



# Precession driven changes in terrestrial organic matter input to the Eastern Mediterranean leading up to the Messinian Salinity Crisis



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## ABSTRACT

Eastern Mediterranean sediments over the past 12 Myr commonly show strongly developed precessional cyclicity, thought to be a biogeochemical response to insolation-driven freshwater input from run-off. The Mediterranean's dominant freshwater source today and in the past, is the Nile, which is fed by North African monsoon rain; other, smaller, circum-Mediterranean rivers also contribute to Mediterranean hydrology. Crucially, run-off through all of these systems appears to vary with precession, but there is no direct evidence linking individual water sources to the biogeochemical response recorded in Mediterranean sediments. Consequently, it is not clear whether the North African monsoon is entirely responsible for the Mediterranean's sedimentary cyclicity, or whether other, precessional signals, such as Atlantic storm precipitation, drive it.

Organic matter in sediments derives from both marine and terrestrial sources and biomarker analysis can be used to discriminate between the two, thereby providing insight into sedimentary and ecological processes. We analysed a wide range of lipids from the Late Miocene (6.6–5.9 Ma) Pissouri section, southern Cyprus, and reconstructed the vegetation supplied to this region by measuring the carbon isotopes of the terrestrial component to identify its geographic source. BIT (Branched-Isoprenoidal-Tetraether) indices reflect changes in the relative abundance of marine vs terrestrial (soil) organic matter inputs, and with the exception of records from the last deglaciation, this work is the first application of the BIT approach to the reconstruction of orbital impacts on sedimentological processes. BIT indices show that the organic matter supplied to Cyprus changed over the course of each precession cycle and was dominantly terrestrial during insolation maxima when North African run-off was enhanced. The  $\delta^{13}\text{C}$  values from these intervals are compatible with tropical North African vegetation. However, the  $\delta^{13}\text{C}$  record indicates that during insolation minima, organic material supplied to southern Cyprus derives from a more arid source region. This is likely to have been aeolian-transported organic matter from the Anatolian Plateau demonstrating that even in Mediterranean sedimentary systems influenced by Nile run-off, there is more than one independent precessional organic matter contribution to the sedimentary cyclicity. Pissouri's organic geochemistry also illustrates a long-term trend towards more saline Mediterranean conditions during the 600 kyr leading up to the Messinian Salinity Crisis.

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## 1. Introduction

Mediterranean sedimentary successions over the last 12 million years (Myr) are commonly dominated by strong cyclicity (Kidd et al., 1978; Rohling et al., 2015). These regular litholog-

ical alternations, comprising some combination of marls, lime-stones, diatomites and organic-rich sapropelic layers, are demonstrably precessional (Hilgen et al., 1997; Sierro et al., 2001) and are thought to be the Mediterranean's biogeochemical response to orbitally-driven variations in freshwater input (Rossignol-Strick, 1985). The largest source of this freshwater variation is derived from the North African monsoon (Rohling et al., 2015 and references therein). Precessional changes shift the position of the Intertropical Convergence Zone (ITCZ) northward during times of insolation maxima (July 65°N; Laskar et al., 2004) and increase the

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intensity of the monsoon in the catchment of northward draining rivers that flow into the Mediterranean (Marzocchi et al., 2015). These insolation-driven changes also affect the vegetation across North Africa (Larrasoña et al., 2013), although model simulations fail to capture the full greening of the Sahara indicated by terrestrial data (Larrasoña et al., 2013 and references therein). Precessional changes in the location and intensity of North Atlantic storm tracks also impact Mediterranean precipitation patterns, principally in the west and along the north Mediterranean margin (Kutzbach et al., 2014; Toucanne et al., 2015).

In the Late Miocene Mediterranean salinity rose significantly and thick, basin-wide evaporites were deposited during the Messinian Salinity Crisis (MSC, 5.97–5.33 Ma; Manzi et al., 2013 and references therein). While deep basinal evaporites have yet to be recovered, those preserved on the Mediterranean margins also show strong cyclicity which is thought to reflect an on-going precessional signal (Flecker et al., 2015), despite extreme environmental conditions. Although the transition to evaporite precipitation in the Mediterranean is synchronous and abrupt at 5.97 Ma (Manzi et al., 2013), geochemical evidence (Flecker et al., 2015 and references therein) as well as water column oxygenation and faunal data (Kouwenhoven et al., 2003; Sierro et al., 2001) indicate that the first environmental precursors of the MSC occurred several million years earlier.

The evidence of precessional-pacing of Mediterranean sedimentation is clear. However, the specific processes that generated the recorded biogeochemical response remain controversial. Debates over the role of freshwater driven productivity versus water column stratification in the generation of organic-rich sapropelic layers (Kidd et al., 1978) endure, while the question of whether deep-sea anoxia, promoted by enhanced export productivity, a freshwater cap, or a combination of both, can transfer the precessional run-off signal from the Eastern to Western Mediterranean Basins as advocated by Rohling et al. (2015), remains untested. One alternative possibility is that the North Atlantic storm-track generates the precessional biogeochemical response in the Western Mediterranean independent of, but in phase with precessional run-off to the Eastern Mediterranean (Toucanne et al., 2015). However, it is not clear whether the storm track precipitation is volumetrically sufficient to generate the biogeochemical response observed.

All of these controversies relate, in part, to the difficulty in identifying the fresh water sources responsible for driving the biogeochemical sedimentary product observed in the Mediterranean Basin. This study uses biomarkers preserved within the sedimentary record as tracers of the freshwater from which they were derived in order to explore and provide new insight into the underlying dynamics of the Mediterranean's sedimentary cyclicity.

Lipid biomarkers have been used to explore changes in the hydrological cycle in a variety of ways. Leaf wax  $\delta^{13}\text{C}$  values differ between  $\text{C}_3$ - and  $\text{C}_4$ -plants where  $\text{C}_3$ -plants have lower  $\delta^{13}\text{C}$  values than  $\text{C}_4$ -plants (O'Leary, 1981) as a result of their different morphology and carbon assimilation biochemistry (Edwards et al., 2010 and references therein). Because the distribution of  $\text{C}_3$ - and  $\text{C}_4$ -plants is governed by a combination of environmental factors, including temperature and aridity (Yang et al., 2014), leaf wax  $\delta^{13}\text{C}$  values provide insight into both past vegetation and climatic conditions. Biomarkers can also provide insight into changes in marine versus terrestrial organic matter (OM) fluxes (Hopmans et al., 2004), from which changes in the OM source can be inferred. Other lipid biomarkers, including isoprenoidal and branched glycerol dialkyl glycerol tetraether (GDGT) lipids can be used to determine sea-surface temperatures ( $\text{TEX}_{86}$  [TetraEther index]; Schouten et al., 2013 and references therein) and land surface temperatures (MBT' [Methylation of Branched Tetraether]/CBT [Cyclisation of Branched Tetraethers]; Weijers et al., 2007; Peterse et al., 2012), providing additional in-

sights into environmental changes. This approach constrains the relative control of temperature versus aridity on  $\text{C}_3/\text{C}_4$  plant abundances.

We have applied these approaches to the Late Miocene Pissouri section on Cyprus (5.98–6.51 Ma), which lies to the north of the Nile delta (Fig. 1). Today, the majority of the monsoon-derived North African run-off reaches the Mediterranean via the Nile which is its largest fluvial system. We extracted and characterised biomarkers from the strongly cyclic Pissouri succession and have used them to reconstruct the influence of Nile water and the precipitation in its catchment. In particular, we use the proportions branched-GDGTs (brGDGTs) to crenarchaeol (BIT index), distributions of  $n$ -alkyl lipids, and the  $\delta^{13}\text{C}$  values of high molecular weight  $n$ -alkanes of terrestrial plant origin, to establish the nature of organic matter supplied to this central eastern Mediterranean region. The ~600 kyr interval preceding the MSC was targeted in order to evaluate the climatic changes that occur during the leading up to the MSC.

## 2. Methods

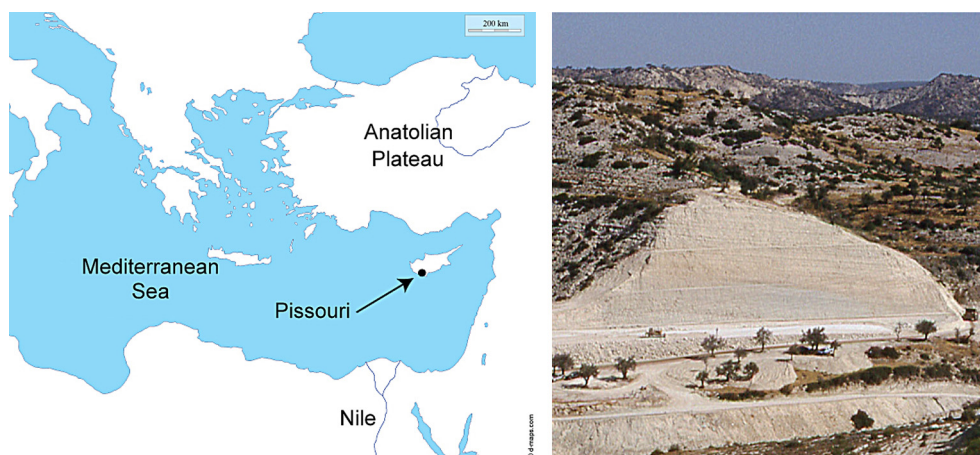
### 2.1. Site description and sampling strategy

The Pissouri basin on southern Cyprus (Fig. 1) is filled with Neogene sediments extending back to the Middle Miocene (Krijgsman et al., 2002). The Messinian succession of the basin comprises regular alternations of limestones and marls (Fig. 2; Krijgsman et al., 2002) where carbonate content (Krijgsman et al., 2002) has been used to distinguish them (e.g. limestone >75%; marl <75%; Sugden and McKerrow, 1962). The cyclicity of the succession has been used to astronomically tune the Pissouri section to orbital solutions (Laskar et al., 2004) using both bio- and magnetostratigraphic tie-points (Fig. 2; Krijgsman et al., 2002). At Pissouri, three biostratigraphic events occur in the 30 m of limestone–marl alternations that directly underlie the gypsum and are studied here (Fig. 2). These biostratigraphic tie points consistently link marl deposition to the area of the orbital curve that includes the insolation maxima (Fig. 2). In addition, three magnetostratigraphic boundaries also occur in this part of the section (Fig. 2) and these are consistent with the tuning based on biostratigraphic tie-points and confirm the lithological phase relationship with the orbital curve (Krijgsman et al., 2002). The marls have been correlated with sapropelic horizons (Krijgsman et al., 2002) that are observed in other Mediterranean successions (e.g. Sorbas; Sierro et al., 2001). In line with standard practice for astronomically tuned Mediterranean successions and consistent with the biostratigraphic and magnetostratigraphic tie points, the middle of each marl (or sapropel) layer is linked to the extremes of the  $65^\circ\text{N}$  summer insolation curve (Sierro et al., 2001; Fig. 2).

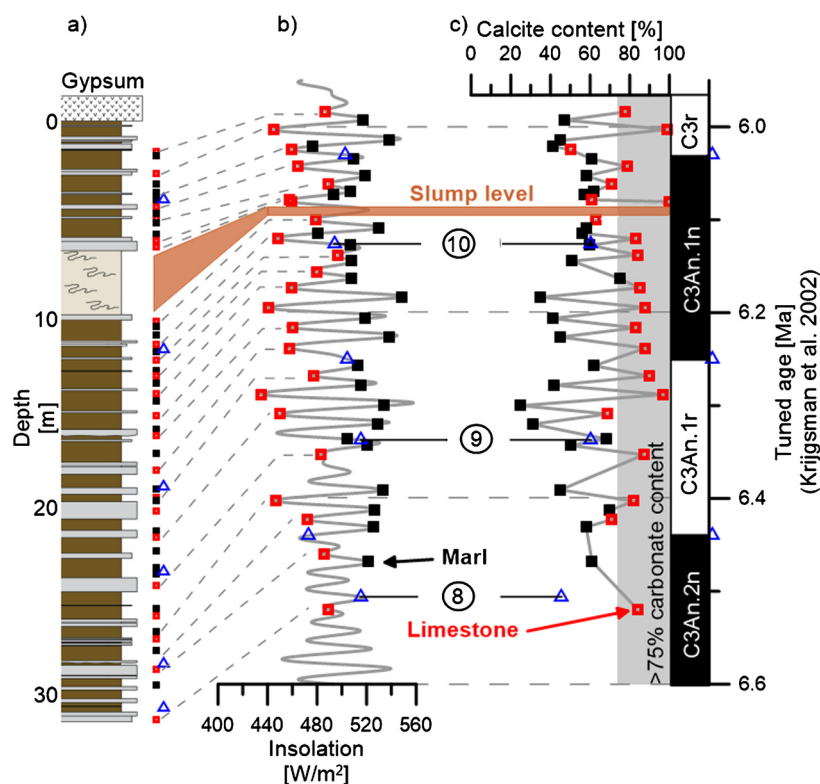
Forty-eight samples were collected in 1998 from the Pissouri section road cut (Kouwenhoven et al., 2006; Krijgsman et al., 2002) before it was sprayed with concrete. Where possible, one sample for every limestone and one for every marl was analysed throughout the section, starting at ~32 m below the gypsum and representing the 600 kyr preceding the MSC (6.52–5.98 Ma; Krijgsman et al., 2002). While this approach does not necessarily show the full range of values in relation to precession, it does allow us to compare data generated during two distinct phases of multiple orbital cycles. The slump layer (7–10 m, Fig. 2) towards the top of the section was not sampled because its age and orbital phasing is uncertain.

### 2.2. Extraction and separation

The sediments (~40 g) were homogenised and extracted via Soxhlet apparatus for 24 h using dichloromethane (DCM):



**Fig. 1.** Map showing the inflow of the Nile into the Mediterranean and the location of the Pissouri section on Cyprus ( $34^{\circ}40'01.9''\text{N}$ ;  $32^{\circ}38'48.8''\text{E}$ ). A photograph of the section before it was sprayed with concrete is given on the right.

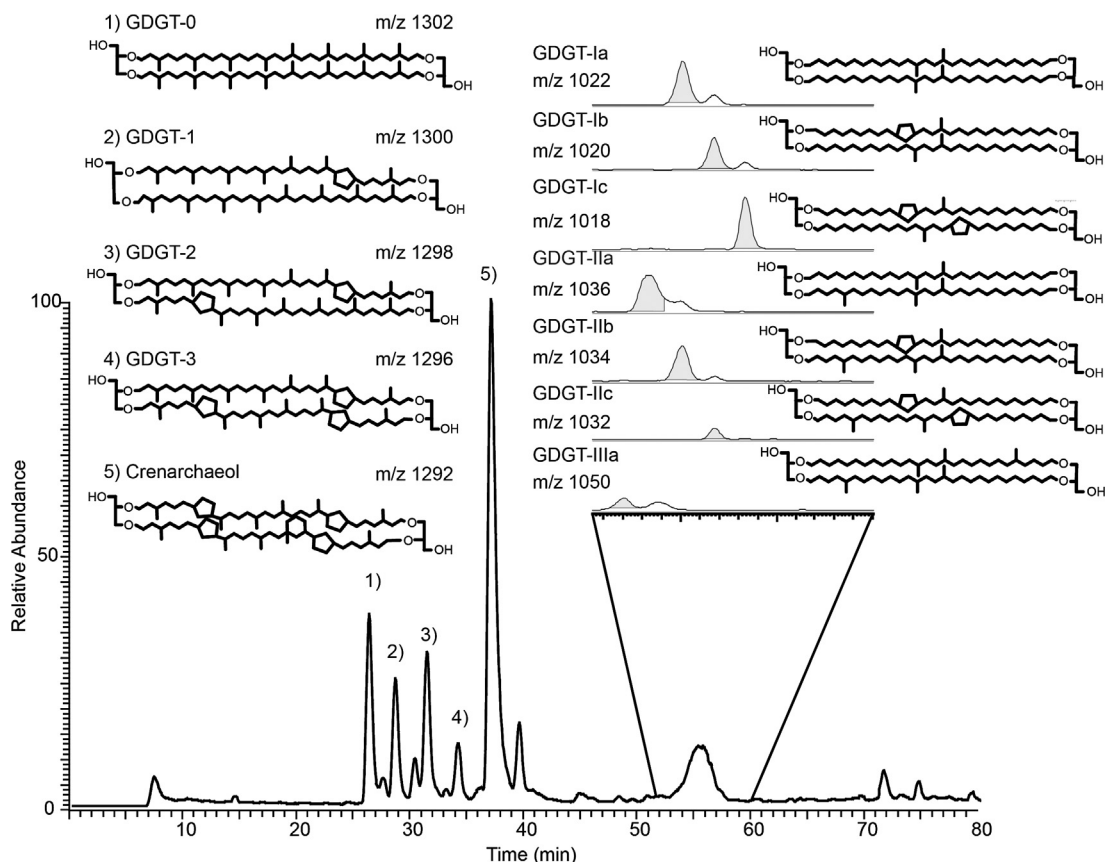


**Fig. 2.** Schematic log of the Pissouri section (a) with alternating harder (grey) and softer (brown) layers alongside (b) the insolation curve (Laskar et al., 2004) and (c) the calcium carbonate content of the sediment which was used for astronomical tuning of the section (Krijgsman et al., 2002). The biostratigraphic tie points (blue triangles) shown from Krijgsman et al. (2002) are: (8) the LO of the *G. miotumida* group (6.506 Ma); (9) the sinistral/dextral coiling change of *Neoglobobquadrina acostaensis* (6.337 Ma); and (10) the first influx (>80%) of sinistral neoglobobquadrinids (6.126 Ma). The palaeomagnetic tie points (blue triangles) are C3r–C3An.1n (6.03 Ma) C3An.1n–C3An.1r (6.25 Ma) C3An.1r–C3An.2n (6.44 Ma; Krijgsman et al., 2002). The vertical grey bar reflects >75% carbonate content, the formal definition of a limestone (Sugden and McKerrow, 1962). A slump layer (brown bar) interrupts the cyclic limestone–marl alternations between 7 and 10 m depth (Krijgsman et al., 2002). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

methanol (MeOH) (2:1 v/v). An aliquot of the total lipid extract (TLE) was separated into two fractions using alumina flash column chromatography: an apolar fraction eluted with 4 ml hexane (Hex)/DCM (9:1 v/v) and a polar fraction eluted with 3 ml DCM:MeOH (1:2 vol/vol). An internal standard (IS) was added to the apolar (androstande 5  $\mu\text{l}$  of 200 ng/ $\mu\text{l}$ ) and polar (hexadecan-2-ol, 5  $\mu\text{l}$ , 207.5 mg/ $\mu\text{l}$  solution) fractions. The polar fraction was derivatised for 1 h at  $70^{\circ}\text{C}$  using 50  $\mu\text{l}$  of BSTFA+TMCS 99:1 (N,O-bis(trimethylsilyl) trifluoroacetamide and trimethylchlorosilane).

### 2.3. GC-MS

Both fractions were analysed using a Thermo Scientific ISQ Single Quadrupole gas chromatography-mass spectrometer (GC-MS). The GC was equipped with a 50 m  $\times$  0.32 mm i.d. fused silica capillary column with an Rtx-1 stationary phase (100% dimethylpolysiloxane, 0.17  $\mu\text{m}$  film thickness; Restek) and programmed from  $70^{\circ}\text{C}$  (held for 1 min) to  $130^{\circ}\text{C}$  at  $20^{\circ}\text{C}/\text{min}$ , then to  $300^{\circ}\text{C}$  (held for 24 min) at  $4^{\circ}\text{C}/\text{min}$ . The MS continuously



**Fig. 3.** Representative HPLC-APCI-MS chromatogram of a Pissouri limestone (R.F.-7018; data table) showing both br- and iGDGTs with their respective  $m/z$ . Note that the brGDGT peak in this particular total ion content trace is dominated by GDGT-Ia.

scanned between mass to charge ratios ( $m/z$ ) 50 and 650 Daltons. The  $n$ -alkanes,  $n$ -alkanols and  $n$ -alkanoic acids were identified by their spectra and quantified in the total ion current.

### 2.3.1. GC-C-IRMS

The  $n$ -alkane  $\delta^{13}\text{C}$  values were determined using an Isoprime 100 GC-combustion-isotope ratio MS (GC-C-IRMS). Apolar fractions were analysed in duplicate, injected via a splitless injector onto a  $50\text{ m} \times 0.32\text{ i.d.}$  fused silica capillary column with an HP 1 stationary phase (100% dimethylpolysiloxane, 0.17 film thickness, Agilent). The temperature programme was the same as for GC-MS analysis. The combustion reactor was maintained at a temperature of  $850^\circ\text{C}$ . Standard notation relative to the Vienna Pee Dee Belemnite (VPDB) was achieved by comparison against a calibrated reference  $\text{CO}_2$  gas. Two thirds of samples had sufficient  $n$ -alkane abundances for  $\delta^{13}\text{C}$  determination.

### 2.3.2. HPLC-APCI-MS

The second aliquot of the TLE was dissolved in A (A = hexane:iso-propanol (IPA) (99:1 v/v)) and passed through a  $0.45\text{ }\mu\text{m}$  PTFE filter. High performance liquid chromatography-atmospheric pressure chemical ionisation-MS (HPLC-APCI-MS) was performed using a ThermoFisher Scientific Accela Quantum Access triple quadrupole MS to analyse the iGDGTs and brGDGTs. Separation was achieved with an Alltech Prevail Cyao column ( $150\text{ mm}$ ;  $2.1\text{ mm}$ ;  $3\text{ }\mu\text{m i.d.}$ ) with a flow rate of  $0.2\text{ ml/min}$ . The initial solvent mix A was eluted isocratically for 5 min, followed by a change in solvent polarity via a gradient to 1.8% IPA over 45 min (Hopmans et al., 2000). After separation, ionisation was performed at atmospheric pressure, and select  $m/z$ : 1302, 1300, 1298, 1296, 1294, 1292, 1050, 1048, 1046, 1036, 1034, 1032, 1022, 1020, 1018, 744, 659 (Fig. 3; Schouten et al., 2013) were scanned to increase

sensitivity and reproducibility. GDGT ratios were calculated from the respective  $\text{M}^+$ -ions chromatograms, and abundances were determined by comparing with a known  $\text{C}_{46}$ -GDGT standard (Huguet et al., 2006). The relative response of the standard and the GDGTs was not determined, such that reported concentrations are strictly semi-quantitative.

### 2.3.3. Proxies

Long-chain  $n$ -alkanes are synthesised by terrestrial vascular plants (Eglinton and Hamilton, 1967). The carbon preference index (CPI) is the ratio of odd-to-even chain lengths of the  $n$ -alkanes and is calculated as follows (Bray and Evans, 1961):

$$\text{CPI} = 0.5 * \left( \frac{\text{C}_{23} + \text{C}_{25} + \text{C}_{27} + \text{C}_{29} + \text{C}_{31} + \text{C}_{33}}{\text{C}_{24} + \text{C}_{26} + \text{C}_{28} + \text{C}_{30} + \text{C}_{32} + \text{C}_{34}} + \frac{\text{C}_{23} + \text{C}_{25} + \text{C}_{27} + \text{C}_{29} + \text{C}_{31} + \text{C}_{33}}{\text{C}_{22} + \text{C}_{24} + \text{C}_{26} + \text{C}_{28} + \text{C}_{30} + \text{C}_{32}} \right) \quad (1)$$

The components of Equation (1) refer to the relative concentrations of  $\text{C}_{22}$  to  $\text{C}_{32}$   $n$ -alkanes. During biosynthesis, higher plants produce high-molecular-weight (HMW) odd-numbered  $n$ -alkanes (Eglinton and Hamilton, 1967), but subsequent diagenesis (and catagenesis) causes homogenisation of the distribution (Bray and Evans, 1961). Consequently, lower CPI values can indicate increased degradation of these terrestrial inputs or a change in their source, whereas values  $>2$  indicate good preservation.

The terrestrial aquatic ratio ( $\text{TAR}_{n\text{-alkanes}}$ ) for the  $n$ -alkanes is calculated as (Bourbonniere and Meyers, 1996):

$$\text{TAR}_{n\text{-alkanes}} = \frac{\text{C}_{27} + \text{C}_{29} + \text{C}_{31}}{\text{C}_{15} + \text{C}_{17} + \text{C}_{19}} \quad (2)$$



This can be used to evaluate sources of OM, because HMW *n*-alkanes are generally produced by terrestrial vascular plants (Eglinton and Hamilton, 1967), and low-molecular-weight (LMW) *n*-alkanes generally derive from aquatic organisms (Meyers and Arnaboldi, 2008 and references therein). Similar ratios can be calculated using *n*-alkanoic acids (Bourbonniere and Meyers, 1996):

$$\text{TAR}_{n\text{-alkanoic acids}} = \frac{C_{26} + C_{28}}{C_{14} + C_{16} + C_{18}} \quad (3)$$

The BIT index is a ratio of the three major brGDGTs to crenarchaeol (Hopmans et al., 2004):

$$\text{BIT} = \frac{(\text{GDGTIa}) + (\text{GDGTIIa}) + (\text{GDGTIIIa})}{(\text{Crenarchaeol}) + (\text{GDGTIa}) + (\text{GDGTIIa}) + (\text{GDGTIIIa})} \quad (4)$$

Crenarchaeol is derived from Thaumarchaeota (Sinninghe Damsté et al., 2002), which represents ~20% of the picoplankton in the ocean, although it also occurs in subordinate abundances in soils (Weijers et al., 2007). BrGDGTs occur in high abundances in terrestrial settings, including soils and peats (Hopmans et al., 2004; Peterse et al., 2012). Therefore, BIT indices close to 1 represent OM originating from a predominantly terrigenous source, whereas low indices represent a Thaumarchaeotal source of GDGTs and by extension a strong aquatic source of OM (Schouten et al., 2013).

For the sea-surface temperature reconstructions, the TEX<sub>86</sub> proxy is defined as (Schouten et al., 2013):

$$\text{TEX}_{86} = \frac{\text{GDGT} - 2 + \text{GDGT} - 3 + \text{Crenarchaeol}'}{\text{GDGT} - 1 + \text{GDGT} - 2 + \text{GDGT} - 3 + \text{Crenarchaeol}'} \quad (5)$$

Various calibrations have been proposed to transform TEX<sub>86</sub> values to sea-surface temperature (SST), including both the original linear (Schouten et al., 2013) and subsequent logarithmic (Kim et al., 2010) relationships. Here, we use the BAYSPAR-calibration, which enables an increased accuracy and precision of reconstructions, due to the inclusion of a spatial element in the calibration (Tierney and Tingley, 2015), as well as more robust Bayesian-derived error estimates. It applies to the modern core-top data and searches for modern analogue locations based on the TEX<sub>86</sub> index (Tierney and Tingley, 2015). Biomarker data (i.e. hopane distributions) indicate that Pissouri OM is thermally immature, justifying the application of this core-top approach to these uplifted marine sediments.

For the land-based temperature reconstructions the MBT'/CBT index is used (Peterse et al., 2012):

$$\text{CBT} = \log \frac{\text{GDGTIb} + \text{GDGTIIb}}{\text{GDGTIa} + \text{GDGTIIa}}$$

$$\text{MBT}' = \frac{\text{GDGTIa} + \text{GDGTIb} + \text{GDGTIc}}{\text{GDGTIa} + \text{GDGTIb} + \text{GDGTIc} + \text{GDGTIIa} + \text{GDGTIIb} + \text{GDGTIIc} + \text{GDGTIIIa}} \quad (6)$$

In soils, the degree of cyclisation of branched GDGTs (reflected in the CBT index) is correlated with pH, whereas the degree of methylation (MBT') is correlated with both pH and Mean Annual Air Temperature (MAAT), resulting in the MBT'/(CBT) soil temperature proxy (Weijers et al., 2007; Peterse et al., 2012). This proxy is commonly applied to marginal marine sediments to reconstruct the terrestrial climate of the catchment area (e.g. Peterse et al., 2012). Since these analysis were completed, a new methodology has been proposed by De Jonge et al. (2014) to include 6-methyl brGDGTs as well as the 5-methyl brGDGTs in the CBT and MBT' proxies. GDGT abundances are low in these Pissouri

samples, so there was insufficient material to enable reanalysis and the application of this new method.

### 3. Results

All samples contained a variety of biomarkers, including both branched (brGDGT) and isoprenoidal (iGDGT) GDGTs (Fig. 3) and a homologous series of *n*-alkanes and *n*-alkanoic acids. Also present was an unusual nonacosan-10-ol which can be derived from conifers (Jetter and Riederer, 1995).

#### 3.1. GDGTs

The Pissouri sediments contain a wide range of br- and iGDGTs (Fig. 3; supplementary data table; Schouten et al., 2013). BIT indices are highly variable in the Pissouri sediments (Fig. 4c), ranging from below 0.1 to 0.85, indicating more than one OM source. These variations are strongly related to lithology, where the marls have significantly higher (Student's paired t-test between BIT values of marls vs limestones  $p < 0.001$ ) BIT indices (0.3 to 0.9) than the limestones (0.1 to 0.6; Fig. 4c). There is a general decline in the limestone BIT indices from 0.6 to 0.05, up section (Fig. 4c). BIT indices from the marls have a more complex pattern, with high BIT indices (0.67–0.90) in sediments older than 6.26 Ma and in those overlying the slump (0.67–0.88) and lower indices in the intervening interval (0.17–0.65). Consequently, the difference in marl-limestone BIT indices is most pronounced at the top and bottom of the section, with a smaller marl-limestone contrast in BIT indices between 6.3 Ma and the slump (Fig. 4c), mainly due to lower values in the marls.

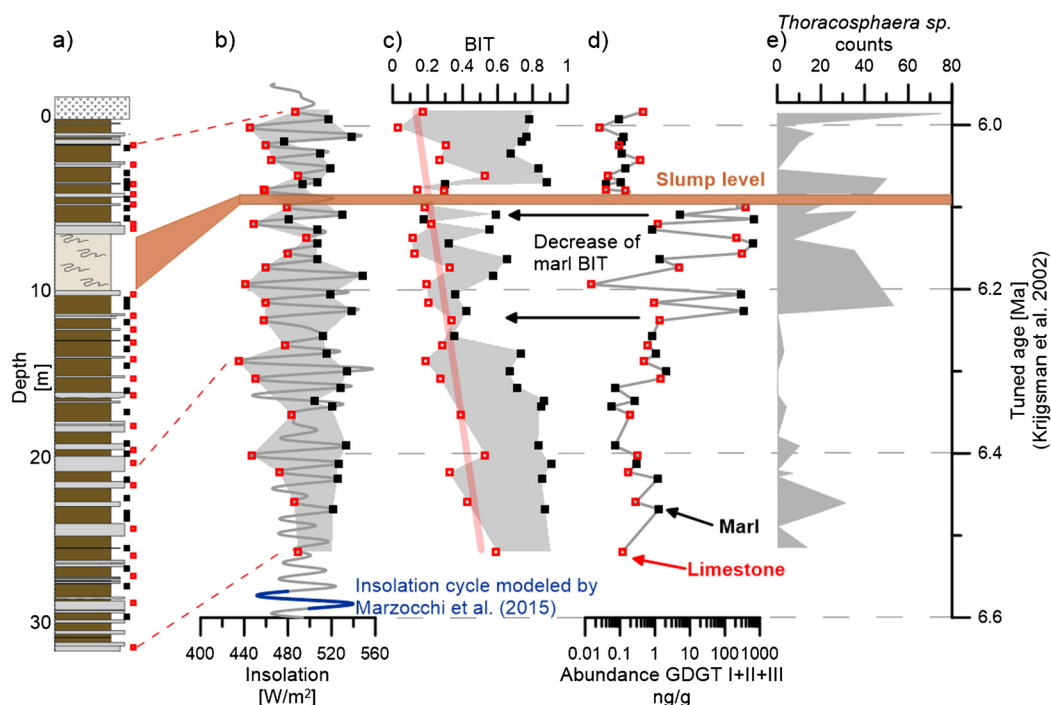
The low BIT-contrast interval immediately below the slump is also characterised by variable i- and brGDGTs abundances (Fig. 4d), with some values approximately two orders of magnitude higher than in the rest of the section.

br- and iGDGTs can also be used to determine terrestrial and sea-surface temperatures (see Section 2.3.3). However, TEX<sub>86</sub> should not be applied to sediments with high BIT indices (>0.4; Schouten et al., 2013 and references therein), and terrestrial temperatures should be treated with caution in samples with low (<0.3, Weijers et al., 2007) BIT indices. In the Pissouri section, 24 samples had BIT indices lower than 0.4, indicating the dominance of marine GDGTs (Hopmans et al., 2004); in these, the TEX<sub>86</sub>-derived SSTs range from 22 to 30 °C (data table) using the BAYSPAR calibration (Tierney and Tingley, 2015). The 32 samples with BIT indices >0.3, yield MBT'/CBT-derived MAAT for the adjacent land ranging from 15 to 28 °C (supplementary data table) based on the calibration of Peterse et al. (2012).

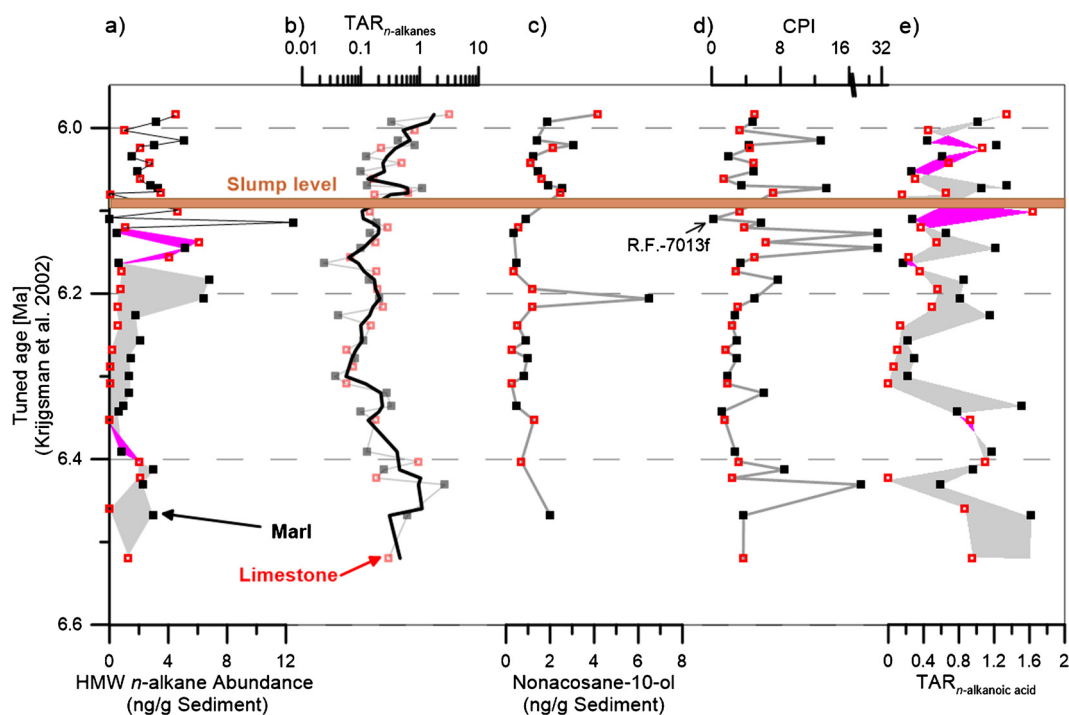
#### 3.2. *n*-alkyl biomarkers

The overall distribution of the *n*-alkanoic acids in the Pissouri samples is bimodal, with a peak at *n*-C<sub>16</sub> and a second peak at *n*-C<sub>28</sub>. In most cases the *n*-C<sub>28</sub> *n*-alkanoic acid is most abundant. The TAR<sub>*n*-alkanoic acid</sub> values vary from 0.7 to 2.2 (Fig. 5e). Commonly the limestones have lower TARs than the marl layers (Fig. 5e), but this difference is less consistent than in the BIT indices (Fig. 4c).

The *n*-alkane distribution is similar to the bimodal *n*-alkanoic acid distribution with one short-chain peak (C<sub>19</sub> although C<sub>15</sub> and C<sub>17</sub> homologues are also abundant) and another long-chain maximum (C<sub>31</sub>). The concentrations of the HMW *n*-alkanes range between 0.6 and 12 ng/g sediment (Fig. 5a), and is generally lower in the limestones. Between 6.35 and 6.15 Ma the offset between the two lithologies is particularly clear, with higher *n*-alkane



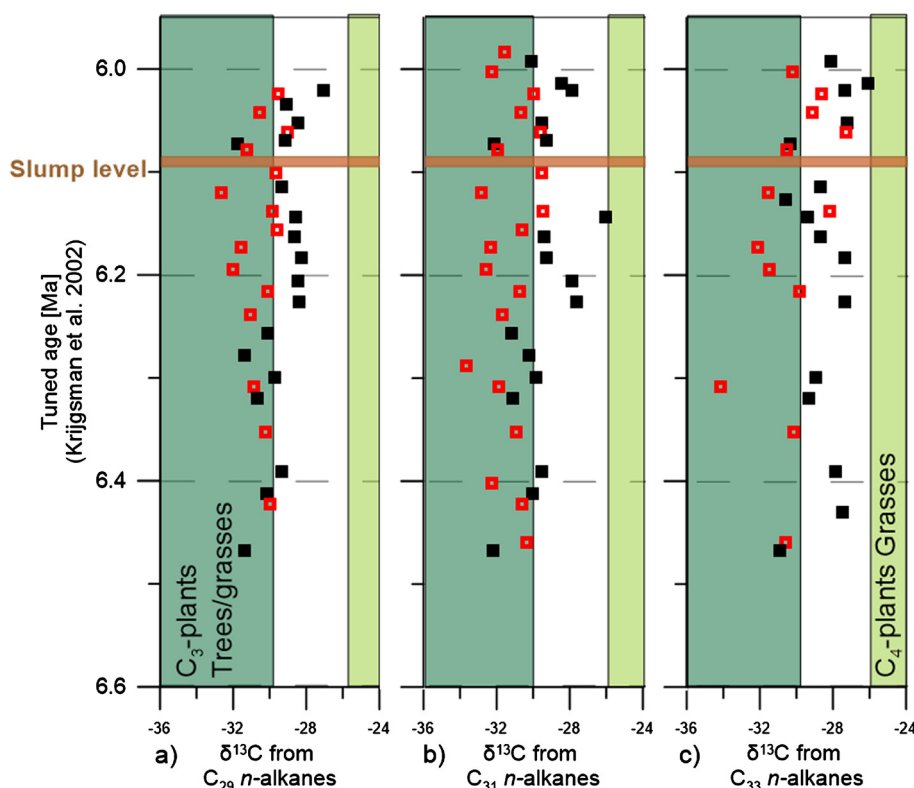
**Fig. 4.** A schematic log (a) of the Pissouri section (Krijgsman et al., 2002) showing sampled levels: limestones (red open squares) and marls (black squares). Aligned with the log are (b) the 65N insolation curve (Laskar et al., 2004) with the specific cycle used for the General Circulation Model simulations indicated in blue (Marzocchi et al., 2015), (c) BIT indices, including the long-term trend through the limestone data, (d) brGDGT abundances and (e) counts for the calcareous dinocyst *Thoracosphaera* sp. (Kouwenhoven et al., 2006). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



**Fig. 5.** Alkyl biomarker records through the Pissouri section including (a) HMW *n*-alkane ( $C_{29} + C_{31} + C_{33}$ ) concentrations; (b) the  $TAR_{n-alkanes}$  with a three point running average; the (c) nonacosane-10-ol concentrations; (d) the CPI of the *n*-alkanes with the excluded sample R.F.-7013f highlighted; and (e) the  $TAR_{n-alkanoic\ acid}$ . Grey shading indicates higher marl values relative to limestones, whereas magenta indicates higher values for limestones than adjacent marls. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

concentrations occurring in the marls (Fig. 5a). The  $TAR_{n-alkanes}$  ratio (data table) varies from 0.05 to 3.16 with an average of 0.36, but unlike the BIT indices, the  $TAR_{n-alkanes}$  (Fig. 5b) show no systematic relationship with lithology, potentially due to the

complex range of biological sources for the LMW components or a diverse range of higher plant inputs (i.e. aeolian vs fluvial inputs) for the HMW components. At the top of the section, the  $TAR_{n-alkanes}$  shift to higher indices, i.e. higher concentra-



**Fig. 6.** Age profiles of the (a)  $C_{29}$ , (b)  $C_{31}$ , and (c)  $C_{33}$   $n$ -alkane  $\delta^{13}C$  values. The stable carbon isotopic composition of leaf waxes is dependent on the biosynthetic pathway of the source plant (O'Leary, 1981). A typical distribution for  $\delta^{13}C$  values of  $C_{29}$ – $C_{31}$   $n$ -alkanes from leaf waxes of  $C_3$  and  $C_4$  plants is given as green shading (modified after Rommerskirchen et al., 2006). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

tions of long chain  $n$ -alkanes indicating stronger terrestrial inputs (Fig. 5b).

With one exception, all sediments have a CPI > 1 (Fig. 5d; supplementary data table) with an average value of 6 and a range from 2 to 8. This indicates a strong predominance of odd-over-even HMW  $n$ -alkanes, compatible with a leaf wax source and good preservation (Bray and Evans, 1961). We have excluded the sample at 6.11 Ma (R.F-7013f; supplementary data table) from further discussion, because of its anomalously low CPI and position close to the slump interval.

Nonacosan-10-ol, which is derived from specific conifers (Jetter and Riederer, 1995), is found throughout the section (Fig. 5c). Concentrations do not vary systematically with lithology, but, with the exception of one highly concentrated sample at 6.2 Ma, the relatively constant Nonacosan-10-ol concentration that characterises the lower part of the succession up to 6.1 Ma is followed by a trend towards higher concentrations (6.08–5.98 Ma).

### 3.3. Carbon isotopes of the long-chain $n$ -alkanes

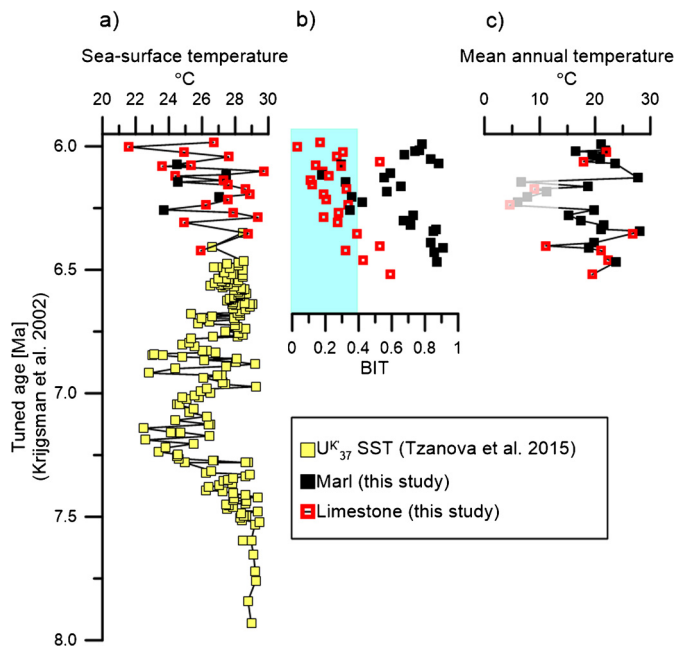
In the Pissouri section,  $\delta^{13}C$  values for the HMW  $C_{29}$ ,  $C_{31}$  and  $C_{33}$   $n$ -alkanes (Fig. 6) range between  $-35\text{‰}$  and  $-26\text{‰}$ . This range is more negative than the  $\delta^{13}C$  values typically associated with leaf waxes from  $C_4$ -plants (Kohn, 2016 and references therein), but is consistent both with  $\delta^{13}C$  values measured on  $C_3$  plants or a mixture of  $C_4$  and  $C_3$  plants (Fig. 6). In the upper part of the section (younger than 6.22 Ma),  $\delta^{13}C$  values for all three long-chain  $n$ -alkanes are consistently lower by 2–3‰ in the limestones than in the marls, with which they are interbedded (Fig. 6). In the lower part of the section low  $n$ -alkane abundances prevented  $\delta^{13}C$  analysis in 10 samples (~21% of dataset). However, where measured,  $n$ -alkanes in limestones have lower  $\delta^{13}C$  values than those in the adjacent marls.

## 4. Discussion

### 4.1. Temperature proxies

As discussed above, GDGTs are used to reconstruct both land and marine palaeo-temperatures. The high variability of marine versus terrestrial source inputs (Fig. 7a) at Pissouri allows both the marine and the terrestrial temperatures to be evaluated but only for specific horizons. Where BIT indices are <0.4 (Fig. 7b; Schouten et al., 2013 and references therein), TEX<sub>86</sub>-derived SSTs are in good agreement with alkenone-derived SST records from Monte dei Corvi, Italy (Tzanova et al., 2015; Fig. 7a). The combined datasets indicate a cooling of the Eastern Mediterranean prior to the onset of the MSC (Fig. 7), consistent with a global Late Miocene cooling. The average temperature of the Eastern Mediterranean today is 20–22 °C, several degrees cooler than that indicated by the average SST for these Late Miocene samples (25.5 °C; Fig. 7a) even when including the TEX<sub>86</sub> uncertainty of 2–3° (Tierney and Tingley, 2015). The long-term temperature change is overprinted by shorter-term variability of 6 °C (Fig. 7). The 12–13 kyr resolution of both our TEX<sub>86</sub> and the alkenone (Tzanova et al., 2015) datasets means that we cannot be sure that this reflects the full range of precessional temperature variability.

The terrestrial MBT'/CBT-derived MAAT records appear to suggest an average temperature of 18 °C with large temperature variations of up to 14 °C (Fig. 7c); much larger than those seen in the SST reconstruction (Fig. 7a). However, the average is lower than that observed today (20 °C) and the large variability is unexpected, even given the large standard error on MBT'/CBT-derived MAAT reconstructions of  $\pm 5$  °C (Peterse et al., 2012). There are several likely reasons for the pronounced variations.



**Fig. 7.** The SST profile (a) derived from GDGTs (TEX<sub>86</sub>, this study) at Pissouri and alkenones (U<sup>K</sup><sub>37</sub>, Tzanova et al., 2015) from Monte dei Corvi, Italy. TEX<sub>86</sub> data are limited to those samples with BIT < 0.4 from Pissouri. Also shown are limestone and marl BIT indices (b; with BIT < 0.4 shaded in turquoise) and (c) limestone and marl MBT'/CBT-derived MAAT (Peterse et al., 2012) for samples with BIT > 0.3. The shaded area in grey marks the decrease in marl BIT area. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

1. New analytical methods have been developed (De Jonge et al., 2014), and these could affect both determination of MBT, indices and the application of appropriate calibrations. We consider this factor to be minor given that much previous work based on the original methods and calibrations still exhibited clear temporal trends.
2. The dataset includes samples with low BIT indices, which could be problematic (Weijers et al., 2007); however, removing those data with BIT below 0.3 yields an average MAAT of 18 °C and a range of 15 to 28 °C, still much larger than expected.
3. Recent work has repeatedly shown that MBT'/CBT indices yield relatively low MAATs in arid settings (Peterse et al., 2012; Yang et al., 2014). Using a recently developed aridity calibration, shifts the temperatures to higher values by ~2 °C (Yang et al., 2014), and given the fact that aridity is likely to have varied in the source region, this could be another source of variability.

Overall, it appears that GDGT-based proxies confirm that the Eastern Mediterranean was warmer during the Late Miocene than it is today, consistent with globally higher temperatures (e.g. Tzanova et al., 2015) and model simulations using elevated pCO<sub>2</sub> (Marzocchi et al., 2015). Elevated MAATs are less clear, given the profound variability in brGDGT indices, but we attribute this to complex changes in sources of brGDGTs and the additional impact of highly variable hydrology (Yang et al., 2014).

#### 4.2. Differences in limestone and marl OM inputs

There is a statistically significant difference between the BIT indices of the limestones and those of the marls ( $P > 0.001$ ; students t-test; Fig. 4c), probably as a result of varying OM sources and preservation controls. The BIT index can be affected by OM degradation, with preferential degradation of marine OM over ter-

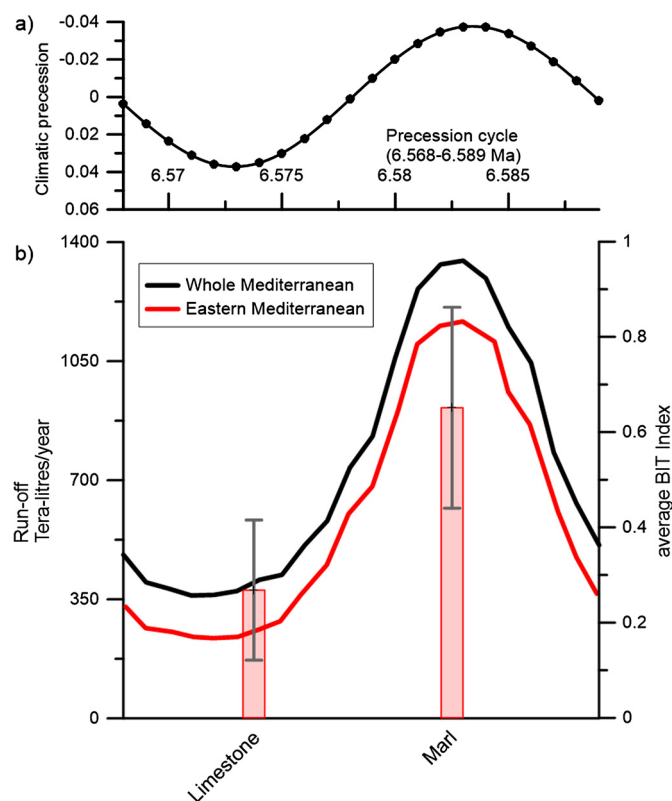
restrial OM shifting BIT indices higher (Huguet et al., 2009). Indeed, enhanced preservation beneath the slump is suggested by the higher GDGT concentrations (Fig. 4d) and this may account for the abrupt shift to lower BIT indices in the marls from this interval (Fig. 4c). In other intervals, however, the GDGT concentrations exhibit much less variability and no systematic change with lithology suggesting that differential OM degradation is not the primary driver of cyclic BIT variations. This is consistent with previous work where Huguet et al. (2009) showed that extreme changes in redox conditions, between oxic and anoxic sediments with the same OM input, can result in changes in the BIT index preserved, but only by up to 0.4. The BIT variations we see here are >0.4 and therefore cannot be entirely attributable to oxic degradation. In addition, the presence of benthic foraminifera in both limestones and marls (Kouwenhoven et al., 2006) suggests that oxygen fluctuations were never extreme enough to induce total anoxia. Elevated proportions of the benthic foraminifera, *Bolivina spathulata* (70–90%), however, indicate that, as early as 7.167 Ma the sediments were also never fully oxic, and changes in redox conditions from less to more severe dysoxia on a precessional time scale are apparent from repeated shifts in benthic foraminifer assemblages after 6.4 Ma (Kouwenhoven et al., 2006 and references therein). Consequently, the redox-driven preservational impact on BIT indices is likely to be small.

Instead, we suggest that variability in the BIT indices reflects changes in the source of OM. The consistently higher indices in the marls (Fig. 4c) can either be produced by greater terrestrial input of brGDGTs, or by a decrease of marine crenarchaeol production. If the accumulation rate is constant, the concentration of the brGDGTs can be used to distinguish between a terrestrial and marine driver of BIT indices (Smith et al., 2012), i.e. more brGDGTs reflect increased terrestrial input. Here, however, not only is there considerable uncertainty in the accumulation rates through the lithological cycle (Nijenhuis and De Lange, 2000), but also, increased fluvially-derived nutrients could both supply additional terrestrial OM (additional brGDGTs) and also stimulate primary productivity, yielding higher concentrations of crenarchaeol (Zell et al., 2015). GDGT concentrations, therefore, cannot be used to distinguish between these two explanations, because accumulation rates cannot be considered constant (variable brGDGT concentrations before the slump; Fig. 4d). However, increased run-off can explain the increased BIT indices in marls due to increased terrestrial OM input (Fig. 4c), while there is no obvious mechanism for an increase in crenarchaeol production during limestone deposition. Therefore, we argue that, the higher marl BIT indices are indicative of elevated terrigenous OM inputs (e.g. Hopmans et al., 2004).

Other OM proxies e.g. TAR<sub>n</sub>-alkanoic acid (Fig. 4c and Fig. 5e) and *n*-alkane concentrations have similar but less consistent relationships with lithology (Fig. 5a and Fig. 6). One explanation for these more ambiguous records is that they are more sensitive to changes in relative preservation and within-cycle sedimentation rate changes than BIT indices. Furthermore, these ratios are comprised of end-members (especially the aquatic end-member) with a broad range of terrestrial and marine sources (Meyers and Arnaboldi, 2008). Finally, it appears that *n*-alkyl lipids derive from both fluvial inputs, which would be expected to track other hydrological indicators, and aeolian inputs (see section 4.3) and this mixture of controls further complicates the interpretation of these data. We therefore suggest that BIT indices best record the changes in OM source (i.e. Strong et al., 2012), but that other biomarkers (e.g. TAR<sub>n</sub>-alkanes; HMW *n*-alkane abundances), also show similar relationships.

As a consequence of the insolation control on lithology (Krijgsman et al., 2002), the BIT indices also vary with insolation and are commonly higher during times of high insolation (Fig. 4c) when





**Fig. 8.** Climate Model-derived run-off into the Mediterranean as a whole (black line) and Eastern Mediterranean (red line) and its relationship with precession-forced insolation over one precession cycle (Marzocchi et al., 2015 and see supplementary material). The bar chart shows the average and standard deviation of the BIT indices in the limestones and the marls throughout the entire section. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the marls were deposited. Numerical simulations with an ocean-atmosphere-vegetation general circulation model (GCM; Marzocchi et al., 2015 and see supplementary material) show a large increase in run-off from North Africa during the precession minimum (insolation maximum; Fig. 8b), which is consistent with elevated terrigenous OM inputs during marl deposition. A substantial component of this North African fluvial input is delivered to the Eastern Mediterranean via the Nile, which could therefore have exerted a strong control on sedimentary processes at Pissouri (Fig. 1). Given the proximity of Cyprus to the Nile cone, the systematic changes in BIT indices with lithology, record the variability of fluvial input to the Eastern Mediterranean.

The relationship between insolation and BIT indices explains only half of its variability ( $R^2 = 0.5$ ) and the relationships are weaker within the individual marl and limestone datasets ( $R^2 = 0.24$ ,  $R^2 = 0.26$  respectively). These low regression coefficients suggest that insolation does not generate a systematic gradational change in BIT indices, but instead describes a bi-modal shift in the BIT-insolation relationship between the extremes of the insolation curve. Samples with  $>0.6$  BIT indices derive from parts of the precession cycles associated with insolation  $>500 \text{ W/m}^2$  (these are all marls) while those with  $<0.3$  BIT indices are associated with insolation of  $<480 \text{ W/m}^2$  (all but one of these are limestones).

#### 4.3. Insolation-driven changes in plant inputs inferred from $n$ -alkane isotopes

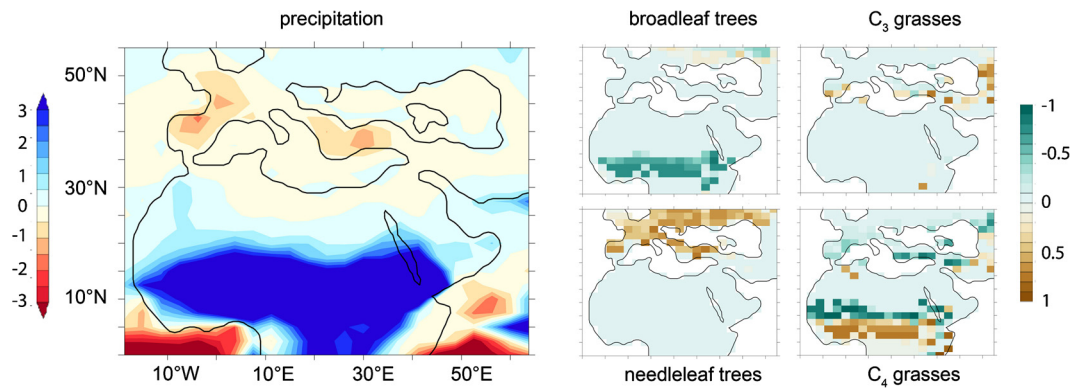
The correlation between BIT indices and astronomically-controlled lithology suggests that both the quantity and the nature of the terrestrial OM being supplied from North Africa could change

as the ITCZ shifts position, reflecting changes in the aridity/humidity of the North African catchment. One mechanism for monitoring this is through  $n$ -alkane  $\delta^{13}\text{C}$  values, which increase in response to the proportion of aridity-adapted  $\text{C}_4$  plants in the depositional system (Schwab et al., 2015). There is clear evidence of a global expansion of  $\text{C}_4$  plants from 8 Ma onwards although this appears to have been delayed by  $\sim 2$  Myr in the Eastern Mediterranean region (Edwards et al., 2010), post-dating the Pissouri section. These observations dictate caution in the interpretation of even cyclic variations in leaf wax  $\delta^{13}\text{C}$  values. If the expansion exclusively post-dated the deposition of the study interval, we would expect little leaf wax  $\delta^{13}\text{C}$  sensitivity. However, this is not the case (Fig. 6). The observed variation in  $\delta^{13}\text{C}$  could therefore result from two interrelated factors: (i) superimposed on this long-term  $\text{C}_3$ - $\text{C}_4$  transition are localised, shorter-term variations, perhaps amplified during the global ecological transition and reflecting regional changes in precipitation and the advantage  $\text{C}_4$ -plants have over  $\text{C}_3$ -plants in semi-arid regions (Rommerskirchen et al., 2006); or (ii) changes in precipitation driving similar carbon isotopic variation, although of smaller magnitude, in  $\text{C}_3$  plants (Diefendorf et al., 2015). Consequently,  $\delta^{13}\text{C}$  values of  $n$ -alkanes are expected to be less negative during insolation minima, i.e. limestone deposition, when the ITCZ shifts further south, due to either more enriched  $\text{C}_3$  plants and/or a higher proportion of  $\text{C}_4$  plants (Fig. 9). However, this is not what we observe in the Pissouri section (Fig. 6), where the  $\delta^{13}\text{C}$  values for the  $n$ -alkanes of the limestones are consistently more negative than most ( $\sim 80\%$ ) of the marls (Fig. 6).

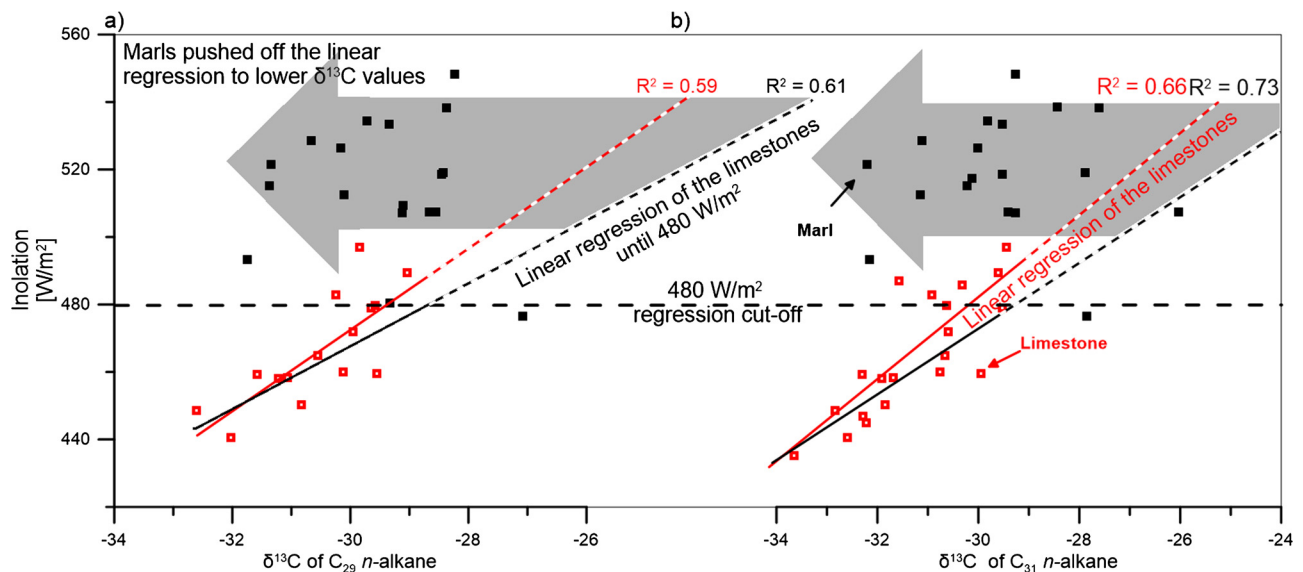
It is possible that rather than recording  $\text{C}_3/\text{C}_4$  vegetation change driven by humidity, the  $n$ -alkane  $\delta^{13}\text{C}$  values of the Pissouri samples are responding to other factors, such as temperature (Yamori et al., 2014) or  $p\text{CO}_2$  variations (Bolton et al., 2016; Freeman and Colarusso, 2001). However, while we cannot be sure that these low resolution records capture the full range of precessional temperature variation, there is little evidence for pronounced temperature fluctuations with insolation (Fig. 7) that could account for the pattern of  $n$ -alkane  $\delta^{13}\text{C}$  observed (Fig. 6). Equally, although there is evidence of  $p\text{CO}_2$  decline during the Late Miocene to Early Pliocene (Bolton et al., 2016), the resolution of this record is far too low (4 samples covering 6–8 Myr) to provide any indication that precessional fluctuations in  $p\text{CO}_2$  could account for the  $n$ -alkane  $\delta^{13}\text{C}$  record at Pissouri.

To explore this further, we have compared our data with inferred insolation assuming little  $p\text{CO}_2$  variations, and this reveals  $\delta^{13}\text{C}$  variations within and between the two lithologies. Within the limestone dataset, there is a clear positive relationship between insolation and the carbon isotopic composition of the long-chain  $n$ -alkanes, especially  $n\text{-C}_{29}$  and  $n\text{-C}_{31}$  (Fig. 10). It appears that at higher insolation (between  $480$  and  $500 \text{ W/m}^2$ ),  $\delta^{13}\text{C}$  values become relatively stable, such that Fig. 10 shows two possible linear regressions for the relationship between insolation and  $n$ -alkane  $\delta^{13}\text{C}$  values based on either all or a subset of the limestone data. The carbon isotopic compositions of the marls do not lie on the same insolation- $\delta^{13}\text{C}$  regression line as the limestones, but deviate from it towards more negative values (Fig. 10) and show no systematic relationship with insolation.

As discussed above, the positive relationship between insolation and  $n$ -alkane  $\delta^{13}\text{C}$  values in the limestones is unexpected, differing with model simulations of decreased rainfall and expansion of  $\text{C}_4$  plants during insolation minima (Fig. 9). By extension, it is apparently inconsistent with our BIT indices which indicated decreased terrestrial OM inputs (i.e. decreased run-off) during insolation minima. Instead, the insolation- $\delta^{13}\text{C}$  relationship during the limestone deposition interval of each precessional cycle (e.g. during precession maxima and insolation minima), can be explained by enhanced aeolian transport of  $n$ -alkanes



**Fig. 9.** Precipitation differences between precession minima (insolation maxima) and precession maxima (insolation minima) across N. Africa and the Mediterranean, simulated using HadCM3L with coupled ocean, atmosphere and vegetation (Marzocchi et al., 2015). The four smaller panels show vegetation changes between precession minima and precession maxima for broadleaf trees, needle leaf trees, C<sub>3</sub>-plants and C<sub>4</sub>-plants (Marzocchi et al., 2015 and see supplementary material).



**Fig. 10.** Cross-plot of insolation versus leaf wax  $\delta^{13}\text{C}$  values, showing the different relationships for the marls (black) and the limestones (red) for (a) C<sub>29</sub> and (b) C<sub>31</sub> *n*-alkanes. The linear regressions of the limestones are indicated (red for all limestones and black for limestones beneath 480 W/m<sup>2</sup>, above which the relationship appears to break down). Both regressions are extrapolated to higher insolation by dashed lines. The offset of the leaf wax  $\delta^{13}\text{C}$  values to lower values in the marls is indicated with the grey arrow. The absolute insolation is derived from astronomical tuning of the Pissouri section (Krijgsman et al., 2002). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

from the northern margin of the Mediterranean which experienced more arid conditions during insolation maxima (Fig. 9; Marzocchi et al., 2015 and see supplementary material). Such an interpretation is reflected in the vegetation changes seen in the GCM simulations in the Anatolian Plateau (Fig. 9); therefore, it appears that the *n*-alkane  $\delta^{13}\text{C}$  records in the limestones document a persistent source from the north that experienced changing vegetation, where, in contrast with North Africa, there was more precipitation during insolation minima than insolation maxima.

This relationship, however, only holds during the extreme part of the precession cycle that includes the insolation minima. We suggest that during this interval, when run-off from North Africa was presumably lower (Fig. 4), Pissouri was largely unaffected by North African run-off and aeolian input dominated the terrestrially-derived organic matter incorporated into the sediment. During insolation maxima, by contrast, the *n*-alkanes from the tropical rain forests of North Africa (Holtvoeth et al., 2003) supplied the Pissouri depositional system via enhanced Nile River input (Fig. 9). This drove the *n*-alkane  $\delta^{13}\text{C}$  values lower (Fig. 10) overprinting the signal from the Anatolian Plateau.

#### 4.4. Long-term trends

Continuous dry environments have been postulated for the lead up to the MSC (Fauquette et al., 2006), inferred from Eastern Mediterranean pollen records. These show an increase in *Pinus* and *Citrus* and a decrease in tropical and sub-tropical taxa, which Fauquette et al. (2006) attribute to sea-level change and associated coastline variations. The increase of nonacosan-10-ol concentrations, which are thought to derive from pine trees (Jetter and Riederer, 1995) at the top of the section could also therefore indicate a sea-level fall (Fig. 5c).

The dry conditions inferred from the pollen data are consistent with the small increase in *n*-alkane  $\delta^{13}\text{C}$  values above the slump layer (Fig. 6), especially in the longest *n*-alkanes (C<sub>33</sub>), that may indicate an increase in the proportion of C<sub>4</sub>-plants due to increasing aridity or an increase in CO<sub>2</sub>; both are favourable for a C<sub>4</sub> plant expansion (Freeman and Colarusso, 2001; Bolton et al., 2016; Huang et al., 2007). It is also consistent with the long-term decrease in limestone BIT indices (Fig. 4c), perhaps suggesting a decrease in run-off during the dry extreme of the precession cycle.

However, the BIT record exhibits additional complexity, with three major trends/shifts in the 600 kyr preceding the MSC: first, the decrease in limestone BIT indices; second, the sharp decrease in marl BIT directly underneath the slump interval; and third, the increase in marl BIT above the slump interval (Fig. 4c). The long-term decrease in BIT indices probably reflects a gradual decrease of terrestrial OM inputs associated with increasing aridity during the 500 kyr before the onset of the MSC, but an increase in cre-narchaeol input cannot be excluded (Fig. 4c). The concentrations of *n*-alkanes also exhibit a long-term decline, but reach their lowest values below the slump layer (Fig. 5b) before increasing in the upper part of the section;  $TAR_{n\text{-alkanoic-acids}}$  exhibit similar trends. Therefore, in the final ~100 kyr before the MSC, there appears to be divergence in the behaviour of different terrigenous OM inputs.

Marine faunal and floral data from Pissouri provides some insights as to the causes of these long-term changes and the environmental conditions that influenced Pissouri. Kouwenhoven et al. (2006) document a collapse in nannoflora at about 20 m (e.g. 6.25 Ma, where the marl BIT indices decline; Fig. 5e) and a recovery just above the slump. Those authors suggest that the abrupt decline in nannoflora may have been caused by a salinity increase. The decline is associated with a sharp increase in the abundance of the calcareous dinocyst: *Thoracosphaera* (Fig. 5e; Kouwenhoven et al., 2006). This dinocyst has been described from the K/T boundary where its abundance immediately after the boundary has suggested that it survived and then exploited a stressful environment as a result of either considerable warming, fluctuation in salinity and pH, or higher CO<sub>2</sub> (Kouwenhoven et al., 2006 and references therein). Of these possible causes, the occurrence of *Thoracosphaera* at Pissouri probably implies higher and more fluctuating salinity conditions from 6.25 Ma onwards, in line with Mediterranean-wide indicators of rising salinity in the lead up to the MSC (e.g. Sierro et al., 2001). An increase in salinity, if driven by decreased freshwater inputs, would be consistent with the decline in BIT indices,  $TAR_{n\text{-alkanes}}$  and nonacosan-10-ol abundances from 6.5 Ma to near the top of the slump at ~6.1 Ma (Fig. 4 and Fig. 5).

Immediately above the slump, *Thoracosphaera* abundance drops and the nannoflora diversity and abundance recovers, suggesting a less extreme environment (Kouwenhoven et al., 2006). Less saline conditions are consistent with the sharp increase in marl BIT indices, an increase in  $TAR_{n\text{-alkanes}}$  indices and the return to pre-6.25 Ma levels of brGDGT abundance above the slump. The high variability in GDGTs and the sharp drop in marl BIT indices at 6.25 Ma (Fig. 4c) are also consistent with fluctuating salinity conditions in the Mediterranean before the onset of the MSC at 5.971 Ma. *Thoracosphaera* exhibits a similar increase immediately before the MSC, again indicating increasing salinity.

Our new data are consistent with a long-term drying in the surrounding catchment, an associated change in vegetation, a decrease in run-off and an associated increase in Mediterranean salinity. However, the expression of these changes varies between proxy records, perhaps suggesting decoupling of simple rainfall, runoff and salinity relationships. Of course, this is to be expected as the MSC is not thought to have been caused solely by climate change but also tectonic changes impacting the Mediterranean Sea's connection to the Atlantic Ocean (Flecker et al., 2015; Achalhi et al., 2016). It appears that a combination of these processes, strongly modulated by orbital forcing, dictated the evolution of Mediterranean climate in the 600 kyr interval leading up to the MSC.

## 5. Conclusions

The analysis of biomarker distributions and carbon isotopic compositions in the Pissouri section reveals a strong relationship with lithology and, therefore, they are also inferred to respond

to precession-driven insolation variation during the 600 kyr preceding the MSC. The sediments deposited during inferred insolation maxima (marls) contain a greater proportion of terrestrially-derived organic matter than those deposited during insolation minima (limestones). BIT indices support the hypothesis that the Eastern Mediterranean experienced large changes in run-off from Northern African catchments during each precessional cycle. Leaf wax  $\delta^{13}C$  values indicate that North Africa is not the only source of terrestrially-derived organic matter and that Pissouri also received material from the northern margin of the Mediterranean, probably the Anatolian Plateau in Turkey via aeolian inputs. The  $\delta^{13}C$  data suggest that this area was characterised by more arid vegetation, which also varied with precession such that more arid conditions prevailed during periods of higher insolation, in line with climate models. This arid material was supplied to Pissouri throughout the succession, but it only dominates the carbon isotope signature of leaf waxes during low insolation when the influence of the Nile was reduced as a result of lower discharge. The long-term trends in the data indicate that the increasing salinity of the looming MSC was already apparent in the Pissouri section in the biomarker data more than 600 kyr before the first gypsum precipitated and was especially clear and influential during the last 100 kyr.

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## Appendix A. Supplementary material

Supplementary material related to this article can be found online at <http://dx.doi.org/10.1016/j.epsl.2017.01.029> or from author: [j.p.mayser@bristol.ac.uk](mailto:j.p.mayser@bristol.ac.uk).

## References

- Achalhi, M., Münch, P., Cornée, J.-J., Azdimousa, A., Melinte-Dobrinescu, M., Quil-lévéré, F., Drinia, H., Fauquette, S., Jiménez-Moreno, G., Merzeraud, G., Moussa, A.B., El Kharim, Y., Feddi, N., 2016. The Late Miocene Mediterranean–Atlantic connections through the North Rifian Corridor: new insights from the Boudinar and Arbaa Taourirt basins (northeastern Rif, Morocco). *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 459, 131–152. <http://dx.doi.org/10.1016/j.palaeo.2016.06.040>.
- Bolton, C.T., Hernandez-Sanchez, M.T., Fuertes, M.A., Gonzalez-Lemos, S., Abrevaya, L., Mendez-Vicente, A., Flores, J.A., Probert, I., Giosan, L., Johnson, J., Stoll, H.M., 2016. Decrease in coccolithophore calcification and CO<sub>2</sub> since the middle Miocene. *Nat. Commun.* 7, 10284. <http://dx.doi.org/10.1038/ncomms10284>.
- Bourbonniere, R.A., Meyers, P.A., 1996. Sedimentary geolipid records of historical changes in the watersheds and productivities of Lakes Ontario and Erie. *Limnol. Oceanogr.* 41, 352–359.
- Bray, E.E., Evans, E.D., 1961. Distribution of normal-paraffins as a clue to recognition of source beds. *Geochim. Cosmochim. Acta* 22, 2–15. [http://dx.doi.org/10.1016/0016-7037\(61\)90069-2](http://dx.doi.org/10.1016/0016-7037(61)90069-2).
- De Jonge, C., Hopmans, E.C., Zell, C.I., Kim, J.-H., Schouten, S., Sinninghe Damsté, J.S., 2014. Occurrence and abundance of 6-methyl branched glycerol dialkyl glycerol tetraethers in soils: implications for palaeoclimate reconstruction. *Geochim. Cosmochim. Acta* 141, 97–112. <http://dx.doi.org/10.1016/j.gca.2014.06.013>.
- Diefendorf, A.F., Leslie, A.B., Wing, S.L., 2015. Leaf wax composition and carbon isotopes vary among major conifer groups. *Geochim. Cosmochim. Acta* 170, 145–156. <http://dx.doi.org/10.1016/j.gca.2015.08.018>.
- Edwards, E.J., Osborne, C.P., Stromberg, C.A., Smith, S.A., Consortium, C.G., 2010. The origins of C<sub>4</sub> grasslands: integrating evolutionary and ecosystem science. *Science* 328, 587–591. <http://dx.doi.org/10.1126/science.1177216>.



- Eglinton, G., Hamilton, R.J., 1967. Leaf epicuticular waxes. *Science* 156, 1322–1335.
- Fauquette, S., Suc, J., Bertini, A., Popescu, S., Warny, S., Taoufik, N., Villa, M., Chikhi, H., Feddi, N., Subally, D., Clauzon, G., Ferrier, J., 2006. How much did climate force the Messinian salinity crisis? Quantified climatic conditions from pollen records in the Mediterranean region. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 238, 281–301. <http://dx.doi.org/10.1016/j.palaeo.2006.03.029>.
- Flecker, R., Krijgsman, W., Capella, W., de Castro Martins, C., Dmitrieva, E., Mayser, J.P., Marzocchi, A., Modestou, S., Lozano, D.O., Simon, D., Tulbure, M., van den Berg, B., van der Schree, M., de Lange, G., Ellam, R., Govers, R., Gutjahr, M., Hilgen, F., Kouwenhoven, T., Lofi, J., Meijer, P., Sierro, F.J., Bachiri, N., Barhoun, N., Alami, A.C., Chacon, B., Flores, J.A., Gregory, J., Howard, J., Lunt, D., Ochoa, M., Pancost, R., Vincent, S., Yousfi, M.Z., 2015. Evolution of the Late Miocene Mediterranean–Atlantic gateways and their impact on regional and global environmental change. *Earth-Sci. Rev.* <http://dx.doi.org/10.1016/j.earscirev.2015.08.007>.
- Freeman, K.H., Colarusso, L.A., 2001. Molecular and isotopic records of C<sub>4</sub> grassland expansion in the Late Miocene. *Geochim. Cosmochim. Acta* 65, 1439–1454. [http://dx.doi.org/10.1016/S0016-7037\(00\)00573-1](http://dx.doi.org/10.1016/S0016-7037(00)00573-1).
- Hilgen, F.J., Krijgsman, W., Langereis, C.G., Lourens, L.J., 1997. Breakthrough made in dating of the geological record. *Eos Trans. AGU* 78, 285. <http://dx.doi.org/10.1029/97eo00186>.
- Holtvoeth, J., Wagner, T., Schubert, C.J., 2003. Organic matter in river-influenced continental margin sediments: the land–ocean and climate linkage at the Late Quaternary Congo fan (ODP Site 1075). *Geochim. Geophys. Geosyst.* 4. <http://dx.doi.org/10.1029/2003gc000590>.
- Hopmans, E.C., Schouten, S., Pancost, R.D., van der Meer, M.T., Sinninghe Damsté, J.S., 2000. Analysis of intact tetraether lipids in archaeal cell material and sediments by high performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometry. *Rapid Commun. Mass Spectrom.* 14, 585–589. [http://dx.doi.org/10.1002/\(SICI\)1097-0231\(20000415\)14:7<585::AID-RCM913>3.0.CO;2-N](http://dx.doi.org/10.1002/(SICI)1097-0231(20000415)14:7<585::AID-RCM913>3.0.CO;2-N).
- Hopmans, E.C., Weijers, J.W.H., Schefuss, E., Herfort, L., Damsté, J.S.S., Schouten, S., 2004. A novel proxy for terrestrial organic matter in sediments based on branched and isoprenoid tetraether lipids. *Earth Planet. Sci. Lett.* 224, 107–116. <http://dx.doi.org/10.1016/j.epsl.2004.05.012>.
- Huang, Y., Clemens, S.C., Liu, W., Wang, Y., Prell, W.L., 2007. Large-scale hydrological change drove the late Miocene C<sub>4</sub> plant expansion in the Himalayan foreland and Arabian Peninsula. *Geology* 35, 531. <http://dx.doi.org/10.1130/G23666A.1>.
- Huguet, C., Hopmans, E.C., Febo-Ayala, W., Thompson, D.H., Sinninghe Damsté, J.S., Schouten, S., 2006. An improved method to determine the absolute abundance of glycerol dibiphytanyl glycerol tetraether lipids. *Org. Geochem.* 37, 1036–1041. <http://dx.doi.org/10.1016/j.orggeochem.2006.05.008>.
- Huguet, C., Kim, J.-H., de Lange, G.J., Sinninghe Damsté, J.S., Schouten, S., 2009. Effects of long term oxic degradation on the TEX<sub>86</sub> and BIT organic proxies. *Org. Geochem.* 40, 1188–1194. <http://dx.doi.org/10.1016/j.orggeochem.2009.09.003>.
- Jetter, R., Riederer, M., 1995. In vitro reconstitution of epicuticular wax crystals: formation of tubular aggregates by long-chain secondary alkanediols. *Bot. Acta* 108, 111–120. <http://dx.doi.org/10.1111/j.1438-8677.1995.tb00840.x>.
- Kidd, R.B., Cita, M.B., Ryan, W.B.F., 1978. The Stratigraphy of Eastern Mediterranean Sapropel Sequences as Recovered by DSDP Leg 42A and Their Palaeoenvironmental Significance. *Init. Rep. DSDP 42A*, pp. 421–443.
- Kim, J.H., van der Meer, J., Schouten, S., Helmke, P., Willmott, V., Sangiorgi, F., Koc, N., Hopmans, E.C., Damsté, J.S.S., 2010. New indices and calibrations derived from the distribution of crenarchaeal isoprenoid tetraether lipids: implications for past sea surface temperature reconstructions. *Geochim. Cosmochim. Acta* 74, 4639–4654. <http://dx.doi.org/10.1016/j.gca.2010.05.027>.
- Kouwenhoven, T.J., Hilgen, F.J., van der Zwaan, G.J., 2003. Late Tortonian–early Messinian stepwise disruption of the Mediterranean–Atlantic connections: constraints from benthic foraminiferal and geochemical data. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 198, 303–319. [http://dx.doi.org/10.1016/S0031-0182\(03\)00482-3](http://dx.doi.org/10.1016/S0031-0182(03)00482-3).
- Kouwenhoven, T.J., Morigi, C., Negri, A., Giunta, S., Krijgsman, W., Rouchy, J.M., 2006. Palaeoenvironmental evolution of the eastern Mediterranean during the Messinian: constraints from integrated microfossil data of the Pissouri Basin (Cyprus). *Mar. Micropaleontol.* 60, 17–44. <http://dx.doi.org/10.1016/j.marmicro.2006.02.005>.
- Krijgsman, W., Blanc-Valleron, M.M., Flecker, R., Hilgen, F.J., Kouwenhoven, T.J., Merle, D., Orszag-Sperber, F., Rouchy, J.M., 2002. The onset of the Messinian salinity crisis in the Eastern Mediterranean (Pissouri Basin, Cyprus). *Earth Planet. Sci. Lett.* 194, 299–310. [http://dx.doi.org/10.1016/S0012-821X\(01\)00574-X](http://dx.doi.org/10.1016/S0012-821X(01)00574-X).
- Kutzbach, J.E., Chen, G., Cheng, H., Edwards, R.L., Liu, Z., 2014. Potential role of winter rainfall in explaining increased moisture in the Mediterranean and Middle East during periods of maximum orbitally-forced insolation seasonality. *Clim. Dyn.* 42, 1079–1095. <http://dx.doi.org/10.1007/s00382-013-1692-1>.
- Larrasoana, J.C., Roberts, A.P., Rohling, E.J., 2013. Dynamics of green Sahara periods and their role in hominin evolution. *PLoS ONE* 8. <http://dx.doi.org/10.1371/journal.pone.0076514>.
- Laskar, J., Robutel, P., Joutel, F., Gastineau, M., Correia, A.C.M., Levrard, B., 2004. A long-term numerical solution for the insolation quantities of the Earth. *Astron. Astrophys.* 428, 261–285. <http://dx.doi.org/10.1051/0004-6361:20041335>.
- Manzi, V., Gennari, R., Hilgen, F., Krijgsman, W., Lugli, S., Roveri, M., Sierro, F.J., 2013. Age refinement of the Messinian salinity crisis onset in the Mediterranean. *Terra Nova* 25, 315–322. <http://dx.doi.org/10.1111/Ter.12038>.
- Marzocchi, A., Lunt, D.J., Flecker, R., Bradshaw, C.D., Farnsworth, A., Hilgen, F.J., 2015. Orbital control on Late Miocene climate and the North African monsoon: insight from an ensemble of sub-precessional simulations. *Clim. Past Discuss.* 11, 2181–2237. <http://dx.doi.org/10.5194/cpd-11-2181-2015>.
- Meyers, P.A., Arnaboldi, M., 2008. Paleooceanographic implications of nitrogen and organic carbon isotopic excursions in mid-Pleistocene sapropels from the Tyrrhenian and Levantine Basins, Mediterranean Sea. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 266, 112–118. <http://dx.doi.org/10.1016/j.palaeo.2008.03.018>.
- Nijenhuis, I.A., De Lange, G.J., 2000. Geochemical constraints on Pliocene sapropel formation in the eastern Mediterranean. *Mar. Geol.* 163, 41–63. [http://dx.doi.org/10.1016/S0025-3227\(99\)00093-6](http://dx.doi.org/10.1016/S0025-3227(99)00093-6).
- O'Leary, M.H., 1981. Carbon isotope fractionation in plants. *Phytochemistry* 20, 553–567. [http://dx.doi.org/10.1016/0031-9422\(81\)85134-5](http://dx.doi.org/10.1016/0031-9422(81)85134-5).
- Peterse, F., van der Meer, J., Schouten, S., Weijers, J.W.H., Fierer, N., Jackson, R.B., Kim, J.-H., Sinninghe Damsté, J.S., 2012. Revised calibration of the MBT-CBT paleotemperature proxy based on branched tetraether membrane lipids in surface soils. *Geochim. Cosmochim. Acta* 96, 215–229. <http://dx.doi.org/10.1016/j.gca.2012.08.011>.
- Rohling, E.J., Marino, G., Grant, K.M., 2015. Mediterranean climate and oceanography, and the periodic development of anoxic events (sapropels). *Earth-Sci. Rev.* 143, 62–97. <http://dx.doi.org/10.1016/j.earscirev.2015.01.008>.
- Rommerskirchen, F., Eglinton, G., Dupont, L., Rullkotter, J., 2006. Glacial/interglacial changes in southern Africa: compound-specific  $\delta^{13}\text{C}$  land plant biomarker and pollen records from southeast Atlantic continental margin sediments. *Geochim. Geophys. Geosyst.* 7, Q08010. <http://dx.doi.org/10.1029/2005gc001223>.
- Rossignol-Strick, M., 1985. Mediterranean quaternary sapropels, an immediate response of the African monsoon to variation of insolation. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 49, 237–263. [http://dx.doi.org/10.1016/0031-0182\(85\)90056-2](http://dx.doi.org/10.1016/0031-0182(85)90056-2).
- Schouten, S., Hopmans, E.C., Sinninghe Damsté, J.S., 2013. The organic geochemistry of glycerol dialkyl glycerol tetraether lipids: a review. *Org. Geochem.* 54, 19–61. <http://dx.doi.org/10.1016/j.orggeochem.2012.09.006>.
- Schwab, V.F., Garcin, Y., Sachse, D., Todou, G., Séné, O., Onana, J.-M., Achoundong, G., Gleixner, G., 2015. Effect of aridity on  $\delta^{13}\text{C}$  and  $\delta\text{D}$  values of C<sub>3</sub> plant- and C<sub>4</sub> graminoid-derived leaf wax lipids from soils along an environmental gradient in Cameroon (Western Central Africa). *Org. Geochem.* 78, 99–109. <http://dx.doi.org/10.1016/j.orggeochem.2014.09.007>.
- Sierro, F.J., Hilgen, F.J., Krijgsman, W., Flores, J.A., 2001. The Abad composite (SE Spain): a Messinian reference section for the Mediterranean and the APTS. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 168, 141–169. [http://dx.doi.org/10.1016/S0031-0182\(00\)00253-4](http://dx.doi.org/10.1016/S0031-0182(00)00253-4).
- Sinninghe Damsté, J.S., Schouten, S., Hopmans, E.C., van Duin, A.C., Geenevasen, J.A., 2002. Crenarchaeol: the characteristic core glycerol dibiphytanyl glycerol tetraether membrane lipid of cosmopolitan pelagic crenarchaeota. *J. Lipid Res.* 43, 1641–1651. <http://dx.doi.org/10.1194/jlr.M200148-JLR200>.
- Smith, R.W., Bianchi, T.S., Li, X., 2012. A re-evaluation of the use of branched GDGTs as terrestrial biomarkers: implications for the BIT index. *Geochim. Cosmochim. Acta* 80, 14–29. <http://dx.doi.org/10.1016/j.gca.2011.11.025>.
- Strong, D.J., Flecker, R., Valdes, P.J., Wilkinson, I.P., Rees, J.G., Zong, Y.Q., Lloyd, J.M., Garrett, E., Pancost, R.D., 2012. Organic matter distribution in the modern sediments of the Pearl River estuary. *Org. Geochem.* 49, 68–82. <http://dx.doi.org/10.1016/j.orggeochem.2012.04.011>.
- Sugden, W., McKerrow, W.S., 1962. The composition of marls and limestones in the Great Oolite Series of Oxfordshire. *Geol. Mag.* 99, 363. <http://dx.doi.org/10.1017/S0016756800058477>.
- Tierney, J.E., Tingley, M.P., 2015. A TEX<sub>86</sub> surface sediment database and extended Bayesian calibration. *Sci. Data* 2, 150029. <http://dx.doi.org/10.1038/sdata.2015.29>.
- Toucanne, S., Angue Minto'o, C.M., Fontanier, C., Bassetti, M.-A., Jorry, S.J., Jouet, G., 2015. Tracking rainfall in the northern Mediterranean borderlands during sapropel deposition. *Quat. Sci. Rev.* 129, 178–195. <http://dx.doi.org/10.1016/j.quascirev.2015.10.016>.
- Tzanova, A., Herbert, T.D., Peterson, L., 2015. Cooling Mediterranean sea surface temperatures during the Late Miocene provide a climate context for evolutionary transitions in Africa and Eurasia. *Earth Planet. Sci. Lett.* 419, 71–80. <http://dx.doi.org/10.1016/j.epsl.2015.03.016>.
- Weijers, J.W.H., Schouten, S., van den Donker, J.C., Hopmans, E.C., Damsté, J.S.S., 2007. Environmental controls on bacterial tetraether membrane lipid distribution in soils. *Geochim. Cosmochim. Acta* 71, 703–713. <http://dx.doi.org/10.1016/j.gca.2006.10.003>.
- Yamori, W., Hikosaka, K., Way, D.A., 2014. Temperature response of photosynthesis in C<sub>3</sub>, C<sub>4</sub>, and CAM plants: temperature acclimation and temperature adaptation. *Photosynth. Res.* 119, 101–117. <http://dx.doi.org/10.1007/s11120-013-9874-6>.
- Yang, H., Pancost, R.D., Dang, X.Y., Zhou, X.Y., Evershed, R.P., Xiao, G.Q., Tang, C.Y., Gao, L., Guo, Z.T., Xie, S.C., 2014. Correlations between microbial tetraether lipids and environmental variables in Chinese soils: optimizing the paleo-



- reconstructions in semi-arid and arid regions. *Geochim. Cosmochim. Acta* 126, 49–69. <http://dx.doi.org/10.1016/j.gca.2013.10.041>.
- Zell, C., Kim, J.-H., Dorhout, D., Baas, M., Sinninghe Damsté, J.S., 2015. Sources and distributions of branched tetraether lipids and crenarchaeol along the Portuguese continental margin: implications for the BIT index. *Cont. Shelf Res.* <http://dx.doi.org/10.1016/j.csr.2015.01.006>.