Chapter 8
Reconstructing Terrestrial Paleoenvironments Using Sedimentary Organic Biomarkers

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Abstract Organic geochemical biomarkers are an increasingly utilized set of tools for reconstructing past terrestrial conditions. These biomarkers can be preserved from all life, representing Archaea, Eukarya, and Bacteria, and are deposited in sediments and soils, and yet they can be separated in the laboratory for analysis of individual compounds from the same sample. Often well preserved in the geologic record, new proxies based on these "molecular fossils" extracted from sedimentary archives have allowed for the reconstruction of a wide array of paleoenvironmental and paleoclimatic conditions, including but not limited to past temperature, vegetation, and hydroclimate. This review provides a brief overview of those molecular proxies that might be most applicable for terrestrial environmental conditions.

Keywords Molecular proxies • Compound specific stable isotopes • Paleoclimate • Biomarkers • Organic geochemistry

Introduction

Organic molecules are used as recorders of past climate and environmental conditions in terrestrial and aquatic settings. These "molecular fossils," or biomarkers as they are commonly called, can survive for millions of years and can be linked to specific source organisms or groups of organisms, or particular biogeochemical processes, such as methane production, anoxia, or photosynthesis (Brocks and Summons 2003; Pancost and Boot 2004; Peters et al. 2005). The biomarker concept is based on the separation of various aquatic, microbial, and terrestrial biomarkers in a heterogeneous mixture of organic compounds within the sediments. These compounds are insoluble in water and often are chemically inert with low volatility, making many of them long-lived in the environment and well-suited for studies of the past where low thermal maturation of the host lithology has occurred (Eglinton and Eglinton 2008). Sedimentary archives often contain a diverse mixture of these compounds, including compounds from organisms with poor preservation potential, which can be used to answer questions about the climate and environment over a wide range of spatial and temporal scales (Peters et al. 2005). Sediments contain a complex aggregate of molecular biomarkers that can be chemically separated and identified in the laboratory to help determine the origin and abundance of the organic matter. Therefore, these compounds can be more useful for the examination of specific environmental conditions, such as using compound specific stable isotopes extracted from sediments rather than bulk sediment organic matter isotope analyses that may include a large mixture of sources (Castañeda and Schouten 2011). Accumulation and preservation of biomarkers most commonly occurs in the soils and sediments of lakes, rivers, and oceans, but they have also been found preserved in speleothems (Blyth et al. 2008), rodent middens (Carr et al. 2010; Chase et al. 2012), ooids (Summons et al. 2013), and rock concretions (Kiriakoulakis et al. 2000). Biomarker distributions, abundances, and stable isotopic values are all used to determine past environmental conditions. Specifically, advances in stable isotope analysis at the compound level (focusing here on $\delta^{13}C$ and δD) are now used to address new and unique paleoenvironmental questions. This chapter is aimed at students and non-specialists with an interest in applying molecular proxy techniques for terrestrial reconstruction. Biomarkers and methods most useful for reconstructing Cenozoic terrestrial environments are given preference here, but excellent reviews of both shorter-lived biomarkers and those specific for unique aquatic conditions are available elsewhere (e.g., Brocks and Summons 2003;

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Castañeda and Schouten 2011; Grice and Eiserbeck 2014; Meyers 1997a; Pancost and Boot 2004; Schouten et al. 2013; Sinninghe Damsté and Schouten 2006).This review focuses on the reconstruction of terrestrial vegetation, hydroclimate (broadly defined as water cycle variability linked to climate shifts), and temperatures preserved in soil, lake, and marine settings.

The connection between biomarkers and their biological precursor has been greatly improved upon in recent times. Through modern sampling, laboratory growth experiments, and culturing, we have established the relationship (in many cases in the form of a mathematical calibration) between many carbon-hydrogen structures preserved in geologic materials and their modern counterpart and identified their original function and source. However, the concept of an organic source for preserved hydrocarbons was not always supported. In the late nineteenth century, multmacromolecular carboniple theories for the abiotic origin of oil were presented. Alfred Treibs (1899–1983), a noted organic geochemist of the time, was the first to support the organic link between molecules from rocks and biota, based on a comparison of rock-extracted hydrocarbons to the structure of plant and algal pigments. In 1934, Treibs observed the striking similarity between a vanadyl-porphyrin separated from black shale and modern eukaryote chlorophyll *a*. He later outlined the breakdown of chlorophyll *a* into the observed sedimentary products (Treibs 1936), and as such became the "Father of Organic Geochemistry" (Kvenvolden 2006). Another early pioneer and contemporary of Treibs, Parker Trask (1899–1961), was a geologist who also made significant early contributions to the field. In an effort to better understand oil reservoirs, Trask made comparisons of organic matter in sedimentary units to source beds of petroleum. The field of organic geochemistry was thus born, yet it wasn't until the 1970s that the term biomarker was coined. An amalgamation of 'biological marker,' it is thought that the term was first used by Wolfgang Seifert, one of Treibs' students employed by Chevron (Gaines et al. 2008). Seifert's early work was on steroids in oils and analytical fingerprinting methods utilizing hopanes as an index of thermal maturity (an effect of high heat and pressure on sedimentary hydrocarbons). The late 1970s and early 1980s became a time of moving away from solely using biomarkers to uncover the biological origin of petroleum products and towards a more complete understanding of the formation and preservation of hydrocarbons. Ultimately, the window these biomarkers provided into Earth's history become more apparent. Significant instrumentation advances (see further discussion below) have rapidly moved the field of organic geochemistry forward, with the ability to measure smaller samples and more complex molecules with greater precision and accuracy.

Organic Matter Preservation

Accumulation and preservation of organic matter occurs overwhelmingly in aquatic environments, both terrestrial (often lacustrine) and marine settings. In fact, terrestrial biomarkers are thought to be preserved preferentially in marine sediments compared to marine lipids (Prahl et al. 2003; Sinninghe Damsté et al. 2002b). However, the vast majority of organic matter does not get preserved at all and is ultimately remineralized and respired as CO_2 (Canfield 1994; Hedges and Keil 1995). The rates and location of organic matter production and its composition can help predict the likelihood of preservation. The oceans cover >70% of the Earth's surface area, but only account for a fraction of the long-term burial of organic matter compared to terrestrial aquatic settings (Dean and Gorham 1998). Furthermore, continental margins, including deltas and continental shelves, which make up ~10% of the ocean's surface area, account for >90% of the organic matter buried in marine settings (Berner 1989; Killops and Killops 2005). Regions with low burial efficiency, such as the pelagic or abyssal zones of the ocean, experience almost full remineralization of organic carbon and thus have sediments with low total organic carbon and low reactivity (Burdige 2007).

Although much emphasis has been placed on carbon cycling in marine settings, the sink of carbon in lakes and wetlands significantly outweighs that of the oceans (Dean and Gorham 1998). Lacustrine sediments commonly contain 10% or greater total organic carbon in sediments, even though they cover <4% of earth's surface area (Carroll and Bohacs 2001; Dean and Gorham 1998; Peters et al. 2005; Tranvik et al. 2009). These terrestrial sites collect and sequester large amounts of carbon from the surrounding watersheds, with the majority accumulating in these basin and not continuing out to sea (Cole et al. 2007), making them an important long-term repository for carbon.

The presence of oxygen is one of the important determinants in preservation of organic matter in aquatic environments. The reduction or complete absence of O_2 can occur in the water column during increased phytoplankton activity when algae consume O_2 during respiration and zooplankton consume O_2 while breaking down organic matter. These sediments favor the preservation of organic matter over oxic environments because low dissolved oxygen (<0.1% dissolved O_2) inhibits the bioturbation, resuspension, and exposure of freshly buried organic matter to aerobic degradation by microbes. Although conditions are less favorable for the decomposition of organic matter, microorganisms in anoxic sediments are able to utilize alternative terminal electron acceptors (e.g., NO^{3-}, Fe^{3+}, SO_4^{2-}), and emphasize the importance of anaerobic oxidation in the subsurface (Calvert and Pedersen 1993). In fact, new research suggests that deep subsurface microbes may

have a larger role in the presence of organic matter, particularly at extreme depths and high water temperatures than previously thought. Anaerobic microbes have been found living at deeper depths and in higher temperatures than previously thought possible (Hallmann et al. 2008; Inagaki et al. 2015; Pikuta et al. 2007), which has implications for long-term organic matter burial and preservation. For example, Inagaki et al. (2015) found microbial communities at ~1.5 to 2.5 km below the seafloor living at temperatures between ~40° to 60°C in the Pacific Ocean off the coast of Japan. These microbial communities are thought to have survived living on coal and hydrogen from the coal-bed they were found associated with at depth (Inagaki et al. 2015).

For the most part, degradation of the organic biomarkers deposited in sediments occurs quickly following burial, within a few hundred years as observed in lacustrine deposits (Cranwell 1981; Meyers et al. 1980). However, the importance of anaerobic oxidation in the subsurface has become evident in light of new analytical methods in recent years. In fact, new research suggests that deep subsurface microbes may have a larger role in the presence of deep ocean organic matter than previously thought. Anaerobic microbes have been found living at deeper depths and in higher temperatures than previously thought possible (Hallmann et al. 2008; Inagaki et al. 2015; Pikuta et al. 2007), which has implications for long-term organic matter burial and preservation. For example, Inagaki et al. (2015) found microbial communities at ~1.5 to 2.5 km below the seafloor living at temperatures between ~40° to 60°C in the Pacific Ocean off the coast of Japan. The microbial communities are thought to have survived living on coal and hydrogen from the coal-bed they were found associated with at depth (Inagaki et al. 2015).

Organic matter that survives scavenging and microbial degradation in the water column and sediments is separated into two classes: bitumen and kerogen, both characterized by their relative resistance to significant further microbial alteration (Wakeham et al. 1997). Biomarkers can be found in kerogen, highly chemically varied organic matter that is insoluble with organic solvents and is often polymerized into large macromolecules (De Leeuw and Largeau 1993; Tegelaar et al. 1989b; Vandenbroucke and Largeau 2007). Bitumen is organic matter defined by its ability to be extracted from geologic materials using organic solvents (Killops and Killops 2005), and contains the majority of molecules used for reconstructing terrestrial paleoenvironments. Excessive heating and pressure during burial thermally alters the organic compounds in both the bitumen and kerogen components. When kerogen breaks down, some of its degradation byproducts form bitumen. This thermal alteration can continue to occur within the deposit, but biomarkers are no longer preserved at temperatures greater than ~150–250°C (Killops and Killops 2005; Peters et al. 2005).

Ultimately, some biomarkers can be preserved for billions of years (Peters et al. 2005). Although lipids often lose functional groups during diagenesis, the remaining hydrocarbon skeleton is then often stable and long-lasting. Some biomarkers, such as deoxyribonucleic acid (DNA) (Coolen and Overmann 1988; D'Andrea et al. 2006) and certain sterols (D'Anjou et al. 2012) are relatively short-lived, surviving only up to a few thousand years and often in unique chemical or temperature settings allowing enhanced preservation potential. Molecules that are much less labile, including lipids and plant structural components such as lignin may still experience some degree of chemical diagenesis (Killops and Killops 2005). The diagenetic fate of some of these lipids will be discussed further later in this chapter.

Organic matter preservation can also occur with inorganic association within the water column, during transportation, or in the upper sediment layers following burial. In these instances, organic matter is afforded a level of protection that allows it to persist longer than exposed organic matter would (Burdige 2007). This may involve actual physical protection mechanisms, such as sheltering organic matter and preventing remineralization or chemical attack (Burdige 2007). For instance, organic matter preservation was higher when molecules were associated with fine grained, rather than coarser grained sediments (Keil et al. 1994; Mayer 1994), providing a link between mineral surface area and preservation (Hedges and Keil 1995). Mayer (1994) noted a proportional relationship between surface area of sediment grains and organic matter abundance, and proposed an adsorption mechanism, termed "mesopore protection," whereby, bacterial enzymes are excluded due to size restriction of pores. Similarly, the monolayer hypothesis postulates that mineral surfaces are coated with the equivalent of a monolayer of carbon which creates a size restriction and enables protection (Hedges and Keil 1995; Mayer 1994, 1999). Although research has uncovered a connection between mineral surface area and organic matter preservation, we still do not have a complete understanding of the mechanisms involved in organic matter protection and burial.

Sampling Locations

The use of organic biomarkers for Cenozoic environmental reconstruction is limited by the availability of sedimentary units for study. Escaping thermal overprinting is a part of this preservation, but aspects of post-burial exposure and outcrop location are important to preservation as well. Outcrop samples are often available and the easiest to obtain, however, these samples pose recovery challenges for

biomarkers because of their exposure. Alteration of organic matter and more specifically biomarkers can occur in outcrop due to oxidation, microbial degradation, and percolation of meteoric water exposing organic matter to further decay (Marynowski et al. 2011; Petsch et al. 2000, 2001a, b; Sageman et al. 2003). Biomarker studies from outcrop require extra care in sampling and interpretation. In a study of a shale weathering profile, Petsch et al. (2001a) used phospholipid fatty acid biomarkers and found that modern microbial communities were subsisting on exposed and ancient (~365 Ma) macromolecular carbon in shale. This implies that exposed organic matter thought to be refractory is actively being assimilated, and thus changed, in outcrops. Active degradation of biomarkers in exposed outcrops is a possibility and that care should be taken at these sampling locations. Indicators of poor preservation or contamination of biomarkers are further discussed below.

Marine or lacustrine sediment core samples are commonly used for terrestrial environment reconstruction. Recovery from deep drill holes alleviates the post-burial weathering and oxidation that outcrop samples experience and thus they often contain higher overall abundances of biomarkers (Peters et al. 2005). However, there are other considerations that must be made with regards to biomarker sampling from cores. Information regarding coring operations (e.g., recovery and disturbances) are critical when sampling for paleoecological reconstructions. Storage of cores for many years could result in oxidation or microbial degradation of biomarkers (Brittingham et al. 2017). These issues can be prevented with advanced storage techniques such as sealing sediment cores in plastic and archiving in cold, dark storage (e.g., Berke et al. 2012b, 2014).

Leaf Waxes of Past Vegetation

Plant leaf waxes lipids are highly refractory in the sediments and preferentially accumulated in aquatic settings prior to deposition compared to other lipids (Cranwell 1981; Schimmelmann et al. 1999; Sinninghe Damsté et al. 2002b; Wakeham et al. 1997), making them a robust and valuable tool for environmental paleoreconstructions (Eglinton and Eglinton 2008). Waxes are a part of the leaf cuticle, acting as a barrier between soft plant tissues and environmental stressors (Shepherd and Griffiths 2006). This epicuticular wax serves to protect the plant from water loss, disease, and ultraviolet radiation (Shepherd and Griffiths 2006).

Vascular plant biomarkers can be used for a wide range of applications in past environment and climate reconstruction. *n*-Alkanes, *n*-alkanols, *n*-alkanoic acids, and wax esters, collectively termed *n*-alkyl lipids (Fig. 8.1), make up leaf waxes and can be produced by both terrestrial and aquatic organisms. However, the number of carbon atoms in these lipids can be used as a general fingerprint for the source organism (Cranwell 1973; Eglinton and Hamilton 1963, 1967). Long-chain *n*-alkyl lipids make up a significant component of the epicuticular coating on vascular plant leaves, and exhibit a strong predominance of odd instead of even number of carbon atoms in a chain (*n*-alkanes) or even over odd carbon number chain length predominance (*n*-alkanoic acids) (Eglinton and Hamilton 1967). While long carbon chain lengths are commonly more dominant in terrestrial higher plants (C_{29}-C_{35} for *n*-alkanes and C_{28}-C_{34} in *n*-alcohols and *n*-alkanoic acids), aquatic algae and microbes are often predominantly composed of shorter chain lengths (C_{17}-C_{21} for *n*-alkanes and C_{16}-C_{22} in *n*-alkanoic acids) (Blumer et al. 1971; Cranwell et al. 1987). The mid-chain length homologues C_{23}-C_{27} (*n*-alkanes) and C_{24}-C_{26} (*n*-alkanoic acids), often produced in smaller quantities by terrestrial higher plants, make up the majority of leaf waxes of aquatic macrophytes (Cranwell 1984; Ficken et al. 2000).

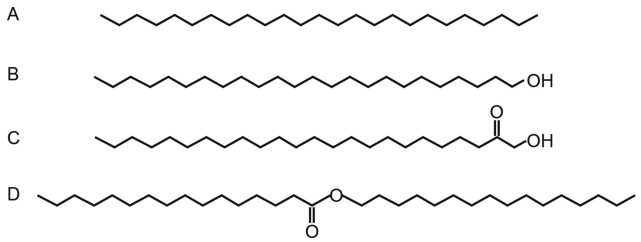

Fig. 8.1 *n*-Alkyl lipid structures found in plants including: A, *n*-alkanes (C_{25} shown); B, *n*-alkanols (C_{24} shown); C, *n*-alkanoic acids (C_{24} shown); and D, wax esters

Though these general guidelines often hold true, there are exceptions that complicate environmental interpretations based upon carbon chain length. For example, Aichner et al. (2010) observed that C_{29} *n*-alkanes, linked most commonly to terrestrial plants, are the dominant *n*-alkane chain length measured from submerged macrophytes in a Tibetan lake while terrestrial plants, such as *Betula* sp., *Sphagnum* moss, and *Salix glauca*, produce an abundance of C_{23} and C_{25} alkanes (Berke et al. 2015b; Bush and McInerney 2013; Sachse et al. 2006). Long-chain alkanes have been linked to aquatic algal sources (Lichtfouse et al. 1994; Metzger et al. 1985; Volkman et al. 1980). Exceptions to the commonly held ideas of plant production suggest that interpretation of *n*-alkyl carbon chain lengths for source identification must proceed with caution.

Angiosperms contribute more *n*-alkanes to the sedimentary record than do gymnosperms (Bush and McInerney 2013; Diefendorf et al. 2011). Diefendorf et al. (2011) found

in a study quantifying modern plant lipids that *n*-alkanes are often too low in quantity to be measured in gymnosperms, while angiosperms contained abundant *n*-alkanes, a finding that has been supported with studies from soils in angiosperm forests (Otto and Simpson 2005; Sachse et al. 2006). In landscapes dominated by gymnosperm forests, other compounds may serve as better biomarkers, though fewer paleoreconstruction studies have been done using these biomarkers (Bastow et al. 2001; Hautevelle et al. 2006; Schouten et al. 2007c). For example, terpenoids, defense compounds produced by gymnosperms and angiosperms to ward off pests, pathogens, and herbivory (Coley 1983; Langenheim 1994; Otto and Wilde 2001), may serve as better gymnosperm biomarkers (Diefendorf et al. 2011; Otto and Simoneit 2001, 2002; Schouten et al. 2007c). These aromatic hydrocarbon compounds were first utilized for terrestrial environmental reconstruction from Jurassic marine sediments off the coast of Australia (van Aarssen et al. 2000) and more studies are exploring the use of aromatic biomarkers and their diagenetic precursors (Otto and Wilde 2001). Recent modern exploration of these biomarkers (Diefendorf et al. 2012) also aids in our understanding of terpenoid formation for paleoreconstruction.

While leaf wax lipids are known to be fairly robust in the sedimentary record, it is clear that degradation via microbial oxidation (e.g., Cranwell et al. 1987) and heating associated with burial (e.g., Chikaraishi and Naraoka 2007) can occur. Observations suggest that *n*-alkyl lipids are not affected equally by degradation. Specifically, *n*-alkanes withstand sedimentary degradation better than *n*-alkanoic acids or *n*-alkanols (Cranwell 1981). Additionally, sedimentary *n*-alkanoic acids and *n*-alkanols can convert to *n*-alkanes as deposits approach and move into the oil generation window (increasing thermal maturity via heating and time since burial), ultimately changing composition and abundances of the leaf wax lipids found in the sedimentary record (Collister et al. 1994a; Gupta et al. 2006, 2007). In addition to changing the lipid found in these sediments, thermal maturity also alters carbon chain length distributions, providing an alteration signature of carbon chain lengths and not original distribution (Bray and Evans 1961). These alterations make paleoenvironmental reconstructions based on *n*-alkyl lipid abundances and carbon chain length distributions in ancient deposits that have undergone some thermal maturity difficult. Though leaf wax lipid distribution and carbon chain lengths are altered with thermal maturity, recent research suggests that stable carbon isotopic values likely remain relatively unaltered. Using hydrous pyrolysis experiments of various modern plants, Diefendorf et al. (2015) found little alteration of $\delta^{13}C$ values (ca. 1‰) prior to or within the early temperatures of the oil generation window (temperatures <250°C).

n-Alkane Distribution

Plants produce different *n*-alkyl lipid types and while the chemical composition is similar, abundances and distribution may vary with respect to environmental conditions, abundances, thicknesses, chain length distribution (Bush and McInerney 2013). Ratios of *n*-alkane carbon chain lengths can be used to provide general information about changing environmental conditions and the relative contributions of plant communities to the lipid pool (Meyers 1997a). One such ratio, P_{aq}, is used to determine non-emergent aquatic macrophyte input to a lake system, differentiated from emergent macrophytes and terrestrial plants. P_{aq} is calculated using the abundance of mid-chain *n*-alkanes (C_{23} and C_{25}) relative to the sum of these mid-chain lengths and longer C_{29} and C_{31} homologues (Ficken et al. 2000). Similarly, the ratio of long, terrestrially-derived *n*-alkanes to short chain, aquatically-derived *n*-alkanes, referred to as the terrestrial to aquatic ratio (TAR) (Meyers 1997b, 2003), measures the abundance of C_{27}, C_{29}, and C_{31} compared to C_{15}, C_{17}, and C_{19}.

Another ratio commonly applied to *n*-alkane homologues with the purpose of gaining plant and environmental information across sampled sites or through time is known as the average chain length (ACL) (Poynter and Eglinton 1990). The ACL is the weighted average of the carbon chain lengths defined as:

$$ACL_{m-n} = \sum_{i=m}^{n} \frac{i[C_i]}{C_i}$$

The *m* and *n* are the shortest and longest chain lengths, respectively, and *i* is the number of carbon atoms in each homologue. For *n*-alkanes, the odd-numbered homologues are used and for *n*-alkanoic acids, the even-numbered homologues are used in this equation. It is used as an approximation of integrated local vegetation and changing vegetation types through time (e.g., Eglinton and Hamilton 1967). However, beyond general tracking of changes in carbon homologue distribution in sedimentary archives, it is still unclear what plant ACL variability reflects. ACL values do not remain constant as leaves develop (Sachse et al. 2009; Tipple et al. 2013) and also are not consistent across all plant groups or functional types, particularly within woody vegetation (Diefendorf et al. 2011). Studies have found that grasses and deciduous trees exhibit homologue preferences (e.g., Cranwell 1973; Rommerskirchen et al. 2006; Vogts

et al. 2009). C_4 grasses (described below) often produce longer homologue lengths than do C_3 grasses, with studies suggesting an abundance of longer chain lengths (higher ACL) being a characteristic of C_4 grasses (Vogts et al. 2012). Furthermore, warmer climates are often associated with longer ACLs (Sachse et al. 2006; Simoneit 1977), which may correspond to a prevalence of C_4 grasses and aridity in some regions (Vogts et al. 2012). Though it is likely that environmental conditions help shape ACL values (e.g., Bush and McInerney 2015; Rommerskirchen et al. 2003), caution must be taken with interpretations that rely on these ratios because there is an incomplete understanding of what drives homologue abundances.

Another commonly used lipid ratio is the carbon preference index (CPI), which looks at the extent of odd to even dominance in *n*-alkanes (Bray and Evans 1961; Marzi et al. 1993). The updated calculation of the original CPI definition (Bray and Evans 1961) by Marzi et al. (1993) is:

$$CPI = \frac{\left(\sum_{odd} C_{17-33}\right) + \left(\sum_{odd} C_{19-35}\right)}{2 \cdot \left(\sum_{even} C_{18-34}\right)}$$

CPI values are often used to distinguish the biological origin and the possible influence of thermal maturity on *n*-alkanes (Bray and Evans 1961; Peters et al. 2005; Tegelaar et al. 1989a). Deposits with high thermal maturity, an abundance of algae or bacteria, degradation of higher carbon chain lengths, or input of petroleum contamination all tend to yield low CPI values, ≤ 1 (Peters et al. 2005). High CPI values have also been found in modern plants (Bush and McInerney 2013; Collister et al. 1994b) and recent sediments (Cranwell 1981).

Leaf Wax $\delta^{13}C$ for Vegetation Reconstruction

All higher plants fall into one of three photosynthetic classifications: C_3 (those that use the Calvin-Benson pathway, ~90% of all plants, including common trees and bushes), C_4 (those that use the Hatch-Slack pathway, many tropical grasses and sedges), or Crassulacean acid metabolism (CAM, those that use a mixture of pathways, most commonly succulents) plants. These pathways describe uptake of atmospheric CO_2 by plants and the efficiency and isotopic fractionation associated with the diffusion and incorporation of their carbon source (O'Leary 1981).

The evolution of C_4 photosynthesis is thought to have developed to help plants cope with the environmental pressure of high aridity and excessively warm conditions that can lead to desiccation in C_3 plants (Ehleringer and Monson 1993; Monson 1989). Interestingly, this metabolic pathway appears to have arisen multiple times through geologic history in over 50 separate evolutionary lineages (Sage 2004; Sage et al. 2011). While the timing of emergence and expansion are debated, multiple lines of evidence indicate that the initial rise of C_4 plants occurred during the Cenozoic, with grasses emerging prior to sedges (Besnard et al. 2009; Sage et al. 2011). Evidence of C_4 photosynthesis in the Carboniferous has not been definitively identified (Cerling 1999), even though low CO_2 and high O_2 environments might have been ideally suited for the initial development of this metabolic pathway (Wright and Vanstone 1991). An expansion of C_4 photosynthesis during the Miocene does not appear to be globally synchronous, however, with evidence suggesting C_4 dominated landscapes occurred earlier in Africa then elsewhere, attributed to some combination of low pCO_2, arid conditions, or shifts in fire regimes (Tipple and Pagani 2007).

The isotopic fractionation between atmospheric carbon from CO_2 and bulk leaf tissue stable carbon isotope ($\delta^{13}C$) values (Farquhar et al. 1989) and compound specific $\delta^{13}C$ values of leaf waxes (Diefendorf and Freimuth 2017; Diefendorf et al. 2010) has been well documented. Carbon isotope use in long-chain leaf wax compounds relates to plant physiology, regional environmental parameters, and large-scale climate and atmospheric controls (Ehleringer et al. 1997; Farquhar et al. 1989). C_3 and C_4 plants both fix carbon using the Calvin-Benson pathway (biochemical reactions within the plants), where CO_2 and a 5-carbon sugar (ribulose bisphosphate, known as RuBP) are converted into 3-phosphoglycerate. This reaction converting CO_2 into sugars is catalyzed by the enzyme ribulose bisphosphate carboxylase oxygenase (RuBisCO), which creates a large fractionation against ^{13}C, 29‰ for higher plants (Farquhar et al. 1989), accounting for lower $\delta^{13}C$ values for plants than $\delta^{13}C$ of CO_2 in the atmosphere.

In C_4 plants, prior to the Calvin-Benson fixation, CO_2 is fixed via carboxylation of phosphoenolpyruvate (PEP) by PEP-carboxylase (PEP-C). C_4 efficiency in CO_2 uptake begins with the enzyme PEP-C, which has a strong preference for CO_2 and not O_2. The 4-carbon acid (oxaloacetate) that is produced is then reduced and moved from the outer cells (mesophyll cells) to the inner cells (bundle sheath cells). CO_2 is then released by decarboxylation, and then Calvin-Benson fixation begins. When temperatures are higher or atmospheric CO_2 concentrations are lower, RuBisCO can react with O_2, the process known as photorespiration, wasting some of the energy generated from photosynthesis. Today, this reduction by photorespiration is 30–40% of the photosynthetic rate (Ehleringer and Monson 1993; Pearcy and Ehleringer 1984). C_4 plants have a physical separation of cells (bundle and mesophyll cells) that keeps CO_2 stomatal uptake and CO_2 fixation separate. Thus, there is a separation of cells where CO_2 is concentrated, keeping nearby cells saturated in CO_2 and ensuring

RuBisCO uses CO_2 instead of O_2, eliminating the energy loss through photorespiration.

In low pCO_2 environments, C_4 plants have an advantage over C_3 plants due to the PEP-C enzyme and CO_2 concentrating mechanism (Ehleringer et al. 1997; Sage et al. 1999; Tipple and Pagani 2007), which leads to ^{13}C enrichment of their tissues compared to C_3 plants (tissues that have less negative $\delta^{13}C$ values because they have more ^{13}C and less ^{12}C). The isotopic difference is related to fractionation associated with RuBisCO discrimination and slow leak of enriched CO_2 from the leaf vascular tissue. C_4 plants decrease their stomatal opening, reducing transpiration while maintaining an equivalent amount of carbon fixation than C_3 plants could in similar environments. Ultimately, this results in higher water use efficiency in C_4 plants than C_3 plants, making C_4 plants better adapted to warmer, more arid conditions (Cerling et al. 1997; Ehleringer et al. 1997; Rommerskirchen et al. 2006). Therefore, C_4 vegetation dominates locations where these adaptations allow survival over C_3 vegetation, such as tropical and subtropical ecosystems (Sage 2004; Sage et al. 2011). However, in higher pCO_2 environments, C_4 vegetation is not favored due to the higher energy requirement necessary for this photosynthetic pathway (Ehleringer et al. 1997). Physiological advantages of C_3 and C_4 plants in different environments and variability of pCO_2 likely played a significant role in vegetation distribution over geologic intervals. For example, it is thought that C_4 vegetation (such as many tropical grasses) dominated the tropics during glacial intervals (Ehleringer et al. 1997). Additionally, within a given environment, C_3 plants seem to respond to the availability of water by producing lower $\delta^{13}C$ values in wetter conditions (Marshall et al. 2007). This may have an impact on vegetation $\delta^{13}C$ reconstructions during wetter and more arid intervals, even if vegetation type does not vary significantly.

The environmental and climate preferences of C_3 and C_4 vegetation allow for reconstruction of environments using stable isotopes. In sediments, this requires compound specific isotopes to distinguish terrestrial vegetation isotope variability from aquatic or bacterial inputs. Variations in $\delta^{13}C$ values have been used to reconstruct past landscape vegetation variability, by means of photosynthetic pathway variability of C_3 plants and C_4 plants. On average, the isotopic signature of plants utilizing these photosynthetic pathways is distinct (Cerling et al. 1997; Dawson et al. 2002; Farquhar et al. 1989). Modern bulk plant isotope values for C_3 are more depleted in ^{13}C (ranging from −19 to −35‰) relative to C_4 plants (ranging from −10 to −16‰) (Cerling and Harris 1999). These isotope values are also reflected in the isotopic values of individual compounds from plants. For example, plants using the C_4 pathway are ^{13}C enriched (more positive $\delta^{13}C$ values) relative to C_3 plants, with an idealized endmember value of ∼−21.4‰ (for the C_{29} *n*-alkane) (Castañeda et al. 2009a). Plants utilizing the C_3 pathway are more ^{13}C depleted (more negative $\delta^{13}C$ values) on average than C_4 plants, with a cited endmember of −34.7‰ (C_{29} *n*-alkane) (Castañeda et al. 2009a).

CAM plants, the third photosynthetic pathway, keep stomata shut during the day but open at night, and isotopically resemble an intermediate of C_3 and C_4 $\delta^{13}C$ values (Deines 1980; Farquhar et al. 1989; O'Leary 1981; Osmond et al. 1973). This method of carbon-fixation is found primarily in arid desert plants, especially succulents. A large input of CAM plant waxes to the sediments would complicate the interpretation of C_3/C_4 vegetation reconstruction, as their intermediate isotope values are difficult to distinguish from these other plants (Osmond et al. 1973). However, overall *n*-alkyl production of CAM plants is low relative to C_3 and C_4 vegetation (Black Jr 1973), and thus reconstructions are expected to be unbiased from the insignificant contributions of CAM *n*-alkyl lipids with intermediate $\delta^{13}C$ values.

Simple two-member C_3/C_4 mixing models have been used to assess the relative contributions of vegetation types to sedimentary sequences (e.g., Berke et al. 2014; Schefuß et al. 2003; Sinninghe Damsté et al. 2011b). These estimates were more recently updated to include a ±20% uncertainty estimate based on a small dataset that showed the variability in endmember values (Castañeda et al. 2009a). A recent paper by Diefendorf et al. (2010) found a large range of $\delta^{13}C$ values for globally-distributed C_3 vegetation. Using >3,000 leaf measurements from trees and shrubs at 105 locations on 5 continents, the authors observed values that range from −34.9‰ to −21‰ for C_3 plants (Diefendorf et al. 2010). This finding suggests that a simple binary model cannot be used to assign a relative percentage of C_3/C_4 vegetation from $\delta^{13}C$ values unless there is other information to constrain vegetation type in a specific environment (Dawson et al. 2002; Diefendorf et al. 2010). While the range of variability of C_3 vegetation precludes the use of a single isotopic endmember, it does not prevent the use of $\delta^{13}C$ values to indicate relative trends in C_3/C_4 vegetation change on the landscape. For example, Cerling et al. (2011), showed that there was a nonlinear relationship between woody cover and $\delta^{13}C$ values of soil organic matter, due to C_3 plants being found in open or wooded environments. A large compilation of $\delta^{13}C$ values indicated a continuum between woody to herbaceous plants and C_3 to C_4 vegetation (Cerling et al. 2011). Following this, Magill et al. (2013a, b) compiled $\delta^{13}C$ values for soil organic matter, bulk leaves and leaf *n*-alkanes for 64 plant species and 288 soils from 289 subtropical and tropical geographic sites. The authors measured lipid-leaf fractionation factors and compared them to soil organic matter-leaf fractionation factors to establish relative C_3 and C_4 contribution shifts through time for hominin sites in northern East Africa (Fig. 8.2). For these sites, the study found the same fractionation between soil organic matter $\delta^{13}C$ (with respect to bulk leaf $\delta^{13}C$) and $\delta^{13}C$ of C_{31}

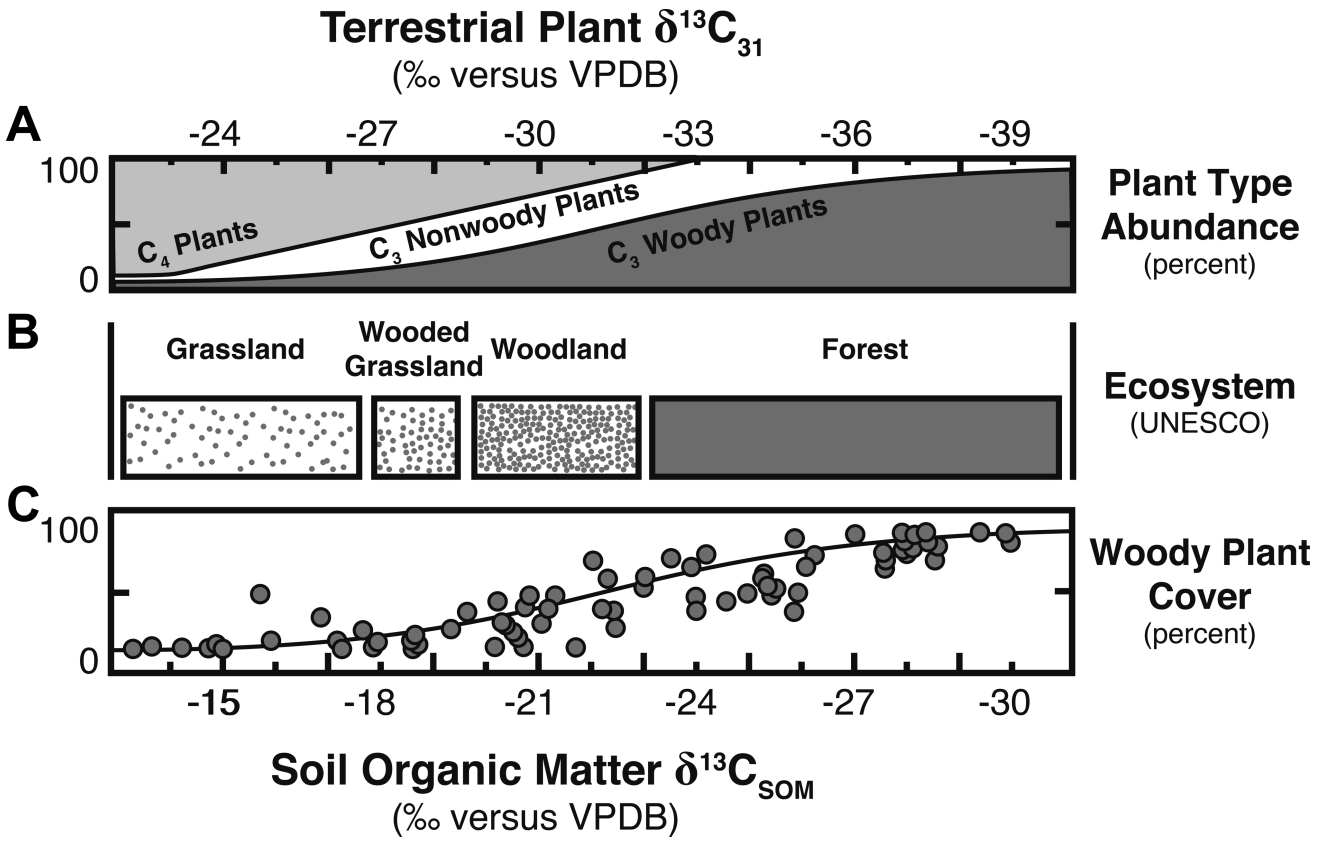

Fig. 8.2 A relationship for the Olduvai region of eastern Africa between $\delta^{13}C$ of soil organic matter (lower axis) and $\delta^{13}C$ of C_{31} plant wax alkane (upper axis) compared to: A, plant type (C_4 plants, C_3 nonwoody plants, and C_3 woody plants); B, UNESCO ecosystem designation; and C, percentage plant woody cover. The isotopic offset (fractionation) for C_3 and C_4 plants relative to soil organic matter were both 9‰, which allowed for this comparison. Reproduced from Magill et al. (2013a)

n-alkane (with respect to bulk leaf $\delta^{13}C$) for both C_3 plants and soils and C_4 plants and soils (9‰). This allows for the estimation of $\delta^{13}C$ of soil organic matter from leaf wax $\delta^{13}C$ and the prediction of woody cover and C_3/C_4 vegetation change from this region using the scheme shown in Fig. 8.2.

Another approach for C_3/C_4 vegetation estimation involved a mixing model but with a constrained third end-member, specifically considering pine (or general evergreen) waxes, for reconstructing vegetation shifts for ~62,000 years from a sedimentary record from Lake Tulane, Florida (Huang et al. 2006). In this case, pollen was used to assist in the reconstruction of vegetation shifts between C_3 and C_4. Huang et al. (2006) showed a 40% increase in C_4 vegetation during the Last Glacial Maximum (LGM, ~21,000 cal years B.P.) compared to the Holocene and attributed the increase to increased regional aridity. Although there is low production of *n*-alkanes from evergreens (Diefendorf et al. 2011), Huang et al. (2006) also showed an isotopic excursion of 4−5‰ interpreted as an increase in pine contribution to the system from the Holocene to present.

In another example, $\delta^{13}C$ was used to reconstruct vegetation variations across North America during the Miocene. In sediments from the Gulf of Mexico (DSDP Site 94), $\delta^{13}C$ of *n*-alkanes illustrated the possible evidence of C_4 vegetation prior to the Middle Miocene, with a significant rise in C_4 vegetation concurrent with the Middle Miocene Climatic Optimum (~14 Ma). Tipple et al. (2010) corrected $\delta^{13}C$ values of sedimentary wax compounds for shifting value of atmospheric CO_2 during the Miocene using reconstructed $\delta^{13}C_{CO2}$ from a benthic foraminifera reconstruction (Tipple et al. 2010; Tipple and Pagani 2010). C_{29} ranged between −26.2‰ and −30.9‰ and C_{31} ranged between −24.8 and −30.2‰. These authors posited that CO_2 wasn't necessarily the driver of C_4 vegetation expansion and that hydroclimate helped grassland expansion in North America (Tipple and Pagani 2010).

Recent research has shown that integration of multiple proxies, comparing biomarker stable isotopes with stable isotopes from other archives, can provide a powerful complimentary dataset of terrestrial changes from both marine and continental archives. Uno et al. (2016) observed $\delta^{13}C$ values from terrestrial leaf wax *n*-alkanes (C_{31} and C_{35}) extracted from a marine sediment core off the east coast of Somalia (in the Somali Basin) with a ^{13}C enrichment beginning at 10 Ma (Fig. 8.3). This was used as evidence for

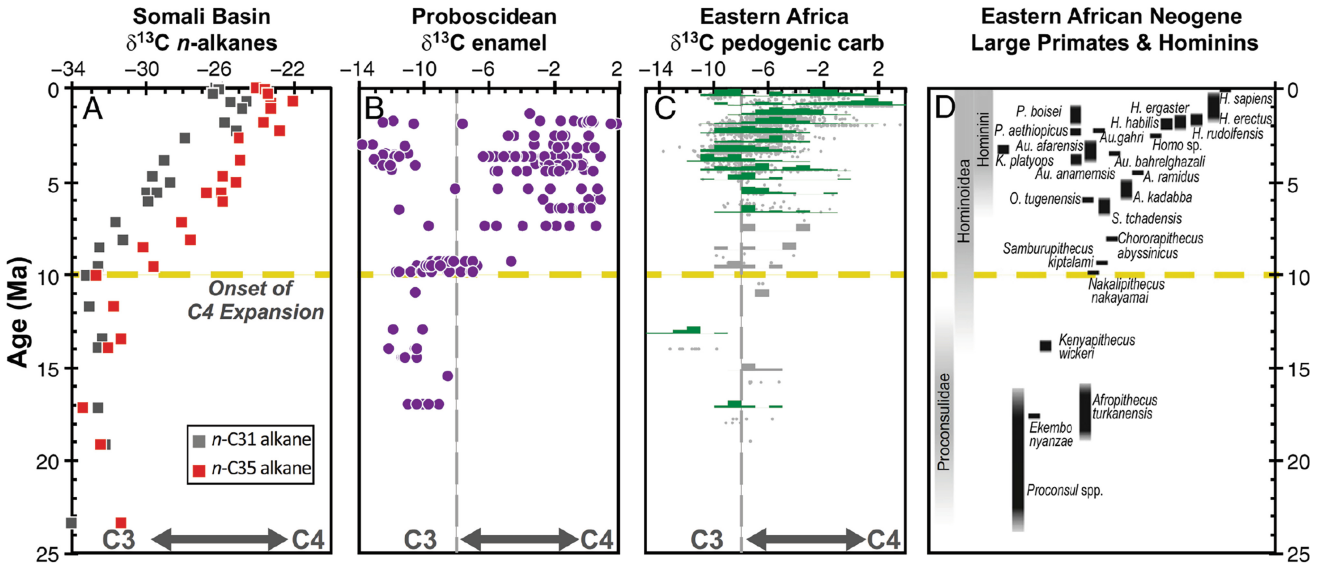

Fig. 8.3 Multiproxy data from eastern Africa showing comparison between leaf wax compound and: A, specific stable isotopes ($\delta^{13}C$ of C_{35} and C_{31} alkanes); B, tooth enamel stable isotopes ($\delta^{13}C$ eastern and central African proboscideans); C, soil carbonate stable isotopes ($\delta^{13}C$ from pedogenic carbonate in eastern Africa); and D, age ranges of Neogene primate taxa from eastern Africa. Reproduced from Uno et al. (2016). Data show good integration of terrestrial lipid isotopes, sourced from leaf wax lipids from marine sediment cores recovered from the Somali Basin, and terrestrial soil and fossil isotopes, sourced from around eastern and central Africa. Uno et al. (2016) interpreted these data as indicating the onset of C_4 vegetation expansion in the region, at 10 Ma, observed in ^{13}C enrichment (3‰) in leaf wax lipids and diet shift to inclusion of C_4 vegetation by proboscideans at this time

the expansion of C_4 vegetation in eastern Africa, alongside data from proboscidean fossil tooth $\delta^{13}C$ values (indicative of a diet shift from C_3 to some C_4 plants), and soil carbonate $\delta^{13}C$ values (a proxy of landscape vegetation). The agreement between these records strengthens the interpretations made and provides evidence of the way that biomarker proxies can complement other geochemical analyses for paleoenvironmental reconstruction.

Reconstructing Patterns of Aridity Using $\delta^{13}C$

In many carbon isotope studies from the tropics and subtropics, $\delta^{13}C$ values of leaf wax compounds have been used as a general indicator of aridity, due the adaptations of C_4 plants to hot, arid conditions (Sage et al. 1999). In these cases, leaf wax $\delta^{13}C$ is used as a general proxy for changes in landscape aridity based on the physiological advantage of the C_4 photosynthesis. C_3 plants dominate the global natural landscape productivity, but C_4 plants play an important role in specific ecosystems (Ehleringer et al. 1997). In the tropics and subtropics, two-thirds of all grasses are C_4 vegetation (Sage et al. 1999). Tropical savannas are also overwhelmingly dominated by C_4 plants, in this case composing more than 90% of the vegetation (Sage et al. 1999). These environmental advantages and skewed landscape distribution allow stable carbon isotopes, specifically proportions of C_4 vegetation, to be used as general proxies of aridity.

Leaf wax compound specific $\delta^{13}C$ is commonly used to reconstruct patterns of aridity across East Africa. For example, Castañeda et al. (2007) and Johnson et al. (2016) used changes in $\delta^{13}C$ of *n*-alkanes from Lake Malawi sediments to reveal aridity patterns in southeastern Africa. A record of $\delta^{13}C$ of the last 23 ka indicated that Lake Malawi was arid during the Last Glacial Maximum (~23 −21 ka), Younger Dryas (~13−11.5 ka), early Holocene (11–7.7 ka) and Little Ice Age (~1300−1850 AD) (Castañeda et al. 2007). Extending the Lake Malawi record back to 1.3 Ma, Johnson et al. (2016) observed a progressive ^{13}C depletion that matched the calcium XRF (X-ray Fluorescence) data, with concentrations of calcium a proxy for open/closed status of the basin (Fig 8.4). Together, these data suggest an increase in wetter conditions in southeastern Africa for the last 1.3 Ma, punctuated by abrupt "megadroughts" seen throughout the record in both proxies of aridity (Johnson et al. 2016). These records would suggest that during drier intervals, the proportion of C_4 vegetation was greater, using the C_3/C_4 relationship for East Africa proposed by Magill et al. (2013a) shown in Fig. 8.2.

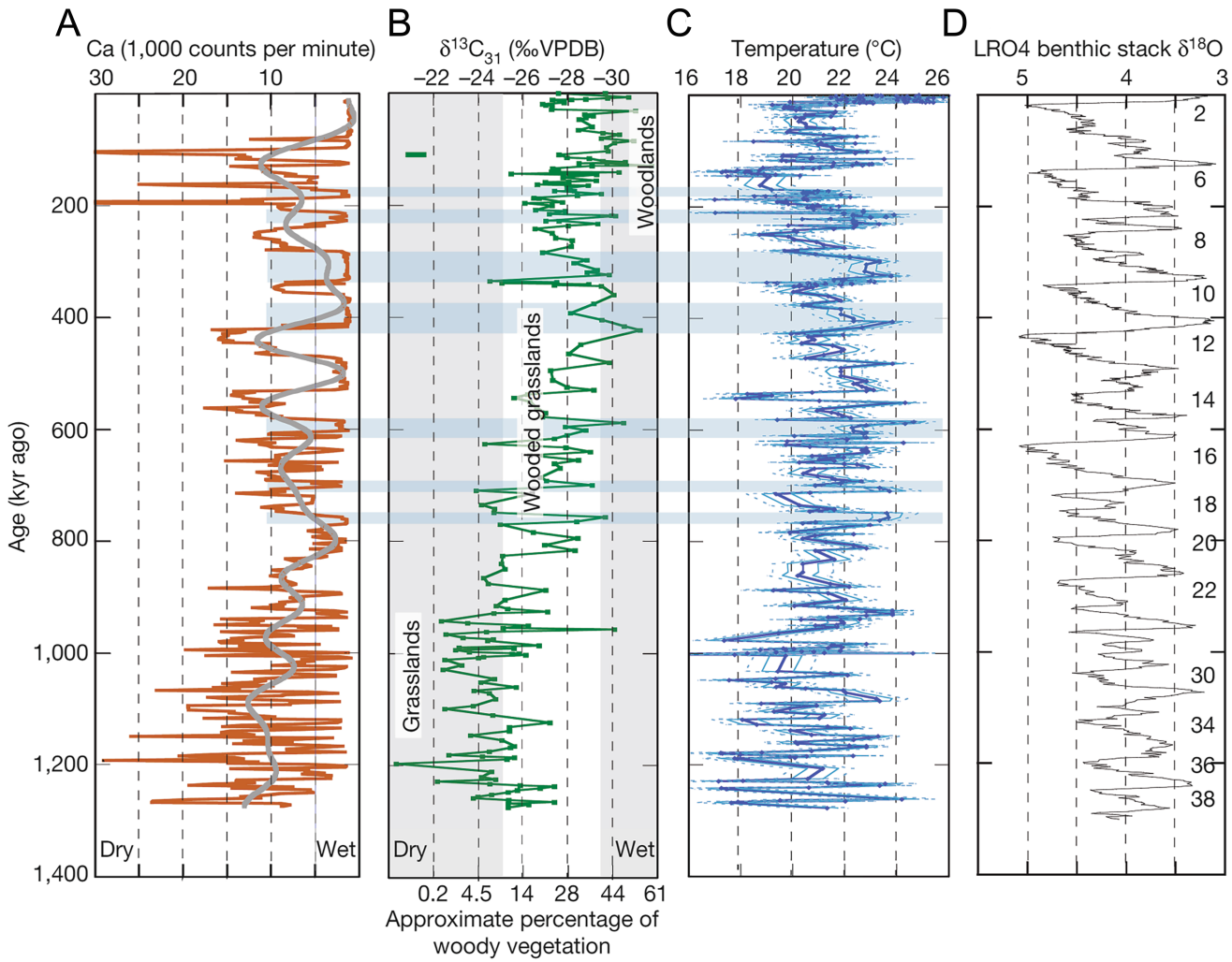

Fig. 8.4 Multiproxy records from Lake Malawi, East Africa, were interpreted to show a change from more arid conditions, to overall wetter climate with long periods of generally stable conditions in hydroclimate over the last 1.3 Ma (reproduced from Johnson et al. 2016). A, calcium concentrations by XRF analysis, with greater variability and higher amplitude shifts between low and high calcium abundances prior to 900,000 years ago indicating wetter and more arid conditions, respectively; B, $\delta^{13}C$ of C_{31} alkane with a trend towards wetter conditions and increased woodlands; C, application of TEX_{86} temperature reconstruction (with 1σ and 2σ uncertainties from analytical and lapse rate corrections in solid and dashed lines, respectively) from a lacustrine setting, with shaded horizontal bars across data panes indicating alignment of warmer, wetter conditions during some of the interglacial periods; D, LR04 stack of Lisiecki and Raymo (2005) showing benthic oxygen isotope stages (glacial proxy indicator) for comparison

δD of Leaf Wax Compounds for Hydroclimate

Evidence of dynamic water processes are preserved in the hydrogen isotopic composition of organic compounds from sediments. Water used in terrestrial plant photosynthesis can be traced back to the composition of meteoric water (Gat 1996), and thus can serve as a paleohydroclimate proxy. The δD values of long-chain, terrestrially-derived sedimentary leaf wax compounds show a strong linear relationship to precipitation δD values across a wide range of environments (Hou et al. 2008; Huang et al. 2004; Sachse et al. 2004). Meteoric water is the ultimate source of the hydrogen values of this organic matter, the isotopic composition of water is significantly modified by biological and physical processes (Sachse et al. 2012). Understanding these processes is critical in the interpretation of δD leaf wax values (Fig. 8.5).

The use of hydrogen isotopes of long-chain leaf compounds avoids complications associated with bulk organic matter hydrogen isotopes analyses. Specifically, establishing the relationship between measured δD of leaf wax compounds and meteoric water may be easier in regions with more well constrained vegetation types or similar fractionations. Long-chain leaf wax hydrogen isotope values (δD) reflect the plant source water with a certain offset that is

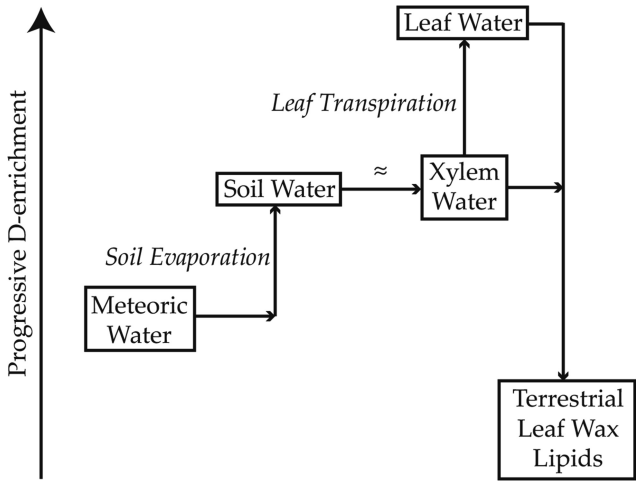

Fig. 8.5 Schematic figure illustrating potential fractionations between plant source water (precipitation) and leaf wax lipids from terrestrial plants used to reconstruct past vegetation and hydroclimate (not drawn to scale). Processes such as soil evaporation and leaf transpiration can act to alter hydrogen isotope values in leaf water, the ultimate source of water used in leaf wax lipid synthesis. Xylem water is often used as a proxy of soil water utilized by the plant due to a lack of fractionation in this uptake. Diagram modified after Sachse et al. (2006) and Smith and Freeman (2006)

dependent on physiological and environmental variables (Chikaraishi and Naraoka 2003; Sachse et al. 2004; Sauer et al. 2001; Sessions et al. 1999). However, even while constraining biosynthetic fractionation by focusing on specific lipids and homologues, there is additional complexity in reconstructing past precipitation based on lipid isotopes (Fig. 8.5). While applications for reconstructing past hydroclimate stress the importance of δD of long-chain leaf waxes as a powerful tool (e.g., Hou et al. 2008; Huang et al. 2004; Sauer et al. 2001), new examinations of modern plants have helped refine the relationship between source water and lipid δD in order to create more quantitative reconstructions of past hydroclimate variability (Sachse et al. 2012).

Variability in environmental water δD values reflects one of the largest determinates of δD values of leaf waxes. The geographic variability in source water can be summarized by the Rayleigh distillation processes (Craig 1961; Dansgaard 1964). Rayleigh distillation fractionates isotopes, with values more negative farther from the source of precipitation. The effects of atmospheric temperature, precipitation amount, latitude, and continentality on meteoric water isotope values (Dansgaard 1964) are reflected in δD values of leaf wax lipids. The temperature effect, predominantly seen outside of tropical regions, results in a strong correlation of isotopic variability and the associated temperature of rainout, such that there is a larger fractionation associated with colder temperatures. The amount effect is most evident in tropical regions where temperature associated fractionation is minor, shows strong correlation between D-depletion and higher rainout rates. Lastly, continentality, or distance the air mass travels from its source, results in a progressive shift towards lower δD values as movement of air masses travel inland. Across a wide range of geographic scales, there is a D-depletion of precipitation as latitude increases (Bowen and Revenaugh 2003; Craig and Gordon 1965).

Hydrogen isotope values of soil water used by plants can also be D-enriched relative to soil water through evaporative effects (Allison et al. 1983; Barnes and Allison 1983), and this process can be expressed in leaf wax lipids (Sachse et al. 2004; Sauer et al. 2001). Effects of soil evaporation are most evident in the uppermost sections of the soil, where the influence of evaporation is the major control on isotopic value beyond source water value. Initial studies of plant tissues found evidence of the influence of evaporation on plant water δD, particularly in desert plants with shallow rooting depths (Ehleringer and Dawson 1992; Ehleringer et al. 1991; Williams and Ehleringer 2000). Plants with deeper rooting depths, such as those in arid environments that are trying to tap into groundwater may not be affected by surface evaporation (Dawson 1993) or may switch between sources depending on the regularity of precipitation events (White et al. 1985). However, it is also important to note that deeply stored groundwater is often an integrated signal of precipitation (Berke et al. 2015a; Ehleringer et al. 1991).

Transpiration can also produce higher δD values in leaf waters relative to xylem and source waters (Feakins and Sessions 2010; Sachse et al. 2006; Smith and Freeman 2006). Fractionation of water isotopes has not been observed during uptake of soil water by roots in most plant types, confirming that xylem water can be used as a proxy for source water (Ehleringer and Dawson 1992; Ehleringer et al. 1991, 1998). However, after initial uptake, xylem water may be modified by water transpiration in the leaves from leaf stomata. The degree of isotope enrichment in plant leaves is controlled by physiological and environmental factors including vapor isotopic composition, relative humidity, and temperature. The relative importance of these processes is still debated, with studies interpreting the majority of meteoric water D-enrichment is from soil evaporation (Hou et al. 2008) or transpiration (Feakins and Sessions 2010; Yang et al. 2009).

In order to quantify the influence of environmental or physiological factors on leaf wax δD, fractionations between source water and lipids are often discussed. Net fractionation (also called apparent fractionation) between source water and plant wax lipid (Fig. 8.5) accounts for evapotranspiration fractionations and any biosynthetic fractionations (Sachse et al. 2006; Smith and Freeman 2006). Sedimentary basins are likely to integrate plant lipids of multiple plant life forms, with a mixture of different biotic fractionations. The differences in isotope fractionation among these plants in some cases may be exacerbated by climate variability across the integrating region. Significant variability in δD

fractionation have been described within a single species and single chain length, from −204‰ to −34‰) (Sachse et al. 2012). These differences can be attributed to some combination of environmental and climate factors, including, but not limited to, microclimate, seasonality of wax synthesis, and canopy position of leaves (Sachse et al. 2012). It is important to compare δD values within a compound class and chain length because significant variability within a pathway has been observed due to large enzymatic effects (Chikaraishi et al. 2004, 2009).

Applications of δD for Hydroclimate Reconstruction

Fractionations in modern environments are understudied, leading to an incomplete understanding of the likely variations between source water and wax δD. In order to improve quantitative reconstruction of past hydroclimate, studies have utilized other means of constraining environmental and physiological variables, with independent vegetation reconstruction using pollen or carbonate analyses (Feakins 2013; Magill et al. 2013b).

While there are still many outstanding questions regarding the environmental and physiological factors influencing δD values of leaf wax compounds, the use of this proxy for qualitative hydroclimate variability continues to grow. For example, applications of δD for hydroclimate reconstruction in the tropics have been used to describe changes in the amount of precipitation through time. One of the earliest records to provide insights into hydroclimate of tropical Africa using δD values of leaf wax compounds was a marine sediment core off the coast of the Congolese River catchment (Schefuß et al. 2005a). This core spanned the last 20,000 years and contained waxes integrated from a large catchment covering much of central Africa. Schefuß et al. (2005b) interpreted the lower δD values of leaf wax compounds as indicating wetter conditions and higher δD values representing drier conditions in the Congolese basin. The authors concluded that the largest influence on the δD values was from the precipitation amount affect. The predominant control on the hydroclimate of central Africa for the last 20,000 years was shifting trade winds that would bring monsoonal moisture to the region and lower δD values (Schefuß et al. 2005a).

In another location from Africa, the variability in a sedimentary δD record from Lake Malawi was the result of shifting sources of region precipitation (Konecky et al. 2011). The 140,000 year record showed changes in atmospheric circulation over southeastern Africa, with lower δD values associated with Lake Malawi moisture derived from the descending limb of the proximal Hadley circulation (Konecky et al. 2011). Lake level proxies and δD values prior to 56,000 years ago show extreme variations in hydroclimate, and then show a shift to more stable, wetter conditions with progressive D-enriched leaf wax *n*-alkanoic acid values.

In high latitude settings, δD values have the potential to inform about both the isotopic composition of rainfall and temperature, because temperature and rain-out history are highly correlated (Dansgaard 1964; Jouzel et al. 1997). Modern measurements constrain the relationship between temperature and δD of precipitation and allowed Feakins et al. (2012) to estimate the isotopic value of paleoprecipitation (∼−50‰) in the middle Miocene from 20 to 15.5 Ma on the margins of Antarctica using long-chain (C_{28}) *n*-alkanoic acids from sediment cores. Their reconstructions suggested that vegetation coverage on the landscape peaked during the warmest intervals during the Middle Miocene, from 16.4 to 15.7 Ma.

Plant Lignin

Lignin is an abundant component of wood and terrestrial plant tissues (Hedges and Mann 1979) and is well preserved in older sediments. Environmental reconstruction studies in need of additional vegetation differentiation and estimates of continental material transportation can be supplemented with lignin data. Of particular importance for paleoreconstruction is the relative abundances of three phenolic polymers and their ratios to one another: syringyl, vanillyl, and cinnamyl (Goni and Thomas 2000) (Fig. 8.6). Cinnamyl phenols are only found in non-woody tissues, in both gymnosperms and angiosperms (Hedges and Mann 1979). Syringyl phenols are overwhelmingly found in nonwoody and woody angiosperms tissues (Hedges and Mann 1979). Vanillyl phenols are the most general, found in all land plants. However, vanillyl phenols are the only lignin phenol found within woody gymnosperm plant tissues. As such, the ratio of cinnamyl/vanillyl phenols can be used for reconstructions of non-woody to woody vascular plant materials. The ratio of syringyl/vanillyl phenols can be used to look at the relative contributions of angiosperm and gymnosperm plants. These ratios have been used to distinguish between woody/non-woody, angiosperm/gymnosperm, and are particularly powerful when used in combination with $\delta^{13}C$ to distinguish between relative shifts in C_3 and C_4 vegetation. However, studies have suggested that while generally resistant to degradation, syringyl and cinnamyl are less resistant to oxic degradation than vanillyl (Goni et al. 1993). Any differential degradation would skew paleoreconstructions, and thus caution should be exercised where oxic degradation may be possible. Additionally, the degree to which this degradation has affected samples can be assessed by studying the ratio of acid phenols to aldehyde phenols (Hedges and Van Green 1982).

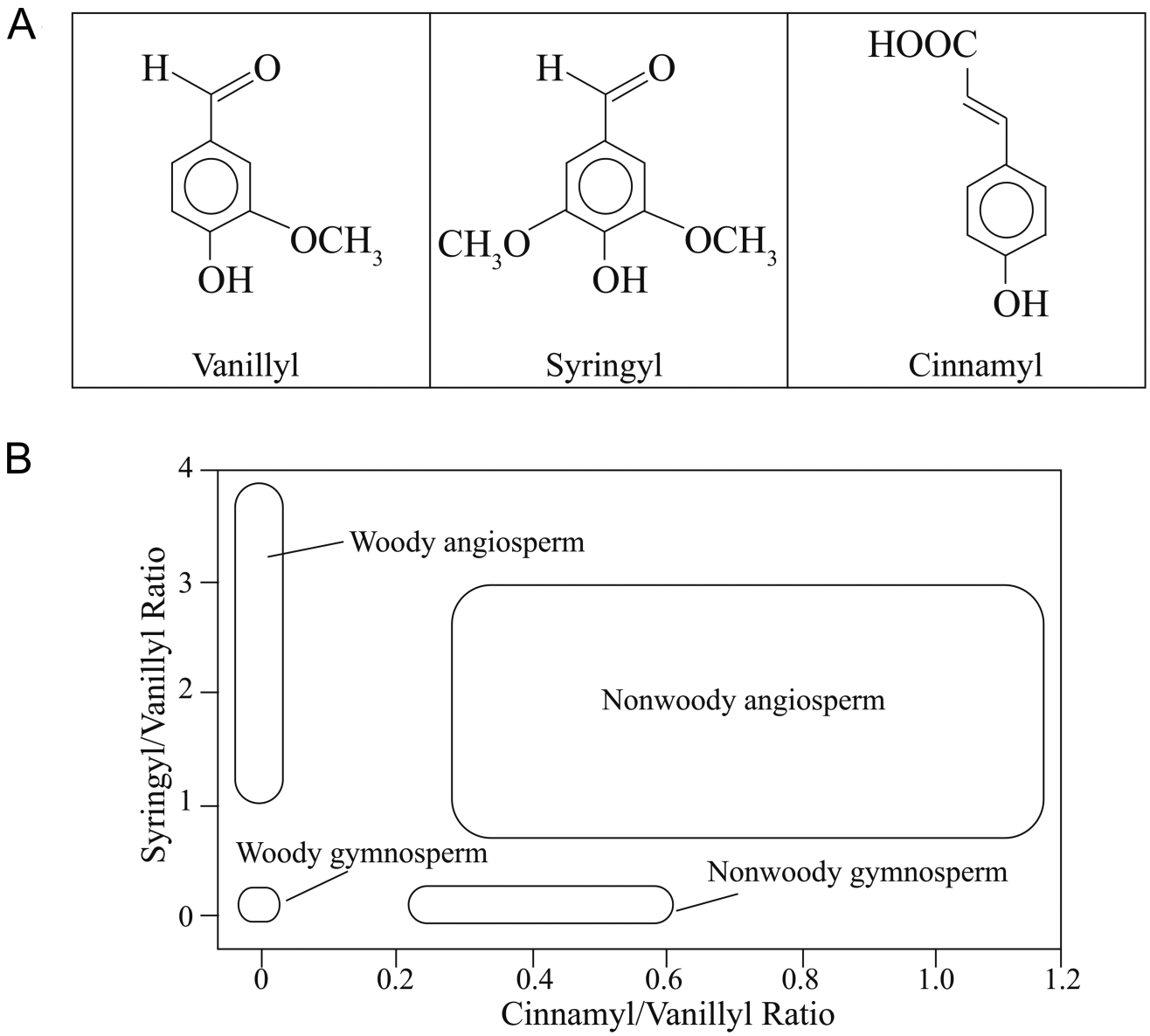

Fig. 8.6 A. Phenolic monomer structures from vascular plant tissues: cinnamyl, syringyl, and vanillyl. B. Lignin phenol crossplot of syringyl/vanillyl ratio to cinnamyl/vanillyl ratio. The crossplot of lignin phenol ratios can be useful in interpreting angiosperm or gymnosperm terrestrial vegetation as well as woody or nonwoody source vegetation. Modified from Hedges and Mann (1979)

Lignin Applications

Early studies of lignin often used phenol abundances to look at the transport of organic matter across the landscape. In the Columbia River drainage basin, lignin phenols were used to examine the accumulation of terrestrial plant matter gathering in the river sediments and study how closely the sedimentary tissues matched with vegetation for the local landscape (Hedges et al. 1984). The authors found large quantities of nonwoody angiosperm tissues in the Columbia River sediments, with vascular land plants composing ~90% of the organic matter found in coastal sediments. For Hu et al. (1999), interpretations of fossil pollen and lignin provided additional information for reconstruction. Their research of Alaskan lake sediments showed a strong similarity between both vegetation proxies; however, as specific pollen types are rich in only certain phenols they suggested that there may be a bias in the lignin record due to preservation. Castañeda et al. (2009b) used $\delta^{13}C$ and distributions of phenols to reconstruct paleovegetation of Lake Malawi sediments since the Late Pleistocene. These authors interpreted the Lake Malawi sedimentary record of cinnamyl/vanillyl phenols as primarily due to inputs of

woody C_3 trees to C_4 non-woody grasses. Lignin results supported the $\delta^{13}C$ values from *n*-alkanes, which showed an increase of C_4 vegetation during dry intervals of the Last Glacial Maximum and Younger Dryas during the Holocene.

Proxies For Terrestrial Paleotemperature

Long-Chain Alkenones

Long-chain alkenones are one of the longest standing organic geochemical proxies for past temperature, but they are not found in all lacustrine settings and so are primarily applied for marine surface temperature reconstructions. Therefore, this review will cover fewer details of this proxy. The reader is encouraged to seek out specific reviews of alkenone temperature calibrations and reconstructions for further information (e.g., Herbert 2001).

The $U^{K'}_{37}$ Index is based on long-chain alkenones found in haptophyte algae that are comprised of C_{37}–C_{39} di- and tri-unsaturated ketones, where the degree of unsaturation has a strong positive relationship with growth temperature (Prahl and Wakeham 1987). The proxy is based on the relative proportions of these unsaturations, which are produced by haptophyte algae, primarily *Emiliania huxleyi*, but also by *Geophyrocapsa oceanica*, *Isochryss galbana*, and *Chrysotila lamellosa*. The $U^{K'}_{37}$ Index is defined as the ratio of abundances of C37:2/(C37:2 + C37:3), and is used to estimate surface water temperatures (Prahl et al. 1988) using a core top calibration that is based on modern sediment $U^{K'}_{37}$ Index values and modern water temperatures. It has been observed that $C_{37:4}$ and C_{38} homologues are the most abundant for lacustrine systems (Pearson et al. 2008; Zink et al. 2001), but more research is necessary to determine if this is a characteristic feature of all lacustrine basins. Marine temperature calibrations cannot be applied to lacustrine alkenone records because of the abundance of $C_{37:4}$ to $C_{37:2}$ provides temperature estimates that are too cold (Zink et al. 2001).

The limited presence of alkenones in lakes regionally as well as through time (Zink et al. 2001) and poor correlation with temperature in many terrestrial locations because of the lack of a broadly applicable calibration (Toney et al. 2010) have made this proxy of somewhat limited use in terrestrial (lacustrine) settings. Additionally, in both marine and lacustrine settings there are regions where it is difficult to apply the $U^{K'}_{37}$ Index because global temperatures are at the upper limit of the temperature range that can be measured using this proxy (~27−28°C) (Herbert 2001). However, terrestrial settings where alkenones have been found and used to reconstruct past temperature continue to increase (e.g., Innes et al. 1998; Jaraula et al. 2010; Toney et al. 2010). Where there is good correlation between lake surface temperatures and mean annual or some seasonal air temperature, alkenones can be used to reconstruct air temperature. This is likely to occur in systems where the haptophyte algae live in the uppermost water column or in systems where the basin is fairly homogeneous in temperature (even if only seasonally). Successful application of lacustrine alkenones is often done after determining the source organism and careful measurement of environmental parameters to establish a unique calibration for the site (D'Andrea et al. 2006, 2011).

Glycerol Dialkyl Glycerol Tetraethers (GDGTs)

Glycerol dialkyl glycerol tetraethers (GDGTs) are a major part of membrane lipids from specific marine and lacustrine Archaea and bacteria. The degree of cyclization (0−3 cyclopentane rings, GDGT I – III) increases with increasing growth temperature of the organisms, which can then be used to reconstruct temperatures for sites where they are found (Schouten et al. 2013 and references therein) (Fig. 8.7). Studies suggest that in hyperthermal environments many different GDGTs are formed from diverse Archaeal populations that do not have the same relationship between cyclization and temperature (Pearson et al. 2004; Schouten et al. 2007b). The phylum Thaumarchaeota and Euryarchaeota (Karner et al. 2001; Lipp et al. 2008) dominate in non-hyperthermal settings. Distinguishing between hyperthermophiles or methanogenic/methanotrophic Euryarchaeota (which do not show this temperature relationship) (Schouten et al. 2007b) and Thaumarchaeota is thought to be possible by looking at GDGT IV (often referred to as crenarchaeol), a GDGT suggested to be specific for Thaumarchaeota (Pitcher et al. 2011). The first application of GDGTs for paleoreconstruction using the TEX_{86} proxy described below was done in marine settings (Schouten et al. 2002), but subsequent confirmation of the presence of GDGTs with a correlation to air temperature in lakes was completed soon after (Blaga et al. 2009; Powers et al. 2004, 2005).

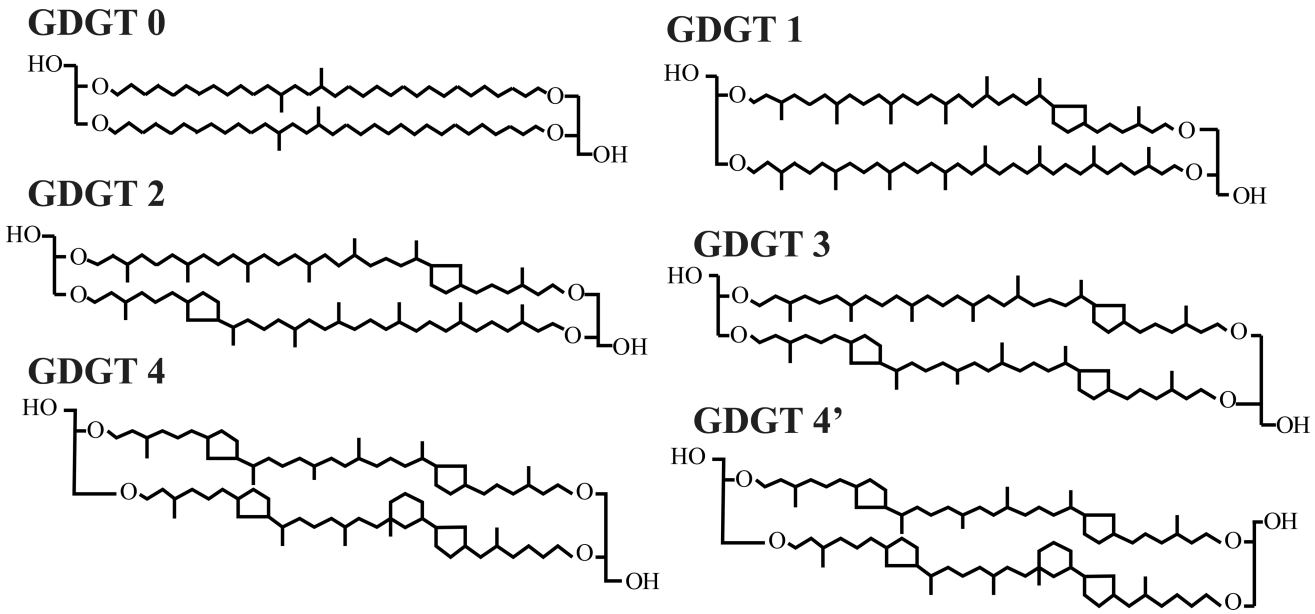

Fig. 8.7 Isoprenoid glycerol dialkyl glycerol tetraether (GDGTs) structures. GDGT 4 is crenarchaeol and GDGT 4' is crenarchaeol region-isomer

GDGTs, like leaf wax compounds, are relatively resistant to degradation, though there is evidence that they can undergo diagenetic alteration with time. Sedimentary evidence suggests that while GDGTs can degrade (Huguet et al. 2008), the TEX_{86} value and therefore the reconstructed temperatures, remain relatively unchanged within proxy error in sediments that have not undergone thermal maturity (Huguet et al. 2009; Schouten et al. 2004; Sinninghe Damsté et al. 2002a). Experimental research shows that more thermally mature sediments (>240°C) would have degraded GDGTs, with inhomogeneous alteration of GDGT structures and an overall decrease in TEX_{86} values (Schouten et al. 2004). Nevertheless, use of TEX_{86} as a temperature proxy has been widely successful since its discovery in 2002, supporting other temperature proxies where there is overlap in analyses, and with wider geographic and time application possibilities than many other paleoclimate proxies (Schouten et al. 2013; Spang et al. 2010).

TEX_{86} Paleotemperature Proxy

The proxy that utilizes the relative abundance of non-thermophilic, aquatically produced GDGTs by Thaumarchaeota is called the TetraEther Index of TetraEthers with 86 carbon atoms (TEX_{86}) (Schouten et al. 2002) (Fig. 8.7). TEX_{86} is an index that was mathematically defined by Schouten et al. (2002) as:

$$TEX_{86} = \frac{[\text{GDGT 2} + \text{GDGT 3} + \text{GDGT 4}']}{[\text{GDGT 1} + \text{GDGT 2} + \text{GDGT 3} + \text{GDGT 4}']}$$

The strong linear relationship between measured water and air surface temperatures suggest they are related and that the former can serve as a proxy for the latter because similar aspects of heating and cooling affect both (Powers et al. 2005). There are a series of different empirical calibrations available, all of which use measured sediment core-tops to determine a regional TEX_{86} value compared to measured water temperatures. One of the more recent global marine core-top calibrations (Kim et al. 2008) utilized 223 core-top sediment samples to create the formula:

$$T = -10.78 + 56.2 \times \text{TEX}_{86} \left(r^2 = 0.94\right)$$

Recent marine calibrations have used specific calibrations for low and high temperature waters, which have been difficult to capture in previous calibrations (Kim et al. 2010). The newest calibration efforts utilize a Bayesian statistical approach (BAYSPAR) to integrate global core-top data (Tierney and Tingley 2014; Tierney and Tingley 2015).

While there is significantly less data in lacustrine TEX_{86} calibrations, the TEX_{86} values from certain large lakes also show good correlation with surface water temperatures. The linear calibration of core-top lake data from Powers et al. (2010a) was based on 12 globally distributed large lakes (r^2 = 0.86) while Tierney et al. (2010a) was based on 13 globally distributed lakes, removing cold water lakes and focusing only on lakes with temperatures today >10°C (r^2 = 0.92). However, when these lacustrine calibrations are combined and cold sites are included (Castañeda and Schouten 2011), a total of 19 lacustrine core-top calibrations yields a good correlation as well in the calibration:

$$T = 54.889 \times \mathrm{TEX}_{86} - 13.363 \left(\mathrm{r}^2 = 0.89\right)$$

In lakes with small watersheds relative to surface area, TEX_{86} appears to reconstruct temperatures well. In many smaller lakes, high amounts of soil or *in situ* bacterial production of GDGTs can result in a poor correlation to water temperature, as seen in a European survey of lakes (Blaga et al. 2009).

There is still uncertainty surrounding the source organisms generating GDGTs within the phylum Thaumarchaeota. Specifically, a lack of cultures of the source organisms has prevented a rigorous test of the correlation versus causation of temperature to increases in cyclopentane moieties. For example, new pure cultures of marine ammonia-oxidizing Archaea (AOA), members of Archaea that show GDGT relationship with temperature is not only strain-specific, but is influenced by concentrations of O_2 (Qin et al. 2015). This ecophysiological response means that for certain marine AOA, O_2 limitation can drive TEX_{86} inferred temperatures warmer than measured temperatures. Additionally, where these organisms live may be an added complication. Studies have suggested there may be small quantities of soil-produced GDGTs that have a different relationship to temperature that can get transported to marine or lacustrine sediments (Weijers et al. 2006b). However, there are additional proxies (i.e., the BIT Index) that may help constrain the possibility of soil isoprenoid influence.

Perhaps a larger concern is aquatic Thaumarchaeota occurrence or movement within the water column. Thought to be primarily nitrifiers, Thaumarchaeota have been noted to occupy a niche in timing during the year that may be related to the presence of ammonium in the water column (Konneke et al. 2005; Wuchter et al. 2006). This can then manifest itself as a seasonal cycle of Thaumarchaeota GDGTs, which may only capture a specific seasonal range of temperature and not an integrated annual temperature (Blaga et al. 2010; Sinninghe Damsté et al. 2009; Woltering 2011). Recent work has suggested that in addition to a seasonal bias, Thaumarchaeota may not restrict their movements to the upper photic zone, and thus represent temperatures not associated with surface waters. In fact, a wide range of studies have observed Thaumarchaeota spread throughout the water column (e.g., Castañeda et al. 2009a; Huguet et al. 2007; Woltering et al. 2012; Woltering 2011). However, a study by Wuchter et al. (2005) using sediment trap data found that GDGTs in sediments are sourced primarily from the upper water column, even though Thaumarchaeota live in much deeper waters. This may help explain how TEX_{86} temperatures are so well-correlated to surface water temperatures. Additional studies are necessary to provide confidence in the correspondence between surface temperatures and GDGT location of lipid generation.

TEX_{86} Applications

Even though there are a significant number of complications surrounding the use of TEX_{86} as a paleothermometer, there have been many successful climate reconstructions across the Cenozoic using this proxy based on comparisons with independent proxies of temperature where available. Many TEX_{86} records have been generated from the East African Rift Lakes, such as from Lakes Malawi (Fig. 8.4), Turkana, Victoria, Albert, Challa, and Tanganyika (e.g., Berke et al. 2012a, b, 2014; Johnson et al. 2016; Powers et al. 2005; Sinninghe Damsté et al. 2012; Tierney et al. 2008; Woltering et al. 2011). These records show some similarities to one another, such as the timing of warming following the Last Glacial Maximum (Sinninghe Damsté et al. 2012), but show variability among lakes including amplitude of temperature shifts, which may be related to regional climate complexities or calibration complexities. The oldest reconstructions using TEX_{86} are from Late Jurassic marine sedimentary deposits (Carrillo-Hernandez et al. 2003; Jenkyns et al. 2012). Further extension into the past is limited by the preservation of the GDGT lipids and likely not due to the presence of Archaea generating these lipids. One interval that has undergone the highest amount of scrutiny is the extreme, abrupt warmth at the Paleocene–Eocene Thermal Maximum (PETM) ($\sim$55 Ma). A significant warming of $\sim$5°C was described using the TEX_{86} paleothermometer at the PETM (Zachos et al. 2006), supported by more recent modeling and TEX_{86} calibrations (Dunkley Jones et al. 2013), which also coincides with a similar warming revealed using $\delta^{18}O$ of planktonic foraminifera. Contemporaneous Arctic Ocean warming of <5°C (Sluijs et al. 2006) and Southern Ocean warming (Sluijs et al. 2011) of $\sim$7°C has also been described using the TEX_{86} paleothermometer. In both high latitude cases, absolute temperature values have been difficult to capture with climate models (Sluijs et al. 2006).

Where available in marine settings, comparisons with other available temperature reconstructions such as $\delta^{18}O$ and Mg/Ca from foraminifera and $U^{K'}_{37}$ match well. Examples of excellently preserved GDGTs have allowed multiproxy temperature comparisons including TEX_{86} and $U^{K'}_{37}$ to be generated from another critical climate interval, the Eocene-Oligocene boundary. A significant 3−5°C cooling was described from globally-distributed marine sediments, with similar trends and magnitudes between TEX_{86} and $U^{K'}_{37}$ (Liu et al. 2009). Further, an Eocene-spanning TEX_{86} record

from the Southern Ocean (Bijl et al. 2009) showed a similar record to the global ocean benthic $\delta^{18}O$ stack of Zachos et al. (2001).

Branched GDGTs

BIT Index: The branched to isoprenoid ratio of tetraethers, called BIT Index (Hopmans et al. 2004) relies on the relative abundances of branched GDGTs (brGDGTs) and crenarchaeol to estimate the ratio of soil to aquatic derived material entering a system (Hopmans et al. 2004) (Fig. 8.8). As such, the BIT Index has been used to estimate water runoff and landscape variability associated with water runoff through time. As previously described, crenarchaeol was thought to be produced solely by aquatic Thaumarchaeota, while brGDGTs are thought to be a marker for soil bacteria (Weijers et al. 2006b). However, our understanding of the physiological relationship of brGDGT proxies to the producer is incomplete as the majority of producing organisms have not been identified or cultured in a laboratory setting. The BIT Index (Hopmans et al. 2004) utilizes the equation:

$$BIT = \frac{[\text{GDGT I} + \text{GDGT II} + \text{GDGT III}]}{[\text{crenarchaeol} + \text{GDGT I} + \text{GDGT II} + \text{GDGT III}]}$$

BIT Index numbers therefore range from 0 (solely aquatically produced) to 1 (solely terrestrially/soil produced). In marine settings, BIT is often very low, unless samples are taken close to terrestrial sources (a river outflow, for example). This has allowed the BIT Index to be used for reconstruction of terrestrial runoff into sedimentary systems. In lacustrine systems, BIT values can often be high. In fact, in surveys of lake systems, >75% were found to have high BIT values, in this case defined as >0.5 (Blaga et al. 2010; Powers et al. 2010). Most of the lakes with lower BIT values are large lakes with smaller watersheds compared to their surface area (Blaga et al. 2010; Powers et al. 2010).

In addition to soil runoff into lakes leading to high BIT values, recent studies have found that brGDGTs are likely also produced within the water column. Suggestions of *in situ* production have come from varied global lakes (e.g., Blaga et al. 2009; Sinninghe Damsté et al. 2009; Tierney and Russell 2009). Observations of brGDGT abundances in deep waters that surpass those found in surface waters (Sinninghe Damsté et al. 2009) may support the idea that brGDGTs are being generated *in situ* by a yet-to-be-determined organism. In this case, care must be taken when using the BIT Index either as a proxy for sediment transport or as an indication of the validity of TEX_{86} temperatures.

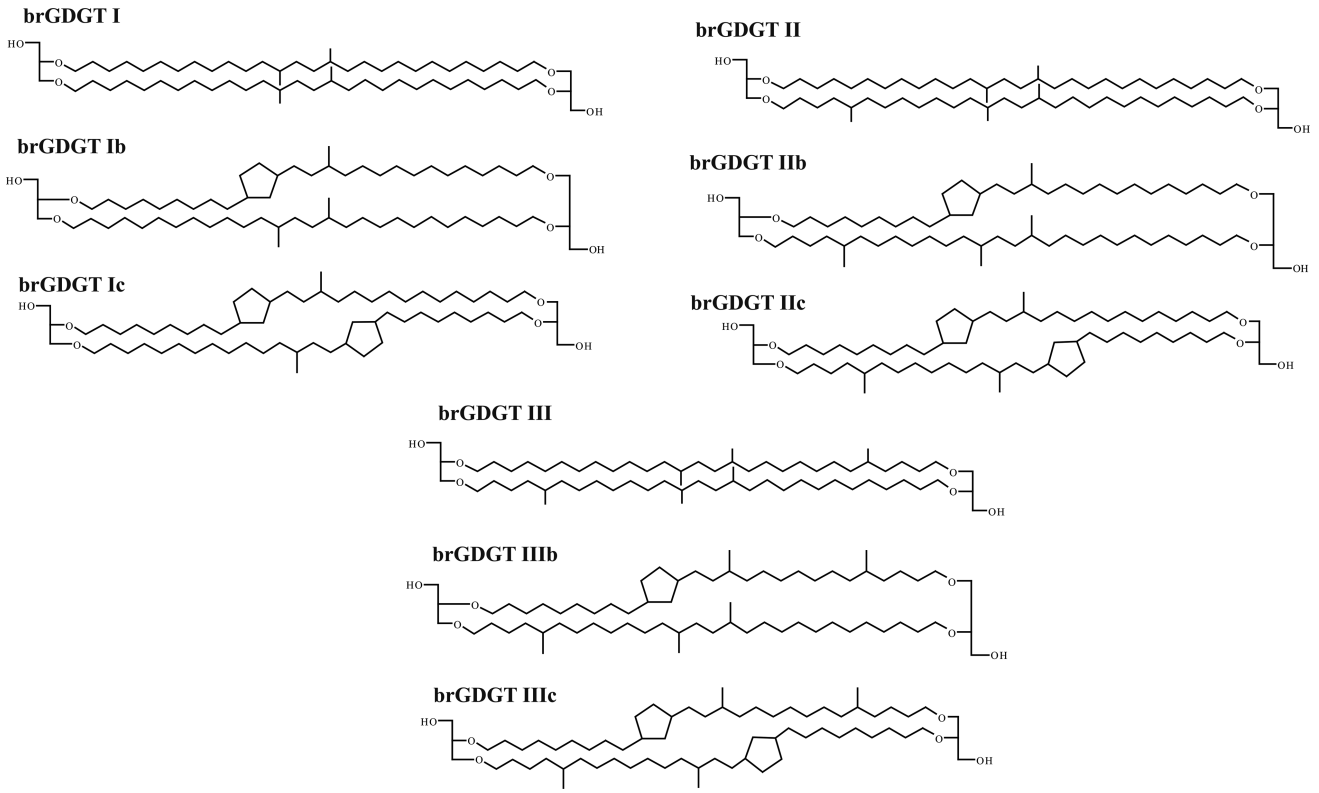

Fig. 8.8 Branched glycerol dialkyl glycerol tetraethers (brGDGT) structures

MBT/CBT: There are two additional proxies that utilize brGDGTs: the methylation of branched tetraethers (MBT) and cyclization of branched tetraethers (CBT) indices (Weijers et al. 2007). The brGDGTs have differing numbers of methyl branches and as many as two cyclopentane structures (Sinninghe Damsté et al. 2000; Weijers et al. 2006a). Initial findings showed that brGDGT distributions had a mathematical relationship to environmental parameters where the soils were collected: MBT providing a proxy of mean annual soil temperature and CBT providing a proxy of pH. Early study of these distributions found that MBT is correlated to soil temperature and also somewhat to soil pH (Weijers et al. 2007). Therefore, CBT, which can be used to reconstruct soil pH, is calculated first, based on the finding that there is a negative, exponential correlation of soil pH with the relative number of cyclopentane ring structures of brGDGTs (Weijers et al. 2007). This can then be used to correct for the effect of soil pH on soil temperature. Soil temperatures often reflect air temperatures, and thus MBT is often used as a proxy for mean annual air temperature (MAAT). The MBT and CBT indices are determined from the following equations, with roman numerals referring to structures found in Fig. 8.8:

$$MBT = \frac{[\text{GDGT I} + \text{GDGT Ib} + \text{GDGT Ic}]}{[\text{GDGT I} + \text{GDGT Ib} + \text{GDGT Ic} + \text{GDGT II} + \text{GDGT IIb} + \text{IIc} + \text{GDGT III} + \text{GDGT IIIb} + \text{GDGT IIIc}]}$$

$$CBT = -\log\left(\frac{(\text{GDGT Ib} + \text{GDGT IIb})}{(\text{GDGT IIb} + \text{GDGT IIc})}\right)$$

Based on a global soil dataset of 134 soils, the uncertainty for the MAAT calculation is ~5°C. The CBT and MBT Indices were defined by Weijers et al. (2007) as:

$$CBT = (3.33 - 0.38 \times \text{pH})$$

$$MBT = (0.122 + 0.187 \times \text{CBT} + 0.20 \times \text{MAAT}).$$

While brGDGTs are known to be bacterial in origin (Weijers et al. 2006a), it is unclear what bacteria are making these structures (Sinninghe Damsté et al. 2011a), which can lead to production uncertainties and difficulties in interpretation of results. Specifically, MBT/CBT looked to be a promising method to reconstruct air temperatures in small lake systems where TEX_{86} did not work because soil input was high. Observations from individual lakes support *in situ* production as well as evidence of transported soils. Blaga et al. (2009) found indication of *in situ* brGDGT production within lakes of varying sizes in Europe. Measuring lacustrine sediments and surrounding catchment soils, Tierney and Russell (2009) found that the pH reconstructed using the CBT index from the sediments of an Indonesian lake reflected soil pH values of the watershed. This site showed abundances of brGDGTs that were higher in lake sediments than in the surrounding catchment soils (Tierney and Russell 2009). This likely resulted in colder reconstructed MBT temperatures than MAAT would predict. Nevertheless, brGDGTs from soil and lake production may still produce reasonable temperature reconstructions. In lakes with water temperatures similar to air temperatures and fairly homogeneous water column temperatures, a mixture of aquatically and soil produced brGDGTs may complicate reconstruction but may still provide a good estimate of temperatures of the past. Studies have found that this mixed signal tends to result in an underestimation of MAAT (Blaga et al. 2010; Sun et al. 2011; Tierney et al. 2010b).

An outstanding question in the use of MBT/CBT is whether global or more regional calibrations are the key to achieve the most accurate paleotemperature reconstruction (Peterse et al. 2009; Sinninghe Damsté et al. 2008). For example, a calibration of East African lakes for MBT/CBT used 46 different sites to establish a regional calibration of brGDGTs to MAAT with an r^2 of 0.94, showing a significant improvement over the global calibration for this region (Tierney et al. 2010b). These authors observe temperature and pH control of brGDGTs in the sediments of East African lakes. However, Peterse et al. (2012), in redefining the global calibration for MBT/CBT, tested whether a local calibration is necessary to improve the MBT/CBT global calibration. Using residual temperatures (measured – reconstructed MAAT) for soils in the global dataset, these authors found a lack of systematic variation associated with specific regions, suggesting that a local calibration would not benefit MBT/CBT reconstructions (Peterse et al. 2012). The updated calibration of Peterse et al. (2012) utilizes the initial dataset with a total of 278 global soils and resulted in new equations with a lower correlation for MBT to MAAT ($r^2 = 0.59$ instead of $r^2 = 0.77$ described in Weijers et al. (2007). The CBT was then defined as: pH = 7.90–1.97 × CBT and MBT (now called MBT' and based on only 7 most abundant brGDGTs): MAT = 0.81–5.67 × CBT + 31.0 × MBT' (Peterse et al. 2012).

Recently, new analytical advances have improved chromatography and have helped to further refine the calibration

of brGDGTs and provided much needed information about the brGDGT structures (De Jonge et al. 2013; Weber et al. 2015; Yang et al. 2015). Importantly, De Jonge et al. (2013) was able to quantify the 5-methyl and 6-methyl isomers of the penta-methylated and hexa-methylated brGDGTs and found that while the 6-methyl isomers were highly correlated with pH, the 5-methyl isomers were not. Thus, the MBT temperature calibration has been freed from the dependency on pH while improving the calibration (De Jonge et al. 2013). Additionally, abundances of the different 6-methyl brGDGTs are correlated positively to one another, suggesting a common biological source in terrestrial deposits (De Jonge et al. 2014), an important discovery in identifying biological controls and origins in addition to appropriate calibrations. Similarly, the presence of 6-methyl brGDGTs lowered the correlation with MAAT in the original brGDGT MBT/CBT calibration, and this improved understanding of structural information and calibrations has reduced error associated with these reconstructions of temperature, with a reduction of the root mean square error (RMSE) from 6.2 to 4.8°C (De Jonge et al. 2014).

MBT/CBT Applications: Using brGDGTs for independent temperature reconstructions and comparing these results to microfossil or pollen generated temperatures resulted in strong correlation between proxies. Bijl et al. (2013) used both MBT and pollen to reconstruct temperatures from the Early Eocene Climatic Optimum (52–50 Ma) to the continental-scale glaciation of Antarctica beginning around 34 Ma. Using marine sediment cores that span the early to middle Eocene from the Wilkes Land Margin, East Antarctica, reconstructed sea-surface temperatures, determined by TEX_{86}, cooled 2−4°C and air temperatures, determined by MBT and corroborated by pollen assemblages, decreased by 4°C (Bijl et al. 2013) during the opening of the Tasmanian Gateway. The cooling suggested by the MBT reconstruction coincides with the termination of the Early Eocene Climatic Optimum (EECO) at ~49−50 Ma and through-flow of the westbound Antarctic Counter Current, supporting the interpretation that the opening of this seaway was influential in the climate events of this transition.

PETM deposits were an initial target of the MBT paleotemperature proxy, but early reconstructions appeared too warm (Weijers et al. 2007). A newer calibration by Peterse et al. (2012) decreased the absolute temperature of these reconstructed temperatures by ~2°C, while the trend remained the same. The PETM MBT temperatures of Peterse et al. (2012) agree with previous estimates of MAAT based on fossil leaf margins (Wing et al. 2005), pollen assemblages, and $\delta^{18}O$ of fossil bone material from the Arctic (Eberle et al. 2010).

Lastly, an example from Pleistocene lacustrine sediments from the American southwest (Valles Caldera, New Mexico) showed significant glacial/interglacial temperature variability utilizing MBT reconstructions of MAAT (Fawcett et al. 2011). MAAT reconstructions from MIS 10−14 (~368 −552 ka) showed swings between warmer interstadials and cooler stadials, which corresponded to pollen assemblage types (such as an increase in *Quercus* and *Juniperus* pollen in warm intervals) and closed/open basin conditions, as illustrated by scanning XRF calcium records (with closed conditions indicated by high calcium) (Fawcett et al. 2011). The alignment of these records and agreed upon interpretation between proxies lends additional credence to the use of MBT for MAAT.

Analytical Approach

Preparation and Extraction

The analytical sections of this chapter are far from a complete guide to all aspects of preparation, analysis, and interpretation of lipid biomarkers. Instead, this section introduces some of the most important analytical concepts with further details available in Killops and Killops (2005) and Peters et al. (2005), essential guides to the field of organic geochemistry.

All analyses described here require that laboratory materials in contact with biomarker archives (e.g., sediments, soils, rocks) must either be purchased at the highest purity grade possible (such as HPLC-grade solvents), or be pre-extracted or distilled (lower solvent grade with initial possible contaminants). Following rinsing (acid wash is not necessary), all glass materials must be placed in a muffle furnace at 400−500°C for at least 4 hours, until possible organic contaminants are removed. Glass and metal materials in contact with sediments can be solvent rinsed with decreasing polarity solvents (for example, methanol followed by hexane). Aluminum foil can either be cleaned of its organic coating by placing in the muffle furnace as above or by solvent rinsing. Chromatographic column gels that can withstand high temperatures (such as alumina and silica) can either be placed in the muffle furnace or solvent rinsed prior to use. No plastics can be utilized with procedures described, though polytetrafluorethylene (PTFE/Teflon) tubing or squirt bottles are often used with satisfactory results. Cutting rock samples or cores must be done with no petroleum based lubricants, relying solely on clean water for blade lubrication. Depending on the preference of the laboratory, nitrile gloves are worn to protect samples from contamination.

However, nitrile gloves are also a source of contamination, especially if wetted with organic solvents, and so care must be taken whether gloves are worn or not to minimize contamination. Sampling from cores should be done near the middle of the core, to avoid possible contamination. This contamination could be from a core liner, especially if plastic, or potentially from drilling fluids (Brocks et al. 2008). This includes drilling fluids recirculating around the cores. However, the middle of the core may be uncontaminated depending on lithology permeability.

Extraction of molecular biomarkers from geologic matrices can be completed using a few different methods. Here the focus will be on methods most often used for general extraction of sediments and soils, with reviews for more specialized extractions, such as intact polar membrane lipids (e.g., Lengger et al. 2012), found elsewhere. Sediments are dried using a freeze dryer to ease grinding and solvent penetration into the material and then homogenized using a solvent-cleaned mortar and pestle or rock pulverizing equipment. A total lipid extract can be isolated from sediment using soxhlet extraction, ultrasonication, accelerated solvent extraction (ASE) or microwave extraction devices. ASE and microwave extraction techniques are both rapid, highly automated, and use much smaller quantities of solvent than more traditional extraction methods such as soxhlet (Kornilova and Rosell-Melé 2003). Often these methods are noted to have higher recovery of analytes (Kornilova and Rosell-Melé 2003; Pastor et al. 1997). However, these methods of lipid extraction involve an initial large cost purchasing these devices, and time required for post-extraction disassembly and cleaning of parts. Additionally some more labile compounds cannot be efficiently extracted using these more "harsh" extraction techniques (e.g., bacteriohopanepolyols (Talbot and Farrimond 2007) and membrane lipids of GDGTs with intact polar head groups (Huguet et al. 2010; Lengger et al. 2012; Pitcher et al. 2009). Soxhlet extraction is slow (inhibited by the number of soxhlet refluxing stations, and the 24−72 hour refluxing time) and require more solvents than other methods described, but is in general a more gentle extraction method. Ultrasonication requires multiple intervals in an ultrasonic bath followed by centrifuging and decanting. The ultimate extraction efficiency of each method is best tested by adding internal standard to samples prior to extraction. In all extraction methods, a mixture of polar and less polar solvents should be used. An extraction blank should be run and checked for contamination for each set of extracted samples.

Lipid Extract Separations

Following organic solvent extraction, a series of chromatographic columns can be run to separate the lipid extract into compound classes based on polarity. Chromatographic columns are often run utilizing gravity and a stationary phase, such as silica or alumina, and organic solvents of increasing polarity, following the principle that only compound classes that can be dissolved in that polarity solvent will be moved from the column bed. Compound classes or groups of similar polarities are then eluted into separate vials (Wakeham and Pease 1992). Analysis of total lipid extracts from geologic materials without column chromatography runs the risk of compound co-elution or a lack of baseline separation during gas chromatography, and thus, poor data quality.

Gas Chromatography

Analysis via a gas chromatograph (GC) works by separating complex mixtures of organic compounds through the interaction with the stationary phase of the fused silica GC column. This necessitates that compounds be GC-amenable, namely, that they are volatile and thermally stable for analysis. Compounds that are not volatile (too polar or containing functional groups) must be chemically altered to be analyzed, usually by derivatization (Meier-Augenstein 2004).

Following column chromatography in the laboratory, there is still a complicated mixture of compounds that can be separated based on different boiling points and polarity. Samples are dissolved in a solvent, commonly hexane or ethyl acetate, and are introduced to the system via syringe. This solvent is then vaporized at the injector. Compounds carried by this vapor elute following their travel and interaction through the column. Retention time depends on the flow rate of the carrier gas (usually H_2 or He), the temperature program, and the physical properties of the GC column

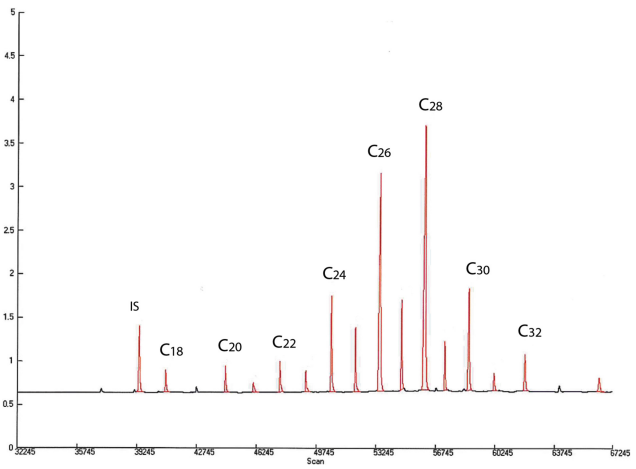

Fig. 8.9 Partial gas chromatogram example showing leaf wax *n*-alkanoic acid, showing even/odd chain length dominance). A known quantity and concentration of internal standard (IS) is added to help quantify compounds by comparison of curve areas

(coating thickness and chemistry, column length, and inner diameter). Compound elution is signified by peaks emerging from the detector, as a plot of time versus intensity, the total series of which is called a chromatogram (Fig. 8.9). Peak height or elution time has no direct relationship to amount of compound or identity of compound without standard or additional analyses. Ideally, increasing column length, slowing flow rate, or otherwise altering the program method so there is more time for compounds to interact with the column may help baseline separation. However, there is a limit to instrumental changes that can be made and still preserve the chromatography.

The GC can be coupled to a series of different detectors. The most common of these is the Flame Ionization Detector (FID). The GC-FID can be used to determine the amount of hydrocarbons exiting the column through pyrolysis by a flame. The resulting area under the peak can be integrated and compared to a known amount of internal standard or standard calibration curves for quantification. GC-FID analysis is highly sensitive with low detection limits. Unlike mass spectrometry (described below), there is little variation of the detector due to compound class, though linearity cannot be assumed.

A GC coupled to a mass spectrometer (GC-MS) allows for the identification of individual compounds of interest. After traveling through the GC column, vaporized compounds are ionized by electron bombardment. This ionization fragments the molecules of the compounds into smaller charged pieces which are then sorted according to their mass to charge ratio using a magnetic field. Often an electron multiplier is necessary to detect these fragments, but once detected, they can be identified based on the way in which they fragmented (charged ions), which serves as a characteristic fingerprint of the molecule in question. A total ion current (TIC) is generated from a full scan spectra of the relative abundance of detected ions as a function of the mass-to-charge ratio. This is similar to an FID trace where all ions are plotted versus time.

One of the most commonly utilized methods for determining the compound specific stable isotopes described in this chapter (δD or $\delta^{13}C$) requires a GC coupled to an Isotope Ratio Mass Spectrometer (GC-IRMS) (Brand 2004; Matthews and Hayes 1978; Sessions 2006). This continuous flow method of compound measurement for light stable isotopes has allowed larger numbers of samples to be run with high precision and accuracy and allows different measurements to be made on the same compound (currently requiring multiple injections and switching between cups and analysis modes as described below). The IRMS contains an electron ionization source, magnetic analyzer, Faraday collector cup array, and acquisition system (Sessions 2006). After passing through the capillary column of the GC, organic compounds enter either enter the combustion or pyrolysis reactor. The combustion reactor is an alumina tube containing Cu, Ni, and Pt wires and is often operated ~900°C. Under oxidizing conditions in the combustion reactor, organic compounds are converted to CO_2 gas. The pyrolysis reactor (also known as thermal conversion) is an open alumina tube and is operated at higher temperatures, often between 1400−1450°C. Organic compounds pass through this reducing reactor and are converted to H_2 gas. After the CO_2 or H_2 passes through the ion source, the resulting gas is then separated by the magnetic analyzer and detected by the Faraday cups. Care must be taken not to fractionate during analysis, which can occur at the inlet if heavy and light isotopes are not moved through the inlet uniformly, or with poor chromatography or peak integration. Typical precision for $^2H/^1H$ analysis is ~2−5‰ and for $^{13}C/^{12}C$ analysis is 0.1−0.3‰ (Sessions 2006).

In order to allow for comparison between systems and across laboratories, measured values must be compared to international, universal standards, Vienna Pee Dee Belemnite (VPDB) for carbon and Vienna Standard Mean Ocean Water (VSMOW) for hydrogen (Coplen 1988; Coplen 2011).

High Performance Liquid Chromatography-Mass Spectrometry

As mentioned, compounds must be amenable or made amenable through derivatization or methylation procedures to be analyzed on a GC. For those compounds that cannot be vaporized at GC inlet temperatures below ~350°C, usually complex and very polar molecules such as phospholipids and GDGTs, high performance liquid chromatography coupled to an MS (HPLC-MS) can be used (Sturt et al. 2004). These less volatile compounds can be carried by a mobile phase that is injected onto the HPLC stainless steel column. This column is filled with fine silica particles (the stationary phase) that help to separate compounds through their interaction (similar to GC columns described previously). Eluting components from the column are introduced to the MS via an interface, most commonly either via electrospray ionization or atmospheric pressure chemical ionization. During electrospray ionization, the analyte solution has a charge applied to it, forcing it from the electrospray needle. Smaller droplets are formed and continue to travel away from the needle until being detected by the MS, which is aided by the charge the analytes carry. Atmospheric pressure chemical ionization, commonly used for GDGT analysis (Schouten et al. 2007a), is not appropriate for thermally labile compounds, instead being used for low mass compounds. At atmospheric pressure, ionization occurs differently from the electrospray technique described above by the introduction of a pneumatic nebulizer that helps to

create ions from the analytes introduced. Solvent molecules in vapor form and initial ions undergo repeated collision, eventually forming analyte ions with little to no fragmentation.

Conclusions

The abundance, diversity, and refractory nature of biomarkers and archives in which they are found make it clear why these chemical fossils have become an important fixture in paleoenvironmental reconstructions. With continual new proxy developments, refined understanding and calibration of existing proxies, and recent analytical advances, biomarker reconstructions of Cenozoic terrestrial environments are likely to continue to serve a critical role in our understanding of past climates and environments. Exciting new developments in biomarker research today are built on decades of foundational exploration, starting with exploration of an organic origin for sedimentary compounds, continuing to the pioneering early work on terrestrial plant wax proxies in the mid-twentieth century, and continuing with ongoing instrumentation and calibration advances, such as those in the realm of the terrestrial temperature proxies of TEX_{86} and MBT. In many cases, there is still much research to be done to uncover linkages between biological sources and environmental response, and what information is necessarily recorded in the lipids that are used for reconstruction. Nevertheless, preserved sedimentary biomarkers provide robust tools to understand Earth's past changes and can be used individually or in multiproxy investigations to provide essential records of the past.

In this chapter, we examined the origins of the term biomarker and the discipline looking to link sedimentary molecules to specific organisms or processes. We explored the wider topic of organic matter preservation, where sediments are likely to contain a record of past environmental variability and the conditions and locations that allow for preferential organic matter preservation and minimal molecular degradation. The chapter discussed some of the biomarker proxies used to reconstruct terrestrial paleoenvironments, and focused on their robustness in the geologic record, first use of the proxy, original and current calibrations, and potential pitfalls in usage. A few applications of each proxy were described to provide examples of how these biomarker proxies have been applied. Reconstructions using leaf wax lipid carbon chain lengths and carbon and hydrogen stable isotopes were described as proxies of landscape change and preservation, aridity and vegetation changes, and hydroclimate variability, respectively. Plant lignin was introduced as a proxy for woody/non-woody and angiosperm/gymnosperm variations. Temperature reconstructions using alkenones, GDGTs, and brGDGTs from haptophyte algae, archaea, and bacteria, respectively, were discussed. Lastly, analytical considerations and instrumentation were discussed as they related to the proxies covered in the chapter.

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