



## Research

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We wish to dedicate this paper to Richard Leakey to honour his 70th birthday and in acknowledgement of his many decades of contributions to the field of human evolution.

# Changes in northeast African hydrology and vegetation associated with Pliocene–Pleistocene sapropel cycles

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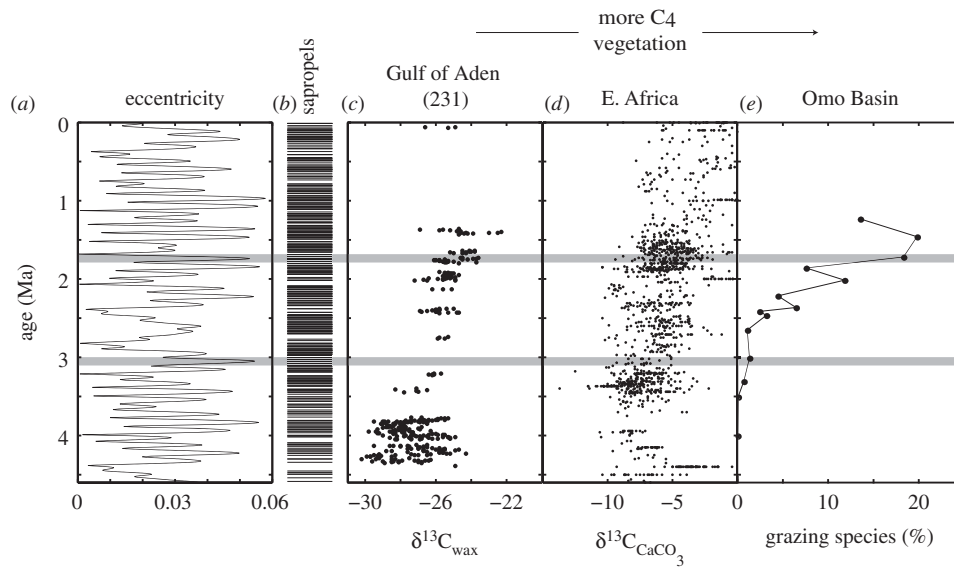
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East African climate change since the Late Miocene consisted of persistent shorter-term, orbital-scale wet–dry cycles superimposed upon a long-term trend towards more open, grassy landscapes. Either or both of these modes of palaeoclimate variability may have influenced East African mammalian evolution, yet the interrelationship between these secular and orbital palaeoclimate signals remains poorly understood. Here, we explore whether the long-term secular climate change was also accompanied by significant changes at the orbital-scale. We develop northeast African hydroclimate and vegetation proxy data for two 100 kyr-duration windows near 3.05 and 1.75 Ma at ODP Site 967 in the eastern Mediterranean basin, where sedimentation is dominated by eastern Sahara dust input and Nile River run-off. These two windows were selected because they have comparable orbital configurations and bracket an important increase in East African C<sub>4</sub> grasslands. We conducted high-resolution (2.5 kyr sampling) multiproxy biomarker, H- and C-isotopic analyses of plant waxes and lignin phenols to document orbital-scale changes in hydrology, vegetation and woody cover for these two intervals. Both intervals are dominated by large-amplitude, precession-scale (approx. 20 kyr) changes in northeast African vegetation and rainfall/run-off. The  $\delta^{13}\text{C}_{\text{wax}}$  values and lignin phenol composition record a variable but consistently C<sub>4</sub> grass-dominated ecosystem for both intervals (50–80% C<sub>4</sub>). Precessional  $\delta\text{D}_{\text{wax}}$  cycles were approximately 20–30‰ in peak-to-peak amplitude, comparable with other  $\delta\text{D}_{\text{wax}}$  records of the Early Holocene African Humid Period. There were no significant differences in the means or variances of the  $\delta\text{D}_{\text{wax}}$  or  $\delta^{13}\text{C}_{\text{wax}}$  data for the 3.05 and 1.75 Ma intervals studied, suggesting that the palaeohydrology and palaeovegetation responses to precessional forcing were similar for these two periods. Data for these two windows suggest that the eastern Sahara did not experience the significant increase in C<sub>4</sub> vegetation that has been observed in East Africa over this time period. This observation would be consistent with a proposed mechanism whereby East African precipitation is reduced, and drier conditions established, in response to the emergence of modern zonal sea surface temperature gradients in the tropical oceans between 3 and 2 Ma.

This article is part of the themed issue ‘Major transitions in human evolution’.

## 1. Introduction

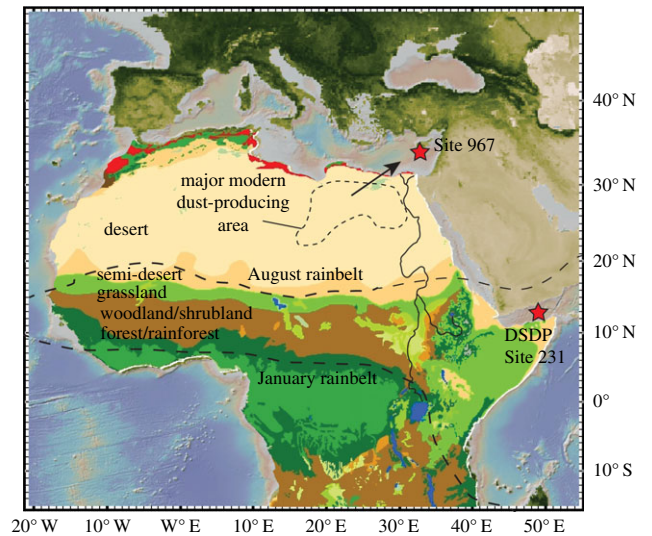
The possible role of Pliocene–Pleistocene changes in East African rainfall and vegetation in shaping faunal change and hominin evolution remains a vigorous area of multidisciplinary research [1–9]. Diverse palaeovegetation proxy records from marine and terrestrial archives document an initial Late Miocene expansion



**Figure 1.** A comparison of East African and Mediterranean palaeoclimate records. Shaded bars indicate the periods analysed in this study. (a) Eccentricity cycles [20]. (b) Mediterranean sapropel (black lines)/carbonate cycles compiled from marine and terrestrial sections [21,22]. (c) Carbon isotope values from plant wax biomarkers from Site 231, Gulf of Aden [12]. (d) Northeast African soil carbonate carbon isotopes [2]. (e) Relative abundance of arid grassland-adapted mammals [1].

of open grassland landscapes, followed by a gradual trend towards increasingly open,  $\text{C}_4$ -rich vegetation until 1–2 Ma, after which the modern savannah grassland expanses were established [2,9–12]. Superimposed on this secular vegetation shift were large-amplitude orbital-scale (approx. 20 ka) variations in North and East African climate and vegetation due to orbital precession insolation forcing of the African summer monsoon [11,13–16]. The interrelationship between the long-term and orbital-scale landscape changes is not well understood because long, continuous archives that capture both signals are rare, despite great efforts to find them [2,5,9,11,17–19]. Feakins *et al.* [11] presented plant wax  $\delta^{13}\text{C}$  data from DSDP Site 231 in the Gulf of Aden and documented that the orbital-scale vegetation cycles were as large or larger than the long-term secular trend.

Our experimental design uses stable H- and C-isotopic analyses of terrestrial plant leaf wax biomarkers preserved in eastern Mediterranean Ocean Drilling Program (ODP) Site 967 for two 100 kyr-duration intervals centred near 3.05 and 1.75 Ma that bracket the East African  $\text{C}_4$  vegetation increase. These windows were selected for their nearly identical orbital eccentricity ranges so that the orbital forcing would be comparable for each interval (figure 1). The goal of this study is to examine the relationship between short-term orbital and long-term secular changes in northeast African climate and vegetation across this Early Pleistocene (3–2 Ma) transition toward more open  $\text{C}_4$  grasslands landscapes. Additionally, several samples were analysed for lignin monomer distributions to constrain the vegetation source of the leaf waxes. We further track orbital-scale Mediterranean surface conditions and sedimentological changes in response to African run-off variability through planktonic foraminiferal  $\delta^{18}\text{O}$  and leaf wax concentration measurements. This study examines whether the East African vegetation transition between 3 and 2 Ma was accompanied by measureable changes in regional vegetation and climate responses to insolation forcing. This study only compares two short 100 kyr windows centred near 3.05 and 1.75 Ma, and thus it cannot address the larger, more encompassing question of whether there were



**Figure 2.** Modern vegetation and precipitation patterns in modern Africa. Vegetation transitions from tropical rainforest at the Equator to bare desert in the north [23]. The monsoonal rainbelt is approximately located in the Sahel during boreal summer and moves southward during austral summer [24]. ODP Site 967 is located in the eastern Mediterranean (red star) in the path of the Nile River outflow. The eastern Sahara (Algeria) is a major source of aeolian dust to the eastern Mediterranean today [25,26].

changes in African climate and vegetation responses to orbital insolation forcing throughout the Pliocene–Pleistocene.

## 2. Background

### (a) Orbital and secular changes in North African climate and vegetation

The large meridional gradient from the humid Equator to the hyperarid Sahara desert in the northern African subtropics is expressed by a distinct zonation of climate and vegetation (figure 2). In northern subtropical Africa, strong monsoonal

circulation prevails, bringing precipitation during the Northern Hemisphere (NH) summer and a pronounced dry season during NH winter. A narrow fringe along the Mediterranean coastline receives wintertime precipitation and follows a typically Mediterranean climate [24]. Equatorial East Africa experiences a bimodal distribution of annual precipitation falling from March–May and October–November as the equatorial rainbelt passes over the Equator twice each year. North African vegetation tracks the precipitation gradient, as evidenced by the strongly zonal character of the rainforest, bushland, grassland and desert vegetation bands (figure 2).

Orbital precession regulates the seasonal distribution of solar radiation at the top of the atmosphere, but does not impact mean annual radiation receipt at a given latitude. During precessional index minima ( $I = e^* \sin(\omega)$ , where  $e$  is eccentricity and  $\omega$  is the longitude of perihelion), summer season insolation is enhanced over northern subtropical Africa and this invigorates summer monsoonal circulation and its northward penetration into the subcontinent [27]. North African palaeoclimate records from palaeolake and marine sediments show that the Early Holocene African Humid Period (11–5 ka BP) was characterized by greater rainfall, reduced dust fluxes, larger lakes, increased grassland cover, reactivated ‘fossil’ river systems, groundwater recharge and high Nile River run-off into the Mediterranean Sea (e.g. [5,28–31]).

The eastern Mediterranean sedimentary record of sapropel deposition documents orbital precession forcing of North African climate since at least 10 Ma [14,21,32,33]. Sapropels are dark, organic-rich sediments (5–10% total organic carbon) that were deposited as a result of bottom-water anoxia due to surface water stratification from enhanced Nile River run-off and greater Mediterranean surface productivity [32–35]. North African run-off during sapropel periods was delivered to the eastern Mediterranean not only by the existing Nile River basin and its large Ethiopian highlands catchment, but also from ‘fossil’ river systems in Libya and Tunisia [36–39]. During North African dry periods, eastern Mediterranean sediments are dominated by carbonate-rich foraminiferal and nannofossil oozes with abundant aeolian dust [40]. A simple proxy for African run-off intensity such as sediment colour (lightness,  $L^*$ ) captures the stark colour contrast between dark sapropel layers and light carbonate sediments [40]. Such records show prominent, precessionally paced bedding cycles that also exhibit characteristic longer-term orbital eccentricity modulation at 100, 400 and 1.2 Myr timescales [33]. Although impressive, the sapropel bedding cycles are not quantitative records of North African run-off because they record only a binary presence/absence of deep ocean anoxia related to variable African river run-off [40]. Aeolian dust supply to the eastern Mediterranean is principally derived from central Algeria and occurs during boreal spring [25,26]. Geochemical analyses of terrigenous sediments at ODP Site 967 document a well-defined aeolian end member composition consistent with this source area [41,42].

Precession-related changes in East African climate and vegetation are also well documented in East African palaeolake and offshore marine sediments. Lake Turkana in northern Kenya was 80 m higher than today during the Early Holocene Humid Period [13], and exhibited precessionally paced lake-level variability between 2.0 and 1.85 Ma [43], with evidence for high lake-level stands during eccentricity maxima [19]. Other East African lake basins such as Lake Challa [44], Lake Tanganyika [45] and the Horn of Africa region [45] were also

more humid at this time. Plant wax biomarker data from palaeolake Olduvai (Tanzania; 1.9–1.8 Ma) record large precessional variations in regional palaeohydrology and vegetation, with local landscapes cycling between closed  $C_3$  woodlands and open  $C_4$  grasslands [15,16].

Secular, long-term changes in East African climate and vegetation towards more arid and open conditions after *ca* 3 Ma have been documented in numerous studies based on both terrestrial and marine sediment sequences (figure 1; [2,9–12,18,46–48]). East African leaf wax biomarker and soil carbonate records (figure 1*c,d*) document a significant shift towards arid-adapted  $C_4$  grasslands near 3 Ma, coincident with peaks in African mammal species richness and major steps in human evolution [8]. Dust flux, soil carbonate, pollen and fossil records document shifts towards open and more arid conditions during this same period near major global climate events, particularly between 3 and 2 Ma [1,7,10,12,18,48–50] (figure 1). Although regional diversity in the timing and spatial patterns of open environments and  $C_4$  expansion is apparent [2], East Africa experienced a broad, long-term trend towards more open landscapes between 4 and 1 Ma [2,9,18,48]. Orbital resolution sampling is not possible for many terrestrial records due to lithostratigraphic changes, limited age control and erosion/non-deposition, so it is challenging to evaluate the relationship between orbital and long-term climate variability for these sequences. For one deep-sea sediment record from the Gulf of Aden, the orbital-scale palaeovegetation amplitude was found to be comparable with the observed overall Pliocene–Pleistocene grassland expansion trend [11].

## (b) ODP Site 967 and Mediterranean paleoclimate records

ODP Site 967 is located in the eastern Mediterranean basin on the northern slope of the Eratosthenes Seamount (34° N, 34° E, 2252 m) [51]. Long records of planktonic foraminiferal  $\delta^{18}O$ , African dust and Mediterranean sea surface temperatures (SSTs) exist for Site 967, allowing the proxy records developed in this paper to be compared with long-term northern African and Mediterranean trends [5,22,52]. Low-resolution alkenone SST estimates for Site 967 show that SSTs were fairly uniform at approximately 23°C before 2.2 Ma, varied between 18 and 20°C from 1.3 to 0.95 Ma, and between 14 and 22°C since 0.5 Ma [53]. An existing 3.2 Myr  $\delta^{18}O_{\text{ruber}}$  record from Site 967 exhibits an orbital-pacing history similar to global  $\delta^{18}O$  records [22]. Strong precessional and obliquity pacing dominates the  $\delta^{18}O_{\text{ruber}}$  signal between 3 and 2 Ma, dominant obliquity pacing between 2 and 1 Ma, and strong eccentricity pacing from 1 to 0 Ma. Shifts in  $\delta^{18}O_{\text{ruber}}$  variability at 2.6 and approximately 0.9 Ma are obvious, indicating that  $\delta^{18}O$  in the Mediterranean recorded high-latitude climate shifts. Mediterranean seawater  $\delta^{18}O$  ( $\delta^{18}O_{\text{sw}}$ ) exhibits very large-amplitude orbital-scale variability (approx. 5‰) compared with open ocean  $\delta^{18}O_{\text{sw}}$ , due to the additional (large) influences of regional temperature, salinity and ice volume changes in the restricted basin [22]. Changes in precipitation–evaporation and river run-off led to salinity decreases of 1–8 practical salinity units (p.s.u.) during sapropel deposition [53,54]. Corresponding changes in  $\delta^{18}O$  of seawater in response to salinity variability are approximately 0.4‰/1 p.s.u. [53,55]. Larrasoña *et al.* [5] constructed a continuous, 3 Myr dust record from Site 967 by measuring haematite concentration variability as a proxy for Saharan dust. Average dust concentrations were found to be

similar between 3 and 1 Ma, with large orbital-scale variability throughout.

### (c) Terrestrial vegetation and climate proxies

The carbon isotope composition of sedimentary leaf wax 'biomarker' compounds provides a rigorous means by which to determine the photosynthetic pathway of the plants that produced them [56]. Plants use three major photosynthetic pathways today:  $C_3$  (Calvin–Benson),  $C_4$  (Hatch–Slack) and Crassulacean acid metabolism (CAM) [57]. The  $C_3$  and  $C_4$  notation refers to the number of carbon atoms in the first photosynthetic compound. Most trees, shrubs and herbs use the evolutionarily ancient  $C_3$  pathway, characterized by relatively inefficient carbon and water utilization. Most tropical grasses and sedges use the  $C_4$  pathway that evolved in response to reduced  $CO_2$  conditions after the Late Oligocene.  $C_4$  plants are distinguished by unique anatomical and biochemical adaptations that result in much more efficient carbon fixation and water utilization. Consequently, even though  $C_4$  plants only represent about 3% of all land plant species,  $C_4$  grasses and sedges represent 80% of terrestrial primary productivity across the warm tropics and subtropics as they are better adapted to seasonal heat and water stress [58].

In this study, we use leaf wax molecules preserved in eastern Mediterranean sediments to infer the mixture of  $C_3$  versus  $C_4$  plants growing on the landscape and the hydrogen isotopic composition of North African rainfall. Leaf waxes are composed of a large variety of very long, straight-chain ( $n$ ) hydrocarbon compounds, including alkanes, carboxylic fatty acids and alcohols, which act as a protective coating on the leaf surface and help maintain water balance within the plant [59]. Leaf wax biomarkers can be transported to lake and marine sediments through fluvial transport, for example, with plant matter in a stream or river, or via aeolian transport [60–62]. The biomarkers and their isotope composition (hydrogen and carbon) are stable over many millions of years [63]. Studies of aerosol leaf wax  $\delta^{13}C$  off the western margin of Africa have concluded that living plant leaf waxes dominated over 'old' leaf waxes associated with soil organic matter entrained by the wind [56,61].

Recent calibration work in North America, Europe and tropical Africa has supported the use of hydrogen stable isotopes from sedimentary leaf wax biomarkers to reconstruct rainfall  $\delta D$  values [60,64–67]. In the tropics and subtropics,  $\delta D_p$  is significantly influenced by the 'amount effect' in addition to temperature effects on Rayleigh fractionation [68]. The amount effect results in very depleted  $\delta D_p$  and  $\delta^{18}O_p$  values during the rainy season in tropical Africa [69]. There is high positive linear correlation ( $r^2 > 0.92$ ,  $p < 0.001$ ) between the  $\delta D$  of precipitation ( $\delta D_p$ ) and the  $\delta D$  of leaf waxes in the tropics and mid-latitudes, making  $\delta D_{wax}$  a good proxy for  $\delta D_p$  in a large variety of environments [67].

An additional proxy for terrestrial vegetation type is sedimentary lignin composition [70]. Lignins are phenolic polymers produced by vascular plants, which are dominantly terrestrial organisms [70]. In order to analyse lignin compounds, the polymers must be oxidized into individual monomers, which include aldehydes, acids and ketones [71]. Conventionally reported compounds are syringyl (S), cinnamyl (C) and vanillyl (V) yields. Gymnosperm and angiosperm vascular plants have different characteristic S/V and C/V ratios from each other and between their woody and non-woody tissues,

making lignins a useful proxy for vegetation source and the presence of terrestrial vegetation in sediments [70,72]. Grasses in particular have very high C/V ratios of greater than unity.

## 3. Methods

### (a) Sampling and age model

We selected eastern Mediterranean ODP Site 967 for this study as it contains a nearly continuous, high-accumulation rate sediment record since the Pliocene, including all known sapropel cycles back to 3.2 Ma [40]. Site 967 is located on the flank of the Eratosthenes Seamount in the eastern Mediterranean and is dominated by African sediment deposition that derives from East Africa via the Nile River as well as from Saharan dust (figure 2; [21,73]). Sedimentation rates at Site 967 have averaged 2.6 cm per kyr since the Early Pliocene [40]. Aeolian dust supply to the eastern Mediterranean is derived principally from eastern Saharan dust sources in Algeria [25,26]

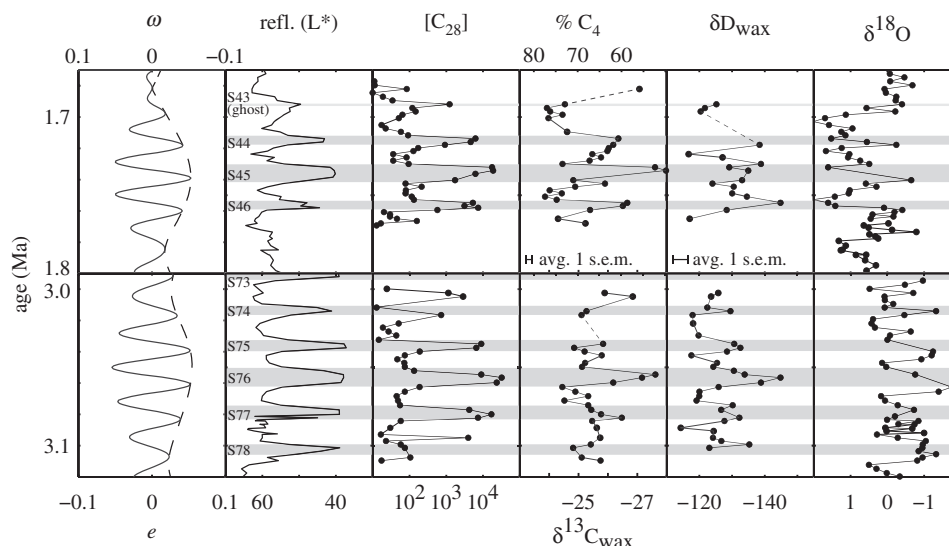
We sampled two 100 kyr-duration intervals centred near *ca* 1.75 and 3.05 Ma. These time periods were selected because they bracket the large increase in East African  $C_4$  vegetation between 3 and 2 Ma (figure 1), and because these specific intervals have similar eccentricity amplitudes and calculated orbital precession forcing (figure 1, grey bars). This sampling allows us to quantify and compare regional hydroclimate and vegetation responses to orbital forcing for these specific time windows. Because the sampling is limited to these windows only, we cannot speak to the larger question of long-term, secular changes in orbital climate variability. ODP Site 967 sediments are composed of nanofossil clays, nanofossil oozes and organic matter-rich sapropels [40]. We sampled ODP Site 967 cores at 5 cm (2.5 kyr) spacing between 51.88 and 53.82 revised metres composite depth (RMCD) and 88.87–91.09 RMCD, or 1.68–1.78 Ma and 3.0–3.1 Ma in age, respectively. The age model of Emeis *et al.* [51] was employed to calculate sample ages via linear interpolation from sample depths on the RMCD scale [40].

To reconstruct regional hydroclimate variability we measured compound-specific hydrogen isotopic ratios from sedimentary leaf waxes ( $\delta D_{wax}$ ). We construct a proxy record of regional vegetation variability by measuring compound-specific carbon isotope ratios in leaf waxes ( $\delta^{13}C_{wax}$ ), and lignin was measured in selected samples to help clarify leaf wax provenance. We also have a qualitative proxy record of monsoonal river run-off to the eastern Mediterranean using  $\delta^{18}O$  from planktonic foraminifera species *Globigerinoides ruber* (white).

### (b) Plant wax biomarker analyses

Ground and homogenized samples were extracted using a Dionex accelerated solvent extractor (ASE) 350 with dichloromethane:methanol (DCM:MeOH) 9:1 at a temperature of 100°C and pressure of 1500 psi (10.34 MPa). Extraction of sufficient leaf wax compounds for gas chromatography (GC) analysis required 15–20 g of nanofossil ooze/clay sediments (hereafter referred to as nanofossil ooze) and approximately 5 g of sapropel sediments. Sediments were mixed with clean Ottawa sand in order to increase sediment permeability during ASE extraction, as nanofossil oozes in particular contain high amounts of clays. Total lipid extracts (TLEs) were spiked with a fatty acid internal standard (*cis*-11-eicosenoic acid) and dried at 30°C under nitrogen gas ( $N_2$ ).

The TLEs were loaded onto a solid-phase aminopropyl (LC-NH<sub>2</sub>) column in 2:1 dichloromethane:isopropanol (2:1 DCM:iso) and separated into neutral, acid and polar fractions (eluted with 4 ml 2:1 DCM:iso; 4 ml 4% acetic acid in ether (v/v); 4 ml methanol, respectively). Separated fractions were blown down to dryness under  $N_2$  gas. The acid fractions were methylated by dissolving the fraction in 0.2 ml of DCM, adding



**Figure 3.** Results: no change in E. Saharan precipitation or vegetation from 3 to 1.67 Ma. Precession and eccentricity pacing are obvious across all proxy records in both time periods [20]. Sediment reflectivity ( $L^*$ ) is a proxy for changes in sedimentology and carbon content and shows strong orbital pacing [40]. Sapropel locations are marked by grey boxes [22,51]. Concentrations of  $C_{28}$  FAMES ( $\text{ng g}^{-1}$  sediment) also closely follow orbital forcing (this study).  $\delta^{13}C_{\text{wax}}$  ( $\text{‰}$  versus VPDB,  $C_{28}$ ) and  $\delta D_{\text{wax}}$  ( $\text{‰}$  versus VSMOW,  $C_{28}$ ), corrected for  $\delta^{13}C_{\text{CO}_2}$  changes and ice volume changes, respectively, exhibit strong precession pacing and similar average values from the older to the younger period (this study), indicating no change in vegetation composition or precipitation between the two times.  $\delta^{18}O_{\text{ruber}}$  (corrected for ice volume changes ( $\text{‰}$  versus VSMOW) measurements show strong precession pacing in the older period but lower frequency variability in the younger time period, indicating increased high-latitude climate influence on Mediterranean surface conditions in the Pleistocene.

1 ml acidified methanol (95:5 v/v mixture of methanol:acetyl chloride) and heating at  $60^\circ\text{C}$  overnight. Deionized water (1 ml) was added to the cool methylation mix and the methylated acids were extracted with hexane to isolate the methyl esters (approx. 1.5 ml hexane, repeated three times). Phthalic acid standards were methylated in parallel for determination and mass-balance correction of  $\delta^{13}\text{C}$  and  $\delta\text{D}$  from the added methyl group. Fatty acid methyl esters (FAMES) were separated from other functionalized FAMES on silica gel columns (Aldrich, 70–230 mesh, 60A; DCM, methanol extracted, activated 2 h at  $200^\circ\text{C}$ ). Samples were loaded in hexane onto the silica gel columns, and three fractions were collected (eluted with 4 ml hexane, 4 ml DCM, 4 ml methanol). The DCM fractions containing the methyl esters were dried gently under  $\text{N}_2$  gas and transferred in hexane to 2 ml glass vials for GC analysis.

To track lithologic and organic carbon variability, we utilized the existing hole-specific colour reflectance ( $L^*$ ) record of Site 967 and measured concentrations of long chain FAMES (figure 3) [40]. Reflectance was calculated for each sample depth via linear interpolation of the published data for each hole (ODP Site 967B and 967C) as appropriate. FAMES were analysed on a gas chromatography–flame ionization detector (GC-FID) for determination of compound abundances using the internal standard. Concentrations of  $n\text{-}C_{28:0}$  FAMES ( $C_{28}$ ) are reported in figure 3, with average replicate nannofossil ooze sample standard deviation (s.d.) of  $0.35$  ( $\text{ng g}^{-1}$  sediment) and replicate sapropel sample s.d. of  $11.17$  ( $\text{ng g}^{-1}$  sed.).

Compound-specific isotope values were analysed on a Thermo Trace GC Ultra coupled to a Thermo Delta V Plus isotope ratio mass spectrometer (GC-irMS) in duplicate to obtain  $\delta\text{D}$  and  $\delta^{13}\text{C}$  values. The GC oven temperature was held at  $60^\circ\text{C}$  for 1.5 min, ramped at  $30^\circ\text{C min}^{-1}$  to  $225^\circ\text{C}$ , then ramped at  $10^\circ\text{C min}^{-1}$  to  $325^\circ\text{C}$  and held for 13 min. Internal laboratory standards (mixture of four FAME peaks of known concentration and unknown isotopic composition) and a standard mix of eight alkyl esters of known isotopic composition ('F8' FAME mix, obtained from Arndt Schimmelmann) were run alongside samples to calibrate isotope values to the Vienna Standard Mean Ocean Water (VSMOW) and Vienna pee dee belemnite (VPDB) scale. Pooled analytical uncertainties

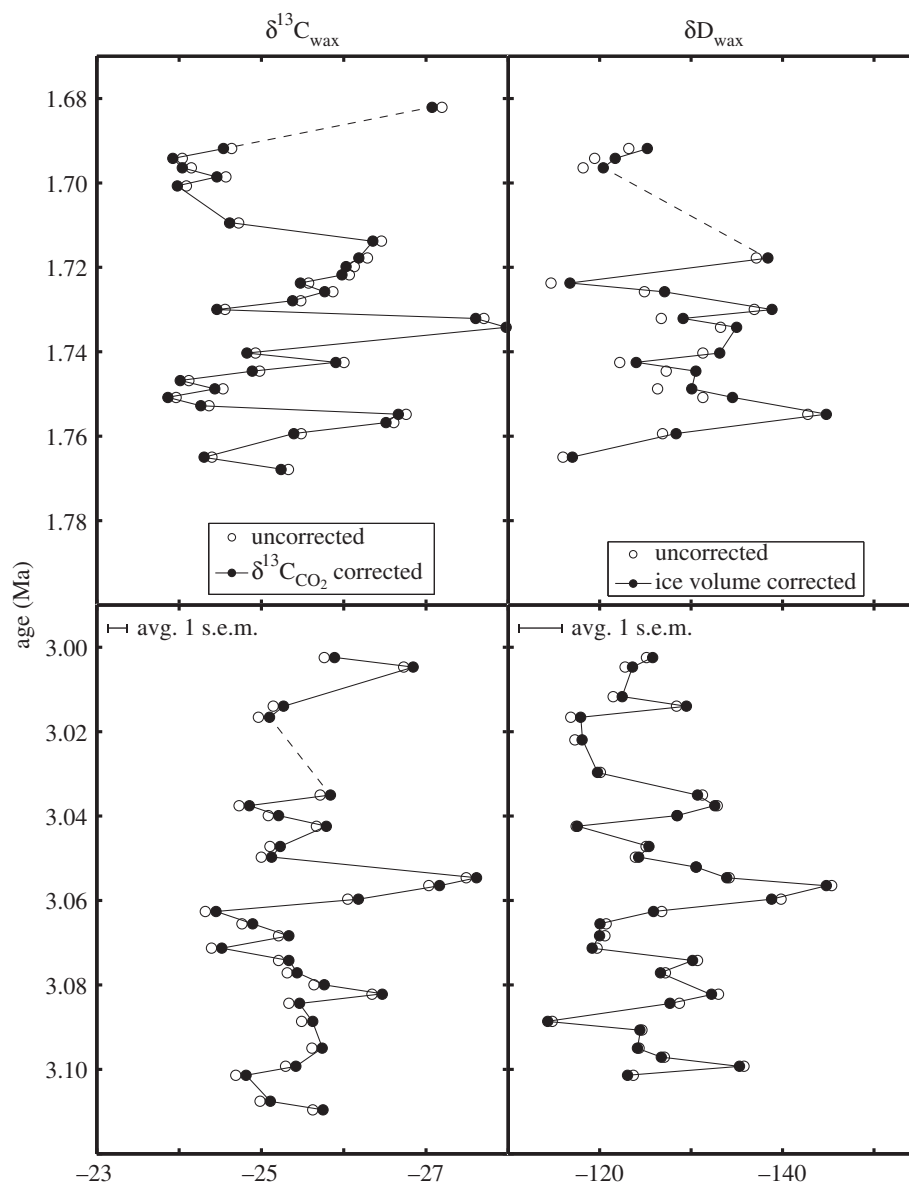
(1 s.e.m.) were calculated after Polissar & D'Andrea [74]:  $\delta D_{\text{wax}}$  and  $\delta^{13}C_{\text{wax}}$  uncertainties were  $4.8\text{‰}$  and  $0.25\text{‰}$ , respectively [74]. Isotope values were adjusted for the methyl group addition through mass-balance corrections based on measurements of phthalic acid methyl esters;  $\delta\text{D}$  and  $\delta^{13}\text{C}$  estimates of methanol stable isotope composition were  $-180 \pm 7.5\text{‰}$  (non-exchangeable H) and  $-25.5 \pm 0.9\text{‰}$ , respectively. Uncertainty in these values is included in the uncertainty estimates above.

Hydrogen isotope measurements are automatically corrected by the operating software for isobaric contributions from  $\text{H}_3^+$  produced in the ion source of the mass spectrometer, monitored daily. The  $\text{H}_3^+$  factor was measured daily during analysis.

Nonlinear effects of isotope values over a range of  $\text{CO}_2$  gas concentrations were observed in  $\delta^{13}C_{\text{wax}}$  analyses in both sample and standard injections. A large range in sample  $C_{28}$  FAME concentrations (figure 3) made it difficult to inject compounds at higher concentrations to counteract this effect. Nonlinear behaviour in  $\delta^{13}\text{C}$  measurements was corrected using repeat measurements of a sample along a large peak area range. While important, uncertainty in these corrections is not large enough to impact the findings of this study.

Changes in ice volume over time affect the isotopic composition of rainfall, which in turn is recorded by  $\delta D_{\text{wax}}$  and  $\delta^{18}O_{\text{ruber}}$  [60,75]. We correct for these changes by using the benthic  $\delta^{18}O$  stack of Lisiecki [76], which records changes in ice volume and deep-sea surface temperatures. We adjust the  $\delta^{18}O_{\text{stack}}$  such that the youngest value is equivalent to modern seawater  $\delta^{18}O$  values. We then divide the adjusted values by the last glacial maximum  $\delta^{18}O_{\text{stack}}$  value ( $1\text{‰}$ ) to scale the stack to the ice volume component of  $\delta^{18}O_{\text{seawater}}$  [77]. Next, we calculate expected  $\delta\text{D}$  changes from ice volume ( $\delta D_{\text{IV}}$ ), using the observed 8:1 relationship of  $\delta\text{D}$  versus  $\delta^{18}O$  in modern precipitation [68,78]. The expected  $\delta D_{\text{IV}}$  ( $\delta_{\text{accepted}}$ ) was interpolated at sample ages, and ice volume-corrected sample  $\delta D_{\text{wax}}$  values ( $\delta D_{\text{IVC}}$ ) were calculated. The effect of this correction on  $\delta D_{\text{wax}}$  is shown in figure 4.

The photosynthetic pathway of the plant, water use efficiency and ambient atmospheric  $\text{CO}_2$   $\delta^{13}\text{C}$  all combine to influence  $\delta^{13}C_{\text{wax}}$  values [80]. We corrected  $\delta^{13}C_{\text{wax}}$  values for changing  $\delta^{13}C_{\text{CO}_2}$  since 3.12 Ma to pre-industrial  $\delta^{13}C_{\text{CO}_2}$  values ( $-6.5\text{‰}$ )



**Figure 4.** Effects of corrections on Site 967 isotope values.  $\delta^{13}\text{C}_{\text{wax}}$  is corrected for changes in atmospheric  $\delta^{13}\text{C}_{\text{CO}_2}$  (3 Myr running average) over time [79].  $\delta\text{D}_{\text{wax}}$  is corrected for changes in  $\delta\text{D}_{\text{precipitation}}$  due to ice volume variability over time [76]. See §3 for details of these corrections.

**Table 1.** Published vegetation end member  $\delta^{13}\text{C}_{\text{wax}}$  data.

veg. type	year collected	$\delta^{13}\text{C}$ atm. $\text{CO}_2$ (mean annual; versus VPDB) <sup>a</sup>	measured compound	$n\text{-C}_{28}^b$ $\delta^{13}\text{C}_{\text{CO}_2,\text{corr.}}$	source
C <sub>4</sub> grass	1997, 1998, 2000	−7.952, −8.048, −8.052	n-acid C <sub>28</sub>	−20.5	Chikaraishi <i>et al.</i> [82,83]
C <sub>4</sub> grass	1989	−7.8	n-alkane C <sub>27</sub>	−21.3	Collister <i>et al.</i> [84]
C <sub>3</sub> angiosperm trees	1999, 2000	−8.035, −8.052	n-acid C <sub>28</sub>	−35.4	Chikaraishi <i>et al.</i> [82,83]
C <sub>3</sub> savannah trees	1992, 2003, 2005	−7.838, −8.161, −8.216	n-alkane C <sub>27</sub>	−31.7	Vogts <i>et al.</i> [85]

<sup>a</sup> $\delta^{13}\text{C}_{\text{CO}_2}$  data are from Francey *et al.* [86] (pre-1978) and Keeling *et al.* [87] (1980–2008).  $\delta^{13}\text{C}_{\text{wax}}$  measurements were corrected to the 1861  $\delta^{13}\text{C}_{\text{CO}_2}$  value of −6.49‰, using the  $\epsilon$  equation.

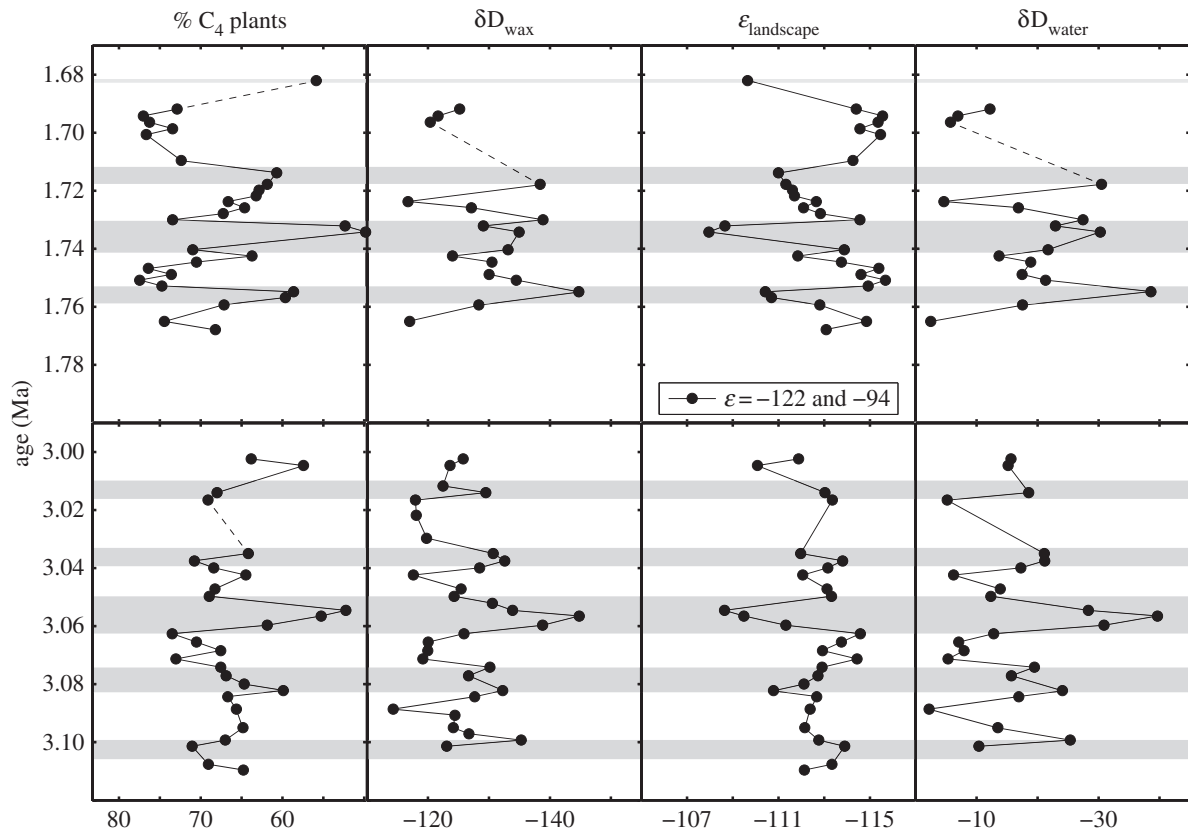
<sup>b</sup>Average  $\epsilon$   $n\text{C}_{27} - n\text{C}_{28}$  is 0.9‰ for C<sub>3</sub> plants, and 1.0‰ for C<sub>4</sub> plants [83].

using the  $\delta^{13}\text{C}_{\text{CO}_2}$  of Tipple [79]. The effect of this correction on  $\delta^{13}\text{C}_{\text{wax}}$  is shown in figure 4.

We examine sedimentary leaf wax provenance using lignin compounds for two samples spanning the largest  $\delta^{13}\text{C}_{\text{wax}}$  range observed in our records. One sapropel and one nannofossil ooze sample, dating to 1.73 and 1.75 Ma, respectively, were analysed for lignin monomers at the Stable Isotope Biogeochemistry

Laboratory, Purdue University. Samples were prepared after [81].

To calculate the percentage of C<sub>4</sub> vegetation present in each sample, a variety of C<sub>4</sub> and C<sub>3</sub>  $\delta^{13}\text{C}$  end members were collected from the literature, corrected to pre-industrial  $\delta^{13}\text{C}$  and used in a variety of linear mixing lines to calculate a range of possible C<sub>4</sub> percentages (table 1). Chikaraishi *et al.* [82]'s fatty acid values for C<sub>4</sub>



**Figure 5.** Potential impacts of vegetation change on plant source water  $\delta D$  ( $\delta D_{\text{water}}$ ) calculations.  $C_4$  vegetation calculations are shown in the first column (calculated via linear interpolation with  $C_3$  and  $C_4$  vegetation end members of  $-35.4\%$  and  $-20.5\%$ , respectively; [83]). The second column shows ice volume-corrected  $\delta D_{\text{wax}}$  ( $\%$  versus VSMOW). The third column displays  $\epsilon_{\text{landscape}}$  calculations from a two-end-member linear mixing model; end member values are indicated in the top (figure 3) [83]. The last column shows  $\delta D_{\text{water}}$  ( $\%$  versus VSMOW) calculations made by correcting  $\delta D_{\text{wax}}$ .  $\delta D_{\text{water}}$  variability is slightly larger amplitude than that of  $\delta D_{\text{wax}}$ . Averages of  $\delta D_{\text{water}}$  are not significantly different from the older to the younger interval.

grass and  $C_3$  angiosperm trees were chosen as the final end members in our analysis [82] (figure 3). We attempted to account for potential effects of vegetation change on  $\delta D_{\text{wax}}$  values using  $\delta^{13}\text{C}_{\text{wax}}$  measurements and modern apparent fractionation factors (epsilon factors,  $\epsilon$ ) (table 1). We selected average  $\epsilon$  values (for  $C_{28}$  n-acids) of  $-94\%$  and  $-122\%$ , respectively, for  $C_3$  angiosperm trees and  $C_4$  grasses measured in modern plants [64], calculated an ' $\epsilon_{\text{landscape}}$ ' (after Magill *et al.* [88]) for each  $\delta D_{\text{wax}}$  measurement using  $C_4$  grass percentage calculations (described above), and applied the  $\epsilon_{\text{landscape}}$  to each  $\delta D_{\text{wax}}$  value to reconstruct  $\delta D_{\text{water}}$  [16,82] (figure 5).

### (c) Planktonic foraminifera analyses

The planktonic foraminifera *G. ruber* (white; 250–300  $\mu\text{m}$ ) was used as a proxy for surface ocean conditions, particularly temperature and salinity. Five to seven whole foraminifera were picked from each sample (250–355  $\mu\text{m}$  fraction), and cleaned with repeated MilliQ water rinses to remove clay and organic matter prior to transfer into acid-cleaned vials for analysis. Nannofossil ooze sediments contained abundant *G. ruber*, but some sapropel sediments were devoid of planktonic foraminifera or did not contain enough *G. ruber* for analysis. Samples were analysed at LDEO for  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  ( $\delta^{18}\text{O}_{\text{ruber}}$  and  $\delta^{13}\text{C}_{\text{ruber}}$ ) on an Isoprime 100 multicarb dual-inlet isotope mass spectrometer; approximately 10% of samples were run in duplicate to assess sample standard deviation (s.d.). International carbonate standard NBS-19 was analysed alongside samples. Long-term s.d. for  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  were 0.06‰ and 0.04‰, respectively, based on repeat analyses of NBS-19. Standard deviation of replicate samples was 0.20‰ and reflects both analytical uncertainty and sampling variability of the distribution of *G. ruber* individuals in a sample.

## 4. Results

### (a) Orbital-scale variability

Precession-scale (approx. 20 kyr) variability is predominant in all measured proxies (figure 3). Figure 3 shows eccentricity-modulated precession calculations, which are very similar for the two periods [20]. Reflectivity of sediments mirrors precession variability with a slight lag (3 kyr, built in from the age model), with more reflective nannofossil ooze sediments deposited near precession maxima and less reflective, sapropelic sediments deposited near precession minima (figure 3). Reflectance amplitudes are similar to eccentricity-modulated precession amplitudes in the younger period, but are less similar in the older period, i.e. reflectance unit minima are relatively constant around 40 ( $L^*$ ).

Concentrations of  $C_{28}$  n-acids vary over four orders of magnitude between high reflectivity sediments and sapropelic sediments, with highest concentrations (greater than  $10^3 \text{ ng g}^{-1}$  sediment) deposited during sapropel intervals (figure 3). These observations are consistent with enhanced organic matter preservation during sapropel formation due to bottom-water anoxia in the Mediterranean Sea [89] and potentially higher local leaf wax production in the humid, vegetated Sahara region. Sedimentary geochemical analysis of these Site 967 sediments indicates significant contributions of Nile-derived sediments during sapropel deposition and this may also have provided another plant wax source vector [42,90], but the several orders of magnitude higher plant wax concentrations cannot be explained by this additional, fractional source.

**Table 2.** Computing Pearson's correlation coefficients and significance for correlation of  $\delta D_{\text{wax}}$  and  $\delta^{13}\text{C}_{\text{wax}}$ .

	<i>r</i> -values	<i>p</i> -values
1.75 Ma $\delta^{13}\text{C}_{\text{wax}}$ versus $\delta D_{\text{wax}}$	0.35	0.20
3.05 Ma $\delta^{13}\text{C}_{\text{wax}}$ versus $\delta D_{\text{wax}}$	0.48	0.02

Large negative excursions of 2–3‰ (approx. 15–20%  $\text{C}_4$ ) and 15–20‰ occur in  $\delta^{13}\text{C}_{\text{wax}}$  and  $\delta D_{\text{wax}}$ , respectively, around and during sapropel sediments in both periods (figure 3), which is consistent with observations of recent precession-forced changes in North African vegetation and rainfall [30,91]. More negative  $\delta^{13}\text{C}_{\text{wax}}$  is indicative of less  $\text{C}_4$  plant abundance during sapropel intervals [80]. Corrections for  $\delta^{13}\text{C}_{\text{CO}_2}$  and ice volume resulted in insignificant adjustments to  $\delta^{13}\text{C}_{\text{wax}}$  and  $\delta D_{\text{wax}}$  (figure 4). Using Pearson's correlation coefficient, we found that  $\delta D_{\text{wax}}$  shows fairly high, very significant positive correlation with  $\delta^{13}\text{C}_{\text{wax}}$  in the older period and slightly lower positive correlation in the younger period, in line with the expected close correspondence between precession forcing and leaf wax stable isotope patterns (table 2). This is also consistent with evidence that sapropels are deposited in response to more humid, vegetated North African conditions and vice versa [5,73,92].

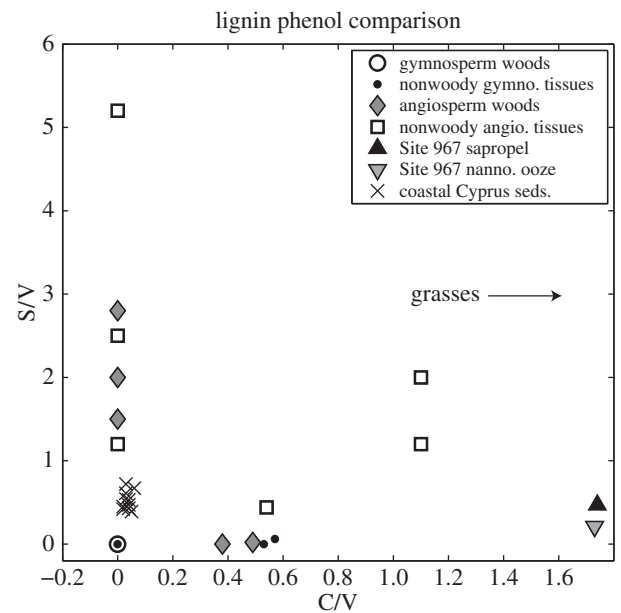
The  $\delta D_{\text{wax}}$  records exhibit variability consistent with precessional forcing of monsoonal rainfall changes [44,67,93]. The amplitude of the Site 967  $\delta D_{\text{wax}}$  cycles is about 20–30‰, which is comparable with the  $\delta D_{\text{wax}}$  amplitude for the Early Holocene African Humid Period in the Gulf of Aden, and Lake Tanganyika and Lake Challa [29]. Our calculations of source water  $\delta D$  ( $\delta D_{\text{water}}$ ) reveal slightly larger variability across precession cycles due to the effect of correcting for vegetation changes from  $\delta^{13}\text{C}_{\text{wax}}$  (§3b), but  $\delta D_{\text{water}}$  is similarly dominated by precession-scale variability (figure 5).

Even though we have only two lignin analyses, these are useful in constraining potential plant wax vegetation source changes between a wet and dry interval. Lignin analyses show very similar S/V and C/V ratios in both the sapropel and nannofossil ooze samples and are consistent with a grass-dominated vegetation source in both time periods (figure 6 and table 3). The nannofossil ooze has a slightly lower S/V ratio than the sapropel, but a nearly identical C/V ratio, consistent with very similar vegetation source. No syringyl aldehyde was detectable in the nannofossil ooze sample; therefore, we can only discuss the vanillyl acid/aldehyde (Ad/Al)<sub>v</sub> ratio here as a measure of lignin preservation. The sapropel sample exhibits much higher (Ad/Al)<sub>v</sub> than the nannofossil ooze, suggesting that lignin compounds in that sample underwent greater oxidation prior to deposition [70].

Variability in  $\delta^{18}\text{O}_{\text{ruber}}$  is similar though not identical to that of sediment reflectivity,  $\text{C}_{28}$  FAME concentrations,  $\delta D_{\text{wax}}$  and  $\delta^{13}\text{C}_{\text{wax}}$  during both periods (figure 3). Negative excursions of approximately 1.5–2‰ occur near sapropel mid-points, consistent with evidence that sapropels are deposited contemporaneously with large decreases in Mediterranean surface salinity due to increased African run-off [22].

### (b) Long-term, secular changes in lipid and foraminifera stable isotope values

A Mann–Whitney/Wilcoxon rank sum test revealed no significant differences in  $\delta^{13}\text{C}_{\text{wax}}$  means between the 3.05 and



**Figure 6.** Site 967 lignin analyses support an eastern Saharan vegetation source. Site 967 lignin phenol data are compared above with published syringyl/vanillyl (S/V) versus cinnamyl/vanillyl (C/V) data from modern plants and a coastal Cyprus marine sediment core located just north of the Eratosthenes Seamount [70]. Grasses exhibit high C/V ratios relative to gymnosperm and angiosperm trees [70]. Site 967 samples exhibit high C/V ratios and relatively low S/V ratios, indicating a grass-dominated vegetation source consistent with eastern Saharan environments.

1.75 Ma windows. This result suggests that the vegetation and palaeohydrologic responses to orbital precession monsoonal forcing were comparable for these two time periods. The results cannot, of course, be used to infer conclusions about unsampled intervals, but they do suggest nearly identical means and variance for these time windows that bracket an important increase in  $\text{C}_4$  vegetation in East Africa [2].

Curiously, the Site 967  $\delta^{13}\text{C}_{\text{wax}}$  results do not indicate a significant increase in  $\text{C}_4$  vegetation for the 1.75 Ma interval compared with the 3.05 Ma interval, as was anticipated given the clear  $\text{C}_4$  increases over this time period noted across many localities in East Africa [2,9] (figure 1). While it is possible our sampling strategy was not sufficient to capture this signal, our data for these two windows show high  $\text{C}_4$  contributions (50–80%  $\text{C}_4$ ) for both intervals, with no significant difference between them. This observation leads to the tentative conclusion that eastern Sahara vegetation evolution may have been separate from that observed in East Africa, a topic that we expand upon below.

The African run-off proxy,  $\delta^{18}\text{O}_{\text{ruber}}$ , does show highly significantly more positive values in the younger period after correcting for ice volume changes (table 4). Orbital-scale variability in  $\delta^{18}\text{O}_{\text{ruber}}$  is also slightly larger in the 1.68–1.78 Ma period relative to the 3.0–3.1 Ma period. Regional and global temperatures have decreased since the Late Pliocene, making this a relevant factor for the Site 967 record (figure 7) [52,95].

## 5. Discussion

### (a) Leaf wax provenance and interpretation of Site 967 $\delta^{13}\text{C}_{\text{wax}}$ and $\delta D_{\text{wax}}$ results

Aeolian dust supply to the eastern Mediterranean is principally derived from central Algeria and occurs during boreal



**Table 3.** Lignin data from this study.

sample	sed. type	$\delta^{13}\text{C}_{\text{wax}}$	S/V	C/V	(Ad/Al) <sub>V</sub>	(Ad/Al) <sub>S</sub>
967B6H4, 147 cm	sapropel	-25.8	0.47	1.74	0.62	0.96
967B6H5, 32 cm	nanno. ooze	-21.5	0.21	1.73	0.32	—

**Table 4.** Average values and standard deviations for measured  $\delta^{13}\text{C}_{\text{wax}}$ ,  $\delta\text{D}_{\text{wax}}$  (ice volume-corrected) and  $\delta^{18}\text{O}_{\text{ruber\_ivc}}$  between 1.68–1.8 Ma and 3.0–3.1 Ma intervals.

	mean 1.75 Ma	mean 3.05 Ma	s.d. 1.75 Ma	s.d. 3.05 Ma
$\delta^{13}\text{C}_{\text{wax}}$	-25.32	-25.58	1.13	0.74
% C <sub>4</sub>	67.63	65.91	7.59	4.98
$\delta\text{D}_{\text{wax}}$	-133.8	-126.3	10.8	6.7
$\delta\text{D}_{\text{water}}$	-19.2	-16.2	10.6	8.9
$\delta^{18}\text{O}_{\text{ruber}}$	0.092	-0.432	0.668	0.577

spring [25,26]. Inorganic geochemical sediment provenance studies of haematite,  $^{87}\text{Sr}$  isotope ratios, and major and minor elements of eastern Mediterranean sediments also suggest a dominantly northeastern Sahara source for dust transported into the eastern Mediterranean [5,73,96]. Wehausen & Brumsack [41], using major element ratios of sapropel sediments from eastern Mediterranean drill sites, found they were mainly (up to 100%) composed of inorganic sediments derived from the Nile River drainage basin, whereas drier (non-sapropel) periods were composed of 50% Nile River sediments and 50% Saharan dust.

A recent study of n-alkane and n-acid compounds in fluvial sediments in the Ganges and Brahmaputra River systems found that vascular plant material is a significant part of river organic carbon, and that these compounds reflect local vegetation composition in their respective drainage basins [97]. They observed a downstream enrichment of  $\delta^{13}\text{C}_{\text{wax}}$  in both compound types that they interpreted as degradation and progressive replacement of fluvial sedimentary leaf wax compounds by mixed C<sub>3</sub>/C<sub>4</sub> vegetation, particularly in the Ganges floodplain where C<sub>4</sub> vegetation dominates. This has important implications for Nile River fluvial leaf wax transport: as the Nile River is over twice as long as the Ganges and Brahmaputra Rivers, it might be expected that leaf wax compounds sourced from Nile headwaters would be completely replaced by downstream and floodplain leaf waxes before being deposited in the eastern Mediterranean Sea.

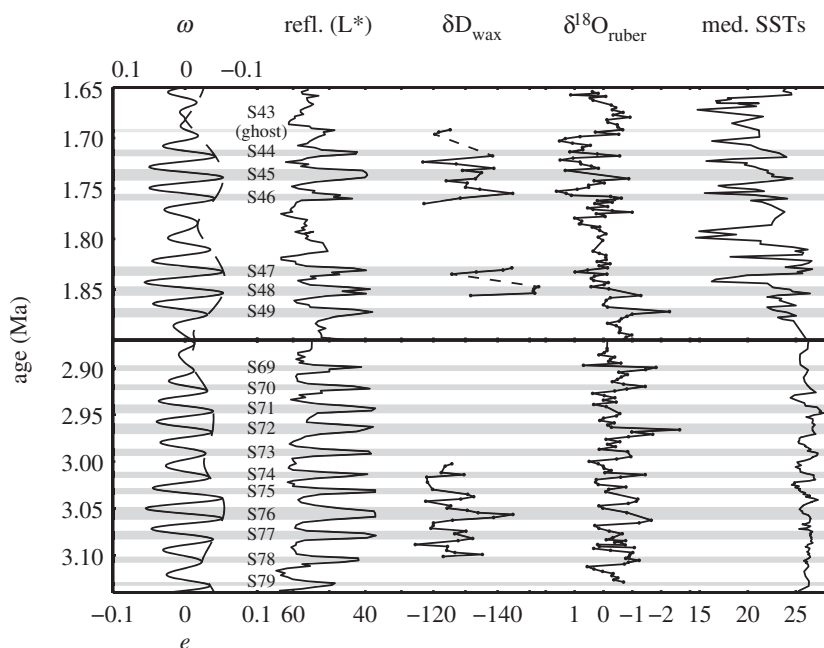
Based on these studies, we suggest that leaf waxes deposited in eastern Mediterranean sediments record a proximal vegetation source area in the eastern Sahara region, with some contribution from fluvially derived leaf waxes. Pollen reconstructions from the African Humid Period indicate that steppe vegetation (grasses, low shrubs) was abundant above approximately 20° N, and that savannah vegetation (annual grasses and scattered trees) was abundant between approximately 10–20° N, indicating an incursion of vegetation into presently bare desert regions [98,99]. This vegetation would have provided abundant additional leaf wax sources to rivers and streams draining into the eastern Mediterranean.

Modern spatial distributions of vegetation and pollen reconstructions of Mid-Holocene vegetation suggest that the Sahara/Sahel regions are dominated by C<sub>4</sub> vegetation during both wet and dry periods [100].

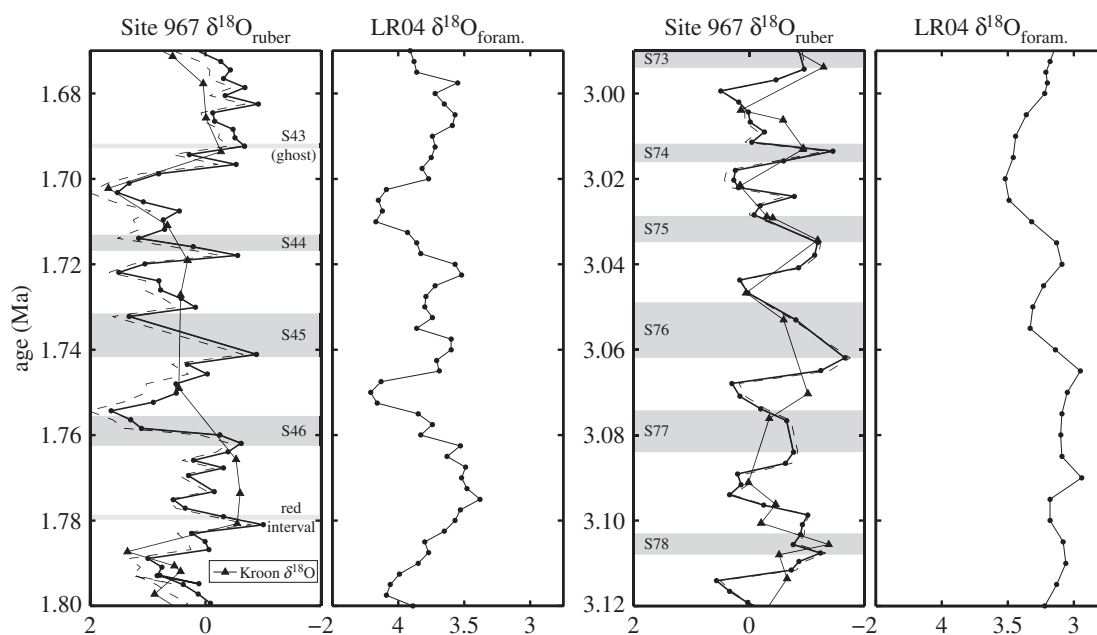
We conclude that C<sub>4</sub> grasses dominated northeast African environments in both the older and younger periods, with higher percentages of C<sub>4</sub> grasses during dry periods and lower percentages during humid periods, as indicated by  $\delta^{13}\text{C}_{\text{wax}}$  and  $\delta\text{D}_{\text{wax}}$  variability. On average, C<sub>4</sub> grasses comprise between 55 and 80% of total vegetation (figure 3), consistent with an eastern Saharan vegetation assemblage [98,101]. Our lignin results provide an additional test of sedimentary leaf wax provenance. High C/V ratios in both samples indicate a non-woody, grass-dominated lignin source [70]. This observation is consistent with high average C<sub>4</sub> grass abundance inferred from  $\delta^{13}\text{C}_{\text{wax}}$  data (figure 4) and supports our conclusion that the eastern Sahara is the dominant source of terrestrial leaf waxes found in eastern Mediterranean sediments.

The observed minima in  $\delta\text{D}_{\text{wax}}$ ,  $\delta^{13}\text{C}_{\text{wax}}$  and  $\delta^{18}\text{O}_{\text{ruber}}$  during sapropel periods are consistent with interpretation of these proxies as a means of tracking African monsoon strength and run-off. Sapropels have been observed to lag precession minima by approximately 3 kyr and correspond to peak monsoon run-off from Africa into the Mediterranean Sea [21,34]. Minima in  $\delta\text{D}_{\text{wax}}$ ,  $\delta^{13}\text{C}_{\text{wax}}$  and  $\delta^{18}\text{O}_{\text{ruber}}$  appear to covary with the L\* proxy for sediment colour reflectance and orbital insolation forcing (figure 3a), which supports our interpretations that  $\delta\text{D}_{\text{wax}}$  tracks African monsoon rainfall to the eastern Sahara region,  $\delta^{13}\text{C}_{\text{wax}}$  records vegetation changes responding to precipitation variability, and  $\delta^{18}\text{O}_{\text{ruber}}$  captures Mediterranean Sea surface salinity and temperature changes in addition to ice volume. Precession-scale  $\delta^{13}\text{C}_{\text{wax}}$  variability ranges approximately 2–4‰ (ca ±15–20% C<sub>4</sub> vegetation), much less than the 14‰ average difference between C<sub>3</sub> and C<sub>4</sub> vegetation, suggesting a mix of C<sub>3</sub> and C<sub>4</sub> vegetation across precessional cycles rather than oscillations between end members [102].

Depletion of  $\delta\text{D}_{\text{wax}}$  and  $\delta^{13}\text{C}_{\text{wax}}$  in response to precession-paced monsoonal precipitation maxima has been observed in numerous records throughout Africa, though some studies have found significant correlations between northern African climate variability and high-latitude climate and ocean circulation variability for the Late Pleistocene [16,45,62,103]. The range in orbital-scale  $\delta\text{D}_{\text{wax}}$  and  $\delta\text{D}_{\text{water}}$  amplitudes (approx. 20‰; figure 5) is reasonable when compared with Late Pleistocene  $\delta\text{D}_{\text{wax}}$  data from tropical Africa. Tierney *et al.* [45] analysed  $\delta\text{D}_{\text{wax}}$  records from the Congo Basin [93], Lake Tanganyika and Lake Challa between 0 and 25 ka, and observed approximately 20–30‰ shifts bracketing the African Humid Period. Collins *et al.* [103] observed orbital-scale  $\delta\text{D}_{\text{wax}}$  variability of approximately 10–15‰ in Late Pleistocene northwest African margin marine sediments [103].



**Figure 7.** Mediterranean Sea surface conditions show increasing high-latitude influence from the Late Pliocene to Mid-Pleistocene. Shown above from left to right are orbital eccentricity (black dotted line) and precession (red line; [20]); sapropel numbers [94]; sediment reflectivity [40];  $\delta D_{wax}$  (this study);  $\delta^{18}O_{ruber}$  (this study) and Mediterranean SSTs [52]. Site 967  $\delta^{18}O_{ruber}$  exhibits two distinct patterns of behaviour in the Late Pliocene and Mid-Pleistocene. During the Late Pliocene period,  $\delta^{18}O_{ruber}$  shows great resemblance to sediment reflectivity and  $\delta D_{wax}$ , varying primarily at approximately 20 kyr frequencies and suggesting that eastern Mediterranean  $\delta^{18}O_{seawater}$  was controlled by salinity variability and African monsoonal run-off. During the Mid-Pleistocene,  $\delta^{18}O_{ruber}$  shows longer periodicities and greatest resemblance to Mediterranean SSTs, strongly implying that SST was the primary control on  $\delta^{18}O_{seawater}$  at this time, with sea surface salinity acting as a secondary control.

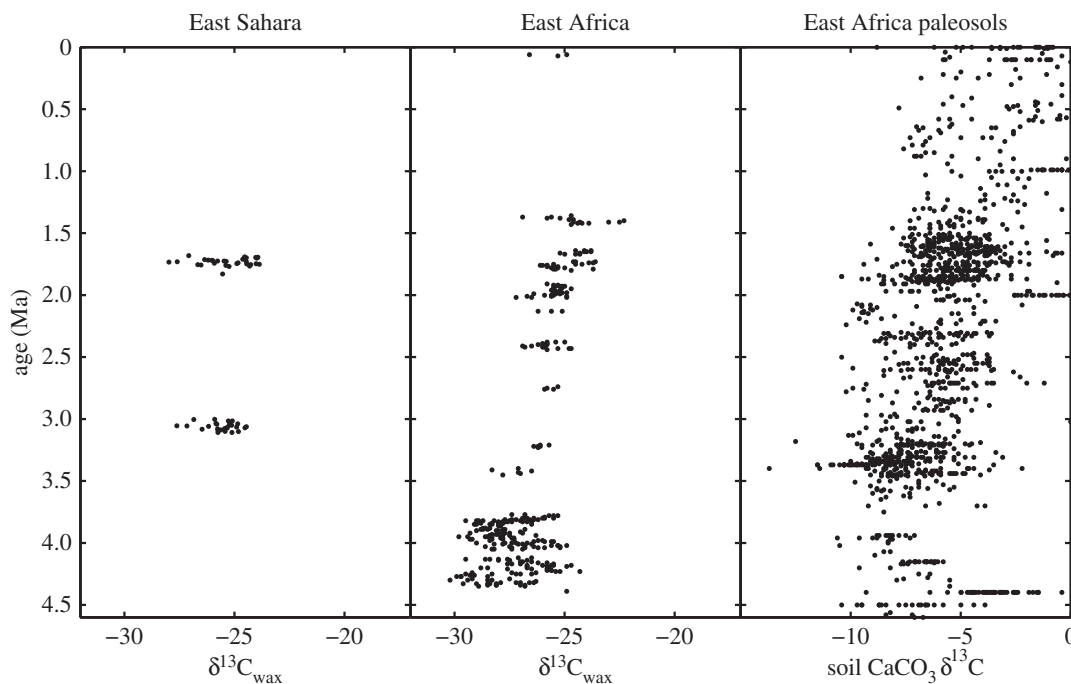


**Figure 8.** Site 967  $\delta^{18}O_{ruber}$  (this study) comparison with previously published Site 967  $\delta^{18}O_{ruber}$  and a global benthic foraminifera  $\delta^{18}O$  stack [22,76]. Sapropel intervals are shown by grey boxes and labelled 'S#'; a possible oxidized sapropel is denoted by a 'red interval' at 1.78 Ma [22,51]. High-resolution sampling at Site 967 (black dots) revealed approximately 20 kyr frequency variability not always captured by low-resolution sampling (black triangles). Site 967  $\delta^{18}O_{ruber}$  more greatly resembles global variability (LR04) from 1.67 to 1.8 Ma than between 3 and 3.12 Ma, suggesting that high-latitude climate has greater influence over Mediterranean surface conditions in the younger interval.

### (b) Comparing Site 967 results with East African palaeoclimate and palaeovegetation records

The eastern Mediterranean Site 967 plant wax  $\delta D_{wax}$  and  $\delta^{13}C_{wax}$  results document clear orbital-scale changes in regional hydrology and vegetation for the eastern Sahara

catchment region. The  $\delta^{13}C_{wax}$  results suggest there was no significant difference in the mean value or variance between the 1.75 and 3.05 Ma intervals that were sampled. Moreover, we show that the values are consistent with an average 65%  $C_4$  contribution for both periods, varying between 55 and 80% at the orbital-scale. The  $\delta D_{wax}$  results similarly



**Figure 9.** Site 967 ( $C_{28}$ )  $\delta^{13}C_{wax}$  are compared with Gulf of Aden DSDP Site 231 ( $C_{30}$ )  $\delta^{13}C_{wax}$  and East African soil carbonate  $\delta^{13}C$  records [2,12]. Soil carbonate data markers (legend) are plotted from north to south and show vegetation data nearest to the Nile River headwaters in Ethiopia, also the primary source region for Nile sediments [105]. Average Site 967  $\delta^{13}C_{wax}$  remains constant between the approximately 3.05 Ma and approximately 1.75 Ma windows, while Site 231  $\delta^{13}C_{wax}$  and palaeosol  $\delta^{13}C$  record a clear shift towards more positive values across the same interval, implying that East Saharan vegetation remained constant while East Africa transitioned towards more abundant  $C_4$  grasslands.

document comparable mean values between the 1.75 and 3.05 Ma intervals, with comparable orbital-scale amplitudes (approx. 20–30‰) under comparable orbital eccentricity and precession forcing (table 4). These amplitudes are comparable to multiple plant wax  $\delta D_{wax}$  records of the Early Holocene African Humid Period [29].

Site 967 records some climate signals that are restricted to the eastern Mediterranean sector. The lower frequency variability (approx. 40 kyr) of  $\delta^{18}O_{rubber}$  in the younger period is likely related to the influence of high-latitude climate on Mediterranean SSTs based on a comparison with a newly developed alkenone record of Mediterranean SST variability [52] (figure 7). Variations in  $\delta^{18}O_{rubber}$  between 1.68 and 1.8 Ma show greater similarity to central Mediterranean Uk'37 SSTs than to sediment reflectivity,  $\delta D_{wax}$  and  $\delta^{13}C_{wax}$ . This supports the assertion that the Mediterranean region shows increasing high-latitude climate influence throughout the Pliocene and Pleistocene [22,52]. However, the similarity of  $\delta^{18}O_{rubber}$  and central Mediterranean SSTs may suggest that SST variability (in excess of 5–8°C for the ca 1.75 Ma interval) increasingly influences Mediterranean  $\delta^{18}O_{rubber}$  over the Pleistocene [22,52]. There is also great similarity between Site 967  $\delta^{18}O_{rubber}$  and the global  $\delta^{18}O$  stack, further indicating that high-latitude climate change influenced Mediterranean surface conditions (figure 8). This implies that Mediterranean surface conditions did not dominantly influence precipitation or vegetation changes in the plant wax catchment area (eastern Sahara) for Site 967, and that precessional monsoonal climate forcing was the predominant climate signal here.

Our results can only speak to the short intervals that were sampled near 3.05 and 1.75 Ma, but they do suggest that there was little change in the monsoonal palaeohydrological or palaeovegetation response to monsoonal circulation changes for these two time intervals which bracket the onset of NH glaciation after 2.8 Ma [104] as well as the shift toward more open,  $C_4$ -dominated landscapes in East Africa [2].

### (c) Regional differences in northeast African vegetation change

We reach the conclusion that there was no change in eastern Saharan hydroclimate or vegetation response to orbital monsoonal forcing for these 3.05 and 1.75 Ma windows. Furthermore, the Site 967  $\delta^{13}C_{wax}$  means and standard deviations for these two windows are indistinguishable (table 4) suggesting (but not proving) that there was no secular change between these windows. This contrasts with the well-documented increase in  $C_4$  vegetation at many, but not all, East African sites and offshore marine sediments [2,9,11,12]. To reiterate, additional sampling is required to confidently assert that the Site 967 results do not conform to the East African vegetation history, but our initial results support this view. Extracting all palaeosol  $\delta^{13}C$  values for these same sampled time periods from the Levin & Naoami [2] database indicates there is a significant increase in mean values between the 1.68 and 1.8 Ma window ( $-5.0 \pm 1.5\%$ ,  $n = 101$ ) and the earlier 3.0–3.15 Ma window ( $-7.6 \pm 1.8\%$ ,  $n = 9$ ; figure 9).

Given the eastern Sahara dust source area provenance for Site 967 and the lack of a significant  $\delta^{13}C_{wax}$  change between our older and younger intervals, we conclude that the eastern Sahara may have had a distinctly different vegetation history from East Africa, and that the well-known  $C_4$  increase in East Africa was, in fact, restricted to this geographical domain. Our results would suggest that the eastern Sahara experienced large-amplitude  $C_3$ – $C_4$  vegetation and palaeohydrological cycles with no evidence for a shift to more open or more varied conditions. By contrast, East African localities, collectively, indicate a marked expansion of  $C_4$  vegetation towards more open landscapes between 3 and 2 Ma [2,9,11,48,106]. Moreover, where detailed, sub-orbital sampling has been performed for lacustrine intervals (palaeolake Olduvai), palaeovegetation inferences from plant wax  $\delta^{13}C$

data indicate full amplitude 0–100%  $C_4$  vegetation cycles tracking orbital precession near 1.9–1.8 Ma [15,16]. If supported by additional analysis, our results suggest that the 3–2 Ma expansion of  $C_4$  vegetation and shift to more open East African landscapes may have been restricted to the East African domain and was not a pan-African vegetation transition. More analysis is required to support this assertion but available data support this view.

One mechanism has been proposed that could explain why East African vegetation shows such a well-defined shift to more open,  $C_4$ -rich vegetation after 3–2 Ma. An emerging view proposes that East Africa became drier in response to changes in tropical ocean temperature gradients during the Early Pleistocene [107,108]. Where tropical SSTs are warmest, such as the IndoPacific warm pool, the overlying atmosphere saturates with water vapour and convects. The resulting rainfall and latent heat release drive a large-scale overturning cell, the Walker circulation. The compensatory dry, descending vertical motions occur where tropical SSTs are coolest, such as the eastern equatorial Pacific and western Indian Ocean where sub-surface waters upwell along the eastern margins of Africa and Arabia.

The emergence of the modern tropical Pacific zonal SST gradient that drives these large-scale tropical climate gradients occurred between 3 and 2 Ma based on multiple SST proxy records [109–112] and was broadly coeval with the transition towards drier and more open,  $C_4$ -rich vegetation in East Africa [108,112]. Northern African aridity was found to be largely unaffected by the emergence of these zonal tropical SST gradients [108], providing a plausible mechanism for different vegetation histories of these two regions.

## 6. Conclusion

Our detailed  $\delta^{13}C_{wax}$  and  $\delta D_{wax}$  analyses of two short (100 kyr) intervals at ODP Site 967 in the eastern Mediterranean basin documents large vegetation and hydroclimate cycles corresponding to orbital precession forcing of African monsoonal circulation and intensity. These intervals were selected for their nearly identical orbital eccentricity and precession amplitude configuration and because they bracket an important transition to more open East African vegetation between 3 and 2 Ma. The amplitude of  $\delta^{13}C_{wax}$  cycles was 3–4‰, corresponding to 50–80%  $C_4$  vegetation. The amplitude of  $\delta D_{wax}$  cycles was about 20–30‰, comparable with  $\delta D_{wax}$  records of the most recent African Humid Period (15–5 ka BP) from ocean and lake archives.

We find no significant difference in means or standard deviations of the  $\delta^{13}C_{wax}$  and  $\delta D_{wax}$  values between the 3.05 and 1.75 Ma intervals, suggesting that vegetation and

hydroclimate responses to precessional monsoonal forcing were comparable for these two periods in the eastern Sahara domain. The lack of a significant increase in  $C_4$  vegetation between these two intervals contrasts with the well-known increase in  $C_4$  vegetation documented for many East African localities and in the Gulf of Aden [2,9,11].

Although further analysis of the Site 967 sediments would be required to confirm this finding, we tentatively conclude that the eastern Sahara dust source region (Algeria) did not experience the remarkable  $C_4$  expansion in the East African domains of Kenya, Tanzania and Ethiopia. We propose that this East African  $C_4$  grassland expansion between 3 and 2 Ma may have been a consequence of the coeval emergence of tropical ocean SST gradients at this time [109–112]. Climate model experiments constrained by palaeoceanographic observations have documented that the broad East African geographical domain would have become drier, and arid-adapted  $C_4$  vegetation would have presumably increased, with the emergence of large zonal tropical SST gradients [108,112].

Our results also speak to a subtle, but important distinction noted in the Feakins *et al.* [11] Gulf of Aden study. Specifically, our results support their conclusion that orbital-scale palaeovegetation and palaeohydrologic variability can be as large, or larger, than long-term, secular shifts. Such observations conform to the very large orbital-scale palaeovegetation and palaeohydrologic variability detected at palaeolake Olduvai (*ca* 1.9–1.8 Ma [15,16]) and thus highlight the relative importance of shorter orbital-scale versus longer secular landscape remodelling influences on early hominin evolution in East Africa [17,113,114].

**Data accessibility.** All organic and isotopic geochemical data for this study have been uploaded as the electronic supplementary material. These data are also available through Interdisciplinary Earth Data Alliance (doi:10.1594/IEDA/100586).

**Authors' contributions.** P.B.d.M., C.R., J.E.T. and P.J.P. conceived the study and supervised the work. C.R., J.E.T., T.F. and P.J.P. acquired the data. All authors analysed and interpreted the data. C.R. and P.B.d.M. wrote the paper and C.R. drafted the figures with input and assistance from all co-authors.

**Competing interests.** We have no competing interests.

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