Soil *n*-alkane δD and glycerol dialkyl glycerol tetraether (GDGT) distributions along an altitudinal transect from southwest China: Evaluating organic molecular proxies for paleoclimate and paleoelevation

Chaoran Wang ^a, Michael T. Hren ^{a,b}, Gregory D. Hoke ^c, Jing Liu-Zeng ^d, Carmala N. Garzione ^e

ABSTRACT

Organic molecular proxies provide a record of the hydrogen isotopic composition of ambient precipitation and paleoenvironmental temperature. In terrestrial settings, two classes of organic biomarkers are commonly utilized to reconstruct these past environmental conditions: straight-chained normal alkanes (*n*-alkanes) and branched glycerol dialkyl glycerol tetraethers (brGDGTs). We measured the $\delta D_{n\text{-alkane}}$ and the ratio of cyclisation and methylation indexes of branched tetraethers (CBT/MBT) from surface soils along a ~1000 km transect along the southeast margin of the Tibetan Plateau to assess how these proxies record changes in climate parameters that are related to elevation, despite variations in ecosystem. Abundance-weighted $\delta D_{nC27-31}$ data record the δD of precipitation across the orogen and indicate minimal change in apparent fractionation ($\epsilon_{n\text{-alkane}/\text{water}} = -133\text{‰}$) with elevation. *n*-Alkane δD mirrors the pattern of Rayleigh distillation of an air mass undergoing rainout as moisture moves from the Bay of Bengal into the SE margin of the Tibetan plateau. Soil tetraether temperatures provide a reliable measure of modern temperature with an MBT/CBT calculated temperature lapse rate ($5.8\text{ }^{\circ}\text{C}/\text{km}$) close to the moist adiabat. These data reflect “ground-level” conditions that are independent of the shape of the orogen or the source of moisture. In combination with a Rayleigh distillation model that incorporates temperature and precipitation isotope data, paired tetraether temperature and leaf wax isotope data produce a reliable record of changes in environmental conditions associated with modern elevation change across the largest orogen. However, some tetraether calibrations may impart systematic temperature bias, most notably in cold or dry climates. These calibration uncertainties can result in underestimates of paleoelevations by up to 1.5 to 2 km. Paired isotopic and temperature paleoelevation reconstructions can minimize errors associated with an individual proxy method, however we recommend that application of tetraether

44 temperature paleoelevation reconstruction take potential calibration biases into account in
45 paleoelevation estimates.

46

1. Introduction

The Tibetan plateau constitutes the largest high elevation landscape on the globe and surface uplift of this landmass impacts global atmospheric circulation, biologic diversity and climate. Numerous approaches have been utilized to constrain the paleoelevation history of this region, including paleobotany (Spicer et al., 2003), carbonate oxygen isotopes (Garzione et al., 2000, 2004; Rowley et al., 2006; Saylor et al., 2009; Hoke et al., 2014), leaf wax isotopes (Polissar and Freeman, 2010; Zhuang et al., 2014) and clumped isotope geothermometry (Huntington et al., 2015). However, proxies that rely on authigenic minerals are generally preserved in semi-arid environments (e.g., soil carbonates) and reflect distillation and rainout processes that operate on a “range scale”, i.e. the shape of an orogen impacts patterns of rainout and distillation (Galewsky, 2009). Moreover, the topographic evolution of an orogen can fundamentally alter climatic conditions on the windward and leeward sides of a range. Thus, proxies whose formation is strongly dependent on climate can prove of modest use in constraining past climatic and topographic change.

Organic molecular biomarkers are produced by a wide variety of biota over the entire range of global climatic conditions and are commonly preserved in sediments and soils over geologic time (Peters et al., 2005). The distribution and stable isotopic composition of organic biomarkers are controlled by ecosystem and environmental parameters and thus record conditions or processes at the time of synthesis (i.e., the growing season). Specifically, organic biomarkers quantitatively record two critical environmental parameters that can be used to constrain paleoenvironmental and paleo-topographic change: (1) the temperature at which molecules are biosynthesized, and (2) the isotopic composition of ambient water at the time of biosynthesis. Both parameters can be related to processes associated with changes in temperature and elevation, namely Rayleigh distillation of an air mass during orographic lifting, cooling and

rainout (Rowley et al., 2001; Rowley and Garzione, 2007). Importantly, organic molecular biomarkers are commonly preserved in sediments through changing ecosystems and depositional environments, enabling a continuous organic molecular record of environmental change during the process of orogenesis.

The hydrogen isotopic composition of individual plant waxes has been shown to reflect changes in environmental water and climate (e.g., Sachse et al., 2006; 2012), and a growing number of studies have examined paired records of plant-derived *n*-alkanes and the distribution of soil-derived glycerol dialkyl glycerol tetraethers (GDGTs) that can be used to quantify temperature (e.g., Peterse et al., 2009; Nieto-Moreno, 2016). Results show that paired application of both proxies better constrains elevation-related variations in precipitation isotopes and temperature of an orogen and helps reconstruction of paleoelevation (Hren et al., 2010; Zhuang et al., 2015). One of the main criticisms of stable isotope paleo-altimetry is that changes in the elevation of a mountain range can change the temperature lapse rate with elevation, the amount and location of precipitation, and the isotopic lapse rate of that precipitation (Roe, 2005; Rowley and Garzione, 2007; Jia et al., 2008; Ehlers and Poulsen, 2009). The combination of temperature and isotopic proxies provides a more reliable reconstruction of changes in environmental parameters that are related to elevation because it can account for changes in temperature at a given locality, producing records of both isotopes of source water at the time of biosynthesis and the temperature of soil environments. While these proxies offer a novel means of constraining environmental conditions over varied spatial or temporal scales, there are few data that examine the fidelity of these proxies over isotopic and elevation gradients on the scale of the Tibetan Plateau. Specifically, do the large-scale changes in ecosystems and climate that accompany large elevation change across the highest orogens bias plant wax hydrogen apparent fractionation and reconstructed tetraether temperature? Furthermore, given the large climatic variations associated

with development of an orogen and orographic rainshadow, does this bias organic molecular proxies?

This study focuses on evaluating the hydrogen isotopic composition of modern plant waxes and the distribution of soil-derived GDGTs across the southeastern margin of the Tibetan Plateau over an elevation span of ~1–4 km. Previous studies have examined the relationship between hydrogen isotopes of plant biomarkers and elevation in the Tibetan plateau region (Bai et al., 2011; 2015) and find a clear relationship between the two. However, Bai et al. (2015) produced apparent fractionation values that are locally consistent, but distinct from studies in other regions. Furthermore, application of this approach to deep time is complicated by variations in apparent fractionation related to uncertain water sources. While Bai et al. (2015) show clear relationships between precipitation and biomarker δD , our data expands the spatial range of data across the Tibetan plateau and provides new constraints on the reliability of a paired organic molecular approach through determination of isotopic composition of precipitation, temperature, and ultimately elevation. When paired with simple Rayleigh distillation models and modern stream water isotope data, these results show that organic molecular proxies reliably mirror modeled changes in temperature and δD associated with rainout across an elevation gradient. Such a paired temperature and isotope approach can reduce uncertainties associated with variations in apparent fractionation and/or moisture source over large spatial gradients.

2. Study area, materials and methods

2.1. Climatic setting and soil collection

We collected modern soils (~500 g) along a N–S transect at the southeastern margin of the Tibetan plateau over several field seasons from 2011 and 2014 (Fig. 1). Our samples span >

1000 km SE–NW distance and a range of environments and forest types. All samples were collected from the upper 5–10 cm of soil in minimally-disturbed areas using a cleaned stainless steel trowel, and collected in a Ziploc storage bag. Sample localities range in elevation from 931 to 4154 m elevation, 250 to 1800 mm/yr precipitation, and mean annual temperatures of ~20.7 °C at low elevations to ~1.6 °C at our highest sites. Climate data is derived from observations of daily mean, minimum and maximum temperature from weather stations throughout southwest China and covers the period of 1951–2004. Climate parameters are not available at individual soil sample sites, however site MAT °C is estimated using the modern temperature–elevation relationship, which is highly correlated and shows a lapse rate of ~5.8 °C/km. China precipitation data is included in the supplementary information (Supplementary Fig. S1). Our samples span one of the largest climatic and environmental gradients measured in a single region to date.

The vegetation of the sample region changes dramatically as a function of the large difference in elevation and monsoon-derived seasonal precipitation across the SE–NW transect. In the southern portion of our sampling area, elevations are low (~1000 m) and the vegetation is dominated by subtropical, evergreen, broad-leaved forests that are heavily influenced by summer monsoonal moisture. To the NW in the Yunnan plateau area, (spanning mid elevations ~2000 m), pine and other coniferous trees are common at elevations up to 1800–2000 m, with seasonally-humid, mixed evergreen-broadleaved forests that are adapted to wet summers and a prolonged cool, dry season from November to April. Above this, in the temperate zone (up to 3350 m), there are abundant shrubs of the azalea and rhododendron family associated with cloud forests of the higher elevation regions and SE margin of the Tibetan plateau. In the highest elevations (up to 4000–4500 m), fir, bamboo and dwarf juniper are common in the mountain regions and vegetation is dominated by alpine vegetation. The highest elevation reaches of our sample areas

span the SE margin of the Tibetan plateau and are situated in an area transitioning from low forest/shrub regions to grasslands of the higher plateau.

2.2. Extraction and separation

All samples were freeze-dried and powdered with a mortar and pestle prior to Soxhlet extraction using a solvent mixture of dichloromethane (DCM):methanol (2:1, v:v). After extraction for 48 h, the total liquid extract (TLE) was dried under a stream of purified N₂ using a Pierce Reacti-Therm III Heating Module. The aliphatic, aromatic and polar fractions were separated by passing over a silica gel column eluting with hexane, DCM and methanol sequentially. The aliphatic fraction was further purified using a modified urea adduction. The methanol fraction containing GDGTs was dried under purified nitrogen gas, dissolved in DCM:methanol (1:1, v:v), and passed through an activated Al₂O₃ column and dried again. The eluted fraction was dissolved in hexane:isopropanol (99:1, v:v) and filtered by 0.7 µm glass microfiber filter prior to analysis for GDGTs (methods after Dirghangi et al., 2013).

2.3. Isotopic analysis of soil n-alkanes

The aliphatic fraction containing *n*-alkanes was analyzed by gas chromatography (GC) using a Thermo-Scientific Trace GC Ultra fitted with flame ionization detector (FID) and a BP-1 column (60 m × 0.25 mm i.d., 0.25 µm film thickness) with helium as the carrier gas at a constant flow rate of 1.0 mL/min. The GC oven was programmed to increase in temperature from 50 °C to 250 °C at a rate of 15 °C/min, then at 6 °C/min to 320 °C and held for 8 min. Compound-specific hydrogen isotope compositions of *n*-alkanes were determined by GC-isotope ratio mass spectrometry using a Thermo GC-Isolink attached to a Thermo Scientific Delta V Plus. GC conditions were similar to conditions for GC analysis except the column was a 30 m × 0.25

mm ID, 0.25 μm film thickness BP-5 fused silica column with a He flow of 1.5 mL/min. The H_3^+ factor was determined daily prior to standard calibration and sample analysis and was between 4.0 and 4.3 over the duration of analysis. H_2 gas with known isotopic composition was used as reference and samples were compared to a known hydrogen isotopic standard (Mix A5) prepared by Arndt Schimmelmann of Indiana University. Hydrogen isotopic compositions of *n*-alkanes represent at a minimum, duplicate analyses of well-resolved peaks. The variability of the Mix A5 over the duration of the analysis was less than 4‰. Reproducibility of *n*-C₂₅–*n*-C₃₁ was always better than 5‰ over a range of sample peak intensities.

2.4. GDGT analysis

The separation and quantification of GDGTs was conducted by high performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry (HPLC-APCI-MS; Agilent 1200 series liquid chromatograph-single quadrupole mass spectrometer) at Yale University, after Schouten et al. (2007) with minor modifications. Samples were run in two batches to test methodologies utilized in Weijers et al. (2007b) and De Jonge et al. (2014). For the first batch, separation was achieved in normal phase on an Alltech Prevail Cyano column (150 mm $\times$ 2.1 mm i.d., 3 μm grain size). The mobile phase was 100% hexane:isopropanol (99:1, v:v) eluted isocratically for 5 min, thereafter eluted with a linear gradient to 7.4% isopropanol at 55 min (held for 10 min). GDGTs were detected in select ion monitoring (SIM) mode to increase the sensitivity, and peak integration was carried out using Agilent Chemstation. A second batch of analyses were performed on the same instrument with two Alltima silica columns in series to better resolve co-eluting peaks that may bias results (150 mm $\times$ 2.1 mm; W. R. Grace & Co., Connecticut, USA). This method employs two columns (after Yang et al., 2015), whereas the method of De Jonge (2014) utilizes four, however for our samples there was good separation of

isomers using this method. brGDGTs were separated isocratically using 98% hexane and 2% isopropanol (IPA) for 120 min, with a flow rate of 0.225 mL/min according to De Jonge (2014).

2.5. Calculation of MBT and CBT indices

MBT and CBT indices were calculated using equations based on the global calibration described by Weijers et al. (2006a, 2007b) and by Peterse et al. (2012). As defined by Weijers et al. (2007b):

$$MBT = \frac{(Ia+Ib+Ic)}{[(Ia+Ib+Ic)+(IIa+IIb+IIc)+(IIIa+IIIb+IIIc)]} \quad (1)$$

$$CBT = -\log \frac{(Ib+IIb)}{(Ia+IIa)} \quad (2)$$

The combination of roman numerals and letters corresponding to the GDGTs and their structures refers to the Weijers et al. (2007b). MBT and CBT indices were used to calculate MAT, using a calibration based on a global dataset where (Eq. 3):

$$MAT = \frac{(MBT-0.122-0.187 \times CBT)}{0.02} \quad (3)$$

Modified MBT' and MAT' were calculated using Eqs. 4 and 5 and provide slight modifications of the original MBT and MAT determinations (after Peterse et al., 2012).

$$MBT' = \frac{(Ia+Ib+Ic)}{[(Ia+Ib+Ic)+(IIa+IIb+IIc)+IIIa]} \quad (4)$$

$$MAT' = 0.81 - 5.67 \times CBT + 31.0 \times MBT' \quad (5)$$

Following the separation method modified by De Jonge (2014), 6-methyl brGDGTs were separated from 5-methyl brGDGTs and MAT_{MR} calculated after De Jonge (2014). 5 and 6-methyl brGDGTs were summed for calculation of MAT and MAT' after Weijers et al. (2007) and Peterse et al. (2012), as these compounds were previously not separated at the time of those temperature transfer functions.

3. Results

3.1. ACL and CPI

Aliphatic fractions from all samples along the elevation gradient in Yunnan Province show strong odd over even predominance with peak normal alkane abundances between n -C₂₃ and n -C₃₃ (representative distributions are displayed in Fig. 2). The carbon preference indices (CPI) were calculated following Marzi et al. (1993), where “A” represents the area of the individual n -alkane peak from the gas chromatogram (Eq. 6). Average chain length (ACL) values were calculated using Eq. 7. Soil ACL values range from 28.3 to 29.9 and the distribution shows dominance of the n -C₂₉ normal alkane, while CPI values are generally high and vary from 2.94 to 14.06 (Table 1).

$$CPI = \frac{1}{2} \left[\frac{(A_{C25} + A_{C27} + A_{C29} + A_{C31})}{(A_{C24} + A_{C26} + A_{C28} + A_{C30})} + \frac{(A_{C25} + A_{C27} + A_{C29} + A_{C31})}{(A_{C26} + A_{C28} + A_{C30} + A_{C32})} \right] \quad (6)$$

$$ACL = \left[\frac{(25 \times A_{C25})(27 \times A_{C27})(29 \times A_{C29})(31 \times A_{C31})}{(A_{C25} + A_{C27} + A_{C29} + A_{C31})} \right] \quad (7)$$

3.2. Soil n -alkanes isotopic composition and GDGT measurements

Long carbon chain n -alkanes show good agreement between δD of n -C₂₇, n -C₂₉ and n -C₃₁ indicating a generally consistent source of n -alkanes in the soils. Mean weighted δD values (n -C₂₇ to n -C₃₁) vary from -150‰ to -260‰ over the range of elevations sampled (Table 1) and show broad correspondence with elevation and mirror surface water hydrogen isotope data along a similar elevation gradient nearby (Hoke et al., 2014).

LC-MS analyses of the polar fractions of soil extracts show a typical terrestrial sediment distribution of GDGTs and is dominated by branched GDGTs. Reconstructed MAT ranges between -2.9 and 27.3 °C for the first batch of analyses following the method of Weijers et al.

(2007b) and 5–20.3 °C using the modified method after calibration of De Jonge (2014).

Differences between the two temperature ranges result primarily from calibration differences rather than analytical differences.

4. Discussion

4.1. Soil *n*-alkane distributions

Fig. 3 shows the relationship between the distributions of long-chain *n*-alkanes preserved in modern soils with elevation. ACL values reflect the dominance of long carbon *n*-C₂₉ and *n*-C₃₁ alkanes in soils across Yunnan; however, these are systematically lower in mid elevation localities compared to both high and low elevation sites. This pattern has been observed in other studies with similar vegetation changes across an elevation gradient (Jia et al., 2008). Over our sample area, the amount of precipitation is dominantly controlled by elevation of the landscape. During monsoonal events, orographic forcing results in cooling of air masses and removal of moisture, such that the highest elevations exhibit the coldest temperatures and lowest precipitation amounts. This change in precipitation has a clear impact on vegetation, with lower and mid-elevation sites dominated by dense forests, while high elevation sites show a shift to cold and drought- tolerant species. The ACL of soil *n*-alkanes is strongly dependent on the type of vegetation as well as environmental variables. Some studies show ACL is consistently higher in grasslands than woodlands (Cranwell et al., 1987; Jia et al., 2008; Duan and He, 2011) while other data indicate ACL is an ambiguous indicator of different plant types (Bush and McInerney, 2013) and can vary with latitudinal/temperature variations (Tipple and Pagani, 2013), with longer chain lengths in warmer climates. Variation in ecosystem-averaged ACL may also reflect plant-specific responses to ambient conditions. Short chain compounds have lower boiling points and higher vapor pressure under ambient conditions and are thus more likely to volatilize under

high temperatures and insolation. Plants at lower elevation could therefore show more long chain *n*-alkanes because of the response to warmer conditions and prolonged growing seasons. While the pattern of ACL change with elevation is observed in multiple localities around the globe, relationships with elevation are not well enough constrained to quantitatively evaluate past changes to a landscape. Most likely, variations in ecosystem and plant distributions are the dominant cause of variability in ACL. Our data do suggest however, that at low to moderate elevations (0–2.5 km), ACL responds to changing temperature conditions, while at higher elevations a shift in vegetation may drive a change to longer chain lengths.

The Carbon Preference Index (CPI) reflects the odd-to-even carbon number predominance of higher plant waxes. *n*-Alkanes are produced through a process of fatty acid elongation to even carbon chain lengths, followed by decarboxylation to produce odd carbon chain *n*-alkanes (Schwender et al., 1996; Sessions et al., 1999; Bush and McInerney, 2013). The synthesis pathway is believed to be the primary cause of high odd carbon abundances in modern leaves, however there remains debate over the pathways of even carbon chain production (Sachse et al., 2012). Carbon preference provides an indication of the origin of alkane components and degradation processes that result in lowering of the CPI (Eglinton and Hamilton, 1967) and degree of microbial soil degradation (Bush and McInerney, 2013). Although it has been suggested that higher plants typically show CPI values > 5 (Eglinton and Hamilton, 1967), modern plants exhibit a tremendous range of CPI values that vary between leaves of a single plant and over the duration of a growing season. Thus, in modern soils, CPI may not be suitable for any quantitative determination of degree of *n*-alkane degradation due to the multiple factors that influence the CPI value (Buggle et al., 2010; Bush and McInerney, 2013).

CPI values of long-chain *n*-alkanes from the surface soils in southwestern China range from 2.94 to 14.06 with a mean value 7.60, and display a generally increasing trend from low to

high elevation (Fig. 3b). The results indicate more odd carbon number *n*-alkanes are preserved under cold and dry climate conditions (Figs. 2 and 3b). There are several possible causes for this pattern. First, changes in ecosystem with elevation may produce a change in the distribution of species with different wax production processes. An alternative is that changes in climate (temperature and precipitation) and the partial pressure of CO₂ uniformly influence biota to result in greater production and/or preservation of odd, long-carbon chains. Finally, variation in temperature may impact the degree or rate of microbial degradation of biomolecules in soils. At low elevations, higher temperatures and precipitation could drive more rapid degradation of primary biomolecules to adjust odd-over-even preference indices.

*4.2. Effect of environmental water on δD values of soil *n*-alkanes*

Cuticular leaf waxes are produced at the surface of plant leaves and serve to minimize water loss and protect from predation (Eglinton and Hamilton, 1967). Waxes from different plant species integrate varied timescales of synthesis related to the period of leaf growth (Tipple et al., 2013) and are integrated into soils during the process of leaf degradation. Thus, soils provide a time and plant-integrated organic molecular record of ecosystems that is well suited for paleoenvironmental reconstruction (Eglinton and Hamilton, 1967; Volkman et al., 1998; Eglinton and Eglinton, 2008). The hydrogen in fully saturated leaf-wax lipids is bonded to carbon chains and generally non-exchangeable below 100–150 °C, preserving primary signatures over long timescales (Sessions et al., 2004). Numerous studies show that the δD of long-carbon chain *n*-alkanes reflects the hydrogen isotopic composition of ambient water (Sauer et al., 2001; Sachse et al., 2004, 2012; Tipple et al., 2011; Tipple and Pagani, 2013). However, data also show that secondary processes related to soil water evaporation, differences in biosynthetic fractionation, or moisture availability can also significantly bias $\delta D_{n\text{-alkane}} - \delta D_{\text{precipitation}}$

relationships (Hou et al., 2008; Polissar et al., 2009; Sachse et al., 2012; Eley et al., 2014; Gamarra et al., 2016).

Several studies have specifically examined the relationship between the isotopic composition of water and elevation to assess precipitation isotopes as an elevation proxy (Garzzone et al., 2000; Poage and Chamberlain, 2001; Rowley et al., 2001; Morrill and Koch, 2002; Ponton et al., 2014), while others have examined plant *n*-alkane data specifically (Jia et al., 2008; Peterse et al., 2009; Polissar et al., 2009). Work by Jia et al. (2008) showed that $\delta D_{n\text{-alkane}}$ values agree with predicted precipitation δD variations along an altitudinal transect of Mt. Gongga, China and Bai et al. (2015) evaluated the relationships between *n*-alkane δD and elevation along tributaries of the Yarlung Tsangpo. Variations in precipitation δD introduced the greatest variability in $\delta D_{n\text{-alkane}}$ followed by secondary effects related to vegetation change. Bai et al. (2015) showed that $\delta D_{n\text{-alkane}}$ values are depleted at higher elevation with a mean lapse rate of $\sim 22\text{‰/km}$, however apparent fractionation between leaf wax isotopes and stream waters show considerably smaller ϵ values than reported here. Uncertainty in apparent fractionation for different systems can complicate application of the $\delta D_{n\text{-alkane}}$ for paleoenvironmental reconstruction. These prior data show that changes in vegetation and ecosystem can fundamentally impact the relationship between plant wax isotopes and ambient water due to variations in stomatal regulation and water use efficiency (Kahmen et al., 2013), differences in soil water evaporation, and potential differences in biosynthetic fractionation (Sachse et al., 2012).

Weighted *n*-C₂₇-*n*-C₃₁ $\delta D_{n\text{-alkane}}$ data from our transect provide an integrated isotopic signal that can minimize potential variations between localities with different sample elevations and ecosystem types. $\delta D_{n\text{-alkane}}$ values across Yunnan show progressive depletion in deuterium with increasing elevation (Fig. 4) and the change in $\delta D_{n\text{-alkane}}$ of *n*-C₂₇-*n*-C₃₁ is $\sim 18\text{‰}$ per km

elevation change. Bai et al. (2015) reported a $\delta D_{n\text{-alkane}}$ lapse rate of $\sim 22\text{‰}$ per km elevation change along two vapor transport pathway transects in the SE Tibetan Plateau, and lapse rate varied spatially. Discrepancies in these lapse rates may derive from differences in moisture pathway or source, or variations in temperature gradients across this region. In particular, evaporation of soil water can impact the magnitude of apparent fractionation between waxes and nearby stream waters. We collected water data from 30 tributaries (sampled in 2006 and 2012) of the Mekong River in Yunnan Province to validate plant wax isotope data as a marker for elevation variability and assess potential regional differences in isotopic fractionation and/or lapse rates along the southeastern margin of the Tibetan plateau. Our data provide a strong complement to existing biomarker $\delta D_{n\text{-alkane}}$ data but provide fundamentally new information that can help to constrain paleoelevation through paired isotope – temperature information.

Ultimately, $\delta D_{n\text{-alkane}}$ reflects the isotopic composition of ambient growth water, not elevation. This can be related to elevation if the sample area is observed to demonstrate systematic Rayleigh distillation due to orographic rainout associated with decreasing temperature and rainout across an orogen. $\delta D_{n\text{-alkane}}$ lapse rates are steeper than the measured lapse rate determined from 30 tributaries of the Mekong river that shows a lapse rate of 14.4‰/km (Fig. 4a). Leaf $\delta D_{n\text{-alkane}}$ values from the highest elevation sites show considerable noise in composition. This may result from uncertainties/mixing of water sources on the high elevation plateau (Bershaw et al., 2012; Xu et al., 2014), variations in plant water use and stomatal regulation, soil water evaporation, or sublimation of snow that results in significant enrichment of surface water. $\delta D_{n\text{-alkane}}$ values are shown to be enriched relative to mean annual water composition in dry areas due to intense evaporation and greater vapor pressure deficit (Hou et al., 2008). Noise in $\delta D_{n\text{-alkane}}$ values from high elevation shows general over-enrichment relative to predictions, highlighting the potential for evaporative enrichment as a source of noise in our data

(Fig. 4). When compared to latitude, both wax and water data show the same general patterns of isotopic variation, highlighting the fundamental controls of precipitation δD on $\delta D_{n\text{-alkane}}$. Comparison of the hydrogen isotopic composition of soil and river water along the same elevation gradient show generally consistent apparent fractionation ($\epsilon_{n\text{-alkane/water}}$) of $-133\text{‰} \pm 15\text{‰}$, indicating that plant waxes consistently reflect precipitation isotopes, independent of the large ecosystem and vegetation changes that are observed over the study landscape (Fig. 4).

Enrichment factors ($\epsilon_{n\text{-alkane/water}}$) refer to the hydrogen isotopic fractionation between leaf lipids and source water and represent the sum of many individual fractionations due to isotope effects on both physical and biochemical processes (Sachse et al., 2012). The isotopic difference between the δD value of the n -alkanes and the δD value of the surface water was calculated using Eq. 8. The average of $\epsilon_{n\text{-alkane/water}}$ ($-133\text{‰} \pm 15\text{‰}$) is similar to the value of surface soil reported by Jia et al. (2008) for Tibetan plateau vegetation and the value of terrestrial plants reported by Mügler et al. (2008). Measured $\epsilon_{n\text{-alkane/water}}$ is larger than shown in Bai et al. (2015), who measured $\epsilon_{n\text{-alkane/water}}$ of $\sim -104.5\text{‰}$. Our $\delta D_{n\text{-alkane}}$ data from SE Yunnan fall along the same isotope–elevation relationship as that shown in Bai et al. (2015), however associated waters are more enriched in deuterium, yielding a larger apparent fractionation. This may represent real differences in plant water utilization and evapotranspiration or differences in offsets between stream water δD and the soil water utilized by plants during biosynthesis, or the water availability in the growing season (Luo et al. 2011). Importantly, despite differences in apparent fractionation, the overall isotope–elevation slope is generally consistent between the two studies. Uncertainties in ϵ on the order of 20–30‰ can impart elevation errors of approximately 1–2 km, given modern lapse rates of 14–22 ‰ per km elevation, however our data show regionally consistent ϵ values. If one can constrain a low elevation $\delta D_{n\text{-alkane}}$ value as well as paleo-moisture source area, it is possible to predict δD values of high elevation samples or more importantly,

reconstruct paleoelevation change, independent of a priori knowledge of apparent fractionation. Our data are summarized with uncertainty with other published enrichment factors in Table 3.

$$\varepsilon_{wax/water} = 1000 \times \left(\frac{\delta_{wax} + 1000}{\delta_{water} + 1000} - 1 \right) \quad (8)$$

4.3. GDGT distribution in modern samples

Branched glycerol dialkyl glycerol tetraethers (brGDGTs) are tetraether lipids with 4–6 methyl groups attached to a carbon backbone containing 0–2 cyclopentane rings formed by internal cyclization in microorganisms (Weijers et al., 2006a). These compounds are produced under anaerobic conditions and are commonly found in soils and sediments from a range of terrestrial systems (Weijers et al., 2006b; 2007b; Dirghangi et al., 2013; Peterse et al., 2014). In nature, microorganisms can modulate the distribution of GDGTs containing different numbers of branches or methyl groups in order to maintain optimal membrane fluidity under differing soil and climatic conditions. The distribution of these compounds is shown to be correlated with mean annual temperature over a range of conditions (e.g., Weijers et al., 2007a; 2007b; Peterse et al., 2012; De Jonge et al., 2014) and has been utilized to reconstruct paleotemperature change over a range of environments and timescales (Schouten et al., 2008; Tierney and Russell, 2009; Birkholz et al., 2013) and as a paleoelevation proxy (Hren et al., 2010). Despite the broad application of the MBT/CBT paleothermometer, recent data shows that the fidelity of this proxy is strongly biased by moisture availability, with poor reliability in arid landscapes (Peterse et al., 2012; Dirghangi et al., 2013; Menges et al. 2014), precisely the areas most likely to preserve other proxy materials such as soil and lake carbonates that are commonly utilized in terrestrial stable isotope paleo-altimetry. Recently, a modified chromatography method was published to separate the 6-methyl brGDGTs that co-elute with the 5-methyl brGDGTs (De Jonge et al., 2013;

2014) and refine pH and temperature estimates. Improved separation may improve precision of measurements however, there is still uncertainty over the how separation of 6- and 5-methyl brGDGTs impacts accuracy of temperature reconstruction (De Jonge et al, 2014, Yang et al., 2015). In particular, recent calibration data suggest potential systematic biases as a function of latitude (most probably temperature) and precipitation (De Jonge et al., 2014) and there is a clear relationship between the offset in reconstructed mean annual temperature using tetraether distribution and actual mean annual temperature and the climate (temperature) (Supplementary Fig. S2). This means that in colder climates, tetraether temperatures are generally overestimated while in hot climates, temperatures are likely underestimated (De Jonge et al. 2014).

We analyzed samples spanning a 3 km elevation gradient across the SE margin of the Tibetan plateau in 2014 utilizing methods and data reduction of earlier approaches (e.g., Weijers et al., 2007; Peterse et al., 2012) and in 2016 using modified methodologies that better separate the methyl brGDGTs (e.g., De Jonge et al., 2014; Yang et al., 2015; Deng et al. 2016). In our soil samples, the most dominant GDGTs are branched GDGTs, while the isoprenoidal GDGTs are generally present in smaller amounts and sometimes undetectable. We reconstructed mean annual temperatures (MAT, MAT' and MAT_{mr}) following methods outlined in Weijers et al. (2007b), Peterse et al. (2012), and De Jonge et al. (2014). These results are listed in Table 2. Data measured in 2014 and 2016 with different columns yielded similar reconstructed temperatures when comparing the same temperature calibration (overall standard deviation is 1.4 °C). Replicate runs both show large temperature differences from low to high elevation across our sample area. MATs calculated using methods of Weijers et al. (2007) and Peterse et al. (2012) both reasonably record the observed temperature variation across the elevation transect, though the Peterse et al. (2012) calibration tends to slightly overestimate temperature at the highest elevation sites. A plot of reconstructed MAT minus actual MAT versus climate

(temperature) shows a linear relationship using the calibration of Peterse et al. (cold climates consistently overestimate temperature; $R^2 = 0.53$), and hot climates underestimate temperature. The calibration of Weijers et al. (2007) shows no systematic bias as a function of temperature. Peterse et al. (2012) recognize that the calibration may underestimate temperature by up to 20 °C in arid regions, which for our study, represents the highest elevation and coldest sites. These sites are characterized by modern MAT near 0 degrees and precipitation of ~500 mm/yr. Thus, calibration biases may result from greater misfit for more arid conditions. In our study area, the original Weijers et al. (2007) calibration underestimates temperature in high elevation sites by several degrees while the Peterse et al. (2012) calibration overestimates by a similar amount. MAT estimates using the De Jonge et al. (2014) produce a narrower range of reconstructed temperatures across our transect, but a significantly shallower temperature lapse than the instrumented record of MAT (3.4 versus 5.8 °C/km elevation). In particular, the reconstructed temperatures at extremely high elevation (above 3500 km) are far warmer than the actual measured temperature in this area and application of this temperature transfer function imparts a large and systematic temperature bias across the climate transect. Such a bias is demonstrated by De Jonge et al. (2014) in their plot of ΔMAT_{MR} versus latitude and MAT, which shows that for climates cooler than 12–15 °C, tetraether temperatures using this calibration overpredict MAT in cold areas and may in some cases, under-predict in warm areas. Deng et al. (2016) suggest that warm season bias may drive this signature. Regardless of cause, these systematic offsets are up to 10 °C over the temperature range of –5 to 25°C. For a modern lapse rate of 5–6 °C/km elevation, this adds up to 2 km of uncertainty in paleoelevation. In studies of paleoelevation, where uplift of an orogen such as the Tibetan plateau may change local temperatures on the order of 20 °C or more over a timescale of several millions to tens of millions of years in association with significant changes to precipitation, a transfer function that is strongly biased by

temperature or precipitation can pose a problem in accurately reconstructing paleotemperature, and thus elevation. A transfer function that is less sensitive to these parameters is preferred. Our data fit well using the equations of Weijers et al. (2007b) and Peterse et al. (2012), with the recognition that each may bias temperature estimates at low and high elevations. We note however, that the original calibration of Weijers et al. (2007b) yields a lapse rate of 5.8 °C/km elevation (equal to the modern observed climate data) whereas the calibration of Peterse et al. (2012) produces a lower lapse rate of 3.3 °C/km. A lower calculated lapse rate is expected when using this calibration due to the linear relationship between ΔMAT (defined as $\text{MAT}' - \text{MAT}$) versus $\text{MAT}_{\text{actual}}$ in both the calibration dataset and our data (Supplementary data). The original calibration of Weijers et al. (2007) is most consistent with observed temperature lapse rates related to moist adiabatic cooling in Yunnan Province and shows no systematic bias in reconstructed temperature across the full temperature range of -5 to 25 °C (Fig. 5).

Calculated MBT/CBT mean annual temperatures are highly correlated to actual modern mean annual temperature (Fig. 5). Several sites show anomalously warm temperatures “JD-1310A” and “JD-1317A” (red). These sites are located at extremely high elevation and characterized by dry conditions. Such overestimates are expected for this proxy, as Dirghangi et al. (2013), Peterse et al. (2012) and De Jonge et al. (2014) all note temperature overestimation for sites with low precipitation (< 500 mm/yr). Thus, high temperatures may reflect bias induced by ambient arid conditions at these localities. In the paleorecord, overestimates of the MBT/CBT-derived temperature due to aridity would result in underestimates of paleoelevation.

4.4. Rayleigh distillation and precipitation isotopes across temperature and elevation gradient

Empirical data from orogens across the globe show predictable changes in temperature and precipitation and/or stream water isotopes with elevation (e.g., Garzione et al., 2000; Poage

and Chamberlain, 2001; Rowley and Garzione 2007; Hren et al., 2012; Feakins et al. 2016). Both can be correlated with elevation, however proxies for each may have their own internal uncertainties. Datasets that show a change in both temperature and isotopic composition of precipitation are thus far more likely to reflect elevation variability than other analytical or proxy complications. The change in temperature and associated change in precipitation $\delta^{18}\text{O}$ or δD during lifting and cooling of an air mass can be calculated using a simple thermodynamic model that evaluates cooling and rainout from an air mass and incorporates key parameters such as starting sea-level temperature and relative humidity (e.g., Rowley et al., 2001; Rowley and Garzione, 2007). Such a model can predict how precipitation isotopes and temperature change as a function of elevation across an orogen, though these results are independent of potential changes to lapse rates that may occur due to surface uplift of an orogen over time (Ehlers and Poulsen, 2009). A well established and heavily utilized model (e.g., Rowley et al., 2001) is shown to reasonably reproduce observed precipitation data (Rowley et al., 2001; Rowley and Garzione, 2007) and has been used in a variety of paleoelevation studies (Mulch et al., 2006; Hren et al., 2009). In mountainous regions such as the SE Tibetan plateau area, precipitation isotope data are sparse, however the isotopic composition of small streams provide a reasonable record of the time-integrated annual composition of meteoric precipitation.

We used the thermodynamic model of Rowley et al. (2001) to calculate predicted temperature and precipitation isotope lapse rates (modeled for $\delta^{18}\text{O}$ and converted to δD) across the modern SE Tibetan plateau and Himalayan front for conditions similar to the moisture source area for Yunnan province. Here, summer moisture is the dominant precipitation and is derived from the SE Asian region including the Bay of Bengal and South China Sea region. The thermodynamic model calculates the change in temperature and precipitation for a given set of starting conditions. This calculated T-isotope path is dependent on the mean annual temperature

at sea level as well as the moisture content of the atmosphere over the source region, however the magnitude of change in temperature and isotopes of precipitation as a function of elevation is generally similar for a range of starting T and RH conditions (e.g., 23–27 °C MAT and 75–90% RH). Rowley et al. (2001) generated the model using precipitation and isotope data from low latitude regions (< 35°N) and in particular, the SE Asian region. Uncertainty in starting temperature and or humidity impact absolute values for T or δD data, but impart smaller variability on the ΔT or $\Delta \delta D$ data. Such an approach is reasonable for the SE Tibetan plateau as it is dominated by moisture derived from the SE and less subject to complex mixing of air masses as seen on the plateau. We used a range of starting sea level mean temperatures of 23–26 °C and relative humidities (85–90%) for the model runs to test variations in T-isotope variability. These starting conditions match annual climatic conditions for the moisture source areas for Yunnan. The model predicts the ΔT and $\Delta \delta D$ with elevation for individual starting conditions. The expected ΔT and $\Delta \delta D$ for starting conditions of 23 °C and 85% RH versus 26 °C and 90% RH are generally consistent for lower elevations (within several ‰ in δD and < 1 °C in T), but deviate by up to 10‰ in calculated $\Delta \delta D$ and 1–1.5 °C in ΔT at elevations of ~3500 m. For simplicity, we show model outputs for 26 °C and 85% RH starting conditions.

Modern stream water δD data from tributaries of the Mekong that range in elevation from < 1000 m to > 5000 m are paired with temperatures that are produced using station temperature–elevation relationships from nearby monitoring stations. These data yield a combined measure of the ΔT and $\Delta \delta D$ for real samples and predicted uncertainties for both temperature and isotope change. This modern station data enables comparison with the predicted ΔT and $\Delta \delta D$ using the Rowley model.

Modern stream water δD data and associated surface MAT (°C) show good agreement with predicted ΔT (°C) and $\Delta \delta D$ based on the simple thermodynamic model of Rowley et al.

(2001) (Fig. 6). Modern data fall closely along the temperature– δD line, where a large ΔT and $\Delta \delta D$ reflects a large change in elevation. Many of the modern water δD data points fall above the predicted $\Delta \delta D / \Delta T$ line (Fig. 6). This may be due to sublimation or evaporation of surface waters in the arid, high elevation localities that contribute to stream waters sampled at lower elevations, or may represent noise in measured stream due to the timing of field sampling.

We calculated ΔT ($^{\circ}C$) and $\Delta \delta D$ based on soil tetraether-calculated temperatures and leaf wax δD . For the ΔT based on organic molecular soil temperature, we used a sea level MAT of $26^{\circ}C$ which is the approximate MAT of coastal regions southeast of our field area and the MBT/CBT calibration of Weijers et al. (2007b) as this is the only calibration to yield a reasonable temperature–elevation lapse. $\Delta \delta D$ was determined using a sea level $\delta D_{n\text{-alkane}}$ of -160‰ , and an assumption of constant apparent fractionation. In combination, soil tetraether temperature and plant wax δD fall close to the predicted temperature–isotope relationship for increasing elevation and within predicted error for these relationships (Rowley et al., 2001). However, noise in the data about the predicted isotope–temperature relationship is on the same order of magnitude as is observed in water data, and likely results from the wide latitudinal and longitudinal variation. In addition, these data do not account for isotopic fractionation occurring during horizontal movement of an air mass, only related to lifting and cooling. The fit of these data could also be biased, in part, by uncertainty in the apparent fractionation due to changing vegetation type across the orogen. However, modern plant data mirror precipitation isotope data, indicating near-consistent apparent fractionation. In total, these data attest to the reliability of paired organic molecular temperature and isotopic proxies for paleoelevation reconstruction.

4.5. Application of $\delta D_{n\text{-alkane}}$ and brGDGTs over broad geographic areas

Previous studies of paired organic molecular temperature and isotopic data evaluated relatively narrow geographic areas (e.g., Peterse et al. (2009) discussed samples along Mt. Kilimanjaro (Tanzania) and Mt. Gongga; Hren et al. (2010) studied samples in northern Sierra Nevada). Our study spans 1 to > 4 km elevation and more than 1000 km of distance, providing one of the largest spatial studies of this kind. Noise in the data may derive, in part, from different timescales of integration for organic molecules in sampled soils, aridity effects on tetraether production and water isotope evaporation, and movement of older sediment downslope to sites of deposition. In particular, in a high elevation and high relief landscape, downslope transport of sediment could significantly mix organics derived from a range of elevations. Terrestrial cosmogenic nuclide data from our study area suggest erosion rates of 0.1–0.3 km/Ma, which translates to 10,000 yr, or shorter, soil residence times (Henck et al., 2011). The erosion rate is roughly related to slope steepness of the landscape and river and type of vegetation. Variation in rates of erosion could increase scatter in organic proxy data due to mixing of materials from a range of elevations or variable soil residence times, with wetter environments (lower elevations) potentially characterized by shorter soil residence times than drier environments, which may integrate longer timescale and glacial/interglacial transitions. Another reason could relate to the feature of regional topography of the sampling area, such as sample sites with higher elevation but located in valley bottom versus lower elevation but situated at a local summit. These differences may account for some of the noise in organic molecular data. However organic data is not substantially noisier than precipitation isotope and climate data and we argue that in this approach, the largest uncertainty in paleoelevation reconstruction derives from uncertainty in paleotemperature or isotopic lapse rates rather than biologic heterogeneity of geochemical signals.

5. Conclusions

We analyzed 36 soil samples along an elevation and climate gradient on the SE margin of the Tibetan plateau to evaluate the applicability of organic molecular temperature and isotope proxies to record ambient environmental conditions. These parameters systematically vary with elevation in the modern earth system and can be related to elevation using simple Rayleigh distillation models. Our data show that vegetation type has a large effect on the distribution of leaf wax compounds produced and preserved in soils and sediments across changing ecosystems, however tetraether paleotemperatures and normal alkane hydrogen isotopes faithfully record changes to climate (temperature) and precipitation isotopes (leaf wax δD) despite these ecosystem variations. In combination, these independent data can be coupled with a Rayleigh distillation model to reliably reconstruct ground-level and range-scale patterns of temperature and precipitation isotopes that are commonly observed in modern orogens. Modern organic molecular data closely fit with modern station and groundwater measurements and thermodynamic predictions for temperature and precipitation isotopes across an elevation span. These results demonstrate the power of organic molecular proxies for paleoenvironmental and paleoelevation reconstruction despite known complexities imparted by vegetation effects on wax records. Environmental variations such as vegetation types, length of growing season and humidity, likely introduce scatter in observed relationships, however data from the SE margin of the Tibetan Plateau show that the paired temperature–isotope approach provides the best opportunity for constraining changes to paleoenvironmental conditions over large elevation gradients. We caution however, that some temperature–tetraether transfer functions may systematically bias temperature estimates across a modern or paleo-orogen by underestimating temperatures at low elevation and overestimating temperatures in high elevation, cold and dry regions. Such a systematic bias could introduce paleoelevations errors on the order of 1.5–2 km.

Care should be used when applying and identifying limitations of different calibrations to reconstruct paleotemperature and thus paleoelevation.

Acknowledgements

The authors thank Y. Zhang for assistance with laboratory analyses. This study was supported by NSF award EAR 1019427 to GDH and EAR 1019762 to CNG. The authors thank M. Pagani and J. Super for assistance with tetraether analyses. The manuscript was greatly improved by suggestions from two anonymous reviewers and Editor in Chief John Volkman

Associate Editor—**Ian Bull**

References

- Bai, Y., Fang, X., Gleixner, G., Mügler I., 2011. Effect of precipitation regime on δD values of soil *n*-alkanes from elevation gradients – Implications for the study of paleo-elevation. *Organic Geochemistry* 42, 838–845.
- Bai, Y., Fang, X., Jia, G., Sun, J., Wen, R., Ye, Y., 2015. Different altitude effect of leaf wax *n*-alkane δD values in surface soils along two vapor transport pathways, southeastern Tibetan Plateau. *Geochimica et Cosmochimica Acta* 170, 94–107.
- Bershaw, J., Penny, S.M., Garzzone, C.N., 2012. Stable isotopes of modern water across the Himalaya and eastern Tibetan Plateau: Implications for estimates of paleoelevation and paleoclimate. *Journal of Geophysical Research: Atmospheres* 117, 1–18.

614 Birkholz, A., Smittenberg, R.H., Hajdas, I., Wacker, L., Bernasconi, S.M., 2013. Isolation and
 615 compound specific radiocarbon dating of terrigenous branched glycerol dialkyl glycerol
 616 tetraethers (brGDGTs). *Organic Geochemistry* 60, 9–19.

617 Buggle, B., Wiesenberg, G.L.B., Glaser, B., 2010. Is there a possibility to correct fossil *n*-alkane
 618 data for postsedimentary alteration effects? *Applied Geochemistry* 25, 947–957.

619 Bush, R.T., McInerney, F.A., 2013. Leaf wax *n*-alkane distributions in and across modern plants:
 620 Implications for paleoecology and chemotaxonomy. *Geochimica et Cosmochimica Acta*
 621 117, 161–179.

622 Cranwell, P.A., Eglinton, G., Robinson, N., 1987. Lipids of aquatic organisms as potential
 623 contributors to lacustrine sediments–II. *Organic Geochemistry* 11, 513–527.

624 Deng, L., Jia, G., Jin, C., Li, S., 2016. Warm season bias of branched GDGT temperature
 625 estimates causes underestimation of altitudinal lapse rate. *Organic Geochemistry* 96, 11–17.

626 De Jonge, C., Hopmans, E.C., Stadnitskaia, A., Rijpstra, W.I.C., Hofland, R., Tegelaar, E.,
 627 Sinninghe Damsté, J.S., 2013. Identification of novel penta- and hexamethylated branched
 628 glycerol dialkyl glycerol tetraethers in peat using HPLC-MS², GC-MS and GC-SMB-MS.
 629 *Organic Geochemistry* 54, 78–82.

630 De Jonge, C., Hopmans, E.C., Zell, C.I., Kim, J., Schouten, S., Sinninghe Damsté, J.S., 2014.
 631 Occurrence and abundance of 6-methyl branched glycerol dialkyl glycerol tetraethers in

632 soils: Implications for palaeoclimate reconstruction. *Geochimica et Cosmochimica Acta* 141,
 633 97–112.

634 Dirghangi, S.S., Pagani, M., Hren, M.T., Tipple, B.J., 2013. Distribution of glycerol dialkyl
 635 glycerol tetraethers in soils from two environmental transects in the USA. *Organic*
 636 *Geochemistry* 59, 49–60.

637 Duan, Y., He, J., 2011. Distribution and isotopic composition of *n*-alkanes from grass, reed and
 638 tree leaves along a latitudinal gradient in China. *Geochemical Journal* 45, 199–207.

639 Eglinton, G., Hamilton, R.J., 1967. Leaf epicuticular waxes. *Science* 156, 1322–1335.

640 Eglinton, T.I., Eglinton, G., 2008. Molecular proxies for paleoclimatology. *Earth and Planetary*
 641 *Science Letters* 275, 1–16.

642 Ehlers, T.A., Poulsen, C.J., 2009. Influence of Andean uplift on climate and paleoaltimetry
 643 estimates. *Earth and Planetary Science Letters* 281, 238–248.

644 Eley Y., Dawson L., Black S., Andrews J. and Pedentchouk N., 2014. Understanding $^2\text{H}/^1\text{H}$
 645 systematics of leaf wax *n*-alkanes in coastal plants at Stiffkey saltmarsh, Norfolk, UK.
 646 *Geochimica et Cosmochimica Acta* 128, 13–28.

647 Ernst, N., Peterse, F., Breitenbach, S.F.M., Syiemlieh, H.J., Eglinton, T.I., 2013. Biomarkers
 648 record environmental changes along an altitudinal transect in the wettest place on Earth.
 649 *Organic Geochemistry* 60, 93–99.

650 Feakins, S.J., Bentley, L.P., Salinas, N., Shenkin, A., Blonder, B., Goldsmith, G.R., Ponton, C.,
651 Arvin, L.J., Wu, M.S., Peters, T., West, A.J., Martin, R.E., Enquist, B.J., Asner, G.P., Malhi,
652 Y., 2016. Plant leaf wax biomarkers capture gradients in hydrogen isotopes of precipitation
653 from the Andes and Amazon. *Geochimica et Cosmochimica Acta* 182, 155–172.

654 Galewsky, J., 2009. Orographic precipitation isotopic ratios in stratified atmospheric flows:
655 Implications for paleoelevation studies. *Geology* 37, 791–794.

656 Gamarra, B., Sachse, D., Kahmen, A., 2016. Effects of leaf water evaporative ^2H -enrichment and
657 biosynthetic fractionation on leaf wax *n*-alkane $\delta^2\text{H}$ values in C_3 and C_4 grasses. *Plant, Cell*
658 *and Environment* 39, 2390–2403.

659 Garzione, C.N., Dettman, D.L., Horton, B.K., 2004. Carbonate oxygen isotope paleoaltimetry:
660 evaluating the effect of diagenesis on paleoelevation estimates for the Tibetan plateau.
661 *Palaeogeography, Palaeoclimatology, Palaeoecology* 212, 119–140.

662 Garzione, C.N., Dettman, D.L., Quade, J., De Celles, P.G., Butler, R.F., 2000. High times on the
663 Tibetan Plateau: Paleoelevation of the Thakkhola graben, Nepal. *Geology* 28, 339–342.

664 Henck, A.C., Huntington, K.W., Stone, J., Montgomery, D.R., Hallet, B., 2011. Spatial controls
665 on erosion in the Three Rivers Region, southwest China. *Earth and Planetary Science*
666 *Letters* 303, 71–83.

667 Hoke, G.D., Liu-Zeng, J., Hren, M.T., Wissink, G.K., Garzione, C.N., 2014. Stable isotopes

668 reveal high southeast Tibetan Plateau margin since the Paleogene. *Earth and Planetary*
 669 *Science Letters* 394, 270–278.

670 Hou, J., D’Andrea, W.J., Huang, Y., 2008. Can sedimentary leaf waxes record D/H ratios of
 671 continental precipitation? Field, model, and experimental assessments. *Geochimica et*
 672 *Cosmochimica Acta* 72, 3503–3517.

673 Hren, M.T., Bookhagen, B., Blisniuk, P.M., Booth, A.L., Chamberlain, C.P., 2009. $\delta^{18}\text{O}$ and δD
 674 of streamwaters across the Himalaya and Tibetan Plateau: Implications for moisture sources
 675 and paleoelevation reconstructions. *Earth and Planetary Science Letters* 288, 20–32.

676 Hren, M.T., Pagani, M., Erwin, D.M., Brandon, M., 2010. Biomarker reconstruction of the early
 677 Eocene paleotopography and paleoclimate of the northern Sierra Nevada. *Geology* 38, 7–10.

678 Huntington, K.W., Saylor, J., Quade, J., Hudson, A.M., 2015. High late Miocene-Pliocene
 679 elevation of the Zhada Basin, southwestern Tibetan Plateau, from carbonate clumped
 680 isotope thermometry. *Geological Society of America Bulletin* 127, 181–199.

681 Jia, G., Wei, K., Chen, F., Peng, P., 2008. Soil *n*-alkane δD vs. altitude gradients along Mount
 682 Gongga, China. *Geochimica et Cosmochimica Acta* 72, 5165–5174.

683 Kahmen, A., Hoffmann, B., Schefuß, E., Arndt, S.K., Cernusak, L.A., West, J.B., Sachse, D.,
 684 2013. Leaf water deuterium enrichment shapes leaf wax *n*-alkane δD values of angiosperm
 685 plants II: Observational evidence and global implications. *Geochimica et Cosmochimica*

686 Acta 111, 50–63.

687 Marzi, R., Torkelson, B.E., Olson, R.K., 1993. A revised Carbon Preference Index. Organic
688 Geochemistry 20, 1303–1306.

689 Menges, J., Huguet, C., Alcañiz, J.M., Fietz, S., Sachse, D., Rosell-Melé, A., 2014. Influence of
690 water availability in the distributions of branched glycerol dialkyl glycerol tetraether in soils
691 of the Iberian Peninsula. Biogeosciences 11, 2571–2581.

692 Morrill, C., Koch, P.L., 2002. Elevation or alteration? Evaluation of isotopic constraints on
693 paleoaltitudes surrounding the Eocene Green River Basin. Geology 30, 151–154.

694 Mügler, I., Sachse, D., Werner, M., Xu, B., Wu, G., Yao, T., Gleixner, G., 2008. Effect of lake
695 evaporation on δD values of lacustrine *n*-alkanes: A comparison of Nam Co (Tibetan
696 Plateau) and Holzmaar (Germany). Organic Geochemistry 39, 711–729.

697 Mulch, A., Graham, S.A., Chamberlain, C.P., 2006. Hydrogen isotopes in Eocene river gravels
698 and paleoelevation of the Sierra Nevada. Science 313, 87–89.

699 Nieto-Moreno, V., 2016. Elevation-dependent changes in *n*-alkane δD and soil GDGTs across
700 the South Central Andes. Earth and Planetary Science Letters 453, 234–242.

701 Peters, K.E., Walters, C.C., Moldowan, J.M., 2005. The Biomarker Guide. Part 1: Biomarkers
702 and isotopes in the environment and human history, second ed. Cambridge University Press.

703 Peterse, F., van der Meer, J., Schouten, S., Weijers, J.W.H., Fierer, N., Jackson, R.B., Kim, J.H.,

704 Sinninghe Damsté, J.S., 2012. Revised calibration of the MBT-CBT paleotemperature
 705 proxy based on branched tetraether membrane lipids in surface soils. *Geochimica et*
 706 *Cosmochimica Acta* 96, 215–229.

707 Peterse, F., van der Meer, M.T.J., Schouten, S., Jia, G., Ossebaar, J., Blokker, J., Sinninghe
 708 Damsté, J.S., 2009. Assessment of soil *n*-alkane δD and branched tetraether membrane lipid
 709 distributions as tools for paleoelevation reconstruction. *Biogeosciences* 6, 2799–2807.

710 Peterse, F., Vonk, J., Holmes, M., Giosan, L., Zimov, N., Eglinton, T.I., 2014. Branched glycerol
 711 dialkyl glycerol tetraethers in Arctic lake sediments: Sources and implications for
 712 paleothermometry at high latitudes. *Journal of Geophysical Research, Biogeosciences* 119,
 713 1738–1754.

714 Poage, M.A., Chamberlain, C.P., 2001. Empirical relationships between elevation and the stable
 715 isotope composition of precipitation and surface waters: Considerations for studies of
 716 paleoelevation change. *American Journal of Science* 301, 1–15.

717 Polissar, P.J., Freeman, K.H., 2010. Effects of aridity and vegetation on plant-wax δD in modern
 718 lake sediments. *Geochimica et Cosmochimica Acta* 74, 5785–5797.

719 Polissar, P.J., Freeman, K.H., Rowley, D.B., McInerney, F.A., Currie, B.S., 2009. Paleoaltimetry
 720 of the Tibetan Plateau from D/H ratios of lipid biomarkers. *Earth and Planetary Science*
 721 *Letters* 287, 64–76.

722 Ponton, C., West, A.J., Feakins, S.J., Galy, V., 2014. Leaf wax biomarkers in transit record river
 723 catchment composition. *Geophysical Research Letters* 41, 6420–6427.

724 Roe, G.H., 2005. Orographic precipitation. *Annual Review of Earth and Planetary Sciences* 33,
 725 645–671.

726 Rowley, D.B., Currie, B.S., 2006. Palaeo-altimetry of the late Eocene to Miocene Lunpola basin,
 727 central Tibet. *Nature* 439, 677–681.

728 Rowley, D.B., Garzione, C.N., 2007. Stable isotope-based paleoaltimetry. *Annual Review of*
 729 *Earth and Planetary Sciences* 35, 463–508.

730 Rowley, D.B., Pierrehumbert, R.T., Currie, B.S., 2001. A new approach to stable isotope-based
 731 paleoaltimetry: Implications for paleoaltimetry and paleohypsometry of the High Himalaya
 732 since the late Miocene. *Earth and Planetary Science Letters* 188, 253–268.

733 Sachse, D., Billault, I., Bowen, G.J., Chikaraishi, Y., Dawson, T.E., Feakins, S.J., Freeman, K.H.,
 734 Magill, C.R., McInerney, F.A., van der Meer, M.T.J., Polissar, P., Robins, R.J., Sachs, J.P.,
 735 Schmidt, H.-L., Sessions, A.L., White, J.W.C., West, J.B., Kahmen, A., 2012. Molecular
 736 paleohydrology: Interpreting the hydrogen-isotopic composition of lipid biomarkers from
 737 photosynthesizing organisms. *Annual Review of Earth and Planetary Sciences* 40, 221–249.

738 Sachse, D., Radke, J., Gleixner, G., 2004. Hydrogen isotope ratios of recent lacustrine
 739 sedimentary *n*-alkanes record modern climate variability. *Geochimica et Cosmochimica*

740 Acta 68, 4877–4889.

741 Sachse, D., Radke, J., Gleixner, G., 2006. δD values of individual *n*-alkanes from terrestrial
 742 plants along a climatic gradient – Implications for the sedimentary biomarker record.
 743 Organic Geochemistry 37, 469–483.

744 Sauer, P.E., Eglinton, T.I., Hayes, J.M., Schimmelmann, A., Sessions, A.L., 2001. Compound-
 745 specific D/H ratios of lipid biomarkers from sediments as a proxy for environmental and
 746 climatic conditions. Geochimica et Cosmochimica Acta 65, 213–222.

747 Saylor, J.E., Quade, J., Dettman, D.L., DeCelles, P.G., Kapp, P.A., Ding, L., 2009. The late
 748 Miocene through present paleoelevation history of southwestern Tibet. American Journal of
 749 Science 309, 1–42.

750 Schouten, S., Huguet, C., Hopmans, E.C., Kienhuis, M.V.M., Sinninghe Damsté, J.S., 2007.
 751 Analytical methodology for TEX₈₆ paleothermometry by high-performance liquid
 752 chromatography/atmospheric pressure chemical ionization-mass spectrometry. Analytical
 753 Chemistry 79, 2940–2944.

754 Schouten, S., Eldrett, J., Greenwood, D.R., Harding, I., Baas, M., Sinninghe Damsté, J.S., 2008.
 755 Onset of long-term cooling of Greenland near the Eocene-Oligocene boundary as revealed
 756 by branched tetraether lipids. Geology 36, 147–150.

757 Schwender, J., Seemann, M., Lichtenthaler, H.K., Rohmer, M., 1996. Biosynthesis of

758 isoprenoids (carotenoids, sterols, prenyl side-chains of chlorophylls and plastoquinone) via
 759 a novel pyruvate/glyceraldehyde 3-phosphate non-mevalonate pathway in the green alga
 760 *Scenedesmus obliquus*. Biochemical Journal 316, 73–80.

761 Sessions, A.L., Burgoyne, T.W., Schimmelmann, A., Hayes, J.M., 1999. Fractionation of
 762 hydrogen isotopes in lipid biosynthesis. Organic Geochemistry 30, 1193–1200.

763 Sessions, A.L., Sylva, S.P., Summons, R.E., Hayes, J.M., 2004. Isotopic exchange of carbon-
 764 bound hydrogen over geologic timescales. Geochimica et Cosmochimica Acta 68, 1545–
 765 1559.

766 Spicer, R.A., Harris, N.B.W., Widdowson, M., Herman, A.B., Guo, S., Valdes, P.J., Wolfe, J.A.,
 767 Kelley, S.P., 2003. Constant elevation of southern Tibet over the past 15 million years.
 768 Nature 421, 622–624.

769 Tierney, J.E., Russell, J.M., 2009. Distributions of branched GDGTs in a tropical lake system:
 770 Implications for lacustrine application of the MBT/CBT paleoproxy. Organic Geochemistry
 771 40, 1032–1036.

772 Tipple, B.J., Berke, M.A., Doman, C.E., Khachatryan, S., Ehleringer, J.R., 2013. Leaf-wax *n*-
 773 alkanes record the plant-water environment at leaf flush. Proceedings of the National
 774 Academy of Sciences 110, 2659–2664.

775 Tipple, B.J., Pagani, M., 2013. Environmental control on eastern broadleaf forest species' leaf

776 wax distributions and D/H ratios. *Geochimica et Cosmochimica Acta* 111, 64–77.

777 Tipple, B.J., Pagani, M., Krishnan, S., Dirghangi, S.S., Galeotti, S., Agnini, C., Giusberti, L., Rio,
778 D., 2011. Coupled high-resolution marine and terrestrial records of carbon and hydrologic
779 cycles variations during the Paleocene–Eocene Thermal Maximum (PETM). *Earth and*
780 *Planetary Science Letters* 311, 82–92.

781 Volkman, J.K., Barrett, S.M., Blackburn, S.I., Mansour, M.P., Sikes, E.L., Gelin, F., 1998.
782 Microalgal biomarkers: A review of recent research developments. *Organic Geochemistry*
783 29, 1163–1179.

784 Weijers, J.W.H., Schouten, S., Hopmans, E.C., Geenevasen, J.A.J., David, O.R.P., Coleman,
785 J.M., Pancost, R.D., Sinninghe Damsté, J.S., 2006a. Membrane lipids of mesophilic
786 anaerobic bacteria thriving in peats have typical archaeal traits. *Environmental*
787 *Microbiology* 8, 648–657.

788 Weijers, J.W.H., Schouten, S., Spaargaren, O.C., Sinninghe Damsté, J.S., 2006b. Occurrence and
789 distribution of tetraether membrane lipids in soils: Implications for the use of the TEX₈₆
790 proxy and the BIT index. *Organic Geochemistry* 37, 1680–1693.

791 Weijers, J.W.H., Schefuss, E., Schouten, S., Sinninghe Damsté, J.S., 2007a. Coupled thermal and
792 hydrological evolution of tropical Africa over the last deglaciation. *Science* 315, 1701–1704.

793 Weijers, J.W.H., Schouten, S., van den Donker, J.C., Hopmans, E.C., Sinninghe Damsté, J.S.,

2007b. Environmental controls on bacterial tetraether membrane lipid distribution in soils.

Geochimica et Cosmochimica Acta 71, 703–713.

Xu, Q., Hoke, G.D., Liu-Zeng, J., Ding, L., Wang, W., Yang, Y., 2014. Stable isotopes of

surface water across the Longmenshan margin of the eastern Tibetan Plateau. *Geochemistry,*

Geophysics, Geosystems 15, 3416–3429.

Yang, H., Lu, X., Ding, W., Lei, Y., Dang, X., Xie, S., 2015. The 6-methyl branched tetraethers

significantly affect the performance of the methylation index (MBT') in soils from an

altitudinal transect along Mt. Shennongjia. *Organic Geochemistry* 82, 42–53.

Zhuang, G., Brandon, M.T., Pagani, M., Krishnan, S., 2014. Leaf wax stable isotopes from

Northern Tibetan Plateau: Implications for uplift and climate since 15 Ma. *Earth and*

Planetary Science Letters 390, 186–198.

Zhuang, G., Pagani, M., Chamberlin, C., Strong, D., Vandergoes, M., 2015. Altitudinal shift in

stable hydrogen isotopes and microbial tetraether distribution in soils from the Southern

Alps, NZ: Implications for paleoclimatology and paleoaltimetry. *Organic Geochemistry* 79,

56–64.

Figure Captions

Fig. 1. Map of modern soil sample locations in southwest China.

Fig. 2. Typical distributions and relative abundances of individual *n*-alkanes in soils from different elevations sampled in this study.

Fig. 3. Variations of: (a) ACL_{27-31} and (b) CPI_{27-31} as a function of elevation. ACL and CPI values are calculated from weighted-mean chain lengths of C_{27} , C_{29} , and C_{31} *n*-alkanes.

Fig. 4. n - C_{27-31} weighted hydrogen isotope composition from soils (circles) and δD of Mekong River tributaries (squares) as a function of: (a) elevation and (b) latitude.

Fig. 5. Comparison of measured MAT $^{\circ}C$ (squares) and soil tetraether (MBT/CBT) reconstructed MAT $^{\circ}C$ (circles) as a function of elevation. Red circles reflect sites that are located east of a regional drainage divide and yield anomalously high MAT.

Fig. 6. Modeled and measured change in temperature and precipitation δD (triangles) and soil tetraether temperature and leaf wax δD (squares) associated with orographic lifting and rainout. Plant wax $\Delta\delta D$ and soil tetraether ΔT ($^{\circ}C$) are shown (squares) with modern water isotope data and measured temperature. Shaded lines represent one standard deviation of uncertainty in ΔT and $\Delta\delta D$ based on the model of Rowley et al. (2001).

830 **Table Captions**

831 **Table 1.** Locations of terrestrial soil samples, elevation, CPI_{27-31} , ACL_{27-31} , stable δD

832 composition of $n\text{-C}_{27}$ to $n\text{-C}_{31}$, δD value of river water and enrichment factor.

833 **Table 2.** Sample information for GDGTs, MBT, CBT, measured MAT and calculated MAT,

834 MAT' and $\text{MAT}_{5\text{ME}}$ data.

835 **Table 3.** Comparison of the enrichment factors in different studies.

Figure 1

Figure 2

Figure 3a

Figure 3b

Figure 4

Figure 5

Figure 6

Table 2. Sample informations of GDGTs, MBT, CBT, measured MAT and calculated MAT, MAT' and MAT_{mr} based on the separation using Weijers and De Jonge methods separately.

	Latitude (°)	Longitude (°)	Elevation (m)	separated by Weijers (2007) method					separated by De Jonge (2014) method				
				MBT	CBT	mMAT (°C)	MAT (°C)	MAT' (°C)	CBT	MBT	MAT (°C)	MAT' (°C)	MAT _{mr} (°C)
CH-11-03	27.5	101.3	2754	0.6	1.2	10.1	13.3	13.0	1.2	0.6	13.4	13.0	8.7
CH-11-04	27.5	101.3	2233	0.7	2.1	13.2	9.4	10.8	2.0	0.7	11.4	11.9	12.3
CH-11-06	27.6	101.0	2362	0.4	0.4	12.4	8.7	10.1	0.4	0.4	8.2	9.7	3.6
CH-11-07	27.7	100.8	2764	0.7	1.3	10.1	14.3	13.8	1.2	0.6	15.0	14.1	9.7
CH-11-08	27.6	100.8	3340	0.4	0.7	6.8	6.6	8.8	0.6	0.4	6.9	9.0	2.6
CH-11-09	27.4	100.8	2538	0.4	0.8	11.4	7.9	9.7	0.8	0.4	8.3	9.9	2.9
CH-11-11	26.9	100.0	2500	0.4	0.3	11.6	9.7	10.8	-	-	-	-	-
CH-11-12	26.9	100.0	1881	0.5	0.8	15.2	9.2	10.5	0.3	0.4	9.4	10.5	5.0
CH-11-14	-	-	2600	0.4	0.0	11.0	13.8	13.5	0.8	0.5	11.7	11.9	8.6
CH-11-15	27.1	99.6	2549	0.5	0.6	11.3	12.6	12.7	0.0	0.3	12.1	12.1	10.3
CH-11-16	26.7	99.9	2459	0.7	1.7	11.9	13.2	13.0	0.5	0.4	14.8	13.9	10.7
CH-11-17	26.6	99.8	2463	0.8	1.7	11.8	17.8	15.9	-	-	-	-	15.9
CH-11-18	26.5	99.8	2357	0.5	0.3	12.4	14.1	13.5	1.6	0.8	16.9	15.2	10.4
CH-11-19	26.5	99.7	2635	0.5	0.6	10.8	12.6	12.5	1.0	0.6	16.9	15.2	10.4
CH-11-20	25.9	99.3	1745	0.5	0.3	16.0	14.3	13.7	1.0	0.6	14.2	13.5	8.0
CH-11-21	25.6	100.1	1265	0.6	0.8	18.7	14.5	13.7	-	-	-	-	-
CH-11-23	25.9	101.8	1171	0.6	0.5	19.3	17.5	15.6	0.5	0.5	16.6	14.9	9.2
TR-1301	33.1	95.2	4146	0.1	0.1	2.1	-2.9	2.9	0.3	0.1	-2.0	3.4	2.3
TR-1302	32.7	95.1	4154	0.2	0.2	2.1	-0.4	4.6	0.2	0.1	-0.6	4.3	0.7
TR-1303	32.4	95.4	3970	0.2	0.2	3.1	5.5	8.2	0.0	0.2	2.8	6.3	3.6
TR-1307	31.2	97.1	3222	0.3	0.3	7.4	8.7	10.4	0.2	0.2	4.2	7.2	2.6
TR-1309	29.9	97.7	3818	0.2	0.2	4.0	5.2	8.1	0.1	0.2	4.1	7.2	3.9
TR-1310	29.4	98.7	3590	0.3	0.3	5.3	5.7	8.5	-	-	-	-	-
TR-1311	28.2	99.3	2248	0.4	0.4	13.1	12.2	12.4	0.4	0.4	8.3	9.8	4.3
TR-1312	27.2	100.0	1971	0.5	0.5	14.7	17.4	15.6	0.4	0.5	13.4	12.9	7.6
JD-13-10A	31.3	98.0	4147	0.3	0.3	2.1	5.8	8.5	0.5	0.3	6.2	8.5	3.1
JD-13-17A	31.8	97.7	3978	0.4	0.4	3.1	9.7	11.0	0.7	0.5	10.6	11.3	6.0
DEQ-13-01	28.5	98.9	3300	0.3	0.3	7.0	6.6	9.0	0.3	0.3	4.0	7.1	2.6
XIS-13-02	22.3	101.3	931	0.7	0.7	20.7	17.3	22.5	1.0	0.8	24.7	20.1	17.3
XIS-13-03	22.7	101.3	956	0.9	0.9	20.5	27.3	15.5	-	-	-	-	-

*mMAT is the calculated mean annual temperature based on the measured temperature from local weather station and elevation.

**MAT is the reconstructed mean annual temperature by the equations in Weijers et al. 2007.

***MAT' is the reconstructed mean annual temperature by the equations revised by Peteres et al. 2012.

****MAT_{mr} is the reconstructed mean annual temperature by the equations revised by De Jonge et al. 2014.