although some level of biological uniformitarianism must be assumed.

Comparison of plant wax carbon isotopes among records for discrete geologic events, such as the Paleocene-Eocene Thermal Maximum, indicate that plant wax carbon isotopes are not only sensitive to carbon cycle perturbations, but are sensitive to vegetation and climate (e.g. Schouten et al., 2007; Diefendorf et al., 2010; McInerney and Wing, 2011; Tipple et al., 2011; Krishnan et al., 2015; Schoon et al., 2015). Other factors, such as lags in the ages of plant wax contributions, may also be important, especially for more recent sediments due to their relatively high temporal resolution (Pearson and Eglinton, 2000; Uchikawa et al., 2008; Galy and Eglinton, 2011; Douglas et al., 2014). Changes in vegetation composition become even more important after the rise of

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angiosperms in the Cretaceous and then the rise of $C_4$ plants in the Miocene (Edwards et al., 2010).

This review focuses on the current state of knowledge about terrestrial plant biomarkers and how this information can be used to better interpret past changes in paleoclimate, paleovegetation and the carbon cycle. Section 2 provides background on the factors that influence plant wax *n*-alkyl lipids. Much of what we know about plant biomarkers is based on *n*-alkanes, although other wax components, such as *n*-alkanoic acids and *n*-alkanols, are also informative, especially for more recent sediments (Feakins et al., 2007; Douglas et al., 2012; Sachse et al., 2012). Section 3 focuses on the factors that influence plant carbon isotopic composition. The organizational structure for the isotope section follows the flow of carbon from the atmosphere to plant biomarkers and on to the integration and preservation of biomarkers in the sedimentary archive (Fig. 1). Section 4 focuses on applications in the paleorecord for interpreting plant carbon isotopes as paleoclimate, paleovegetation and atmospheric $\delta^{13}C$ proxies. Section 5 provides a summary, remaining gaps and future directions. The review does not include the hydrogen isotopic composition of plant wax components, which has been reviewed recently (Sachse et al., 2012; Sessions, 2016).

## 2. Plant wax *n*-alkyl lipids

The cuticular wax of terrestrial vascular plant leaves is composed of long chain *n*-alkyl compounds, including *n*-alkanes, *n*-alkanoic acids, *n*-alkanols and *n*-esters (Eglinton et al., 1962; Eglinton and Hamilton, 1967; Kolattukudy et al., 1976; Jetter et al., 2000). Many of these compounds can be found on the surface of other plant organs, but in almost all cases, the concentration of long chain wax components is significantly lower than in the corresponding leaves. Therefore, based on concentration and biomass, the leaf wax from trees, shrubs and grasses can generally be assumed to be the dominant source of wax delivered to sediments (e.g. Mueller et al., 2012; Gamarra and Kahmen, 2015). We recommend the term 'plant wax' when referring more generally to wax components sourced from plants, especially when the components are found in soils and sediments, and the term 'leaf wax' when specifically referring to lipids sampled directly from leaves.

Plant waxes are biosynthesized in the acetogenic pathway from acetyl coenzyme-A (acetyl CoA) to produce short chain *n*-alkanoic acids that are then elongated through repeated additions of acetyl CoA. *n*-Alkanoic acids are then converted to *n*-alkanes by enzymatic decarboxylation or to *n*-alkanols by reduction (Eglinton and Hamilton, 1967; Kolattukudy et al., 1976; Kolattukudy, 1996; Kunst and Samuels, 2003; Chikaraishi et al., 2004b). The functions of plant waxes are diverse and include forming a barrier to inhibit water loss, acting as a barrier to chemicals, and providing protection from disease and ultraviolet light (Eglinton and Hamilton, 1967; Kerstiens, 1996; Riederer and Schreiber, 2001; Muller and Riederer, 2005; Bargel et al., 2006; Riederer and Muller, 2006; Jetter and Riederer, 2016). The reader is referred to the extensive review of cuticular wax function by Koch and Ensikat (2008) and references therein.

Relatively few studies of plant waxes in extant plants have compared the relative abundance *and* carbon isotopic composition of two or more compound classes (Chikaraishi et al., 2004b; Chikaraishi and Naraoka, 2006, 2007; Rommerskirchen et al., 2006; Vogts et al., 2009; Gao et al., 2015). Surveys of plant waxes in extant plants indicate that the molecular composition and concentration vary among plant species for *n*-alkanes and *n*-alkanols. In contrast, the total concentration of *n*-alkanoic acids is more consistent among trees and shrubs (Polissar and Freeman, 2010; Diefendorf et al., 2011, 2015b; Bush and McInerney, 2013; Polissar et al., 2014).

In general, long chain *n*-alkane abundances (i.e. *n*-$C_{25}$ and above) are typically higher in angiosperm trees and shrubs than in many gymnosperms, and more specifically, conifers (Fig. 2; Table 1; Supplementary material, Table E-1). However, it is critical to take into account which conifers are present within a plant community. For example, Pinaceae and the Taxodioid group in Cupressaceae synthesize low concentrations of *n*-alkanes, whereas Araucariaceae, Podocarpaceae, Taxaceae and the Cupressoideae and Callitroideae groups within Cupressaceae have *n*-alkane concentrations that rival those of angiosperms (Fig. 3). Much of this variation among conifer families can be attributed to the evolutionary history of conifers (Diefendorf et al., 2015b).

Evaluating the contributions of plant waxes from conifers is important as conifer assemblages change in time and space. For example, today in North America, most conifers are Pinaceae and thus have low concentrations of *n*-alkanes. Other species, such as *Juniperus communis* and *Thuja plicata*, are important and have high concentrations, but are typically not the dominant species within an ecosystem (Diefendorf et al., 2011, 2015b; Tipple and Pagani, 2013). However, in the past, this was not always the case. For example, *Juniperus* was common in upland environments in the Oligocene Creede Formation (Axelrod, 1987; Wolfe and Schorn, 1990), so could be the dominant source of long chain *n*-alkanes.

Other growth forms and groups, besides woody angiosperms, produce significant quantities of plant waxes (Fig. 2). For example, *n*-alkanes are found in grasses, succulents, herbs and ferns (Lytle et al., 1976; Rommerskirchen et al., 2006; Vogts et al., 2009; Bush and McInerney, 2013; Carr et al., 2014). Cycads do not, however, appear to be a significant source (Vane et al., 2014). Aquatic macrophytes, including submerged, floating and emergent growth

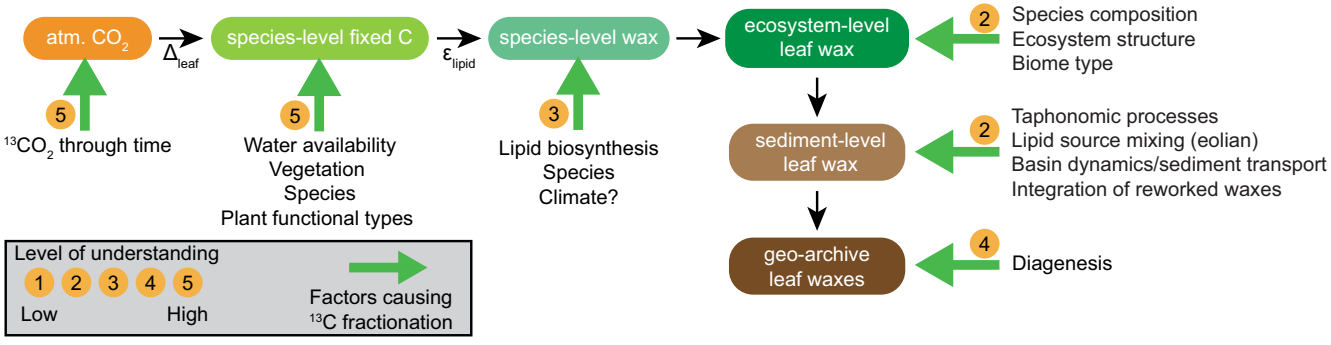


**Fig. 1.** Schematic of factors that influence the carbon isotopic composition of plant waxes following the flow of carbon from atmospheric $CO_2$ into leaves and on to preservation in the geologic record. Factors that influence bulk leaf tissue and plant wax carbon isotopic fractionation are indicated with green arrows. Level of community understanding is based on *n*-alkanes and will be lower for other wax constituents (i.e. *n*-alkanols and *n*-alkanoic acids).

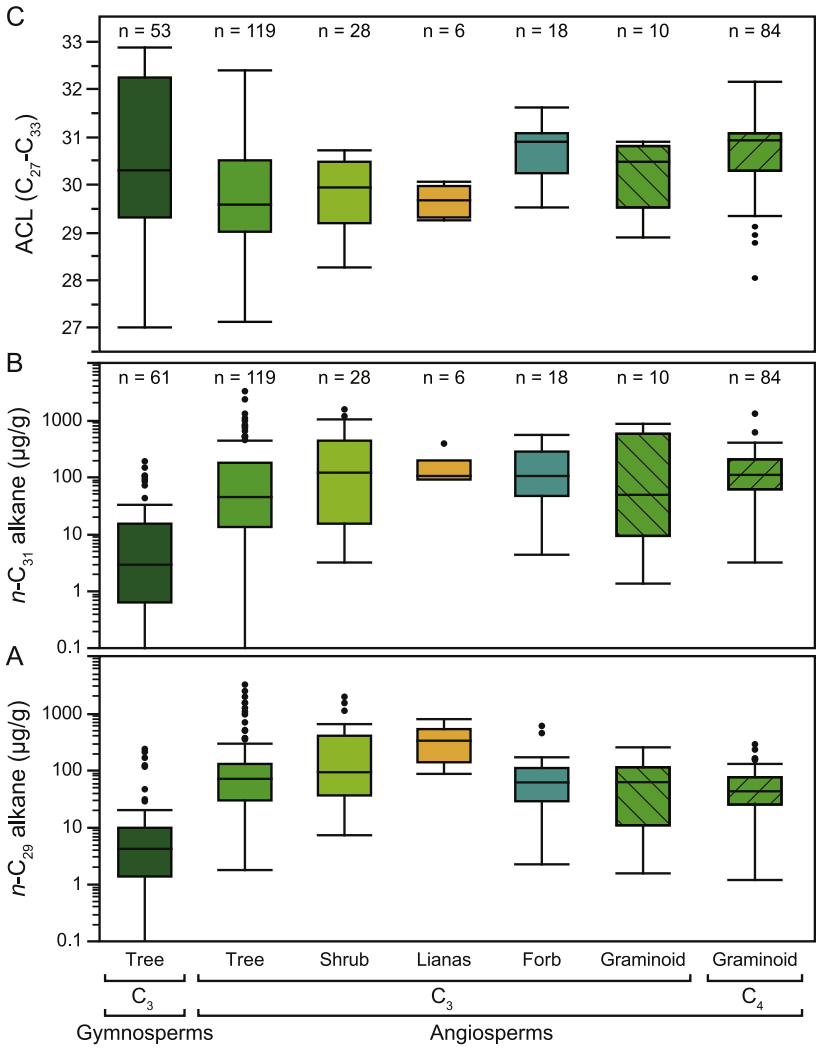


**Fig. 2.** Plant wax concentration of (A) *n*-$C_{29}$ alkane, (B) *n*-$C_{31}$ alkane and (C) ACL ($C_{27}$–$C_{33}$) for different growth forms separated by major taxonomic group and photosynthetic pathway. The *n*-alkane concentration is in µg/g dry leaf and is plotted on a log scale. The number of samples in each group is indicated and *n* is the same for both *n*-alkane chain lengths. There is a strong phylogenetic signal in the gymnosperm *n*-alkane concentration and ACL data that explains much of the observed variation (see Fig. 3). Box and whisker plots show the median, upper and lower quartiles, and maximum and minimum values, with outliers shown as dots. The data are a compilation from many studies (Collister et al., 1994b; Lockheart et al., 1997, 1998; Chikaraishi and Naraoka, 2003, 2007; Conte et al., 2003; Chikaraishi et al., 2004b; Nguyen Tu et al., 2004b; Bi et al., 2005; Krull et al., 2006; Rommerskirchen et al., 2006; Vogts et al., 2009; Bezabih et al., 2011; Diefendorf et al., 2011, 2015b; Duan and He, 2011; Mortazavi et al., 2012; Garcin et al., 2014; Badewien et al., 2015; Bush and McInerney, 2015).

**Table 1**
*n*-Alkane concentration and ACL for different plant functional types.

| Phylogeny[b] | Pathway | Form | *n*-Alkanes (µg/g leaf)[a] | | | | | | | | | | | | | | | ACL$_{(C27–C33)}$ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $C_{27}$ | | | $C_{29}$ | | | $C_{31}$ | | | $C_{33}$ | | | $C_{35}$ | | | | | |
| | | | Mean | 1σ | *n* | Mean | 1σ | *n* | Mean | 1σ | *n* | Mean | 1σ | *n* | Mean | 1σ | *n* | Mean | 1σ | *n* |
| A | $C_3$ | Forb | 22 | 19 | 18 | 114 | 155 | 18 | 625 | 1398 | 18 | 80 | 138 | 18 | 12 | 24 | 13 | 30.6 | 0.6 | 18 |
| | | Graminoid | 18 | 15 | 10 | 81 | 84 | 10 | 242 | 353 | 10 | 54 | 82 | 10 | 4 | 5 | 7 | 30.2 | 0.8 | 10 |
| | | Lianas | 16 | 11 | 6 | 362 | 263 | 6 | 152 | 112 | 6 | 9 | 4 | 6 | 1 | 1 | 6 | 29.7 | 0.3 | 6 |
| | | Shrub | 34 | 36 | 28 | 322 | 472 | 28 | 290 | 395 | 28 | 41 | 82 | 28 | 2 | 3 | 27 | 29.8 | 0.8 | 28 |
| | | Tree | 40 | 64 | 119 | 207 | 455 | 119 | 188 | 404 | 119 | 60 | 152 | 119 | 13 | 71 | 99 | 29.7 | 1.1 | 119 |
| | $C_4$ | Graminoid | 39 | 56 | 84 | 56 | 48 | 84 | 159 | 173 | 84 | 108 | 122 | 84 | 41 | 51 | 70 | 30.7 | 0.7 | 84 |
| G | $C_3$ | Tree | 7 | 17 | 61 | 20 | 47 | 61 | 20 | 39 | 61 | 51 | 101 | 61 | 27 | 55 | 61 | 30.5 | 1.6 | 53 |

[a] Data sources are listed in Fig. 2 caption and reported in Table E-1.
[b] Angiosperms (A), gymnosperms (G); the gymnosperms are all conifers, except for 2 *Ginkgo biloba* samples.

habits, also produce *n*-alkyl lipids, with *n*-alkanes being the most diagnostic compound class to distinguish aquatic from terrestrial plant sources (Ficken et al., 2000). Aquatic macrophytes are typically dominated by mid-chain homologs (Baas et al., 2000; Ficken et al., 2000; Nott et al., 2000) and can be a major source of *n*-$C_{23}$ and *n*-$C_{25}$ in sediments (Aichner et al., 2010). Generally, aquatic plants favor production of longer *n*-alkane homologues in emergent relative to submerged or floating growth forms (Mead

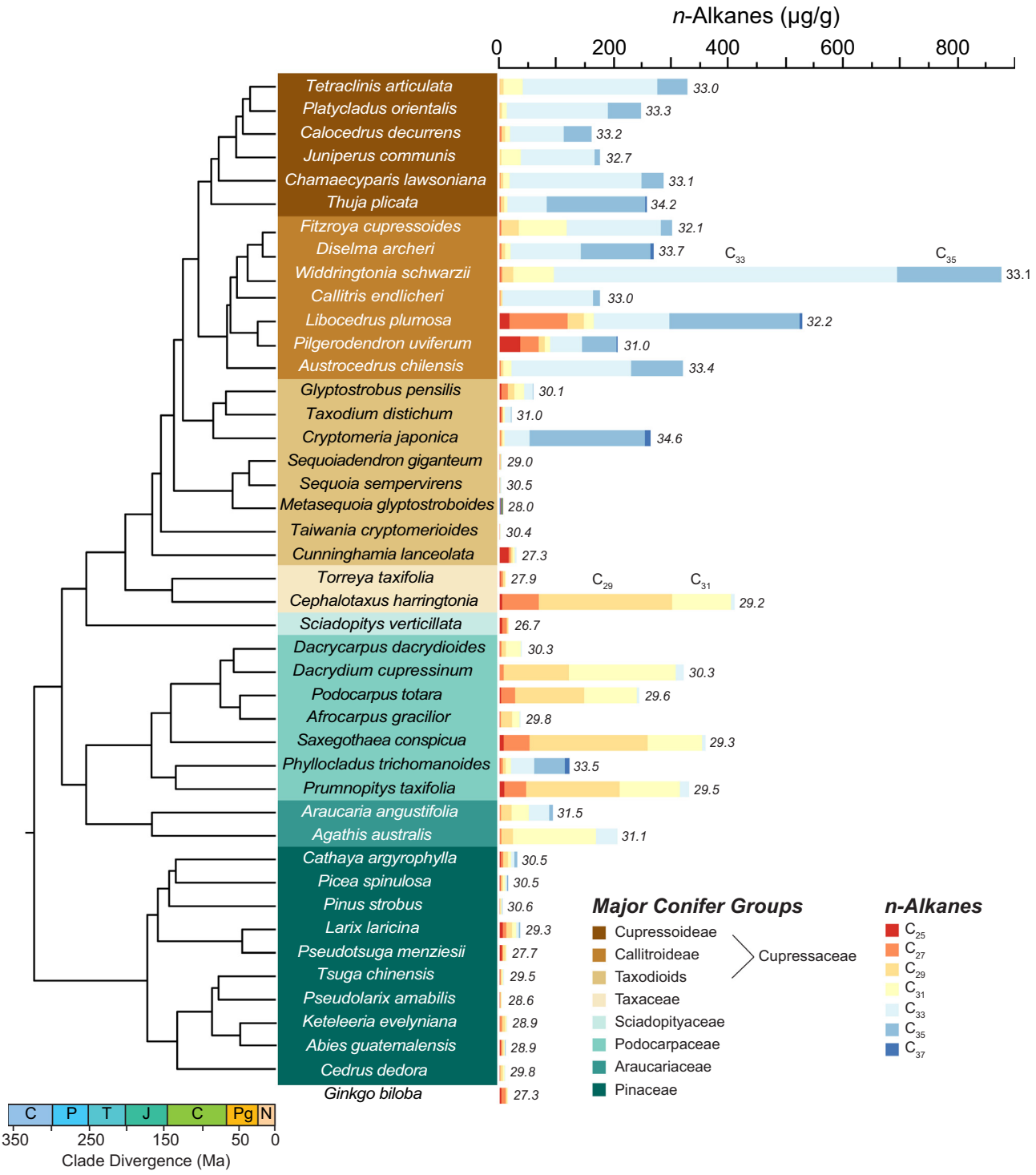


**Fig. 3.** *n*-Alkane concentration for conifer species and *Ginkgo biloba* collected at the same site in Berkeley, California. Conifer species are grouped into major clades with a DNA sequence-based phylogeny age-calibrated using the fossil record (Rai et al., 2008; Leslie et al., 2012). The *n*-alkane concentration (µg/g dry leaf) is reported for odd *n*-alkanes from $C_{25}$ to $C_{37}$ with ACL values indicated ($C_{25}$–$C_{37}$). Box and whisker plots show the median, upper and lower quartiles, and maximum and minimum values, with outliers shown as dots. Figure is redrawn from Diefendorf et al. (2015b).

et al., 2005). The *Sphagnum* moss species, common along bog shorelines, are also generally dominated by *n*-$C_{23}$ and *n*-$C_{25}$ (Baas et al., 2000; Nott et al., 2000). However, significant inter-species variation has been observed, with certain species maximizing at *n*-$C_{31}$ (Baas et al., 2000; Bingham et al., 2010) or favoring higher *n*-$C_{31}$ alkane concentration on relatively dry growth substrates such as hummocks (Nichols et al., 2006).

Several metrics have been developed to distinguish aquatic and terrestrial sources using plant wax distributions in sediments. These include the $P_{aq}$ ratio of *n*-alkane homologues to quantify the contribution of submerged and floating macrophytes relative to emergent and terrestrial plants (Ficken et al., 2000) and, in peat bogs, *n*-$C_{23}$/*n*-$C_{25}$ ratio values to reflect bog wetness (Bingham et al., 2010), and the ratio of *n*-$C_{23}$ to *n*-$C_{31}$ (Nott et al., 2000) or

$n$-$C_{33}$ (Pancost et al., 2002) to indicate the proportion of *Sphagnum* input. Application of these and other indicators based on plant wax molecular distribution must consider whether the depositional setting and plant source influence these metrics. Emergent and terrestrial plants contain 5–10× higher lipid concentration than submerged species (e.g. Aichner et al., 2010). Therefore, terrestrial plants remain the focus of this review as the dominant source of long chain $n$-alkyl lipids in sediments.

The composition of $n$-alkanes also varies between $C_3$ and $C_4$ grasses. For example, $C_4$ grasses tend to have higher concentrations of longer chain $n$-alkanes ($C_{31}$, $C_{33}$, $C_{35}$) than $C_3$ grasses and woody angiosperms (Krull et al., 2006; Rommerskirchen et al., 2006; Vogts et al., 2009; Garcin et al., 2014). The compositional differences between $C_3$ and $C_4$ grasses are important in the context of evaluating Miocene and younger sediments (Edwards et al., 2010).

The composition of $n$-alkanes is commonly compared among plants using the average chain length (ACL):

$$\mathrm{ACL}_{m-n} = \sum_{i=m}^{n} \frac{i[C_i]}{[C_i]}$$

where $m$ and $n$ represent the shortest and longest chain length, respectively. ACL has been used in geologic studies as both a vegetation and climate proxy. However, studies of extant plants indicate that both biology and climate may influence ACL values (see reviews by Freeman and Pancost, 2014; Bush and McInerney, 2015), but not always in clear or consistent ways. For example, Hoffmann et al. (2013) found that climate influenced ACL in opposite ways for two plant genera along the same climate transect in Australia. At a global scale, temperature does not appear to be a strong control on ACL (Fig. 4), likely because of differences in ACL among species. Although no clear patterns emerge when comparing ACL with angiosperm phylogeny, conifer ACL has a strong phylogenetic signal (Diefendorf et al., 2015a). Very long chain $n$-alkanes ($C_{33}$, $C_{35}$) may be a good indicator for $C_4$ grasses in grassland ecosystems (Vogts et al., 2012). However, it is important to highlight that some conifers also make very long chain $n$-alkanes ($C_{33}$ to $C_{37}$; Fig. 3) (Diefendorf et al., 2015b).

Based on our current knowledge about the factors that influence plant wax concentration, interpreting long chain $n$-alkanes as vegetation indicators should be done cautiously with respect to phylogeny, climate and biome (Diefendorf et al., 2011, 2015b; Freeman and Pancost, 2014; Chevalier et al., 2015). Nonetheless, in some biomes or biome transitions through time, it may be possible to exploit differences in chain length distributions between plant groups to constrain plant wax source and to provide group specific $\delta^{13}C$ values. For example, it may be possible to use $n$-$C_{29}$ alkane as a $C_3$ biomarker and $n$-$C_{33}$ and $n$-$C_{35}$ alkanes as $C_4$ biomarkers to reconstruct $C_3/C_4$ plant cover (e.g. Garcin et al., 2014). This may also be useful when evaluating the rise of $C_4$ plants in the Miocene (e.g. Freeman and Colarusso, 2001; Uno et al., 2016a,b).

## 3. Controls on plant carbon isotopes and waxes

This section provides a basis for the factors that control the carbon isotopic composition of plant waxes from the source of carbon, atmospheric $CO_2$, to the integration and deposition of plant lipids in sediments. The organization of this section follows the pathway outlined in Fig. 1. Although this section is not exhaustive, it provides a solid basis for the factors that influence sedimentary plant wax $\delta^{13}C$ values.

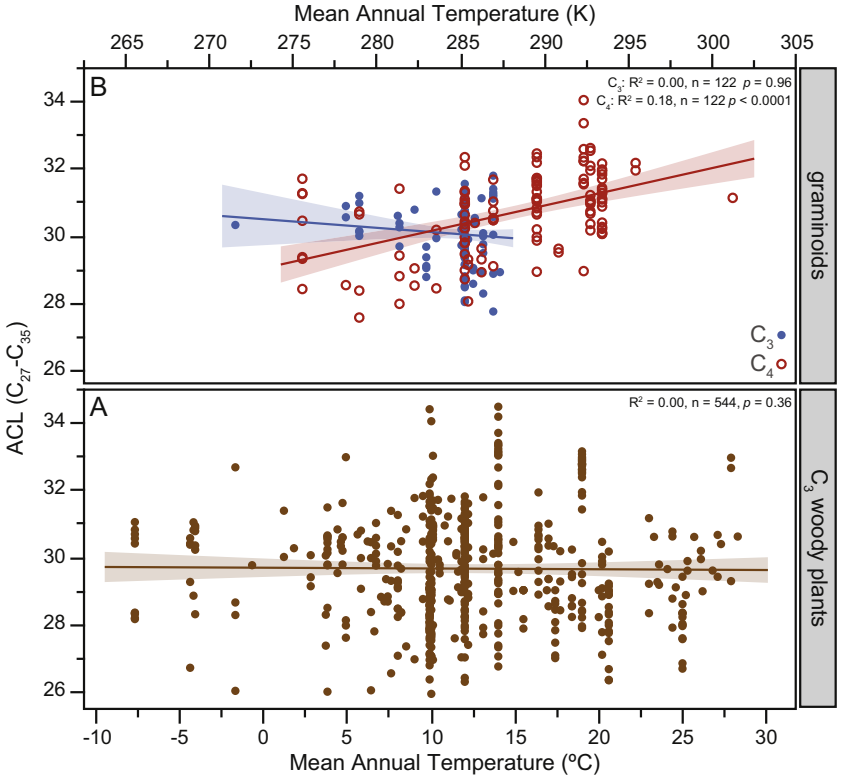


**Fig. 4.** ACL ($C_{27}$–$C_{35}$) as a function of mean annual temperature (°C and K) for (A) $C_3$ woody plants (including angiosperms and gymnosperms) and (B) $C_3$ and $C_4$ graminoids. A least squares regression line is plotted for reference with the 95% confidence of fit shaded for each line. MAT is not a predictor of ACL for woody plants or $C_3$ graminoids. However, it is a predictor of ACL for $C_4$ graminoids and explains 18% of the variability in ACL. Data are compiled from Bush and McInerney (2013) for the references therein that included geographic position, and from Diefendorf et al. (2011, 2015b). MAT is derived from the 1981–2010 long term mean GHCN Gridded V2 data provided by the NOAA/OAR/ESRL PSD, Boulder, Colorado, USA, from their web site at www.esrl.noaa.gov/psd/.

### 3.1. Atmospheric $^{13}CO_2$

In any analysis of plant $\delta^{13}C$ values from the geologic past, it is critical to constrain changes in the $\delta^{13}C$ value of atmospheric $CO_2$ ($\delta^{13}C_{atm}$) as this sets the starting isotopic composition for plant carbon. The best practices for estimating past $\delta^{13}C_{atm}$ values were explored by Tipple et al. (2010). Based on their recommendations, it is best to determine $\delta^{13}C_{atm}$ from carbonate $\delta^{13}C$ values of benthic foraminifera sampled from marine cores. The approach requires estimates of ocean temperature values to correct for fractionation. To that end, Tipple et al. (2010) provided $\delta^{13}C_{atm}$ values from benthic foraminifera for the Cenozoic. For studies that focus on more recent sediments (< 50 ka), ice core $^{13}CO_2$ records are preferable as they are at a higher resolution than marine sediments and provide a more direct sampling technique.

Over the Cenozoic, $\delta^{13}C_{atm}$ varies by up to 2‰ (Fig. 5) and thus significantly deviates from the preindustrial value of −6.5‰. The changes reflect both long term and short term changes in the carbon cycle as a function of carbon source (volcanism, weathering of organic C), burial, weathering and changes in marine photosynthetic fractionation (Kump and Arthur, 1999). Constraining these factors is especially important during periods of large change, such as during the Miocene and early Paleogene. The former is especially important for studies evaluating changes in $C_3$ and $C_4$ vegetation based on $\delta^{13}C$ values (Tipple et al., 2010). Constraining $\delta^{13}C_{atm}$ during periods of rapid carbon cycle perturbations, such as the hyperthermal events in the early Eocene, is more challenging given the rapid feedbacks and imbalances in the carbon cycle that occur on short timescales (McInerney and Wing, 2011; Bowen, 2013).

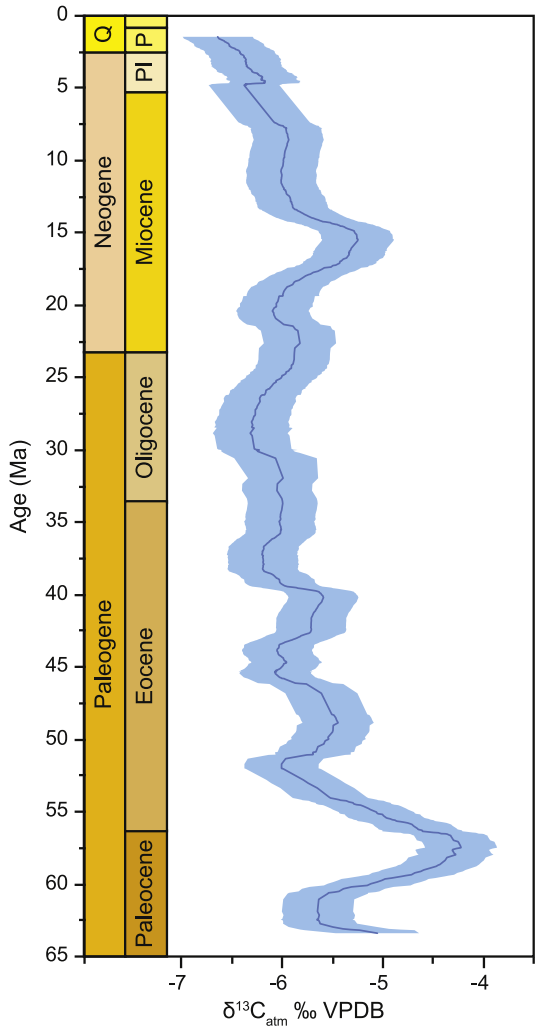


**Fig. 5.** The $\delta^{13}C$ value of atmospheric $CO_2$ estimated for the Cenozoic and based on the $\delta^{13}C$ of benthic foraminifera. The blue line is a $3 \times 10^6$ yr running average and the shaded box is the 90% confidence interval. Figure is redrawn from Tipple et al. (2010).

### 3.2. Plant carbon isotopes

During photosynthetic carbon fixation, atmospheric $CO_2$ is converted to sugars by the enzyme Rubisco, which fractionates strongly against $^{13}C$. Plant carbon therefore has lower $\delta^{13}C$ values than atmospheric $CO_2$ and other inorganic carbon reservoirs in ocean waters, sediments and rocks. Along with Rubisco, other factors influence the size of the fractionation. Photosynthetic pathway is the largest of these. Plants that use the $C_3$ carbon fixation pathway (Calvin-Benson) have the largest net fractionation, whereas plants that use the $C_4$ carbon fixation pathway (Hatch-Slack) have the smallest net fractionation. Plants using Crassulacean acid metabolism (CAM) have intermediate fractionation, ranging from large to small during daytime and nocturnal $CO_2$ fixation modes, respectively (O'Leary, 1981). Other factors such as plant physiology, climate and ecology have considerable influence on net photosynthetic fractionation and are discussed below in the context of photosynthetic pathway.

#### 3.2.1. $C_3$ plants and $^{13}C$ photosynthetic fractionation

In the $C_3$ pathway, Rubisco (O'Leary, 1981), along with other factors (diffusion, mesophyll and stomatal conductance), results in a large net isotope effect (e.g. Farquhar et al., 1989). The fractionation is commonly expressed as discrimination ($\Delta_{leaf}$) where:

$$\Delta_{leaf} = \frac{\delta^{13}C_{atm} - \delta^{13}C_{leaf}}{1 + (\delta^{13}C_{leaf}/1000)} \quad (1)$$

Recent recommendations for the reporting of stable isotopic fractionation prefer the usage of $\alpha$ or $\varepsilon$ notation (Coplen, 2011). Importantly, $\Delta_{leaf}$ is related to $\varepsilon$ notation where:

$$\Delta_{leaf} = \varepsilon_{atm\text{-}leaf} = \left(\frac{\delta^{13}C_{atm} + 1000}{\delta^{13}C_{leaf} + 1000} - 1\right)10^3 \quad (2)$$

and is therefore an exact measure of $\alpha$. $\Delta_{leaf}$ should not be confused with the common geologic usage of $\Delta$ as the difference between $\delta_a$ and $\delta_b$, which is not exact and should not be used. Therefore, the usage of $\Delta_{leaf}$ is appropriate given the large body of ecological literature. For clarity, when reporting $\Delta_{leaf}$ values, the $\Delta_{leaf}$ equation should be provided.

The $\Delta_{leaf}$ of $C_3$ plants is often expressed using the reduced linear relationship of Farquhar et al. (1982) that incorporates fractionation associated with C fixation and the ratio of the $CO_2$ concentration (as partial pressures) in the internal stomatal cavity ($C_i$) to the atmosphere ($C_a$) as:

$$\Delta_{C_3\text{-}leaf} = a + (b - a)\frac{C_i}{C_a} \quad (3)$$

where $a$ is the fractionation associated with $CO_2$ diffusion in air through the stomatal opening (4.4‰; O'Leary, 1981) and $b$ the net fractionation associated with fixation. The term $b$ in Eq. 3 is not a constant, as is often assumed, as it reflects the balance between fractionation by Rubisco and PEP carboxylase (Farquhar and Richards, 1984). Values for $b$ therefore vary between plants and experiment procedures, but are often assumed to range between 27‰ and 30‰ (e.g. Farquhar et al., 1989; Lloyd and Farquhar, 1994). $C_i$ depends on the flux of $CO_2$ into the leaf and is regulated by stomatal conductance and the flux of $CO_2$ removed by C fixation

through assimilation (Farquhar et al., 1982). $C_i$ can be directly proportional to $\Delta_{leaf}$ when $C_a$ and $b$ are held constant, but see Seibt et al. (2008). $\Delta_{leaf}$ generally decreases with decreasing water availability as stomatal conductance is decreased and water use efficiency is increased (Farquhar et al., 1989), although changes in $C_a$ and mesophyll conductance may complicate the relationship (Ehleringer and Cerling, 1995; Warren and Adams, 2006; Seibt et al., 2008). $\Delta_{leaf}$ is sensitive to many factors in addition to water availability, including plant type, light intensity, nutrient status, canopy effects and $CO_2$ partial pressure (Farquhar et al., 1989; Diefendorf et al., 2010; Schubert and Jahren, 2012; Cernusak et al., 2013; Graham et al., 2014).

As mentioned above, $\Delta_{leaf}$ is strongly related to water availability for $C_3$ plants (e.g. Ehleringer et al., 1992; Stewart et al., 1995; Warren et al., 2001; Bowling et al., 2002; Prentice et al., 2011b). Diefendorf et al. (2010) identified mean annual precipitation (MAP) as the strongest single control on $\Delta_{leaf}$ in a global dataset ($R^2$ 0.55, $p < 0.0001$). In that study, they found that MAP was a stronger predictor of $\Delta_{leaf}$ than other climate and geographic indicators alone (temperature, humidity, soil available moisture, latitude, etc.). When the influence of altitude is included, the relationship increases to $R^2$ 0.61 ($p < 0.0001$). In a different study, Kohn (2010) found a similar relationship between $\Delta_{leaf}$ and MAP ($R^2$ 0.59), when also taking into account altitude and latitude (see also Freeman et al., 2011). Although these patterns emerge in large global datasets, it is important to note that large $\Delta_{leaf}$ ranges in $C_3$ plants are common, even at a single site. For example, several sites in the tropics have a range of ca. 8‰ among species, even when sampling is restricted to sun-exposed upper canopy leaves (note the range in $\Delta_{leaf}$ at ca. 3000 mm/yr in Fig. 6). Much of this variation among species can be explained by genetic variability or small scale changes in water availability (Ehleringer et al., 1991; Bonal et al., 2000). However, it is unclear how this range in values at a single site would influence plant carbon isotopes integrated in the sedimentary record.

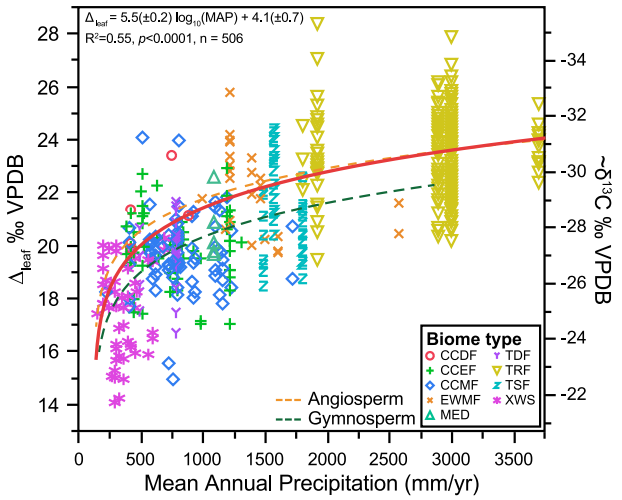


**Fig. 6.** A compilation of leaf discrimination ($\Delta_{leaf}$) values surveyed from the literature as a function of mean annual precipitation (MAP). $\Delta_{leaf}$ was averaged for individuals of a species (3310 total) at each site, resulting in a total of 570 species-site combinations, 334 species and 75 families at 105 geographic sites. MAP accounts for 55% ($p < 0.0001$) of the variability in $\Delta_{leaf}$ in a linear regression model (when MAP is log transformed to account for the log relationship of MAP to $\Delta_{leaf}$). Altitude is also an important predictor of $\Delta_{leaf}$ and increases $R^2$ to 0.61 ($p < 0.0001$) in a multiple linear regression model. The relationship between MAP and $\Delta_{leaf}$ is shown for angiosperms and gymnosperms and indicates higher average $\Delta_{leaf}$ in angiosperms. The right *y*-axis indicates the corresponding approximate $\delta^{13}C$ values. Points are coded by biome: tropical rain forest (TRF), evergreen warm mixed forest (EWMF), tropical seasonal forest (TSF), cool-cold deciduous forest (CCDF), cool-cold evergreen forest (CCEF), cool-cold mixed forest (CCMF), tropical deciduous forest (TDF), xeric woodland scrubland (XWS). Figure is redrawn from Diefendorf et al. (2010).

$\Delta_{leaf}$ varies among different plant types. For example, conifers have lower $\Delta_{leaf}$ values than angiosperms (Leavitt and Newberry, 1992; Brooks et al., 1997; Murray et al., 1998). The difference between angiosperm and conifer $\Delta_{leaf}$ grown at the same site (to remove environmental influences on $\Delta_{leaf}$) is ca. 2.7‰ (Diefendorf et al., 2010). Importantly, the difference is not fixed because the relationship between $\Delta_{leaf}$ and MAP is unique for angiosperms and conifers, likely reflecting the different growth strategies and water use efficiency of these two major taxonomic groups. At the ecosystem scale, $\Delta_{leaf}$ significantly varies among biomes (Fig. 6), reflecting the importance of climate and vegetation (Lloyd and Farquhar, 1994; Kaplan et al., 2002; Diefendorf et al., 2010).

### 3.2.2. Atmospheric $CO_2$ concentration and $C_3$ plants

The effect of $pCO_2$ on $\Delta_{leaf}$ has been of interest to isotope geochemists for some time given the relationship between fractionation and $C_a$ in Eq. 3. Unfortunately, the relationship between $pCO_2$ and $\Delta_{leaf}$ has remained enigmatic (e.g. Körner et al., 1991; Ehleringer and Cerling, 1995; Polley et al., 1995). This may have something to do with the timescales at which these measurements are made (Beerling and Royer, 2002; Franks and Beerling, 2009), or because water availability is often unconstrained (Körner, 2007). On very short timescales, plants optimize leaf gas exchange, thereby modifying $C_i/C_a$, to increase assimilation and water use efficiency (Lammertsma et al., 2011). However, on timescales of decades, plants respond to changing $pCO_2$ by adjusting optimal stomatal size and density and by regulating the rate of carbon fixation to maintain optimal set points, including internal to external $CO_2$ concentration ($C_i/C_a$) (Woodward, 1987; Ehleringer and Cerling, 1995; Woodward and Kelly, 1995; Beerling, 1996; Franks and Beerling, 2009; Franks et al., 2014), which results in a complex response of $C_i$ relative to $C_a$ (Seibt et al., 2008; Voelker et al., 2016). Indeed, recent growth chamber studies have identified a strong relationship between $pCO_2$ and $\Delta_{leaf}$ for plants grown over one life cycle (Schubert and Jahren, 2012). However, studies that have focused on long term changes (> 100 yr) in stomatal conductance and $\Delta_{leaf}$ have observed a small decrease in $\Delta_{leaf}$ as $pCO_2$ increases (Peñuelas and Azcónbieto, 1992; Bert et al., 1997; Duquesnay et al., 1998; Arneth et al., 2002; Saurer et al., 2004).

On much longer timescales, Diefendorf et al. (2015a) found that Paleogene $\Delta_{leaf}$ values are consistent with water availability and taxonomic group and do not appear to respond to change in $pCO_2$. If $C_i/C_a$ is largely maintained over long timescales (Franks and Beerling, 2009; Franks et al., 2014), despite a large change in $pCO_2$, then that would explain the similarity in fossil $\delta^{13}C$ values over long periods of Earth history (Deines, 1980; Peters-Kottig et al., 2006), despite large changes in $pCO_2$ (Berner and Kothavala, 2001). The stability of terrestrial plant carbon isotope values over geologic time thus may indicate that optimum $\Delta_{leaf}$ values are maintained on long geologic timescales, as suggested by Ehleringer and Cerling (1995).

### 3.2.3. $C_4$ plants and $^{13}C$ photosynthetic fractionation

Plants utilizing the $C_4$ photosynthetic pathway incorporate a $CO_2$ concentrating mechanism, along with biochemical and structural modifications, to ultimately fix C using the Calvin-Benson cycle. In this pathway, $CO_2$ is converted to aqueous $HCO_3^-$ and then fixed via carboxylation of phosphoenolpyruvate (PEP) by PEP-carboxylase (PEP-C) to produce the four carbon acid, oxaloacetate. Oxaloacetate is then reduced to either malate or aspartate and is then exported from the outer mesophyll cells to the inner bundle-sheath cells. In the bundle-sheath cells, the acids are decarboxylated, thereby releasing $CO_2$, which is then fixed in the Calvin-

Benson cycle. This process results in a high concentration of $CO_2$ that saturates Rubisco, so a greater proportion of C is fixed compared with $C_3$ photosynthesis (Hatch, 1987). Photorespiration is also reduced or eliminated in the $C_4$ pathway because PEP-C has a high affinity for $CO_2$ and not $O_2$, thereby excluding $O_2$ transfer to the bundle-sheath cells. Because of these biochemical and structural modifications, $C_4$ plants have a physiological advantage over $C_3$ plants at present day atmospheric $CO_2$ concentration, especially in hot, high-light, and dry environments (Sage et al., 1999). The rise and evolution of $C_4$ plants in the Miocene has been reviewed extensively (Tipple and Pagani, 2007; Edwards et al., 2010).

The fractionation that occurs during $C_4$ photosynthesis has been described by Farquhar (1983):

$$\Delta_{\text{C4-leaf}} = a + (b_4 + b\phi - a)\frac{C_i}{C_a} \quad (4)$$

and is related to the small fractionation associated with carboxylation by PEP-C (−5.7‰; $b_4$), Rubisco ($b$), the proportion of $CO_2$ that leaks out of the bundle-sheath cell ($\phi$), the diffusion of $CO_2$ in air ($a$), and the internal to atmospheric $CO_2$ concentration ($C_i/C_a$). Because only a small amount of $CO_2$ leaks out of the bundle-sheath cell, the isotope effect of Rubisco is not fully expressed, so total fractionation is small (Farquhar et al., 1989). $\Delta_{\text{leaf}}$ variation in $C_4$ plants is related to which enzymes are utilized in oxaloacetate conversion, which varies among species, and to variation in the leakiness of the bundle-sheath cells among different $C_4$ plant species (Hattersley, 1982; Farquhar, 1983). As a result, carbon isotopic fractionation in $C_4$ plants is very tightly controlled and results in a small range in $\delta^{13}C$ values as compared to $C_3$ plants (Fig. 7) (Tipple and Pagani, 2007).

### 3.2.4. CAM plants and $^{13}C$ photosynthetic fractionation

While CAM plants are most abundant in the subtropics and tropics, their range extends to temperate zones and high latitudes (Teeri et al., 1978; Lüttge, 2004). Their physiology is adapted to stress tolerance rather than ecosystem dominance (Lüttge, 2004), so CAM biomass productivity is low vs. $C_3$ or $C_4$ plants (Black, 1973). Therefore, $C_3$ and $C_4$ terrestrial plants would be the major contributors to sedimentary long chain $n$-alkyl lipid records for almost all ecosystems and biomes. The reader is referred to detailed information regarding $^{13}C$ fractionation in CAM and aquatic plants described by Farquhar et al. (1989).

### 3.3. $^{13}C$ fractionation during plant wax biosynthesis

The fractionation that occurs during lipid biosynthesis is related to isotope effects associated with numerous biochemical steps involved in the creation of complex lipids from basic organic precursor molecules. Biosynthetic fractionation is also controlled by the starting isotope composition of the carbon source, precursor availability and downstream reaction pathways (Hayes, 2001; Chikaraishi et al., 2004a,b). Fractionation during biosynthesis is commonly measured using $\varepsilon_{\text{lipid}}$ notation:

$$\varepsilon_{\text{lipid-leaf}} = \left(\frac{\delta^{13}C_{\text{lipid}} + 1000}{\delta^{13}C_{\text{leaf}} + 1000} - 1\right) \times 10^3 \quad (5)$$

where the carbon isotopic composition of the lipid of interest is compared with bulk leaf tissue (Chikaraishi et al., 2004b). Measuring biosynthetic fractionation as a function of the carbon isotopic compositions of lipid and leaf has the advantage that the complexities of fractionation during photosynthesis, which are controlled by the many factors outlined above, is treated separately. This has many advantages for paleo applications utilizing the carbon isotopic composition of lipids as a measure of plant carbon and/or $\Delta_{\text{leaf}}$.

For $n$-alkanes, the range of $\varepsilon_{n\text{-alkanes-leaf}}$ can be large (ca. 10‰) among species, even when grown under similar climate (Fig. 8, Tables 2 and E-1). On average, $\varepsilon_{n\text{-C29 alkane-leaf}}$ values for $C_3$ angiosperms are −5.2‰ for trees, −6.0‰ for graminoids, −7.3‰ for forbs and −7.4‰ for shrubs, whereas $C_4$ graminoids have much more negative values at −9.3‰. Although data are sparse, there is some suggestion that the $^{13}C$ fractionation is similar for CAM and $C_4$ plant $n$-alkanes and $n$-alkanoic acids (Collister et al., 1994b; Chikaraishi et al., 2004a).

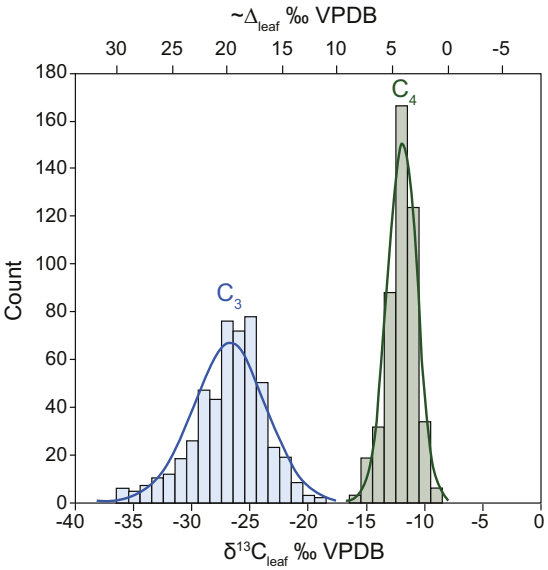


**Fig. 7.** Histogram of plant $\delta^{13}C$ values with $C_3$ and $C_4$ distributions indicated. For reference, approximate $\Delta_{\text{leaf}}$ values are shown (assuming $\delta^{13}C_{\text{atm}}$ −8‰). The larger $\delta^{13}C$ spread in $C_3$ plants is related to the strong environmental and biological factors that influence fractionation. The figure is redrawn from Tipple and Pagani (2007) and reports data from Cerling and Harris (1999).

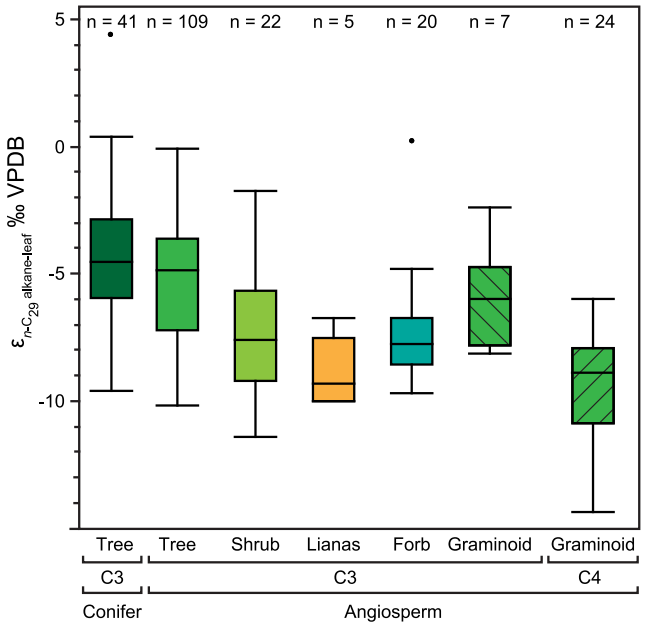


**Fig. 8.** Carbon isotope fractionation during $n$-alkane biosynthesis for $n$-$C_{29}$ ($\varepsilon_{n\text{-C29 alkane-leaf}}$) for different growth forms separated by major taxonomic group and photosynthetic pathway. The number of samples in each group is indicated. The data are a compilation from studies listed in Fig. 2. Additional information is found in Tables 2 and E-1.

**Table 2**
*n*-Alkane biosynthetic fractionation factor ($\varepsilon_{n\text{-alkanes-leaf}}$) for different plant functional types.

| Phylogeny[b] | Pathway | Form | Lat. zone[c] | $\varepsilon_{n\text{-alkanes-leaf}}$ ‰ VPDB[a] | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | $C_{27}$ | | | $C_{29}$ | | | $C_{31}$ | | | $C_{33}$ | | | $C_{35}$ | | |
| | | | | Mean | 1σ | *n* | Mean | 1σ | *n* | Mean | 1σ | *n* | Mean | 1σ | *n* | Mean | 1σ | *n* |
| A | $C_3$ | Forb | All | −6.9 | 2.6 | 11 | −7.3 | 2.1 | 20 | −8.0 | 2.0 | 20 | −8.2 | 1.9 | 16 | 1.1 | | 1 |
| | | | Temperate | −8.7 | | 1 | −7.2 | 0.7 | 2 | −6.5 | 1.4 | 2 | −6.9 | 0.2 | 2 | | | 0 |
| | | | Trop./sub. | −6.7 | 2.7 | 10 | −7.3 | 2.3 | 18 | −8.2 | 2.0 | 18 | −8.4 | 1.9 | 14 | 1.1 | | 1 |
| | | Graminoid | All | −4.8 | 2.5 | 6 | −6.0 | 2.0 | 7 | −6.3 | 1.1 | 7 | −6.3 | 1.2 | 7 | | | 0 |
| | | | Temperate | −5.7 | 0.7 | 3 | −6.6 | 0.9 | 4 | −5.8 | 0.2 | 4 | −6.1 | 1.1 | 4 | | | 0 |
| | | | Trop./sub. | −4.0 | 3.6 | 3 | −5.1 | 2.9 | 3 | −7.1 | 1.3 | 3 | −6.6 | 1.5 | 3 | | | 0 |
| | | Shrub | Trop./sub.[d] | −5.7 | 2.3 | 9 | −7.4 | 2.2 | 22 | −7.4 | 2.2 | 19 | −7.0 | 1.9 | 10 | −9.0 | | 1 |
| | | Tree | All | −4.3 | 2.1 | 93 | −5.2 | 2.4 | 109 | −5.6 | 2.5 | 104 | −5.4 | 2.7 | 71 | −3.4 | 2.7 | 15 |
| | | | Temperate | −4.1 | 1.6 | 32 | −4.6 | 2.2 | 35 | −5.1 | 2.2 | 31 | −5.8 | 1.8 | 14 | −6.1 | 4.0 | 2 |
| | | | Trop./sub. | −4.4 | 2.4 | 61 | −5.5 | 2.5 | 74 | −5.8 | 2.7 | 73 | −5.3 | 2.9 | 57 | −2.9 | 2.3 | 13 |
| | $C_4$ | Graminoid | All | −8.4 | 1.6 | 17 | −9.3 | 2.1 | 24 | −9.0 | 1.9 | 25 | −8.3 | 1.5 | 20 | −8.9 | 1.5 | 6 |
| | | | Temperate | | | | −10.5 | 2.3 | 2 | −9.4 | 2.6 | 2 | −7.7 | 0.2 | 2 | −8.3 | 0.8 | 2 |
| | | | Trop./sub. | −8.4 | 1.6 | 17 | −9.2 | 2.2 | 22 | −9.0 | 1.9 | 23 | −8.3 | 1.6 | 18 | −9.2 | 1.8 | 4 |
| | | Forb | Trop./sub.[d] | −11.1 | 0.2 | 2 | −11.1 | 0.7 | 2 | −10.3 | 1.2 | 3 | −11.0 | 2.7 | 2 | | | 0 |
| G | $C_3$ | Tree | All | −4.2 | 2.7 | 32 | −4.2 | 2.7 | 41 | −4.5 | 2.5 | 44 | −3.7 | 2.4 | 43 | −3.0 | 2.8 | 29 |
| | | | Temperate | −3.7 | 2.8 | 26 | −3.8 | 3.0 | 31 | −4.3 | 2.6 | 34 | −3.4 | 2.4 | 33 | −2.5 | 2.8 | 19 |
| | | | Trop./sub. | −6.1 | 0.6 | 6 | −5.3 | 1.4 | 10 | −5.1 | 1.9 | 10 | −4.5 | 2.3 | 10 | −3.9 | 2.8 | 10 |

[a] Data sources are listed in Fig. 1 caption and reported in Table E-1.
[b] Angiosperms (A), gymnosperms (G); the gymnosperms are all conifers, except for 2 *Ginkgo biloba* samples.
[c] Temperate (temp.), tropical/subtropical (trop./sub.).
[d] All lianas, shrubs, and forbs are tropical and/or subtropical.

Recent studies have suggested that these values vary among climates, with greater fractionation in tropical and subtropical plants than temperate plants (Magill et al., 2013; Freeman and Pancost, 2014). Indeed, this is true when evaluating among all $C_3$ plants. For example, $C_3$ angiosperms have greater *n*-$C_{29}$ alkane fractionation in tropical and subtropical latitudes (−6.2‰) than in temperate latitudes (−4.9‰; *t*-test, $p = 0.002$). However, when comparing individual growth forms among latitudinal zones, these differences diminish. For example, for the largest group of growth forms, temperate angiosperm $C_3$ trees, values are −5.5‰ in tropical and subtropical latitudes and −4.6‰ in temperate latitudes. The means between these zones are different (*t*-test, $p = 0.05$). The comparisons between zones appear to be related to the presence or absence of specific growth forms. For example, shrubs, lianas and forbs are nearly all from tropical and subtropical latitudes and have on average more negative $\varepsilon_{n\text{-C29 alkane-leaf}}$ values than trees.

Within temperate zones, conifer trees have smaller $\varepsilon_{n\text{-C29 alkane-leaf}}$ values (−2.5‰) than angiosperm trees (−4.5‰) growing at the same site (Diefendorf et al., 2011). However, that study reported on conifer species that were largely biased to North America. Among a larger set of plants, conifers appear to have greater fractionation (−4.2‰) and are more similar to angiosperm trees (−5.2‰; Table 2, Fig. 8), although mean values are different (*t*-test, $p = 0.04$). In a more recent study, Diefendorf et al. (2015b) analyzed a large set of conifer species, including many subtropical and Southern Hemisphere conifers, all growing at the same site in California. In that study, the authors measured a wide range in $\varepsilon_{n\text{-alkanes-leaf}}$ values among conifer families (Fig. 9) with the smallest fractionation in Pinaceae (pines), medium fractionation in Cupressaceae (junipers and relatives) and large fractionation in Taxaceae (yews). An analysis of $\varepsilon_{n\text{-alkanes-leaf}}$ values indicates that there is a significant phylogenetic signal in the data. Therefore, it seems likely that carbon metabolism or subtle biochemical variation among conifer clades are responsible, at least in part, for this pattern (Diefendorf et al., 2015b).

Freeman and Pancost (2014) suggest that differences in carbon storage and allocation could explain differences among plant types, growth strategy and climate regions. Indeed, many factors are likely responsible for the wide variation in observed $\varepsilon_{n\text{-alkanes-leaf}}$. Disentangling species from climate effects on $\varepsilon_{n\text{-alkanes-leaf}}$ will be challenging, given the wide differences in plant functional type among biomes and latitudinal zones. Evaluating climatic influences on $\varepsilon_{n\text{-alkanes-leaf}}$ must be done with careful consideration for variations in plant functional type and may also require phyloge-

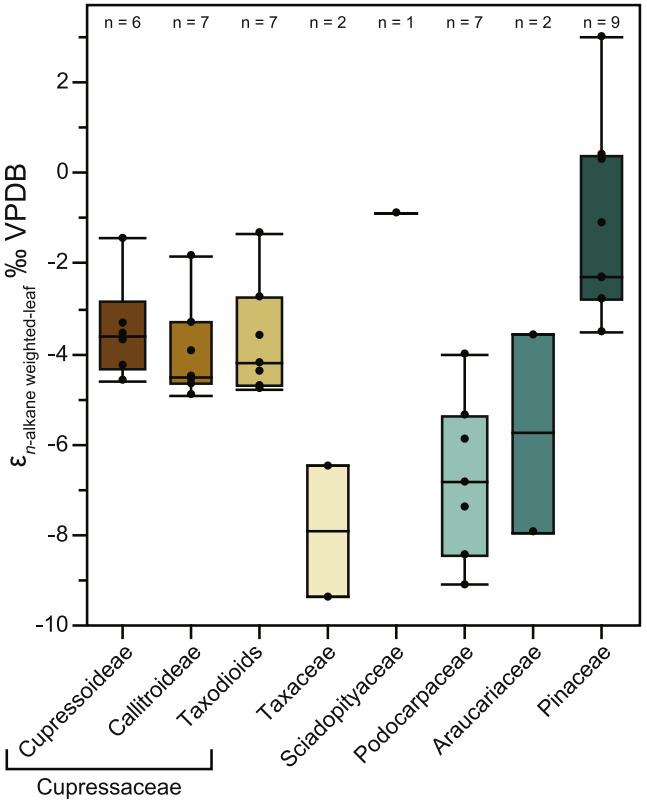


**Fig. 9.** Carbon isotope fractionation during *n*-alkane biosynthesis for conifers. Values are weighted by *n*-alkane concentration ($\varepsilon_{n\text{-alkane weighted-leaf}}$). There is a strong phylogenetic signal in conifer $\varepsilon_{n\text{-alkane weighted-leaf}}$. Box and whisker plots show the median, upper and lower quartiles, and maximum and minimum values. Each black dot represents a different species. Box color and order matches those of Fig. 3. Figure is redrawn from Diefendorf et al. (2015b).

netic control (Diefendorf et al., 2015b). This is especially important for trying to determine if coordinated variations in $\Delta_{leaf}$ and $\varepsilon_{n\text{-alkanes-leaf}}$ exist.

The biosynthetic fractionation factors for *n*-alkanoic acids have been measured for only a few species (Chikaraishi et al., 2004a; Chikaraishi and Naraoka, 2006; Dungait et al., 2008) and future studies are required to fill in this gap.

### 3.4. Waxes from plants to sediments to rocks

While recent advances have improved understanding of environmental and biological factors that control the production of plant wax $\delta^{13}C$ values, relatively little is known about the processes that ultimately determine the integration and transport of plant waxes and their isotopic composition to sedimentary archives. Freeman and Pancost (2014) caution about the simplifying assumption of equal integration of all plant sources within catchments, stressing the importance of understanding the factors and processes that control terrestrial organic matter delivery to sediments. In this section, the review focuses on the three dominant modes of plant wax transport: waxes attached to leaves, aeolian waxes, and erosion and deposition of soil-derived waxes. The relative contribution of plant waxes carried by each of these pathways will vary with the geologic setting and type of sedimentary record (i.e. terrestrial, lacustrine, or marine). Ultimately, the variability in climate and in vegetation composition underlies both spatial and temporal patterns in the $\delta^{13}C$ values of terrestrial plant lipids both today and in the past, and understanding how these signals are transferred to and integrated in sediments is critical.

#### 3.4.1. Integration and transport of leaves and plant waxes to sediments

Leaves are integrated in ecosystems and deposited in sediments (Burnham, 1989, 1994; Greenwood, 1991; Ellis and Johnson, 2013). However, due to taphonomic processes, the species composition in a forest is not necessarily reflected in the forest litter and sediments. Leaves are transferred from biomass to sediments when they fall to the forest floor, creating autochthonous assemblages (i.e. swamps) or are transported by wind and water creating allochthonous assemblages (i.e. lakes, ponds, stream deposits; Greenwood, 1991). The composition of leaf litter in allochthonous assemblages tends to be swamped by leaves from the adjacent canopy (Burnham, 1989). In temperate forests, the leaf litter tends to be dominated by the most abundant tree species, but not necessarily in tropical forests (Brasell et al., 1980; Greenwood, 1991; Burnham et al., 1992; Ellis and Johnson, 2013). Trees with the largest basal area also tend to have greater representation in sediments than trees with smaller basal areas, despite having a greater number of stems within the forest stand (Ellis and Johnson, 2013). Within that context, leaf litter is likely biased to the top of the canopy due to higher biomass, production rate, higher photosynthetic rate and shorter leaf lifespan (Parker et al., 1989; Reich et al., 1991; Wright and Cannon, 2001), which is supported by fossil assemblages (e.g. Graham et al., 2014). When leaves are transported by wind, smaller and lighter leaves tend to travel further than larger and heavier leaves (Spicer, 1981). Importantly, leaves entering lakes tend to be dominated by woody vegetation surrounding the lake (McQueen, 1969; Drake and Burrows, 1980; Spicer and Wolfe, 1987; Greenwood, 1991). If leaf wax transport to sediments were primarily on the leaf surface, then these taphonomic processes would provide a filter on which species' leaf waxes enter the sedimentary record.

Surveys of lake sediments and vegetation suggest that leaf wax input from angiosperm trees may dominate the lacustrine sedimentary record, at least for *n*-alkanes (Cranwell et al., 1987; Sachse et al., 2006, 2012; Diefendorf et al., 2011; Tipple and Pagani, 2013). There is also an indication that leaf waxes from grasses are under-represented in the lacustrine sedimentary record due to differences in leaf wax production and transport between grasses and trees, which has important implications for reconstructing changes in $C_3$ and $C_4$ vegetation based on leaf wax $\delta^{13}C$ (Seki et al., 2010; Garcin et al., 2014; Schwab et al., 2015), but is highly dependent on leaf wax chain length. In contrast, plant wax $\delta^{13}C$ values in marine sediments are sensitive to the relative abundance of both $C_3$ tree/shrub and $C_4$ grass/sedge vegetation (Collins et al., 2011; Vogts et al., 2012), perhaps because marine records integrate waxes over regional to continental scales via wind-blown dust and riverine networks.

Thus, some consideration of vegetation, through pollen, macrofossil, or phytolith analysis can further inform interpretation of sediment plant wax $\delta^{13}C$ values. However, variable production, sourcing and dispersal can also bias records of each of these vegetation proxies (Prentice, 1985). Taphonomic effects, combined with plant wax production and carbon isotope differences among growth forms (grasses, trees, shrubs), major taxonomic groups (angiosperms and gymnosperms) and species can complicate the interpretation of sedimentary plant wax $\delta^{13}C$ values, especially during transitions in vegetation and climate or when comparing depositional environments.

#### 3.4.2. Aeolian sources of plant waxes

Plant waxes can either be ablated from the leaf surface by wind and other abiotic stressors, including rain, heat and UV radiation (Baker and Hunt, 1986; Simoneit et al., 1988; Shepherd and Wynne Griffiths, 2006) or adsorbed to smoke particles from biomass burning (Standley and Simoneit, 1987; Simoneit, 2002). These particulate waxes can be transported great distances by winds as aerosols or on dust surfaces (Simoneit, 1977; Simoneit et al., 1977; Duce and Gagosian, 1982; Simoneit and Mazurek, 1982; Bird et al., 1995; Huang et al., 2000). Plant wax aerosols can be deposited either via dry deposition, when particles fall out of the air, or by wet deposition, when aerosols and dusts are scrubbed from the air during precipitation events, the latter being quantitatively more important (Eichmann et al., 1979, 1980). In marine sediments, aeolian plant waxes can be the dominant source of sedimentary plant waxes, especially when riverine input is limited or nonexistent (Schefuß et al., 2003, 2004). In North America, many studies have identified African plant wax aerosols, documenting the great distances plant waxes travel (e.g. Huang et al., 2000). From spring to fall, the majority of aerosol plant waxes are sourced from local to regional scales and the composition reflects atmospheric mixing and advection of storm systems (Conte and Weber, 2002; Conte et al., 2003). During winter months in North America, plant wax aerosols are much lower in concentration and will reflect long distance transport (Meyers and Hites, 1982).

Meyers and Hites (1982) measured long chain *n*-alkanes and *n*-alkanoic acids in rain and snow to determine the importance of aeolian plant waxes as contributors to sedimentary plant waxes in Lake Michigan. Importantly, they estimated that the maximum contribution of aerosol *n*-alkanes from rain and snow to sedimentary *n*-alkanes was 15%. They also found that the flux of *n*-alkanoic acids was much lower than *n*-alkanes by a factor of ca.10. They argue that aeolian sources of plant waxes are important in lakes with low productivity and low terrigenous input, but likely have a minimal influence on lakes with high productivity and high sedimentation. Therefore, at sites with a high terrestrial input, the locally derived plant waxes would likely swamp the aeolian-sourced plant waxes.

#### 3.4.3. Plant wax reworking and temporal resolution

Riverine transport of reworked terrestrial plant waxes is another major pathway for delivery of waxes and other terrestrial

biomarkers to marine and lake sediments. Rivers transport ca. 0.4 Gt/yr of dissolved and particulate organic carbon (OC) to the marine realm (Hedges and Oades, 1997) with a preservation efficiency in sediments of ca. 20–40% (Blair and Aller, 2012). Of this total riverine OC flux, the particulate flux is comprised of ca. 0.16 Gt/yr biogenic and 0.04 Gt/yr petrogenic carbon (Galy et al., 2015). Total riverine transported OC represents a mixture of sources, but is generally dominated by an input from soil (Hedges and Oades, 1997). Aeolian transport of OC is estimated to be < 0.1 Gt/yr and may be a more significant source of terrestrial OC in the open oceans (Hedges and Oades, 1997).

The source attribution, transport and composition of fluvially transported plant waxes may be influenced by multiple factors, including catchment vegetation cover, seasonality, degradation, and particle sorting and trapping in river sediments (Pancost and Boot, 2004). In tropical and subtropical settings, the stable isotopic composition of plant waxes in-transit in soils and riverine particulate organic matter (POM) reflect δD gradients in precipitation and elevation (Galy and Eglinton, 2011; Ponton et al., 2014). The δD composition of $n$-$C_{28}$ alkanoic acid in riverine POM reflects the average upstream catchment area at each successive point along an elevation gradient in a tributary network to the Amazon (Ponton et al., 2014). In contrast, in central Madagascar, the $\delta^{13}C$ composition of riverine OC is biased toward riparian $C_3$ plants despite the catchment being dominated by $C_4$ grasslands (Marwick et al., 2014). The isotopic composition of riverine OC and terrestrial plant biomarkers can also change between wet and dry seasons, reflecting different fluvial sourcing or OC mobilization mechanisms during wet and dry flow regimes (Marwick et al., 2014; Ponton et al., 2014). Further, the concentration and relative abundance of terrestrial plant $n$-alcohols and $n$-alkanoic acids in fluvial suspended sediments fluctuate with seasonal change in discharge from the Congo River, while those of the $n$-alkanes are more stable (Hemingway et al., 2016). These studies highlight the importance of accounting for variation in the seasonal or storm-driven fluxes and resulting composition of terrestrial plant biomarkers sourced by fluvial networks, especially in sedimentary records that span periods of hydrologic change.

OC transported by rivers generally comprises one or more carbon pools that range in age from modern (fresh biomass production), to pre-aged (through some residence time in soil) and ancient (eroded from sedimentary rocks; Hedges and Oades, 1997; Blair et al., 2003). Each of these pools may also have distinct $\delta^{13}C$ values, thereby influencing both the age and the carbon isotopic record of sedimentary bulk OC (Blair et al., 2003; Bataille et al., 2013). The relative contribution of modern, pre-aged and ancient OC delivered to marine sediments can be controlled not only by ecosystem productivity and source reservoir concentration, but also by the geology and morphology of different drainage basins. Based on the $\delta^{13}C$ and $^{14}C$ composition of OC in a fluvial/marine system on the coast of California, Blair et al. (2003) suggested that tectonically active basins have shorter residence times and deliver greater portions (up to 50%) of ancient carbon to marine sediments than at passive margins. This was supported by a global compilation of $^{14}C$ ages of riverine particulate OC that found that low-relief passive margins and corresponding rivers with smaller suspended load (i.e. less efficient weathering) delivered a greater portion of modern OC (Blair and Aller, 2012).

While basin geology and morphology may be important controls on the age and $\delta^{13}C$ composition of riverine transported OC, the same controls may not pertain for terrestrial plant biomarkers because lipids make up < 1% of TOC (de Leeuw et al., 1995; Blair and Aller, 2012). Therefore, it is likely that there are discrepancies between the age/source of bulk OC and specific biomarkers. With the advent of compound-specific radiocarbon analysis (CSRA; Eglinton et al., 1996) it became possible to directly measure the $^{14}C$ age of biomarkers, including terrestrial plant waxes, in source material, during riverine transport and in associated sediments. Mixing models of the $\delta^{13}C$ and $\Delta^{14}C$ composition of waxes can be used to determine plant wax residence times and fractional contributions of modern (biomass), pre-aged (soil), and ancient (petrogenic) wax sources. Most studies have applied CSRA to fluvial/marine systems, although several recent studies have investigated the source and age offsets of terrestrial plant waxes in lake sediments and in soils (see below).

For marine sediments, the continental residence time of terrestrial (i.e. long chain, > $n$-$C_{25}$) plant waxes (including $n$-alkanes and $n$-alkanoic acids) ranges from < 100 yr (Pearson and Eglinton, 2000; Pearson et al., 2001) to more than ca. 10,000 yr (Eglinton et al., 1997; Drenzek et al., 2007). This wide range of residence times is likely controlled by complex factors, but it is apparent that they vary among sites as a function of climate, geology and morphology. Plant wax transport pathways and the composition of source reservoirs within a catchment can affect sedimentary wax age. For instance, long chain $n$-alkanes in sediments from the Black Sea, where OC is largely transported by rivers, had $^{14}C$ age values of 500–1130 yr BP, whereas sediments from the Arabian Sea, which has a significant aeolian input, had an $n$-$C_{29}$ alkane age of 7200 yr BP, possibly due to wind erosion of pre-aged waxes from a source reservoir of desiccated lake sediment (Eglinton et al., 1997). In a study of Congo River basin sediments, a hydrologic control on the reworking of leaf waxes was identified (Schefuß et al., 2016). The authors used $^{14}C$ ages of $n$-alkanes, in combination with methanogen biomarkers as evidence for wetlands, to identify changes in the storage of plant waxes within the Congo Basin. Schefuß et al. (2016) highlight that, even in flat tropical basins, plant waxes can be stored for thousands of years before being remobilized, due to hydrological change, into marine depositional environments.

The shortest terrestrial $n$-alkane residence time (75 yr) was observed in the Santa Monica Basin, where an estimated > 80% of sedimentary waxes were derived from modern plants, with remaining fractions derived from shales and petroleum (Pearson and Eglinton, 2000). This is consistent with the estimated average residence time of carbon in the upper 1 m of soil humus (80 yr; Hedges and Oades, 1997). A later study of sediments in the same region determined significantly longer terrestrial residence times for $n$-$C_{28}$ alkanoic acid, from 1200 yr in the Santa Barbara Basin to 750–1090 yr in the Santa Monica Basin (Mollenhauer and Eglinton, 2007). In contrast, Kusch et al. (2010) observed younger $n$-alkanoic acid age relative to $n$-alkanes in Black Sea sediments, either due to greater recalcitrance of $n$-alkanes or their potential 'contamination' with ancient petrogenic sources. Variable $^{14}C$ ages among terrestrial biomarkers and among $n$-alkanes and $n$-alkanoic acids in the same sediments (Eglinton et al., 1997; Smittenberg et al., 2004; Feng et al., 2013) are a further indication that the sourcing, transport and preservation of biomarker records is variable among depositional settings and perhaps among lipid compound classes.

The longest terrestrial plant wax residence times have been observed at high latitudes, ranging from 4000–5500 yr for soil-derived sedimentary $n$-alkanes from the Saanich Inlet of the Pacific Northwest (Smittenberg et al., 2004) up to > ca. 10,000 yr for Arctic marine sediments (Drenzek et al., 2007). Protracted storage of total OC (TOC) in permafrost results in TOC in Arctic surface sediments with ages of 7000–16,750 yr BP (Goñi et al., 2005). Likewise, sedimentary plant wax ages correlated positively with percent cover of discontinuous permafrost in a transect of Eurasian Arctic rivers, likely because discontinuous permafrost facilitates mobilization of deep, pre-aged waxes in permafrost (Feng et al., 2013). Additionally, evidence from sub-Arctic Scandinavia suggests that there may be selective preservation of pre-aged, mineral-bound lipid pools

derived from permafrost relative to younger, more degradable lipids from upper soil layers and peat (Vonk et al., 2010). Therefore, climate can influence the source reservoir composition, extent of pre-aging and resulting ages of plant waxes and other terrestrial biomarkers in marine sediments.

In the Eel River basin in coastal northern California, insights from CSRA suggest that, even in this tectonically active margin where bulk TOC export is thought to be rapid and dominated (ca. 50%) by shale-derived ancient carbon (Blair et al., 2003), *n*-alkanoic acids have residence times of several thousand years. Therefore, pre-aged lipids may contribute to sedimentary carbon ages to a greater extent than previously estimated (Drenzek et al., 2009). Similarly, (Galy and Eglinton, 2011) found evidence that different pools of OC have significantly different residence times in the Ganges-Bramaputra river system. While the age of *n*-alkanoic acids ranged from 50–1300 yr, bulk OC was > 15,000 yr on average, suggesting that up to 20% of the OC transported by the river system is made up of a much older, more refractory pool of carbon.

Several studies have applied CSRA to examine the residence times and transport mechanisms of terrestrial plant waxes within lake catchments. The observed age offsets of plant waxes in lake sediments are generally smaller than for marine sediments; however the former varies among depositional settings and may be sensitive to the extent of soil erosion and transport to a basin. For instance, in a karstic sinkhole pond in Hawaii, the $^{14}C$ ages of $n$-$C_{27}$–$n$-$C_{33}$ alkanes in sediments were not significantly offset from the ages of macrofossils (Uchikawa et al., 2008). It is important to note that this is a relatively small (0.005 $km^2$) and hydrologically closed basin fed by groundwater and precipitation. Further, the catchment has poor soil development and is in a low gradient coastal plain. Thus, the sedimentary waxes are likely transported as aerosols or directly as leaves or leaf litter, which would result in minimal terrestrial residence time prior to deposition.

In another lake with no surface water input in Switzerland, terrestrially derived $n$-$C_{27}$–$n$-$C_{31}$ alkanes ranged from 120 to 1000 (mean 396) $^{14}C$ yr older than sediments deposited prior to 3100 cal yr BP (Gierga et al., 2016). However, in younger (400 cal yr BP) sediments, the age offset of waxes relative to sediments increased to 3570 and 7390 $^{14}C$ yr BP. An increased contribution of pre-aged plant waxes in recent sediments may reflect increased erosion of soils in the drainage basin following intensified forest clearing and agricultural development in the area. Thus, erosion and surface runoff may be important factors in mobilizing pre-aged waxes that would otherwise be retained on the landscape. In arid settings, aeolian transport of reworked dunes and dust may be an important source of significantly pre-aged *n*-alkanes in hypersaline lakes (Bray et al., 2014).

In tropical settings, long chain $n$-$C_{26}$–$n$-$C_{32}$ alkanoic acids in core top sediments from four karstic Mexican and Central American lakes had $^{14}C$ ages from 20–520 yr BP (Douglas et al., 2014). The oldest waxes were found in larger lake catchments and at sites with lower mean annual precipitation. Soils within the largest catchment contained *n*-alkanoic acids that had increasing $^{14}C$ ages with depth, suggesting that sedimentary lipids are sourced from older soils. In this lake, *n*-alkanoic acids showed an increasing age offset (up to 1200 yr) relative to sediments at greater depth.

These studies suggest that the residence time of terrestrial plant waxes in lake sediments may be linked to catchment and lake size, soil development, landscape erosion/stabilization, surface water input, hydrologic controls, and aridity. Further work is needed to test these and other basin-specific controls on the residence times of terrestrial plant biomarkers in lacustrine sediments in order to extract more accurate climate information from plant waxes in terrestrial records. Testing biomarker-specific age-depth offsets and lag times may be especially important when using plant wax isotopes as climate/vegetation proxies as they may have lower resolution than other proxies (e.g. authigenic carbonate).

In contrast to marine and lake systems, CSRA analysis of *n*-alkanes and *n*-alkanoic acids from a Quaternary loess-paleosol sequence found good age agreement between plant waxes and corresponding stratigraphic horizons (Häggi et al., 2014), suggesting negligible age offsets for plant waxes in paleosols. This information is helpful for paleoenvironmental reconstruction for the Miocene through the late Quaternary (Freeman and Colarusso, 2001; Zech et al., 2012; Magill et al., 2013; Uno et al., 2016a,b). However, it is important to be aware of possible OC reworking when paleosols are formed on alluvial deposits (Baczynski et al., 2013, 2016).

#### 3.4.4. Plant wax alteration and diagenetic fate

In lacustrine and marine water columns, *n*-alkanes, *n*-alkanoic acids and *n*-alkanols are refractory and are preferentially concentrated relative to other lipids (e.g. Meyers and Ishiwatari, 1993; Wakeham et al., 1997; Hoefs et al., 2002; Sinninghe Damsté et al., 2002; Prahl et al., 2003). After plant waxes are buried in lacustrine and marine sediments, alteration occurs rapidly. For example, studies of recent lake sediments suggest that alteration occurs in less than a few hundred years (Meyers et al., 1980; Cranwell, 1981). Once these compounds make it through the most active zone of degradation within the sediment column, they are relatively resistant to microbial degradation (e.g. Meyers and Ishiwatari, 1993; Wakeham et al., 1997). Of the *n*-alkyl lipids, *n*-alkanes are more resistant to degradation than the *n*-alkanoic acids and *n*-alkanols (Cranwell, 1981). It is important to note that almost all field-based studies must assume that the source of plant waxes remains constant through time. This poses a challenge for identifying the effects of alteration and diagenesis as source variation may also be important in explaining downcore plant wax variation. Thus, alteration may not be the only factor causing changes in *n*-alkyl lipids (Powell and McKirdy, 1973). Nevertheless, microbial oxidation of *n*-alkanes is evident from the presence of *n*-alkan-2-ones in lacustrine sediments. In two different studies, chain length distributions of *n*-alkan-2-ones matched those of co-occurring *n*-alkanes, indicating that microbial oxidation had occurred (Cranwell et al., 1987; Rieley et al., 1991). In addition to oxidation, it is likely that some plant waxes are incorporated into a bound fraction (Cranwell, 1981). Overall, the diagenetic alteration and preservation of plant waxes is determined largely by sediment composition, oxygen exposure (Hartnett et al., 1998) and burial (Meyers and Ishiwatari, 1993), as is also true for other biomarkers and bulk OC (Powell and McKirdy, 1973; Hedges and Prahl, 1993; Hedges and Oades, 1997; Wakeham et al., 1997). In marine settings, terrestrial biomarkers, such as *n*-alkanes, will be preferentially preserved relative to marine biomarkers, such as alkenones (e.g. Hoefs et al., 2002; Sinninghe Damsté et al., 2002; Prahl et al., 2003).

For the plant waxes preserved following deposition, burial and early diagenesis, additional changes occur as sediments are heated. During heating, sedimentary plant waxes undergo changes in composition and concentration. For immature sediments, *n*-alkanoic acids and *n*-alkanols are polymerized to insoluble macromolecules through diagenetic alteration. Evidence for this polymerization and formation of kerogen comes from extensive use of confined and hydrous pyrolysis experiments using fresh leaf or arthropod tissue (Stankiewicz et al., 2000; Gupta et al., 2007a; Gupta, 2014; Diefendorf et al., 2015c) and from observations made on fossils (e.g., Mösle et al., 1997, 1998). For immature sediments and rocks, *n*-alkanes appear to be rather immobile. This was evidenced by molecular studies of fossil leaf cuticle and co-occurring sediment matrix from the Miocene Clarkia Formation (Huang et al., 1995; Logan et al., 1995). In these studies, *n*-alkanes in the cuticle were distinct from the sediment matrix and it was concluded that *n*-

alkanes were not migrating into the sediment matrix. With increasing maturity (oil generation), *n*-alkanes, including both short and long chain, are produced. They are likely generated from polymerized macromolecules (such as *n*-alkanoic acids, *n*-alkanols, cutan and cutin) and kerogens preserved during fossilization of leaves (Collister et al., 1994a; Eglinton, 1994; Finch and Freeman, 2001; Gupta et al., 2006, 2007a,b; Gupta, 2014). As maturity progressively increases, the odd/even preference is lost and the average chain length decreases (e.g., Bray and Evans, 1961; Brooks and Smith, 1967). Because the diagenetic alteration of *n*-alkyl lipids results in compositional changes, it becomes increasingly challenging to extract information from chain length distributions with increasing maturity and the possibility of *n*-alkyl lipids generated from non-plant wax sources increases (e.g. Tegelaar et al., 1989).

Despite significant loss of plant waxes following burial and subsequent diagenetic alteration, their carbon isotopic composition remains unaltered. For example, in a 23 yr litter bag field experiment with *Calluna vulgaris* (heather), Huang et al. (1997) found that long chain *n*-alkane $\delta^{13}C$ values were unchanged despite a 90% loss in *n*-alkanes. Other studies have suggested that *n*-alkanes are altered (e.g. Nguyen Tu et al., 2004a, 2011; Chikaraishi and Naraoka, 2006), but contributions of *n*-alkanes from other sources often cannot be ruled out or the starting composition is not well constrained. These conflicting results highlight the challenges of field-based studies due to possible reworking and multiple sources of *n*-alkanes within soils (e.g. Lichtfouse, 2011). Upon heating and increasing maturity, *n*-alkane $\delta^{13}C$ values appear to not be altered up to the pre-oil generation window. This was determined with hydrous pyrolysis experiments using fresh leaves from *Acer rubrum* (red maple), *Platanus occidentalis* (American sycamore), *Pinus sylvestris* (Scots pine) and *Taxodium distichum* (bald cypress) at various temperatures (Diefendorf et al., 2015c). In that study, changes in long chain *n*-alkane $\delta^{13}C$ values were not observed until hydrous pyrolysis temperature increased to 250 °C and above. Above that temperature, the magnitude and direction of the $\delta^{13}C$ change was variable among species and chain lengths, but generally was $\leqslant$2‰. The differences among species are curious and suggest that other *n*-alkyl lipids are converted to *n*-alkanes, thereby influencing their isotopic composition. Overall, the small changes in $\delta^{13}C$ values are consistent with other studies that have identified progressive alteration of *n*-alkane $\delta^{13}C$ values with increasing maturity, with the greatest changes occurring in the mid to upper oil window (Bjorøy et al., 1992; Clayton and Bjorøy, 1994). For oils, there appears to be no evidence for isotopic fractionation for long chain *n*-alkanes ($\geqslant C_{19}$) during biodegradation, even for oils with extreme biodegradation (Sun et al., 2005). Thus, for geologic studies focused on sediments and rocks with immature to early oil window maturity, long chain *n*-alkane $\delta^{13}C$ values should be unaltered or only slightly altered (ca. 1‰).

## 4. Paleorecord applications

Interpreting sedimentary carbon isotope values of bulk OM and plant waxes as signals of paleoclimate, paleovegetation, or carbon cycle changes is complicated by competing influences of climate, vegetation and atmospheric $\delta^{13}C$. The interpretation also depends, at least in part, on the depositional environment in which plant waxes are preserved. In records from lakes, ponds, swamps and soils, plant waxes are likely sourced from a local signal. However, the spatial integration of plant waxes reflected in any environment will depend on how waxes are transported from plant to sediment (Section 3.4.1) and if waxes are inherited from distal aeolian sources (Section 3.4.2). In records from marine sediments, plant waxes will likely be integrated from a large spatial extent and represent multiple biomes and climates. Thus comparing $\delta^{13}C$ values through time, across space and between terrestrial and marine sediments is difficult without additional information. By utilizing the large body of information about the factors that influence plant carbon isotopes, and by combining with independent proxies, it is now possible to reduce uncertainty and improve the interpretations made from plant carbon isotopes preserved in the sedimentary record. This section has been divided into three parts, each focusing on the most common interpretations made from plant $\delta^{13}C$ values.

### 4.1. Plant carbon isotopes as water indicators

Interpreting changes in $C_3$ plant $\delta^{13}C$ values as water availability or as precipitation indicators has become common in the literature. As discussed above, water availability has a strong control on $C_3$ plants and its influence on their stomatal conductance, which in turn influences $C_i$, and thus plant fractionation during photosynthesis. However, plant type, vegetation, photosynthetic pathway and many other factors also influence $C_i$ and therefore modify $\Delta_{leaf}$. This has important consequences for interpreting plant $\delta^{13}C$ values. For plant $\delta^{13}C$ values to be useful as a proxy for water availability, competing factors must also be accounted for when interpreting sedimentary $\delta^{13}C$ values. For times and regions where the researcher is confident that $C_4$ plants are absent, extracting a water signal from plant $\delta^{13}C$ values can be done if vegetation and $\delta^{13}C_{atm}$ are constrained. Paleovegetation can be identified using pollen and macrofossils, ideally from published studies, or possibly estimated from global biome models (e.g. Kaplan et al., 2003; Prentice et al., 2011a). For studies that utilize plant waxes, additional precautions can be made by knowing which conifer families are present in a geographic region of interest and utilizing *n*-alkane chain lengths that are most likely angiosperm or conifer in origin. In lithologies with good OM preservation, plant terpenoids can be utilized as taxon specific biomarkers and can be compared with *n*-alkane $\delta^{13}C$ values (e.g. Schouten et al., 2007).

When interpreting plant $\delta^{13}C$ values through time intervals when plant communities remain constant, plant $\delta^{13}C$ values are likely to represent water availability, at least qualitatively. Kohn (2010) provides a paleoprecipitation proxy based on observed changes in plant $\delta^{13}C$ values with changes in mean annual precipitation. If vegetation is constant, then this approach may prove useful given that the $\delta^{13}C$ values of individual species are tightly correlated with water availability, although different species have unique relationships (e.g. Prentice et al., 2011b). Given the wide variability in plant $\delta^{13}C$ values at a given site and unique species-level relationships between $\Delta_{leaf}$ and water availability, the uncertainty in precipitation estimates based on plant $\delta^{13}C$ values is likely to be large, especially if the plant community changes through time (Freeman et al., 2011). Depending on the application and required uncertainty, the approach may nevertheless prove useful (Cernusak et al., 2013), but will require constraining uncertainty in paleoprecipitation estimates.

Recently, Sikes et al. (2013) used a multiproxy approach to identify changes in aridity during the most recent deglaciation in New Zealand from maar sediments. By combining plant wax $\delta^{13}C$ values with pollen, biomarkers for fire, and sea surface temperature, they were able to identify a shift from cold and dry conditions during the Last Glacial Maximum to a seasonally warm and wet climate. This interpretation was based on several negative shifts in plant wax $\delta^{13}C$ values. By using pollen and other indicators, the authors were able to rule out other possible controls on the plant wax $\delta^{13}C$ values. In a similar study off the coast of Africa, Hoetzel et al. (2013) combined pollen data with plant wax $\delta^{13}C$ values to better constrain the timing of the rise of $C_4$ grasses in a context of increasing fire, as evidenced from charcoal grains.

### 4.2. Plant carbon isotopes as vegetation indicators

The use of plant $\delta^{13}C$ values as proxies for paleovegetation is commonplace for interpreting changes in $C_3$ and $C_4$ vegetation through time and across space (France-Lanord and Derry, 1994; Freeman and Colarusso, 2001; Tipple and Pagani, 2010; Feakins et al., 2013; Uno et al., 2016a,b). $\delta^{13}C$ values of plants have also been utilized to identify sources of OM as conifer derived or angiosperm derived for time periods prior to the rise of $C_4$ plants. The approach works best at single localities where differences in water availability are minimal, and is often used on specific substrates such as wood, peat and resins (e.g. Murray et al., 1998; Bechtel et al., 2002, 2008).

Utilizing plant $\delta^{13}C$ values as proxies for $C_3/C_4$ vegetation relies on the observation that $C_4$ plants are mostly grass species, so changes in $\delta^{13}C$ values should be a good indicator of the relative abundance of forests to grasslands through time. However, a significant challenge with this approach is the fact that many grasses use the $C_3$ pathway. Additionally, ecosystems do not transition linearly from $C_3$ plant dominated to $C_4$ plant dominated, but rather reflect a patchy continuum of ecosystems from closed canopy forests to open $C_4$ grasslands, each with a unique range of $\delta^{13}C$ values, rather than a continuous gradient (Freeman and Pancost, 2014). For example, forests are composed of trees, shrubs and herbaceous plants, and can be considered $C_3$. Because forests represent a combination of species, each with unique $\Delta_{leaf}$ values, a large range in $\delta^{13}C$ values is expected (up to 8‰ in tropical rainforests; Bonal et al., 2000) that cannot be characterized by a single value. As forests open up and the proportion of woody plants decreases, grasslands emerge. The proportion of $C_3$ and $C_4$ vegetation within grasslands varies and the controls on the vegetation composition are complex, but appear to be related to water availability, as well as other factors (e.g. Edwards et al., 2010; Edwards and Smith, 2010). To address these complexities in modeling vegetation change from plant $\delta^{13}C$ values, Cerling et al. (2011) and Magill et al. (2013) have suggested using ecosystem classifications (i.e. forest, woodland, grassland) and defining appropriate ranges of $\delta^{13}C$ values for each ecosystem type (Fig. 10). This approach is elegant in that it acknowledges the complexities of vegetation and climate among ecosystems, although more information may be extracted than type alone. For example, Ladd et al. (2014) used a multivariate approach that allows the leaf area index to be constrained, although the approach may be challenging for paleo studies where several of the input variables would be difficult to constrain (i.e. temperature, precipitation, seasonality, etc.).

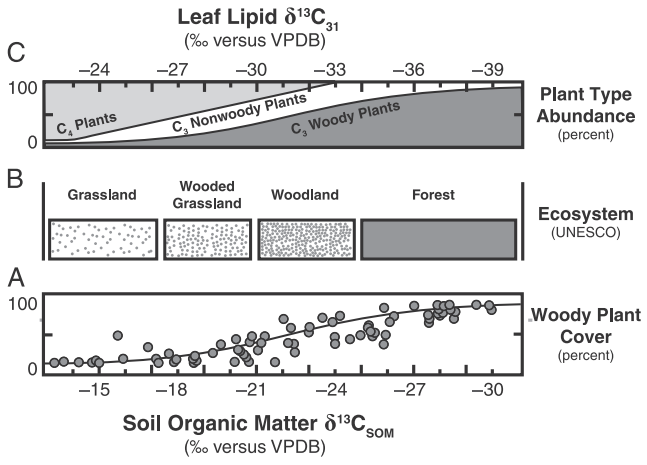


**Fig. 10.** $\delta^{13}C$ values of $n$-$C_{31}$ alkane and soil organic matter (SOM) for different African ecosystem types. Figure is reproduced from Magill, C.R., Ashley, G.M., Freeman, K.H., 2013. Ecosystem variability and early human habitats in eastern Africa. Proceedings of the National Academy of Sciences 110, 1167–1174.

Many substrates preserve a signature of plant $\delta^{13}C$ values and thus $C_3/C_4$ vegetation patterns. These include fossil cuticles, soil OM, phytoliths, pedogenic carbonate nodules, herbivore tooth enamel, pollen and plant waxes. Each of these substrates has its own inherent challenges and caveats for extracting a primary vegetation signal. For example, $\delta^{13}C$ values of soil are shifted to higher values (by up to 6‰) during pedogenesis (Wynn et al., 2005; Wynn, 2007). For plant waxes, challenges arise because different growth forms produce different amounts of plant waxes with different $\varepsilon_{n\text{-alkanes-leaf}}$ values (Figs. 2 and 8). Garcin et al. (2014) approached this problem by taking into account the $n$-alkane concentration differences among plant functional types, along with their $\delta^{13}C$ values, for different chain lengths and latitudes (Fig. 11). By combining that information into a model, the authors were able to estimate % $C_4$ vegetation. This approach includes an estimate of uncertainty, a significant advancement over previous studies. By combining $\varepsilon_{n\text{-alkanes-leaf}}$ and concentration values with the approach in Garcin et al. (2014), ecosystem types could be defined (Cerling et al., 2011; Magill et al., 2013) to avoid the challenges of single end member values, and therefore the method should reduce uncertainty in ecosystem characterization. Regardless of the approach used, it is critical to evaluate $n$-alkane $\delta^{13}C$ shifts for different chain lengths. For example, Uno et al. (2016a) were able to document a shift from $C_3$ to $C_4$ vegetation during the Neogene by carefully examining the increases in concentration and $\delta^{13}C$ values of $n$-$C_{33}$ and $n$-$C_{35}$ alkanes.

The use of plant wax $\delta^{13}C$ values as a screening tool to identify possible bias in plant wax $\delta D$ interpretations because of changes in the proportion of $C_3$ and $C_4$ plants, and associated differences in hydrogen isotopic fractionation may prove useful for hydroclimate reconstruction (Collins et al., 2013; Vogts et al., 2016).

### 4.3. Plant carbon isotopes as recorders of carbon isotope excursions

The carbon isotopic composition of plants and plant biomarkers is linked to atmospheric $\delta^{13}C$. Therefore, plant carbon isotopes can provide a past record of carbon cycle changes and can be especially useful for periods when the marine record is absent or is not in isotopic equilibrium with the atmosphere (Hayes, 1993; Koch, 1998; Hayes et al., 1999; Kump and Arthur, 1999; Bowen, 2013). However, the key to reconstructing past changes in the carbon cycle from plant $\delta^{13}C$ values is accounting for the fractionation between plants and atmospheric $CO_2$. For $C_4$ plants, fractionation is relatively easy to predict as their $\Delta_{leaf}$ values are not sensitive to water and the total measured range is rather small (Fig. 7). Thus, predicting $\delta^{13}C_{atm}$ from $C_4$ plants is rather simple. In contrast, the many factors that affect $C_3$ plant $\Delta_{leaf}$ values make predicting $\delta^{13}C_{atm}$ much more complicated, as additional information is necessary to constrain $\Delta_{leaf}$ values (e.g. Diefendorf et al., 2010, 2015a).

In order to predict $\delta^{13}C_{atm}$ values from $C_3$ plants, Diefendorf et al. (2010) suggested an approach that takes into account the various controls on both $\Delta_{leaf}$ and $\varepsilon_{lipid}$. In that study, the authors utilized paleoprecipitation estimates from fossil leaves to correct for the influence of precipitation on $\Delta_{leaf}$ with modern calibrations (Diefendorf et al., 2010; Kohn, 2010). For other studies, water availability could be constrained with other paleoprecipitation proxies or by limiting sampling to wet depositional environments. In the Diefendorf et al. (2010) study, vegetation was constrained again with fossil leaves to inform the $\Delta_{leaf}$ calibration and to identify which $\varepsilon_{lipid}$ values to use. In this type of approach, it is critical to make some constraints on vegetation and then to make informed decisions about which plant biomarkers or $n$-alkane chain lengths to use. Based on the evidence at the time, Diefendorf et al. (2010)

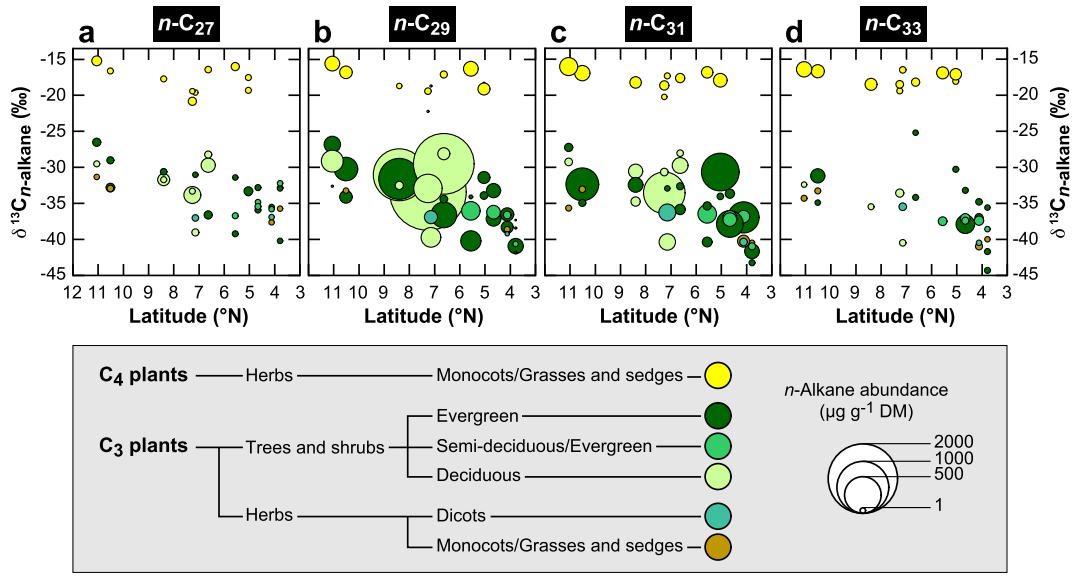


**Fig. 11.** $\delta^{13}C$ values of different plant functional types sampled along a latitudinal transect in Cameroon for *n*-$C_{27}$ (A), *n*-$C_{29}$ (B), *n*-$C_{31}$ (C), and *n*-$C_{33}$ alkanes (D). Reprinted from Reconstructing $C_3$ and $C_4$ vegetation cover using *n*-alkane carbon isotope ratios in recent lake sediments from Cameroon, Western Central Africa, 142, Garcin, Y., Schefuß, E., Schwab, V.F., Garreta, V., Gleixner, G., Vincens, A., Todou, G., Séné, O., Onana, J.-M., Achoundong, G., and Sachse, D., 2016, with permission from Elsevier.

assumed an equal production of *n*-alkanes between conifers and angiosperms for *n*-$C_{31}$ alkane. However, based on more recent studies (Diefendorf et al., 2011, 2015b; Bush and McInerney, 2013), we now know that many conifers produce a low concentration of *n*-alkanes compared with co-occurring angiosperms, and can thus be ignored in many, but not all, ecosystems.

A significant challenge with the approach above, or any similar approach, is making sure that modern calibration data, for $\Delta_{leaf}$ and $\varepsilon_{lipid}$, are appropriate and do not overestimate the true range in values for a given ecosystem, thereby leading to an overestimation of calculated uncertainty for past $\delta^{13}C_{atm}$. As indicated in the previous sections, there is a wide range of biomarker concentration (Fig. 2), $\Delta_{leaf}$ (Fig. 6) and $\varepsilon_{lipid}$ (Fig. 8) among species and growth forms. Determining how this species level information is integrated and averaged in the sedimentary record is unclear and presents a significant gap for the community.

The carbon isotope excursion (CIE) at the onset of the Paleocene-Eocene Thermal Maximum (PETM) presents one of the best examples of a global carbon cycle perturbation that is recorded by plant $\delta^{13}C$ values. Measurements of the PETM CIE from plant lipids varied worldwide among 16 sites with values ranging from −7.6‰ to −2.8‰ (McInerney and Wing, 2011). Much of the variability might be explained by concomitant changes in water availability and vegetation (Wing et al., 2009; McInerney and Wing, 2011), but other factors such as sampling resolution, time averaging and reworking of *n*-alkanes must also be important. To date, only a limited number of studies have been able to investigate and/or control these other factors when estimating the CIE (Bowen et al., 2004; Pagani et al., 2006; Schouten et al., 2007; Handley et al., 2008; Diefendorf et al., 2010; Tipple et al., 2011; Baczynski et al., 2013, 2016; Krishnan et al., 2015; Schoon et al., 2015). These studies highlight the importance of having independent indicators of climate and vegetation change and also highlight the importance of having many records for a single carbon cycle perturbation. Reconciling plant biomarker-based CIEs is important for understanding the PETM, but equally important for providing insights into understanding how plants record changes in $\delta^{13}C_{atm}$. By making careful consideration of the factors that influence sedimentary biomarker $\delta^{13}C$ values, more certain interpretations of plant $\delta^{13}C$ values can be made.

## 5. Conclusions

Since the first publications reporting plant waxes (e.g. Eglinton et al., 1962; Eglinton and Hamilton, 1963, 1967), the number of studies utilizing sedimentary plant waxes has skyrocketed. Indeed, plant biomarkers, including plant waxes, and their carbon isotopes provide records of carbon cycling, paleovegetation and paleoclimate. Much progress has been made to understand what controls biomarker concentration and $^{13}C$ fractionation during photosynthesis and lipid biosynthesis in modern plants. This has improved our ability to interpret sedimentary biomarkers. However, it is clear from these modern studies that interpreting a signal from sedimentary biomarker $\delta^{13}C$ values, without controlling additional factors that influence plant $\delta^{13}C$, will be problematic. If competing factors are constrained, then it is possible to extract the most information from the geologic record, while also reducing uncertainty in these interpretations. Below we summarize current knowledge of plant waxes and identify major gaps and future directions.

### 5.1. Summary points

- Plant waxes and their carbon isotopes provide records of past carbon cycling, paleovegetation and paleoclimate. Much progress has been made to understand controls on plant wax concentration and $^{13}C$ fractionation in modern plants. Insights from these modern studies demonstrate that interpreting sedimentary biomarker $\delta^{13}C$ values necessitates constraining factors that influence plant $\delta^{13}C$ values. These include the $\delta^{13}C$ composition of past atmospheric $CO_2$, changes in plant community and associated fractionation, and past hydrological shifts that influence $^{13}C$ fractionation during photosynthesis.
- Distinct plant wax abundance, chain length distribution and $^{13}C$ fractionation among species, growth habits and physiology may influence plant wax signals in sediments. Past source vegetation and growth conditions may be characterized using additional vegetation proxies, plant biomarkers and sediment properties.
- Differences in $^{13}C$ fractionation among $C_3$ and $C_4$ vegetation are encoded in plant wax $\delta^{13}C$ values, providing a tool for interpreting past ecological shifts and the rise of $C_4$ plants. Careful determination of the plant wax $\delta^{13}C$ values and chain(s) of interest

are needed when ascribing changes in the $C_3$ and $C_4$ plant community. A promising way to address the challenges inherent in single end member values is to characterize $^{13}C$ fractionation diagnostic for a range of ecosystems.
- When using sedimentary plant wax and other biomarkers, it is important to consider the sourcing, transport and preservation in each specific depositional system (e.g. lacustrine, marine, soil) and climate/biome (e.g. tropical, temperate, boreal). The various processes controlling the integration of waxes into sediments will impact the abundance, isotope composition and sensitivity (i.e. attenuation of a climate signal via lipid mixing) of the record. By selecting sites that maximize the signal to noise, more meaningful information can be obtained.

### 5.2. Gaps and future directions

- Understanding of the abundance, transport, preservation, fractionation and biological function of different wax compound classes is in its infancy. More studies are critically needed (with both modern and geologic based approaches) to assess and quantify how different *n*-alkyl compounds respond to and record biological and climatic change. This is a potential source of additional information about past environments and plant ecology that is waiting to be extracted.
- Relatively few studies have compared the relative abundance and stable isotopic composition of two or more *n*-alkyl lipid compound classes either in extant plants (Rommerskirchen et al., 2006; Chikaraishi and Naraoka, 2007; Vogts et al., 2009; Gao et al., 2015) or in sediments (Freeman and Colarusso, 2001; Feakins et al., 2007; Uno et al., 2016a). Therefore, sedimentary *n*-alkane and *n*-alkanoic acid records are frequently interpreted without respect to potential differences in production, isotopic fractionation, transport and preservation. This leaves several critical questions unresolved. For instance, do *n*-alkanes and *n*-alkanoic acids record divergent, related, or equivalent records? Is there additional information that can be gained by measuring both? For example, do the two compound classes record disparate components of a catchment, possibly at different temporal scales? A concerted effort to compare compound classes needs to be undertaken in the coming years to address these questions.
- Residence times and sedimentary age offsets for terrestrial plant biomarkers in marine and lake sediments vary widely among sites. Advancing understanding of basin-specific controls on biomarker sources, transport pathways and residence times will be critical for developing accurate biomarker based reconstructions of climatic and environmental change.
- Modern calibrations are the primary source of information about the mechanisms that explain the $\delta^{13}C$ values produced in plant waxes. However, it is a challenge to reconcile the temporal and spatial scales of calibrations with those of the sediment record. The development of scaling relationships between the plant, forest, and sediment are needed. Future calibration studies could greatly advance progress if they include multiple plant wax compounds and include wax concentration. But, greater effort should be placed on developing scaling relationships that connect extant plant wax to the sediment.
- Collaboration with plant physiologists and those studying plant evolution and paleobotany may be a promising way to help understand the origins and functions of plant waxes through geologic time. There may be further unexplored evolutionary or phylogenetic explanations for observed differences in plant wax abundance and composition among plant groups.
- Combining the $\delta^{13}C$ and $\delta D$ records of plant waxes in sediments may yield greater information about past climate, but to date, few studies have measured both stable isotopes.
- Future studies would greatly benefit from enhanced treatment of uncertainty. For example, $C_3/C_4$ vegetation studies often assume single end member values, yet it is clear from the literature, especially for $C_3$ plants, that there is a range of possible values. By adopting more rigorous methods for treating uncertainty (e.g. Polissar and D'Andrea, 2014; Tierney and Tingley, 2014), the community could assign uncertainties and probabilities to our predictions.

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.orggeochem.2016.10.016.

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