

## Sedimentary lipid biomarkers in the magnesium rich and highly alkaline Lake Salda (south-western Anatolia)

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### ABSTRACT

Lake Salda, located in south-western Anatolia, is characterized by the presence of living stromatolites and by a low diversity of both phytoplankton and zooplankton due to high pH and magnesium concentration. The most abundant, free sedimentary lipids of the uppermost five centimetres of the lake sediments were studied as potential environmental biomarkers, and proxies based on glycerol dialkyl glycerol tetraethers (GDGT) were tested in this extreme environment. Dinosterol and tetrahymanol are potentially relevant biomarkers for the dinoflagellate *Peridinium cinctum* and ciliates, respectively.  $C_{20:1}$  and  $C_{25:2}$  highly branched isoprenoid (HBI) alkenes, and  $n-C_{17}$  alkane and  $n-C_{17:1}$  alkene are considered to represent, respectively, diatoms and Cyanobacteria, that are involved in the formation of stromatolites. *Crenarchaeol* is assumed to be derived mainly from *Thaumarchaeota* thriving in the lake. Allochthonous organic material is represented by long-chain *n*-alkanes and *n*-alkanols derived from land plant leaf waxes, as well as branched GDGTs produced by soil bacteria. The latter may also be produced partly in the water column and/or the surface sediment of the lake. Branched GDGT-derived lake calibrations for water pH provide estimates close to observations, but estimated lake water/air temperature are lower than observed values.  $TEX_{86}$  (tetraether index of tetraethers consisting of 86 carbons), a proxy based on isoprenoid GDGTs, potentially allows estimating mean annual lake surface temperature. Interestingly,  $C_{22}$  to  $C_{25}$  1,2 diols, which have a yet unknown origin, were found for the first time in lake sediments. This study represents the first investigation of sedimentary lipid distribution in an alkaline and magnesium-rich lake in Anatolia, and provides a basis for future biomarker-based paleoenvironmental reconstruction of Lake Salda.

Key words: Sedimentary biomarkers; GDGTs; 1,2 diols; alkaline; Lake Salda.

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### INTRODUCTION

Extreme environments are key for studying the limits and adaptation of living organisms and their related organic molecular proxies (or biomarkers). Lake Salda located in south-western Anatolia represents such an environment with a pH between 8 and 10 and water strongly enriched in magnesium (300 ppm Mg) (Braithwaite and Zedef, 1996; Kazanci *et al.*, 2004). The lake is characterized by the presence of living hydromagnesite ( $Mg_3(CO_3)_4(OH)_2 \cdot 4H_2O$ ) stromatolites (microbialites) in the coastal, shallow (<6 meter water depth, mwd) and sheltered areas (Braithwaite and Zedef, 1996; Russell *et al.*, 1999; Shirokova *et al.*, 2011). A biofilm, comprised mainly of green filaments of Cyanobacteria and diatoms, completely covers the submerged portions of the microbial mounds (Braithwaite and Zedef, 1996; Shirokova *et al.*, 2011). As suggested by Braithwaite and Zedef (1994, 1996), diatoms, such as *Navicula* sp. and *Cymbella* sp., and more especially Cyanobacteria, such as *Lyngba* sp., *Gloeocapsa* sp. and *Synechococcus* sp., are involved in the nucleation and precipitation of hydromagnesite. The acidic polysaccharide on the cell surface of these microorganisms can bind cations (Ca, Mg), which may act as nu-

cleation sites for authigenic carbonate precipitation. Cyanobacteria are further responsible for the photosynthetic alkalization of the microenvironment due to their ability to use bicarbonate ( $HCO_3^-$ ) as primary source of inorganic carbon and release hydroxide ions ( $OH^-$ ). This increases the pH of the microenvironment around the cell as well as the fluid supersaturation state, and provides favourable nucleation sites for Mg hydrous carbonate precipitation (Thompson and Ferris, 1990; Braithwaite and Zedef, 1996; Russell *et al.*, 1999; Shirokova *et al.*, 2011). Lake Salda is one of the few modern environments on Earth where hydrous Mg-carbonates are the dominant precipitating minerals.

The low diversity of organisms thriving in Lake Salda due to its chemically extreme conditions represents an excellent opportunity to investigate the potential sources of the main lipid biomarkers found in the uppermost centimetres of the lake sediments. Such an approach will also provide biomarkers and associated proxies for potential paleoenvironmental reconstructions based on sediment cores from Lake Salda. Indeed, preliminary dating on a sediment core from Lake Salda (S. Akçer-Ön, unpublished data) reveal the possibility to reconstruct paleoenviron-

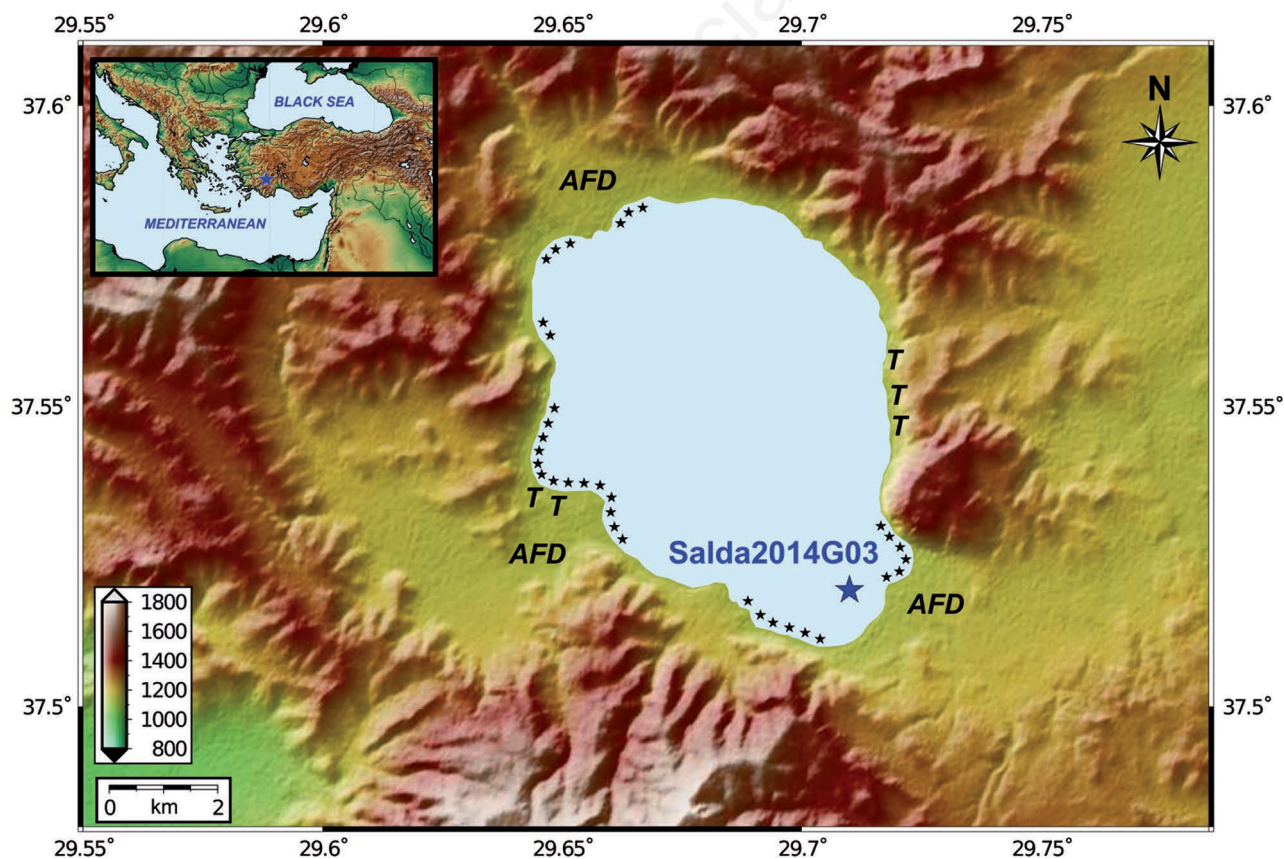
ments over the last 5000 years at a high temporal resolution (sedimentation rates of 0.5 to 1 mm/year) and without interruption in a region, which is archeologically rich and very sensitive to climate change, but lacking long and continuous paleoclimate records (Jones *et al.*, 2006; Tayanç *et al.*, 2009; Roberts *et al.*, 2012; Tudryn *et al.*, 2013).

## SETTING

Lake Salda (Fig. 1) is located at an altitude of 1140 meters above sea level (asl) in south-western Anatolia (Braithwaite and Zedef, 1996; Kazancı *et al.*, 2004). It belongs to the carbonate type of saline lakes and is included in the mixo-oligohaline, alpha oligohaline category (salinity of 0.2 to 2.3 psu) (Hammer, 1986; Bulger *et al.*, 1993). The surface area of Lake Salda is 45 km<sup>2</sup> and the water depth ranges between 0 and 110 m, but reaches 200 m in several local depressions. The 148 km<sup>2</sup> drainage basin comprises ultramafic (mainly serpentinized ophiolite) and karstified carbonate rocks. The high Mg concentration in the lake water results from the flow of meteoric waters

through the Yeşilova ultramafic rocks and ultramafic-derived sediments from extensive alluvial fan deltas located south-east, south-west and north-west of the lake. While numerous streams and groundwater inflows feed the lake in winter, there are no surface outlets and the lake level is strongly affected by evaporation in summer. Although the lake is a closed system, it is perennial and the water level is dependent on climate and the balance between inflow and evaporation (Braithwaite and Zedef, 1996). After Kazancı *et al.* (2004), the water level fluctuates ca. 50 cm annually due to the hydraulic relationship of the lake with karstic aquifers, extensive evaporation during summer months, and irrigational use of the surrounding groundwater sources.

At the altitude of Lake Salda, mean air temperature ranges between 3°C in January and 25°C in July, with an annual mean of 13°C. The yearly average rainfall is 430 mm (with most rainfall during the winter months), while annual potential evaporation averaged 1150 mm (Braithwaite and Zedef, 1996; Turkish State Meteorological Service, [www.mgm.gov.tr](http://www.mgm.gov.tr)). Available data for the year



**Fig. 1.** Topography (meter above sea level) around Lake Salda and location of core Salda2014G03 (37°31'10 N; 29°42'35 E; 42 meter water depth). Black stars, hydromagnesite stromatolites in water; T, terraces consisting of hydromagnesite sedimentary rocks (based on Russell *et al.*, 1999); AFD, alluvial fan delta. Inset: location of the study area.

1998 (Kazanci *et al.*, 2004) show that the water column of Lake Salda was stratified in summer (between May and July) and overturned in spring (April) and autumn (October). The water column was always oxic (dissolved oxygen  $\geq 5 \text{ mg l}^{-1}$ ) and pH varied between 8.3 and 9.7, but was always  $\geq 9.2$  at the surface. The lake surface temperature in that year ranged between  $8^\circ\text{C}$  in April,  $17^\circ\text{C}$  in May,  $22^\circ\text{C}$  in July and  $17^\circ\text{C}$  in October, and the mean annual temperature was  $16^\circ\text{C}$  (Kazanci *et al.*, 2004).

As suggested by Braithwaite and Zedef (1996), the lake sediments are probably made partly of a mixture of carbonate and hydromagnesite derived from erosion of ancient stromatolites and terrace deposits consisting of hydromagnesite sedimentary rocks located in the eastern and western shores of the lake. However, at the coring site most of the terrigenous sediment is very likely derived from the erosion and transport of soil material by runoff and rivers from the alluvial fan delta located south-east (Fig. 1).

As a result of relatively low nutrient levels, high pH values and high concentrations of magnesium and chloride, Lake Salda is poor in phytoplankton and zooplankton assemblages with Shannon's diversity indices of 0.4 and 0.7, respectively (Tab. 1) (Kazanci *et al.*, 2004). Notably, the phytoplankton is dominated by a single species of dinoflagellates, *Peridinium cinctum*, with a mean abundance of 60%, but increasing to  $>99\%$  during the summer months (Kazanci *et al.*, 2004).

## METHODS

### Sediment core

The 86 cm-long Salda2014G03 core was taken with a gravity corer in the southern part of Lake Salda (Fig. 1) at a water depth of 42 m in July 2014. The sediment is made of dark gray and dark green to olive green silty to muddy layers (cm-scale). The results presented here are based on the topmost 5 cm of the core ( $n=5$ ).

### Elemental analysis

Total carbon (TC) and total nitrogen (TN) were analyzed by means of an EA3000 series elemental analyzer (EuroVector). Total inorganic carbon (TIC) was determined with a multi EA 2000 elemental analyzer (Analytik Jena). Total organic carbon (TOC) was calculated by the subtraction of TIC from TC values with an error bar of  $\pm 0.1\%$ . The C/N ratio was defined as the molar ratio between TOC and TN:  $C/N = (\text{TOC}/12) / (\text{TN}/14)$ .

### Lipid analysis

The dried and homogenized sediment (1-2 g dry weight) was extracted three times using 10 ml of a mixture of dichloromethane and methanol (DCM/MeOH; 9:1), ultrasonicated for 10 min and centrifuged for 5 min.

After the addition of four internal standards (squalane, nonadecan-2-one,  $5\alpha$ -androstane- $3\beta$ -ol and a  $C_{46}$  glycerol trialkyl glycerol tetraether), the total lipid extract of the sediment was separated into four fractions using hexane (F1 fraction containing hydrocarbons), hexane/DCM (F2 fraction containing esters), DCM (F3 fraction containing ketones) and DCM/MeOH (F4 fraction containing GDGTs, sterols and alcohols).

After filtration through a PTFE syringe filter ( $0.45 \mu\text{m}$ ), the F4 fraction has been analysed by high performance liquid chromatography atmospheric pressure chemical ionization mass spectrometry (HPLC APCI-MS; ThermoScientific Dionex Ultimate 3000 UHPLC system coupled to a ThermoScientific MSQ Plus). Separation of the individual GDGTs was achieved on a Prevail Cyano column (Grace,  $3 \mu\text{m}$ ,  $150 \text{ mm} \times 2.1 \text{ mm}$ ) maintained at  $35^\circ\text{C}$ . Using a flow rate of  $0.25 \text{ ml min}^{-1}$ , the gradient of the mobile phase was first held isocratic for 5 min with 100% solvent A (*n*-hexane/isopropanol; 99:1), followed by a linear gradient to 90% solvent A and 10% solvent B (*n*-hexane/isopropanol; 90:10) within 20 min, followed by a linear gradient to 100% solvent B at 40 min (method modified after Liu *et al.*, 2012a). The column was cleaned by back flushing with 100% solvent B for 5 min at  $0.6 \text{ ml min}^{-1}$ . Finally, the column was equilibrated with 100% solvent A for 10 min. GDGTs were detected using positive-ion APCI-MS and selective ion monitoring (SIM) of their  $[M+H]^+$  ions (Schouten *et al.*, 2007) with APCI source conditions as follows: nebulizer gas 45 psi, corona current +5  $\mu\text{A}$ , probe heater temperature  $600^\circ\text{C}$  and cone voltage 130 V. Compound concentrations were estimated using the relative response factor (0.9 for the period of analysis) between the  $C_{46}$ -GTGT standard (obtained from D.H. Thompson, Purdue University) and pure crenarchaeol (obtained from A. Pearson, Harvard University). Hydroxylated isoprenoid GDGTs (OH-GDGTs) were detected in the respective selected ion monitoring (SIM) range of the isoprenoid GDGTs: OH-GDGT-0 in the  $m/z$  1300 SIM scan, OH-GDGT-1 and 2OH-GDGT-0 in the  $m/z$  1298 SIM scan, and OH-GDGT-2 in the  $m/z$  1296 SIM scan (Liu *et al.*, 2012c).

After the derivatization of F4 with a mixture of pyridine and *N,O*-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TCMS) for 1 hour at  $60^\circ\text{C}$ , the F1, F3 and F4 fractions were analyzed by gas chromatography (GC; multichannel TraceUltra GC; ThermoScientific) for lipid quantification, and by gas chromatography / mass spectrometry (GC/MS; HP6890 Series GC system coupled to a HP5973 Mass Selective Detector; Agilent) for lipid identification. The first channel (1) of the TraceUltra GC consisted of a split/splitless inlet, a DB-5 MS  $30 \text{ m} \times 0.32 \text{ mm} \times 0.25 \mu\text{m}$  capillary column and a flame ionization detector (FID) detector. The second channel (2) consisted of an on-column inlet, a DB-5 MS  $60 \text{ m} \times 0.32 \text{ mm} \times 0.1 \mu\text{m}$  column and a FID

detector. F1 and F4 fractions were analyzed on channel 1 with a temperature program from 40°C to 290°C at 4°C/min followed by a 20 min plateau. On channel 2, F3 fractions were measured with a temperature program starting with a first ramp from 60°C to 290°C at 25°C/min and a second ramp from 290°C to 320°C at 2°C/min followed by a 30 min plateau. Hydrogen was used as carrier gas for both channels. The ChromCard software was used to visualize the chromatograms.

For peak identification, the fractions were analyzed by GC-MS. The GC system was equipped with a split/splitless injector and a DB-5 MS 60 m x 0.25 mm x 0.25 µm capillary column. The GC temperature program was from 60°C to 290°C at a rate of 25°C/min and from 290°C to 320°C at a rate of 2°C/min followed by a 30 min plateau. Helium was used as carrier gas. The electron impact ionization mode conditions of the MS were as follows: ion energy 70eV; ion source temperature 230°C; mass range

**Tab. 1.** Abundances and diversity (Shannon's diversity index) of the phytoplankton and zooplankton assemblages of Lake Salda. The data are from Kazanci *et al.* (2004), Braithwaite and Zedef (1996) and Shirokova *et al.* (2011).

	Mean abundance (%)	Mean total abundance* (%)	Reference
<b>Phytoplankton</b>			
<u>Cyanophyta</u>			
<i>Chroococcus turgidus</i>	12	6	Kazanci <i>et al.</i> , 2004
<i>Gleocapsa aeruginosa</i>	<0	<0	Kazanci <i>et al.</i> , 2004
<i>Microcystis aeruginosa</i>	<0	<0	Kazanci <i>et al.</i> , 2004
<i>Oscillatoria limosa</i>	<0	<0	Kazanci <i>et al.</i> , 2004
<i>Lyngbya majuscula</i>	nd	nd	Braithwaite and Zedef, 1996
<i>Cladophora sp.</i>	nd	nd	Braithwaite and Zedef, 1996
<i>Rhizoclonium sp.</i>	nd	nd	Braithwaite and Zedef, 1996
<i>Synechococcus sp.</i>	nd	nd	Shirokova <i>et al.</i> , 2011
<u>Chlorophyta</u>			
<i>Cosmarium sp.</i>	<0	<0	Kazanci <i>et al.</i> , 2004
<i>Micrasterias rotata</i>	12	6	Kazanci <i>et al.</i> , 2004
<i>Pediastrum boryanum</i>	<0	<0	Kazanci <i>et al.</i> , 2004
<i>Scenedesmus quadricauda</i>	<0	<0	Kazanci <i>et al.</i> , 2004
<u>Bacillariophyta</u>			
<i>Coscinodiscus sp.</i>	12	6	Kazanci <i>et al.</i> , 2004
<i>Cyclotella ocellata</i>	<0	<0	Kazanci <i>et al.</i> , 2004
<i>Stephanodiscus astrea</i>	<0	<0	Kazanci <i>et al.</i> , 2004
<i>Navicula sp.</i>	nd	nd	Braithwaite and Zedef, 1996
<i>Cymbella sp.</i>	nd	nd	Braithwaite and Zedef, 1996
<i>Pinnularia sp.</i>	nd	nd	Braithwaite and Zedef, 1996
<i>Amphora sp.</i>	nd	nd	Braithwaite and Zedef, 1996
<i>Surirella sp.</i>	nd	nd	Braithwaite and Zedef, 1996
<u>Dinophyta</u>			
<i>Ceratium hirundinella</i>	3	2	Kazanci <i>et al.</i> , 2004
<i>Peridinium cinctum</i>	60	30	Kazanci <i>et al.</i> , 2004
<u>Euglenophyta</u>			
<i>Euglena acus</i>	<0	<0	Kazanci <i>et al.</i> , 2004
<i>Trachelomonas sp.</i>	<0	<0	Kazanci <i>et al.</i> , 2004
Diversity index=0.4			Kazanci <i>et al.</i> , 2004
<b>Zooplankton</b>			
<u>Ciliata</u>			
<i>Holotricha sp. 1</i>	13	6	Kazanci <i>et al.</i> , 2004
<i>Holotricha sp. 2</i>	13	6	Kazanci <i>et al.</i> , 2004
<i>Tintinnopsis lacustris</i>	13	6	Kazanci <i>et al.</i> , 2004
<i>Vorticella sp.</i>	13	6	Kazanci <i>et al.</i> , 2004
<u>Rotifera</u>			
<i>Brachionus urceolaris</i>	25	13	Kazanci <i>et al.</i> , 2004
<i>Brachionus plicatilis</i>	13	6	Kazanci <i>et al.</i> , 2004
<u>Crustacea</u>			
<i>Nauplius larvae</i>	13	6	Kazanci <i>et al.</i> , 2004
Diversity index=0.7			Kazanci <i>et al.</i> , 2004

\*Abundance considering both the phytoplankton and the zooplankton; nd, not determined.

50-550  $m/z$ ; electron multiplier voltage 1600V. The ChemStation software was used for the visualization of mass spectra. Structural identification was determined by mass spectral interpretation of the ion fragmentation and comparison with published mass spectra.

## RESULTS

### TC, TIC and TOC relative concentrations

The mean relative concentrations and standard deviations ( $n=5$ ) for TC, TN, TIC and TOC are  $6.2\pm 1.2\%$ ,  $0.3\pm 0.0\%$ ,  $3.8\pm 1.1\%$  and  $2.4\pm 0.0\%$ , respectively. The mean value of the C/N ratio is  $10.4\pm 0.9$ . In order to account for the potential degradation of organic matter and/or dilution by the supply of inorganic material to the sediment, TOC was used to normalize the concentrations of the lipid compounds ( $\mu\text{g g}^{-1}$  TOC).

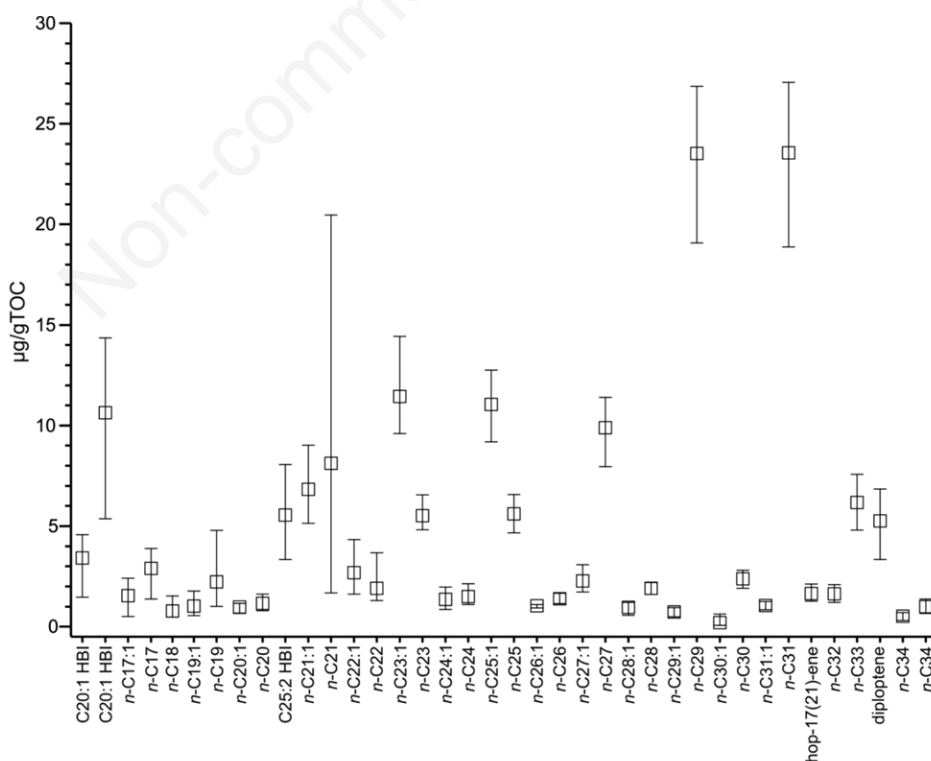
### Hydrocarbons and alkenones

$n\text{-C}_{17}$  to  $n\text{-C}_{34}$  alkanes and  $n\text{-C}_{19:1}$  to  $n\text{-C}_{31:1}$  alkenes are present in the surface sediments of Lake Salda (Fig. 2).  $n\text{-C}_{29}$  and  $n\text{-C}_{31}$  alkanes are the most abundant compounds with mean concentrations of ca.  $60 \mu\text{g g}^{-1}$  TOC, followed by  $n\text{-C}_{23:1}$  and  $n\text{-C}_{25:1}$  alkenes with mean concentrations of

ca.  $29 \mu\text{g g}^{-1}$  TOC. The  $\text{C}_{20:1}$  I,  $\text{C}_{20:1}$  II and  $\text{C}_{25:2}$  HBI alkenes were identified with mean concentrations ranging between 8 and  $26 \mu\text{g g}^{-1}$  TOC. Diploptene (hop-22(29)-ene) and its regioisomer (hop-17(21)-ene) amount to 13 and  $4 \mu\text{g g}^{-1}$  TOC, respectively.  $n\text{-C}_{17:1}$  alkene is also present in relatively low concentration ( $4 \mu\text{g g}^{-1}$  TOC). The mean value of the Carbon Preference Index (CPI; Kolattukudy, 1976) based on the  $n\text{-C}_{24}$  to  $n\text{-C}_{34}$  alkanes is 8.3. The mean value of the Average Chain Length (ACL; Cranwell, 1973) based on the  $n\text{-C}_{25}$  to  $n\text{-C}_{33}$  odd-numbered alkanes is 29.4. Long-chain alkenones are present in the surface sediment (0-1 cm) only.  $\text{C}_{37:4\text{Me}}$ ,  $\text{C}_{37:3\text{Me}}$ ,  $\text{C}_{37:2\text{Me}}$ ,  $\text{C}_{38:4\text{Et}}$ ,  $\text{C}_{38:3\text{Et}}$ ,  $\text{C}_{38:2\text{Et}}$  and  $\text{C}_{39:3\text{Me}}$  alkenones were identified. The concentrations range between 4 and  $45 \mu\text{g g}^{-1}$  TOC (*not shown*).

### Sterols, stanols, diols and $n$ -alkanols

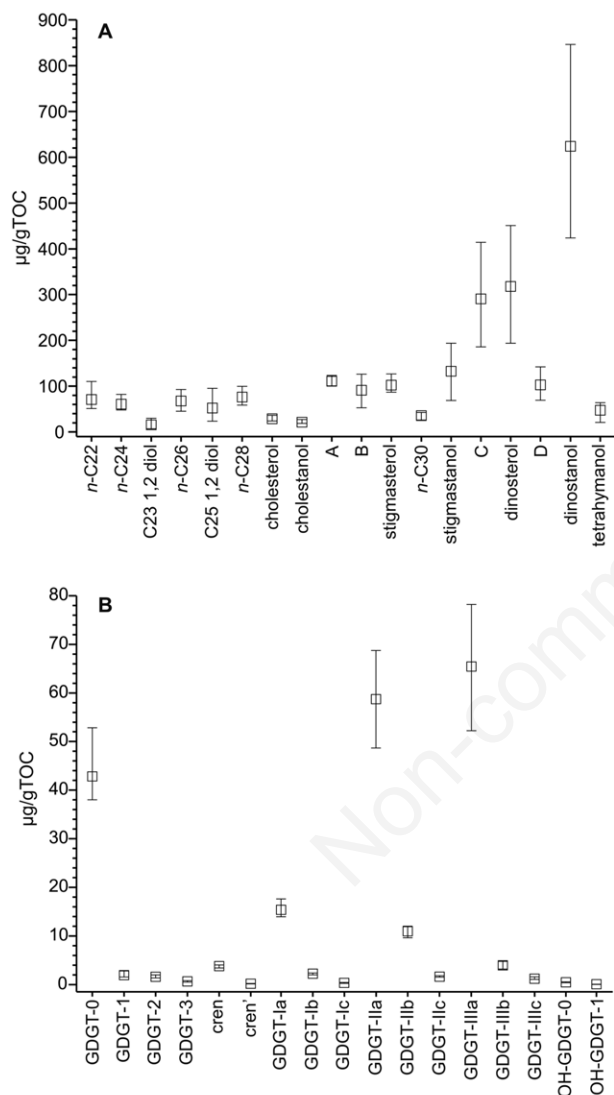
The most abundant sterols (Fig. 3) are  $4\alpha,23,24$ -trimethyl- $5\alpha(\text{H})$ -cholestan- $3\beta$ -ol ( $\text{C}_{30}$ ; dinostanol) with a mean concentration of  $624 \mu\text{g g}^{-1}$  TOC,  $4\alpha,23,24$ -trimethyl- $5\alpha$ -cholest- $22\text{E-en-}3\beta$ -ol ( $\text{C}_{30}$ ; dinosterol); and  $4\alpha,23,24$ -trimethyl-cholesta- $5,22$ -dien- $3\beta$ -ol ( $\text{C}_{30}$ ; compound C in Fig. 3) with concentrations around  $305 \mu\text{g g}^{-1}$  TOC.  $24$ -Ethyl- $5\alpha(\text{H})$ -cholestan- $3\beta$ -ol ( $\text{C}_{29}$ ; stigmastanol) has a mean concentration of  $133 \mu\text{g g}^{-1}$  TOC.  $4\alpha,24$ -Dimethyl- $5\alpha(\text{H})$ -



**Fig. 2.** Box plot (maximum, minimum and mean values) of the concentrations (normalized to TOC) of the most abundant hydrocarbons present in the upper 5 cm of Lake Salda sediments ( $n=5$ ).

cholest-22E-en-3 $\beta$ -ol ( $C_{29}$ ; compound B in Fig. 3), 24-ethyl-cholesta-5,22E-dien-3 $\beta$ -ol ( $C_{29}$ ; stigmasterol), 4 $\alpha$ -methyl-24-ethyl-5 $\alpha$ -cholest-22E-en-3 $\beta$ -ol ( $C_{30}$ ; compound D in Fig. 3) and 4 $\alpha$ -methyl-5 $\alpha$ (H)-cholestan-3 $\beta$ -ol ( $C_{28}$ ; compound A in Fig. 3) have concentrations of ca. 100  $\mu\text{g g}^{-1}$  TOC. Cholest-5-en-3 $\beta$ -ol ( $C_{27}$ ; cholesterol) and 5 $\alpha$ (H)-cholestan-3 $\beta$ -ol ( $C_{27}$ ; cholestanol) are present, but in rela-

tively low concentrations (21–29  $\mu\text{g g}^{-1}$  TOC). The concentrations of even-numbered,  $n$ - $C_{22}$  to  $n$ - $C_{30}$  alkanols are ranging between 36 and 76  $\mu\text{g g}^{-1}$  TOC. Two diols,  $C_{23}$  and  $C_{25}$  1,2 diols (Supplementary Fig. 1), and tetrahymanol ( $C_{30}$ ) are present with mean concentrations of 15, 52 and 48  $\mu\text{g g}^{-1}$  TOC, respectively. Tentatively identified  $C_{22}$  1,2 and  $C_{24}$  1,2 diols (Supplementary Fig. 1) were found in trace amounts.



**Fig. 3.** Box plots (maximum, minimum and mean values) of the concentrations (normalized to TOC) of (A) the most abundant  $n$ -alkanols, sterols, stanols and diols (A: 4 $\alpha$ -Methyl-5 $\alpha$ (H)-cholestan-3 $\beta$ -ol; B: 4 $\alpha$ ,23,24-Trimethyl-5 $\alpha$ (H)-cholest-22E-en-3 $\beta$ -ol; C: 4 $\alpha$ ,23,24-Trimethyl-cholesta-5,22-dien-3 $\beta$ -ol, D: 4 $\alpha$ -Methyl-24-Ethyl-5 $\alpha$ -cholest-22E-en-3 $\beta$ -ol), and (B) the most abundant isoprenoid and branched GDGTs (cren, crenarchaeol; cren', crenarchaeol regioisomer; see Supplementary Fig. 2 for molecular structures) present in the upper 5 cm of Lake Salda sediments ( $n=5$ ).

### Isoprenoid and branched GDGTs

The distribution of GDGTs is dominated by the branched GDGT-IIa and -IIIa, and the isoprenoid GDGT-0 with mean concentrations of 59, 66 and 43  $\mu\text{g g}^{-1}$  TOC, respectively (Fig. 3; Supplementary Fig. 2). The branched GDGT-Ia and -IIb have concentrations of 15 and 10  $\mu\text{g g}^{-1}$  TOC, respectively. The other branched and isoprenoid GDGTs, including crenarchaeol, have concentrations below 4  $\mu\text{g g}^{-1}$  TOC. While OH-GDGT-2 and 2OH-GDGT-0 were not detected, OH-GDGT-1 and OH-GDGT-0 were present in low amounts (<0.5  $\mu\text{g g}^{-1}$  TOC).

### DISCUSSION

#### Lipids potentially derived from terrestrial organisms

Long-chain, odd-numbered  $n$ - $C_{23}$  to  $n$ - $C_{33}$  alkanes and even-numbered  $n$ - $C_{22}$  to  $n$ - $C_{30}$  alkanols are known to be derived from the wax coating the leaves of higher land plants (Eglinton and Hamilton, 1967). The relatively high concentrations of these lipids (60–80  $\mu\text{g g}^{-1}$  TOC) are probably related to an important input of terrestrial organic matter by wind or rivers. The mean CPI value of 8.3 further suggests a significant contribution of recent organic material (Mazurek and Simoneit, 1984). A mean ACL value of 29.4 indicates a vegetation-type dominated by graminoids, but chain-length distributions are highly variable within plant groups, such that chemotaxonomic distinctions between grasses and woody plants are difficult to make. There may be however a relationship between longer chain lengths and warmer and drier environments (Bush and McInerney, 2013).

The high concentrations (65  $\mu\text{g g}^{-1}$  TOC) of branched GDGT-IIa and -IIIa may also be related to the input of terrestrial material as it is assumed that branched GDGTs are biosynthesized by acidobacteria thriving in soils (Weijers *et al.*, 2006a, 2009; Sinninghe Damsté *et al.*, 2011, 2014). Furthermore, the high C/N value of the sediments implies that the organic matter is mainly derived from land-plants (Meyers *et al.*, 1997). We suggest therefore that branched GDGT-IIa and -IIIa can be considered, together with  $n$ - $C_{29}$  alkane,  $n$ - $C_{31}$  alkane,  $n$ - $C_{26}$  alkanol, and  $n$ - $C_{28}$  alkanol leaf-wax lipids, as biomarkers for the aeolian and/or potamic input of terrestrial organic material in the sediments of Lake Salda. However, *in situ* production of

branched GDGTs within lakes is known (Tierney and Russell, 2009, 2010; Pearson *et al.*, 2011; Loomis *et al.*, 2011, 2012, 2014; Buckles *et al.*, 2014) and cannot be excluded as additional source for Lake Salda (see paragraph *GDGT-based proxies*).

### Lipids potentially derived from phytoplankton or zooplankton

The lipid fraction of the sediments is highly dominated by sterols and especially dinostanol, dinosterol and 4 $\alpha$ ,24-dimethyl-5 $\alpha$ (H)-cholest-22E-en-3 $\beta$ -ol, but also 4 $\alpha$ -methyl-5 $\alpha$ (H)-cholestan-3 $\beta$ -ol and 4 $\alpha$ ,24-dimethyl-5 $\alpha$ (H)-cholest-22E-en-3 $\beta$ -ol (Fig. 3; Tab. 2). These sterols were detected in the dinoflagellate *Peridinium cinctum* (Robinson *et al.*, 1985; Tab. 2), which strongly dominates the phytoplankton community of Lake Salda (>98 % of the listed phytoplankton in May, June and July 1998; Tab. 1) (Kazanci *et al.*, 2004). Dinosterol has been found also in dinoflagellate resting cysts (Robinson *et al.*, 1985; Volkman, 1986; Kokinos *et al.*, 1998; Volkman *et al.*, 1998; Amo *et al.*, 2010). Cholesterol, although usually considered to be an animal sterol, is also common in diatoms and dinoflagellates (Volkman, 1986; Kokinos *et al.*, 1998; Amo *et al.*, 2010) such as *Peridinium cinctum*, where it was found in high amounts (ca. 50% of total sterols; Tab. 2) (Robinson *et al.*, 1985). Stigmasterol is commonly associated with higher plants (Volkman, 1986), but a number of microalgae also produce this sterol. The diatom *Amphora* sp., which is present in Lake Salda (Braithwaite and Zedef, 1996), produces almost exclusively stigmasterol (Volkman *et al.*, 1986) and, therefore, may be considered as a source organism.

The stanols found in Lake Salda sediments can originate from direct biogenic input and/or from bacterial hydrogenation (double bond reduction) of sterols.

Cholesterol can be biosynthesized by diverse aerobic organisms, including some phytoplankton, zooplankton and macrophytes (Nishimura and Koyama, 1977; Robinson *et al.*, 1985; Volkman, 1986) and stigmasterol may be produced by vascular plants (Nishimura and Koyama, 1977; Leeming and Nichols, 1998). Dinostanol is found in some phytoplankton including dinoflagellates such as *P. cinctum* (Tab. 2) (Robinson *et al.*, 1985; Volkman *et al.*, 1998; Amo *et al.*, 2010). Dinostanol is also very abundant in resting cysts of the dinoflagellate *Peridinium umbonatum* var. *inaequale* (Amo *et al.*, 2010). However, in oxidative water columns and surface sediments such as in Lake Salda, stanols may also originate from hydrogenation of the parent sterols by aerobic bacteria (Gagosian *et al.*, 1982; Owen *et al.*, 1983; Naghibi *et al.*, 2002; Rontani *et al.*, 2009).

Sterol/stanol ratios may help determining if the primary source of the stanols is from specific source organisms or by bacterial conversion. In Lake Salda surface sediments, the dinosterol/dinostanol ratio is below unity (0.50 $\pm$ 0.03), what indicates a higher production of dinostanol by organisms and/or a substantial hydrogenation of dinosterol in the water column and/or at the water/sediment interface (Fig. 4). The cholesterol/cholestanol ratio is above unity (1.35 $\pm$ 0.11), due to either a high production of cholesterol, and/or its partial hydrogenation. Constant ratios with increasing sediment depth result either from a very low hydrogenation of dinosterol and cholesterol in the sediment, suggesting that hydrogenation occurs mainly in the water column, or sediment homogenization related to bioturbation. On the contrary, the stigmasterol/stigmastanol ratio decreases from 1.4 to 0.6 with sediment depth due to an increase in stigmastanol concentration (Fig. 4). Such an increase may result from a change in the source organism(s) and/or a higher hydrogenation of stigmasterol with depth. The different behav-

**Tab. 2.** Percentages of the main sterols found in surface sediments from lakes Salda and Kinneret, and in the dinoflagellate *P. cinctum* (Robinson *et al.*, 1985).

Sterol	Lake Salda	Lake Kinneret	<i>P. cinctum</i>
cholest-5-en-3 $\beta$ -ol (cholesterol)	3	6	52
5 $\alpha$ (H)-cholestan-3 $\beta$ -ol (cholestanol)	2	6	2
24-methyl-cholest-5-en-3 $\beta$ -ol (campesterol)	nd	7	1.5
24-ethyl-cholesta-5,22E-dien-3 $\beta$ -ol (stigmasterol)	8	4	nd
24-ethyl-5 $\alpha$ (H)-cholestan-3 $\beta$ -ol (stigmastanol)	6	15	nd
4 $\alpha$ -methyl-5 $\alpha$ (H)-cholestan-3 $\beta$ -ol (compound A)	9	2	2
4 $\alpha$ ,24-dimethyl-5 $\alpha$ (H)-cholest-22E-en-3 $\beta$ -ol (compound B)	4	3	1
4 $\alpha$ ,23,24-trimethyl-cholesta-5,22-dien-3 $\beta$ -ol (compound C)*			
4 $\alpha$ ,23,24-trimethyl-5 $\alpha$ (H)-cholest-22E-en-3 $\beta$ -ol (dinosterol)*	32	46	24
4 $\alpha$ ,23,24-trimethyl-5 $\alpha$ (H)-cholest-17(20)-en-3 $\beta$ -ol	nd	1	14
4 $\alpha$ ,23,24-trimethyl-5 $\alpha$ (H)-cholestan-3 $\beta$ -ol (dinostanol)	36	9	<0.5

\*Co-eluting compounds in Robinson *et al.* 1985; nd, not detected.

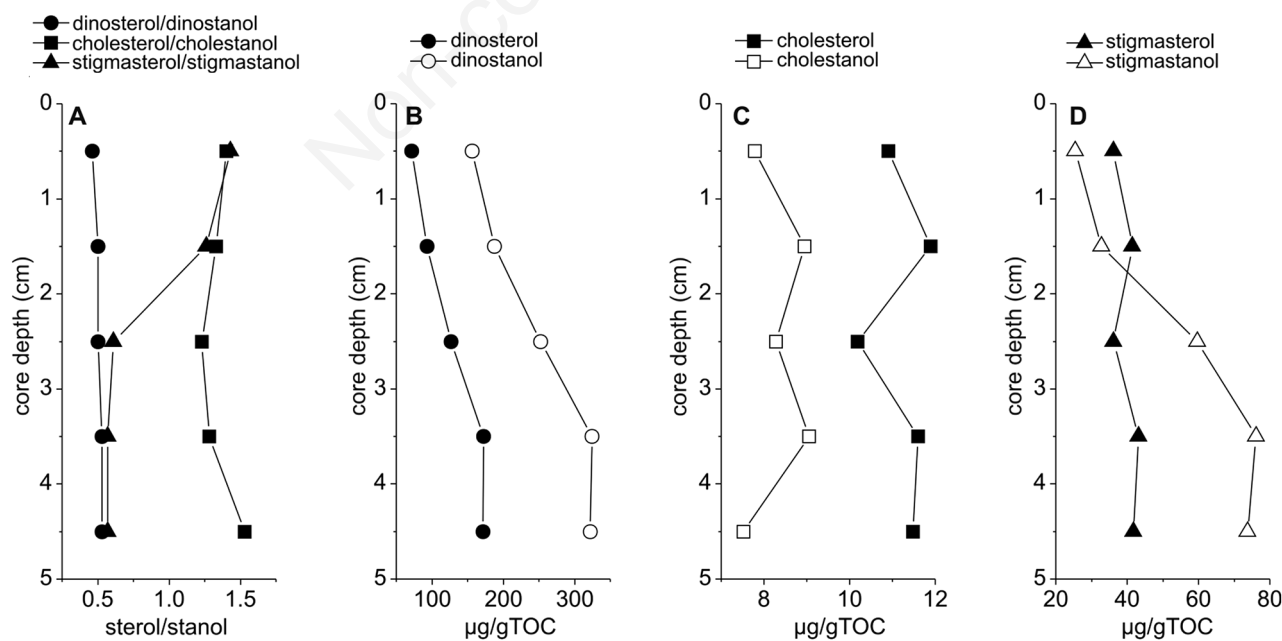
our of the stigmasterol/stigmastanol ratio suggests that stigmasterol may have a different source organism than dinosterol and cholesterol.

The distribution of sterols and stanols in Lake Salda is comparable to Lake Kinneret surface sediments (Tab. 2) (Robinson *et al.*, 1985). Lake Kinneret (or Sea of Galilee, Jordan Rift Valley) is a freshwater, alkaline lake (pH 7-9) with an anoxic hypolimnion (Serruya, 1971; Serruya *et al.*, 1974). As well as for Lake Salda, the phytoplankton is dominated by the dinoflagellate *P. cinctum*, potentially because the optimum growth of this organism occurs at high pH (Lindström, 1984). Therefore, except for stigmasterol and stigmastanol, the major sterols and stanols found in Lake Salda surface sediments are very likely derived from a mixture of hydrogenation and direct input of sterols produced by *P. cinctum*.

$C_{20}$  and  $C_{25}$  HBI alkenes are produced by diatoms (Volkman *et al.*, 1994; Rowland and Robson, 1990).  $C_{20:1}$  (I and II) and  $C_{25:2}$  HBI alkenes have been found in several lakes in the United Kingdom (Barrick *et al.*, 1980; Roland and Robson, 1990).  $C_{25:2}$  HBI is produced by freshwater diatoms of the *Navicula* genus (Belt *et al.*, 2001), which is present in Lake Salda, but the other listed diatoms (Tab. 1) cannot be excluded as potential producers. The origin of the  $C_{20:1}$  HBIs is unknown, but Rowland *et al.* (1985) found both  $C_{20:1}$  and  $C_{25:2}$  HBIs in the freshwater diatoms *Enteromorpha* sp. However, this genus has not been identified in Lake Salda. As lakes with high pH are often associated with poor diatom preservation (Barker, 1992) and

as silica is present only in very low concentration in Lake Salda water column (<1 ppm; Braithwaite and Zedef, 1996), implying rapid diatom shell dissolution,  $C_{25:2}$  HBI alkene may represent a unique tool to reconstruct the variability in diatom abundance. The long-chain alkenones from Lake Salda present a distribution pattern (Tab. 3), whose only known algal precursor is the prymnesiophyte algae *Chrysotila lamellosa* (Rontani *et al.*, 2004; Sun *et al.*, 2007). A similar pattern has been found in sediments from worldwide freshwater lakes (Zink *et al.*, 2001; Toney *et al.*, 2011) including the soda (pH 9.8) and saline (21.6 psu) Lake Van located in eastern Turkey (Thiel *et al.*, 1997; Randlett *et al.*, 2014). As well, 4 $\alpha$ -methyl-24-ethyl-5 $\alpha$ -cholest-22E-en-3 $\beta$ -ol (Fig. 3) has been found in relatively high abundance (up to 50 % of total sterols) in some marine prymnesiophyte algae of the genus *Pavlova* (Volkman *et al.*, 1990). However, no prymnesiophytes have been listed in the phytoplankton community of Lake Salda (Kazanci *et al.*, 2004).

Tetrahymanol (gammaceran-3 $\beta$ -ol) may be produced by the anaerobic phototrophic purple bacterium *Rhodospseudomonas palustris* (Kleeman *et al.*, 1990). While no data exist on the bacterial community of Lake Salda, *R. palustris* was found in anoxic sediments of Lake Akşehir, an alkaline lake located in Konya, south-western Anatolia (Çetinkaya Dönmez *et al.*, 1999). However, 2-methyl tetrahymanol, which is also present in *R. palustris*, was lacking in Lake Salda sediments. Furthermore, Lake Salda is always oxic, which most likely precludes *R.*



**Fig. 4.** Depth profiles of ratios (a) and concentrations of dinosterol and dinostanol (b), cholesterol and cholestanol (c), and stigmasterol and stigmastanol (d).



*palustris* from thriving in this environment. Tetrahymanol is also found in ciliates (Harvey and McManus, 1991), which commonly graze at interfaces of stratified water bodies (Sinninghe Damsté *et al.*, 1995; Ten Haven *et al.*, 1989). While different ciliates are found in Lake Salda with total abundances of ca. 6% (Tab. 1), none of those are known to produce tetrahymanol. To conclude, we suggest using C<sub>25:2</sub> HBI alkene, dinosterol (or dinostanol) and tetrahymanol as specific biomarkers for Lake Salda productivity including, respectively, phytoplankton (diatoms), mixotrophic plankton (dinoflagellates) and zooplankton (ciliates).

### Lipids potentially derived from aquatic Archaea or Bacteria

A number of genus of the phylum Cyanophyta (Cyanobacteria), which have been listed in Lake Salda (Tab. 1) (Kazanci *et al.*, 2004; Braithwaite and Zedef, 1994; Shirokova *et al.*, 2011), are potential producers of *n*-C<sub>17</sub>, *n*-C<sub>18</sub> and *n*-C<sub>19</sub> alkanes and corresponding alkenes (Coates *et al.*, 2014; Liu *et al.*, 2013). *n*-C<sub>19:1</sub> alkene has been found in *Synechococcus* sp. (Coates *et al.*, 2014), which has been identified in Lake Salda (Tab. 1). *Lyngbya* sp., *Oscillatoria* sp. and *Gloeocapsa* sp. (identified in Lake Salda) produce *n*-C<sub>17</sub> alkane and *n*-C<sub>17:1</sub> alkene abundantly (up to 50% of the total hydrocarbons) (Matsumoto *et al.*, 1996; Liu *et al.*, 2013). *Microcystis aeruginosa* (identified in Lake Salda) is also known to produce *n*-C<sub>17</sub> and *n*-C<sub>18</sub> alkanes (Cardoso *et al.*, 1983; Walsh *et al.*, 1998). *Oscillatoria* sp. and *Microcystis aeruginosa* also produce *n*-alkenes including *n*-C<sub>23:1</sub> and *n*-C<sub>25:1</sub> (Cardoso *et al.*, 1983; Matsumoto *et al.*, 1990). Nevertheless, higher plants and some green algae cannot be excluded as other potential producers of *n*-alkenes (Cardoso *et al.*, 1983; Gelpi *et al.*, 1970; Paoletti *et al.*, 1976; Cranwell *et al.*, 1990).

Although diploptene and its regioisomer hop-17(21)-ene may derive from ferns, lichens and higher plants, they reflect very likely a bacterial input (Ourisson *et al.*, 1979) in Lake Salda sediments. While hop-17(21)-ene may also

derive from dehydration of diplopterol (not found in the sediments) during sample workup (Sessions *et al.*, 2013), diploptene is present in various types of bacteria and occurs in diverse environments (Rohmer *et al.*, 1984; Ourisson *et al.*, 1987). It has also been detected in Cyanobacteria such as *Lyngbya aestuarii*, *Nostoc* sp., and *Chroococcus turgidus* (Bird *et al.*, 1971; De Rosa *et al.*, 1971). Indeed, *Chroococcus turgidus* is abundant in Lake Salda and *Lyngbya* sp. has been identified (Tab. 1) (Kazanci *et al.*, 2004; Braithwaite and Zedef, 1996).

Isoprenoid GDGTs (Supplementary Fig. 2) have been found in many lakes worldwide and in cultures of Thaumarchaeota (Castañeda and Schouten, 2011; Schouten *et al.*, 2013; Pearson and Ingalls, 2013; Elling *et al.*, 2014, 2015), and crenarchaeol is considered to be specific for the phylum Thaumarchaeota (Pitcher *et al.*, 2011). Thaumarchaeota are hence assumed to be the primary source of isoprenoids GDGTs in Lake Salda. However, while ammonia-oxidising archaea from Marine Group I Thaumarchaeota were found in high abundance in brackish to saline alkaline lakes (Lanzen *et al.*, 2013), the archaeal distribution in the water column of Lake Salda is unknown. The high amount of GDGT-0 relative to crenarchaeol in Lake Salda sediments (mean GDGT-0/crenarchaeol ratio of 11; Fig. 3) may indicate a contribution from methanotrophic Euryarchaeota (Blaga *et al.*, 2009; Schouten *et al.*, 2013). Indeed, a recent study has shown that the archaeal diversity in microbialites from a temperate, freshwater lake located in Canada was dominated by Euryarchaeota, mainly affiliated with methanogenic taxa (Chan *et al.*, 2014). However, there is no evidence of methane production in Lake Salda (Braithwaite and Zedef, 1996). Isoprenoid GDGTs were also found in worldwide soils, although in relatively low amounts (Weijers *et al.*, 2006b, 2007; Coffinet *et al.*, 2014), and thus a terrestrial contribution cannot be completely excluded. OH-GDGT-0 and OH-GDGT-1 have been identified in worldwide lake sediments (Liu *et al.*, 2012b, 2012c). OH-GDGTs were found in an extremophile Euryarchaeota culture (*Methanothermococcus thermolithotrophicus*) and are present in different strains of planktonic Thaumarchaeota (Liu *et al.*, 2012b; Elling *et al.*, 2014, 2015).

To conclude, different lipids derived from cyanobacteria, such as *n*-C<sub>17</sub> alkane, *n*-C<sub>17:1</sub> alkene or diploptene, may be used to estimate the presence (and extent) of stromatolites in Lake Salda in the past. Crenarchaeol and OH-GDGTs can be used as proxy for the presence of Thaumarchaeota (Schouten *et al.*, 2013).

### Lipids with unknown origin

Long-chain alkyl diols are found in marine, brackish, and lacustrine sediments (Robinson *et al.*, 1985; Volkman *et al.*, 1983; Rowland and Robson, 1990). They are known

**Tab. 3.** Percentages of alkenones found in the surface sediments of lakes Salda (this study) and Van (Thiel *et al.*, 1997), and in the haptophyte *Chrysolita lamellosa* HAP 17 (Rontani *et al.*, 2004).

Alkenone	Lake Salda	Lake Van	<i>C. lamellosa</i> HAP 17
C37:4Me	14	45	5
C37:3Me	43	20	45
C37:2Me	7	5	11
C38:4Et	8	6	2
C38:3Et	16	14	13
C38:2Et	4	6	22
C39:3Me	8	5	1

to be derived from eustigmatophytes and other microalgae such as diatoms (Volkman *et al.*, 1998; Versteegh *et al.*, 1997). The C<sub>23</sub> and C<sub>25</sub> 1,2 diols, which were also found in mud volcano sediments from the Black Sea, have a yet unknown origin (Stadnitiskaia *et al.*, 2007). Interestingly, the cell membrane of the aerobic, heterotrophic bacterium *Thermomicrobium roseum* (phylum Chlorobacteria, or Chloroflexi), which grows at high pH, is made of a series (C<sub>18</sub> to C<sub>24</sub>) of straight chain and branched 1,2 diols replacing glycerolipids (Pond *et al.*, 1986; Pond and Langworthy, 1987; Wu *et al.*, 2009). However, in *T. roseum* the C<sub>22</sub> to C<sub>24</sub> 1,2 diols are in low concentrations relative to the other 1,2 diols, and the C<sub>25</sub> 1,2 diol is absent. Long-chain 1,2 diols have also been found in other bacteria be-

longing to the phylum Chlorobacteria (Lagutin *et al.*, 2015; Sorokin *et al.*, 2012; Wait *et al.*, 1997). While Chlorobacteria have been observed in a soda lake and in tundra soils (Dimitriu *et al.*, 2008; Costello and Schmidt, 2006), their presence in Lake Salda is unknown. The C<sub>22</sub> and C<sub>24</sub> 1,2 diol may derived also from land as they have been identified in the petal wax of a plant from the Asteraceae family (Buschhaus *et al.*, 2013). As far as we know, the present study is the first one to report on the presence of C<sub>22</sub> to C<sub>25</sub> 1,2 diols in lake sediments.

### GDGT-based proxies

Different proxies based on GDGTs (Tab. 4) have been developed to reconstruct pH and surface temperature in

**Tab. 4.** Indices based on branched and isoprenoid GDGTs, and global calibration curves for soil and water pH, surface air temperature, and lake water/air temperature estimation. Roman numerals refer to structures in Supplementary Fig. 2.

Eq.	Indices and calibrations	r <sup>2</sup>	SEE	Sample	Reference
1	BIT=(GDGT-Ia+GDGT-IIa+GDGT-IIIa) / (crenarchaeol+GDGT-Ia+GDGT-IIa+GDGT-IIIa)				Hopmans <i>et al.</i> , 2004
2	TEX <sup>86</sup> =(GDGT-2+GDGT-3+crenarchaeol isomer) / (GDGT-1+GDGT-2+GDGT-3+crenarchaeol isomer)				Schouten <i>et al.</i> , 2002
3	MBT=(GDGT-Ia+GDGT-Ib+GDGT-Ic) / (GDGT-Ia+GDGT-Ib+GDGT-Ic+GDGT-IIa+GDGT-IIb+GDGT-IIc+GDGT-IIIa+GDGT-IIIb+GDGT-IIIc)				Weijers <i>et al.</i> , 2007
4	MBT'=(GDGT-Ia+GDGT-Ib+GDGT-Ic) / (GDGT-Ia+GDGT-Ib+GDGT-Ic+GDGT-IIa+GDGT-IIb+GDGT-IIc+GDGT-IIIa)				Peterse <i>et al.</i> , 2012
5	CBT=-log [(GDGT-Ib+GDGT-IIb) / (GDGT-Ia+GDGT-IIa)]				Weijers <i>et al.</i> , 2007
6	Soil pH=(3.33-CBT) / 0.38	0.7	0.7	Global soils	Weijers <i>et al.</i> , 2007
7	Soil pH=7.90-1.97 x CBT	0.7	0.8	Global soils	Peterse <i>et al.</i> , 2012
8	Soil pH=8.68-2.21 x CBT	0.7	0.9	Chinese soils	Yang <i>et al.</i> , 2014
9	Soil pH=8.13-1.89 x CBT	0.6	0.9	Global soils	Yang <i>et al.</i> , 2014
10	Lake water pH=8.98-1.72 x CBT	0.4	0.8	Global lake sediments	Sun <i>et al.</i> , 2011
11	Lake water pH=(CBT-2.79) / -0.29	0.7	-	USA lake sediments	Schoon <i>et al.</i> , 2013
12	Lake water pH=10.32-3.03 x CBT	0.8	0.7	African lake sediments	Tierney <i>et al.</i> , 2010
13	Lake water pH=7.97+2.78 x CBT	0.8	0.3	Tibetan lake sediments	Günther <i>et al.</i> , 2014
14	Air/lake water T (°C)=6.80-7.06 x CBT+37.09 x MBT	0.6	5.2	Global lake sediments	Sun <i>et al.</i> , 2011
15	Air /lake water T (°C)=55.010 x MBT-6.055	0.7	-	NZ lake sediments	Zink <i>et al.</i> , 2010
16	Air /lake water T (°C)=-6.567 x CBT+12.228	0.7	-	NZ lake sediments	Zink <i>et al.</i> , 2010
17	Air /lake water T (°C)=-3.84+9.84 x CBT+5.92 x MBT'	0.6	1.2	Tibetan lake sediments	Günther <i>et al.</i> , 2014
18	Air /lake water T (°C)=50.47-74.18 x fr <sub>GDGTIIIa</sub> -31.60 x fr <sub>GDGTIIa</sub> - 34.69 x fr <sub>GDGTIa</sub>	0.9	2.2	African lake sediments	Tierney <i>et al.</i> , 2010
19	Air /lake water T (°C)=22.77-33.58 x fr <sub>GDGTIIIa</sub> -12.88 X fr <sub>GDGTIIa</sub> - 418.53 x fr <sub>GDGTIc</sub> +86.43 x fr <sub>GDGTIb</sub>	0.9	1.9	African lake sediments	Loomis <i>et al.</i> , 2012
20	* Air /lake water T (°C)=47.4-53.5 x fr <sub>GDGTIIIa</sub> -37.1 x fr <sub>GDGTIIa</sub> - 20.9 x fr <sub>GDGTIa</sub>	0.8	2.4	Global lake sediments	Pearson <i>et al.</i> , 2011
21	Air /lake water T (°C)=20.9-20.5 x fr <sub>GDGTIIIa</sub> -12.0 x fr <sub>GDGTIIa</sub> - 98.1 x fr <sub>GDGTIb</sub>	0.9	2.0	Global lake sediments	Pearson <i>et al.</i> , 2011
22	Lake water T (°C)=49.032 x TEX <sup>86</sup> -10.989	0.9	3.1	Global lake sediments	Castañeda and Schouten, 2015
23	Air T (°C)=7.5+16.1 x MBT-1.2 x CBT	0.9	1.8	Chinese soils	Yang <i>et al.</i> , 2014
24	Air T (°C)=(MBT-0.122-0.187 x CBT) / 0.02	0.8	4.8	Global soils	Weijers <i>et al.</i> , 2007
25	Air T (°C)=0.81-5.67 x CBT+31.0 x MBT'	0.6	5.0	Global soils	Peterse <i>et al.</i> , 2012
26	Air T (°C)=20.9-13.4 x fr <sub>GDGTIIIa</sub> -17.2 x fr <sub>GDGTIIa</sub> -17.5 x fr <sub>GDGTIb</sub> + 11.2 x fr <sub>GDGTIc</sub>	0.9	1.7	Chinese soils	Yang <i>et al.</i> , 2014

SEE, standard error of estimates; fr, fractional abundance; NZ, New Zealand; \*calibration based on summer temperature.

soils and lakes (see for review Castañeda and Schouten, 2011; Schouten *et al.*, 2013). These proxies are based on different indices: cyclization of branched tetraethers (CBT; Weijers *et al.*, 2007) and methylation of branched tetraethers (MBT and MBT'; Weijers *et al.*, 2007; Peterse *et al.*, 2012), branched and isoprenoid tetraether (BIT; Hopmans *et al.*, 2004) and TEX<sub>86</sub> (Schouten *et al.*, 2002). The CBT and MBT/CBT (MBT'/CBT) indices are used to reconstruct pH in soils and water, and surface air and water temperature, respectively, and TEX<sub>86</sub> is a proxy for water temperature. The BIT index estimates the relative proportion of soil-derived organic matter in the sediments (Hopmans *et al.*, 2004). In Lake Salda sediments, the BIT index is high (0.97) indicating a high input of soil organic material in agreement with the relatively high amounts of higher plant leaf wax lipids in the sediments.

Assuming that branched GDGTs are produced exclusively in soils, the application of four CBT calibrations based on soils (Tab. 4; Weijers *et al.*, 2007; Peterse *et al.*, 2012; Yang *et al.*, 2014) results in soil pH mean values of 6.4 to 7, *i.e.*, lower than observations (mean soil pH 7.8±0.4; n=10; Tab. 5) (Yeşilova Chamber of Agriculture, unpublished data). Estimated mean air temperature using four soil-based calibrations (Weijers *et al.*, 2007; Peterse *et al.*, 2012; Yang *et al.*, 2014) ranges between 0.2 and 8.4°C, *i.e.* below observed mean annual air temperature around Lake Salda. Seasonality, inadequate calibrations,

or the potential production of branched GDGTs in the water column and/or the surface sediments of Lake Salda may explain the differences between estimated and observed soil pH and mean air temperature values. Using four CBT calibrations based on lake sediments (Tierney *et al.*, 2010; Sun *et al.*, 2011; Schoon *et al.*, 2013; Günther *et al.*, 2014), the mean water pH values range between 7.7 and 10.0 (Tabs. 4 and 5). The estimations obtained with Tierney *et al.* (2010) calibration and, especially, with Günther *et al.* (2014) calibration, which is based on alkaline lakes, are closest to the observed water pH of Lake Salda. This suggests that branched GDGTs containing cyclopentane rings (Supplementary Fig. 2) may be produced mainly in the lake.

The estimated mean annual lake surface temperature obtained with the global lake TEX<sub>86</sub> calibration from Castañeda and Schouten (2015) is 16.3°C, *i.e.*, a value very close to the observed mean annual surface temperature of the lake (Tab. 5). This suggests not only that isoprenoid GDGTs are produced *in situ*, but also that methanogens, if present, do not represent a significant additional source of isoprenoid GDGTs, as this would result in an overestimation of TEX<sub>86</sub>-derived water temperatures (Blaga *et al.*, 2009). As well, high BIT values may indicate that TEX<sub>86</sub> estimated temperatures can be biased towards higher values due to a contribution of isoprenoid GDGTs derived from soils (Weijers *et al.*, 2006b). It remains however un-

**Tab. 5.** GDGT-based temperature and pH estimates for Lake Salda surface sediments compared to observed data. See calibration curves in Tab. 4.

Calibration	Parameter	Estimation		Observation
		Surface sediment	Mean ±SD (n=5)	
6 Weijers <i>et al.</i> , 2007	Soil pH	6.9	6.8±0.1	7.8
7 Peterse <i>et al.</i> , 2012	Soil pH	6.5	6.4±0.1	7.8
8 Yang <i>et al.</i> , 2014	Soil pH	7.1	7.0±0.1	7.8
9 Yang <i>et al.</i> , 2014	Soil pH	6.8	6.7±0.1	7.8
10 Sun <i>et al.</i> , 2011	Water pH	7.8	7.7±0.1	9.2
11 Schoon <i>et al.</i> , 2013	Water pH	7.8	7.7±0.1	9.2
12 Tierney <i>et al.</i> , 2010	Water pH	8.2	8.1±0.1	9.2
13 Günther <i>et al.</i> , 2014	Water pH	9.9	10.0±0.1	9.2
14 Sun <i>et al.</i> , 2011	Air/water T (°C)	6.5	5.7±0.5	16
15 Zink <i>et al.</i> , 2010	Air/water T (°C)	0.8	0.2±0.4	16
16 Zink <i>et al.</i> , 2010	Air/water T (°C)	7.7	7.3±0.2	16
17 Günther <i>et al.</i> , 2014	Air/water T (°C)	3.8	4.8±0.2	16
18 Tierney <i>et al.</i> , 2010	Air/water T (°C)	6.5	5.3±0.7	16
19 Loomis <i>et al.</i> , 2012	Air/water T (°C)	1.5	1.5±0.3	16
20 Pearson <i>et al.</i> , 2011	Air/water T (°C)	10.9	9.9±0.6	16
21 Pearson <i>et al.</i> , 2011	Air/water T (°C)	6.9	6.7±0.1	16
22 Castañeda and Schouten, 2015	Water T (°C)	16.3	16.4±1.8	16
23 Yang <i>et al.</i> , 2014	Air T (°C)	8.7	8.4±0.1	13
24 Weijers <i>et al.</i> , 2007	Air T (°C)	-6.4	-7.4±0.6	13
25 Peterse <i>et al.</i> , 2012	Air T (°C)	0.9	0.2±0.4	13
26 Yang <i>et al.</i> , 2014	Air T (°C)	8.2	7.9±0.1	13

clear if and to what extent  $TEX_{86}$  values are biased by terrestrial inputs (Castañeda and Schouten, 2011; Schouten *et al.*, 2013; Inglis *et al.*, 2015).

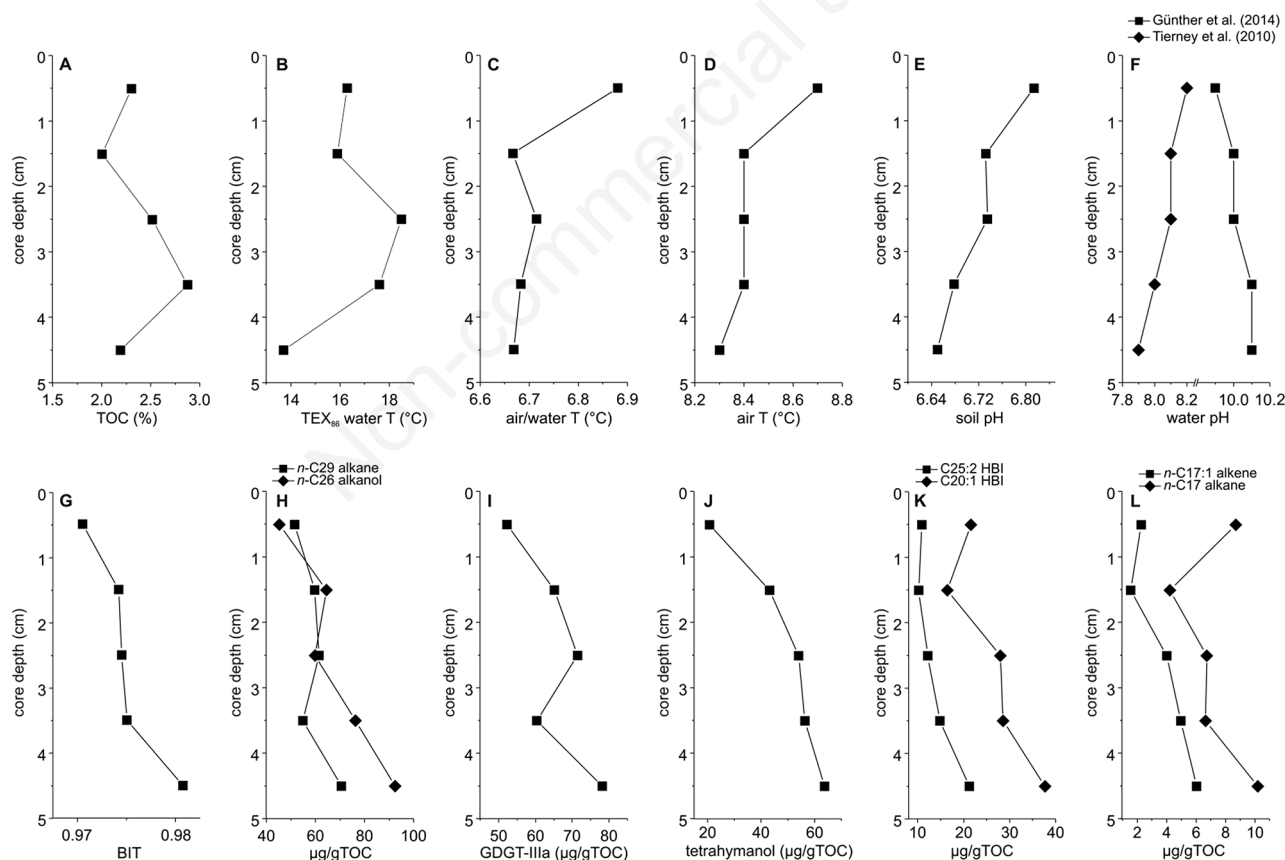
### Downcore profiles of representative biomarkers

Despite the low depth coverage of the records, TOC and GDGT-based proxies were plotted as a function of depth on Figs. 4 and 5, together with lipids having a specific terrestrial or aquatic source. Terrestrial input is represented by the BIT index and the concentrations of  $n$ -C<sub>29</sub> alkane,  $n$ -C<sub>26</sub> alkanol and branched GDGT-IIIa. Dinosterol (Fig. 4) and tetrahymanol are assumed to be relevant biomarkers for dinoflagellates (mainly *Peridinium cinctum*) and ciliates, respectively. The HBI alkenes,  $n$ -C<sub>17</sub> alkane and  $n$ -C<sub>17:1</sub> alkene are considered as representative for, respectively, diatoms and cyanobacteria involved in the formation of stromatolites. Generally, the proxy records related to lake productivity and terrestrial inputs

are decreasing bottom-up. On the contrary, both water ( $TEX_{86}$ ) and air/water temperature records are increasing despite large differences in temperature absolute values and amplitudes. A similar trend is apparent in the proxy records for soil and water pH although the changes are minor (<0.4). In relation to increasing water temperatures, the present pattern may reflect a decrease in rainfall amount, an increase in lake evaporation and a lake level drop resulting in a decay of stromatolites. However, considering the small amount of samples, a more detailed proxy interpretation and discussion is hazardous and may be misleading. These preliminary downcore results emphasize the high potential of organic proxies for the paleoenvironmental reconstruction of Lake Salda.

### CONCLUSIONS

This study represents the first investigation of sedimentary lipid distribution in an alkaline and magnesium-



**Fig. 5.** Depth profiles of (A) TOC, (B)  $TEX_{86}$  lake water temperature [Castañeda and Schouten (2015) calibration], (C) air/water lake temperature [Pearson *et al.* (2011) lake calibration], (D) surface air temperature [Yang *et al.* (2014) soil calibration], (E) soil pH [Yang *et al.* (2014) soil calibration], (F) lake water pH [Tierney *et al.* (2010) and Günther *et al.* (2014) lake calibrations], (G) BIT index, and of the concentrations (normalized to TOC) of biomarkers considered as representative for different terrestrial (H, I) and aquatic (J, K, L) organisms (see text for details).

rich lake from south-western Anatolia. The low diversity in phytoplankton and zooplankton allows assigning potential source organisms to the main lipids present in the sediments, including dinoflagellates for dinosterol and other sterols, ciliates for tetrahymanol, diatoms for  $C_{25:2}$  HBI, Cyanobacteria for  $n-C_{17}$  alkane and  $n-C_{17:1}$  alkene and Thaumarchaeota for crenarchaeol. Lipids such as  $n-C_{29}$  alkane and  $n-C_{26}$  alkanol, which are compounds of land plant leaf waxes, are considered as proxies for terrestrial inputs. Branched GDGTs are probably produced in both soils and lake water (or sediments). Branched GDGT-based proxies for water pH provide reasonable estimates. However, estimated soil pH, air temperature and lake water/air temperature are lower than observed values. Seasonal biases and the uncertainty of absolute temperature estimates may explain these differences.  $TEX_{86}$  water temperature estimates reflect the observed mean annual lake surface temperature. The results based on the present approach provide a basis for future biomarker-based paleoenvironmental reconstructions of Lake Salda.

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#### REFERENCES

- Amo M, Suzuki N, Kawamura H, Yamaguchi A, Takano Y, Horiguchi T, 2010. Sterol composition of dinoflagellates: different abundance and composition in heterotrophic species and resting cysts. *Geochem. J.* 44:225-231.
- Barker P, 1992. Differential diatom dissolution in Late Quaternary sediments from Lake Manyara, Tanzania: An experimental approach. *J. Paleolimnol.* 7:235-251.
- Barrick RC, Hedges JI, Peterson ML, 1980. Hydrocarbon geochemistry of the Puget Sound region-I: Sedimentary acyclic hydrocarbons. *Geochim. Cosmochim. Acta* 44:1349-1362.
- Belt ST, Masse G, Allard WG, Robert JM, Rowland SJ, 2001. Identification of a C-25 highly branched isoprenoid triene in the freshwater diatom *Navicula sclesvicensis*. *Org. Geochem.* 32:1169-1172.
- Bird CW, Lynch JM, Pirt SJ, Reid WW, Brooks CJW, Middleditch BS, 1971. Steroids and squalene in *Methylococcus capsulatus* grown on methane. *Nature* 230:473-474.
- Blaça CI, Reichart G-J, Heiri O, Sinninghe Damsté JS, 2009. Tetraether membrane lipid distributions in water-column particulate matter and sediments: A study of 47 European lakes along a north-south transect. *J. Paleolimnol.* 41:523-540.
- Braithwaite CJR, Zedef V, 1994. Living hydromagnesite stromatolites in Turkey. *Sediment. Geol.* 92:1-5.
- Braithwaite CJR, Zedef V, 1996. Hydromagnesite stromatolites and sediments in an alkaline lake, Salda Golu, Turkey. *J. Sediment. Res.* 66:91-1002.
- Buckles LK, Weijers JWH, Verschuren D, Sinninghe Damsté JS, 2014. Sources of core and intact branched tetraether membrane lipids in the lacustrine environment: Anatomy of Lake Challa and its catchment, equatorial East Africa. *Geochim. Cosmochim. Acta* 140:106-126.
- Bulger AJ, Hayden BP, Monaco ME, Nelson DM, Ray MGC, 1993. Biologically based estuarine salinity zones derived from a multivariate analysis. *Estuaries* 16:311-322.
- Bush RT, McInerney FA, 2013. Leaf wax  $n$ -alkane distributions in and across modern plants: implications for paleoecology and chemotaxonomy. *Geochim. Cosmochim. Acta* 117:161-179.
- Buschhaus C, Peng C, Jetter R, 2013. Very-long-chain 1,2- and 1,3-bifunctional compounds from the cuticular wax of *Cosmos bipinnatus* petals. *Phytochemistry* 91:249-256.
- Cardoso JN, Gaskell SJ, Quirk MM, Eglinton G, 1983. Hydrocarbon and fatty acid distributions in Rostherne lake sediment (England). *Chem. Geol.* 38:107-128.
- Castañeda IS, Schouten S, 2011. A review of molecular organic proxies for examining modern and ancient lacustrine environments. *Quaternary Sci. Rev.* 30:2851-2891.
- Castañeda IS, Schouten S, 2015. Corrigendum to "A review of molecular organic proxies for examining modern and ancient lacustrine environments". *Quaternary Sci. Rev.* 125:174-176.
- Çetinkaya Dönmez G, Öztürk A, Çakmakçı L, 1999. Properties of the *Rhodospseudomonas palustris* strains, isolated from an alkaline lake in Turkey. *Turk. J. Biol.* 23:457-463.
- Chan OW, Bugler-Lacap DC, Biddle JF, Lim DS, McKay CP, Pointing SB, 2014. Phylogenetic diversity of a microbialite reef in a cold alkaline freshwater lake. *Can. J. Microbiol.* 60:391-398.
- Coates RC, Podell S, Korobeynikov A, Lapidus A, Pevzner P, Sherman DH, Allen EE, Gerwick L, Gerwick WH, 2014. Characterization of cyanobacterial hydrocarbon composition and distribution of biosynthetic pathways. *Plos One* 9:e85140.
- Coffinet S, Hugué A, Williamson D, Fosse C, Derenne S, 2014. Potential of GDGTs as a temperature proxy along an altitudinal transect at Mount Rungwe (Tanzania). *Org. Geochem.* 68:82-89.
- Costello EK, Schmid SK, 2006. Microbial diversity in alpine tundra wet meadow soil: novel *Chloroflexi* from a cold, water-saturated environment. *Environ. Microbiol.* 8:1471-1486.
- Cranwell PA, 1973. Chain-length distribution of  $n$ -alkanes from lake sediments in relation to post-glacial environmental change. *Freshwater Biology* 3: 259-265.
- Cranwell PA, Jaworski GHM, Bickley HM, 1990. Hydrocarbons, sterols, esters and fatty acids in six freshwater chlorophytes. *Phytochemistry* 29:145-151.
- de Rosa M, Gambacorta A, Minale L, Bu'lock J, 1971. Bacterial triterpenes. *J. Chem. Soc. Chem. Commun.* 12:619-620.
- Dimitriu PA, Pinkart HC, Peyton BM, Mormile MR, 2008. Spatial and temporal patterns in the microbial diversity of a meromictic soda lake in Washington State. *Appl. Environ. Microbiol.* 74:4877-4888.

- Eglinton G, Hamilton RJ, 1967. Leaf epicuticular waxes. *Science* 156:1322-1335.
- Elling FJ, Könneke M, Lipp JS, Becker KW, Gagen EJ, Hinrichs K-U, 2014. Effects of growth phase on the membrane lipid composition of the thaumarchaeon *Nitrosopumilus maritimus* and their implications for archaeal lipid distributions in the marine environment. *Geochim. Cosmochim. Acta* 141:579-597.
- Elling FJ, Könneke M, Mußmann M, Greve A, Hinrichs K-U, 2015. Influence of temperature, pH, and salinity on membrane lipid composition and TEX<sub>86</sub> of marine planktonic thaumarchaeal isolates. *Geochim. Cosmochim. Acta* 171:238-255.
- Gagosian RB, Smith SO, Nigrelli GE, 1982. Vertical transport of steroid alcohols and ketones measured in a sediment trap experiment in the equatorial Atlantic Ocean. *Geochim. Cosmochim. Acta* 46:1163-1172.
- Gelpi E, Schneider HJ, Mann J, Oró J, 1970. Hydrocarbons of geochemical significance in microscopic algae. *Phytochemistry* 9:603-612.
- Günther F, Thiele A, Gleixner G, Xu B, Yao T, Schouten S, 2014. Distribution of bacterial and archaeal ether lipids in soils and surface sediments of Tibetan lakes: implications for GDGT-based proxies in saline high mountain lakes. *Org. Geochem.* 67:19-30.
- Hammer UT, 1986. Saline lake ecosystems of the world. *Mono-graphiae Biologicae*. 59. Springer, Amsterdam: 616 pp.
- Harvey HR, McManus GB, 1991. Marine ciliates as a widespread source of tetrahymanol and hopan-3 $\beta$ -ol in sediments. *Geochim. Cosmochim. Acta* 55:387-3390.
- Hopmans EC, Weijers JWH, Schefuss E, Herfort L, Sinninghe Damsté JS, Schouten S, 2004. A novel proxy for terrestrial organic matter in sediments based on branched and isoprenoid tetraether lipids. *Earth Planet. Sc. Lett.* 224:107-116.
- Inglis GN, Farnsworth A, Lunt D, Foster GL, Hollis CJ, Pagani M, Jardine PE, Pearson PN, Markwick P, Galsworthy AMJ, Raynham L, Taylor KWR, Pancost RD, 2015. Descent toward the Icehouse: Eocene sea surface cooling inferred from GDGT distributions. *Paleoceanography* 30:1000-1020.
- Jones M, Roberts N, Leng M, Turkes M, 2006. A high-resolution late Holocene lake isotope record from Turkey and links to North Atlantic and monsoon climate. *Geology* 34:361-364.
- Kazancı N, Girgin S, Duge M, 2004. On the limnology of Salda Lake, a large and deep soda lake in southwestern Turkey: future management proposals. *Aquat. Conserv.* 14:151-162.
- Kleemann G, Poralla K, Englert G, Kjøsén H, Liaaen-Jensen S, Neunlist S, Rohmer M, 1990. Tetrahymanol from the phototrophic bacterium *Rhodospseudomonas palustris*: first report of a gammacerane triterpene from a prokaryote. *Microbiology* 136:2551-2553.
- Kokinos JP, Eglinton TI, Goñi MA, Boon JJ, Martoglio PA, Anderson DM, 1998. Characterization of a highly resistant biomacromolecular material in the cell wall of a marine dinoflagellate resting cyst. *Org. Geochem.* 28:265-288.
- Kolattukudy PE, 1976. *Chemistry and Biochemistry of Natural Waxes*. Elsevier, Amsterdam: 495 pp. Lagutin, K, MacKenzie, A, Houghton, KM, Stott, MB, Vyssotski, M, 2015. Novel long-chain diol phospholipids from some bacteria belonging to the class *Thermomicrobia*. *Lipids* 50:303-311.
- Lanzen A, Simachew A, Gessesse A, Chmolowska D, Jonassen I, Ovreas L, 2013. Surprising prokaryotic and eukaryotic diversity, community structure and biogeography of Ethiopian soda lakes. *Plos One* 8:e72577.
- Leeming R, Nichols PD, 1998. Determination of the sources and distribution of sewage and pulp-fibre derived pollution in the Derwent estuary, Tasmania, using sterol biomarkers. *Mar. Freshw. Res.* 49:7-17.
- Lindström K, 1984. Effect of temperature, light and pH on growth, photosynthesis, and respiration of the dinoflagellate *Peridinium cinctum* fa. *westii* in laboratory cultures. *J. Phycol.* 20:212-220.
- Liu A, Zhu T, Lu X, Song L, 2013. Hydrocarbon profiles and phylogenetic analyses of diversified cyanobacterial species. *Appl. Energ.* 111:383-393.
- Liu X-L, Lipp JS, Schroeder JM, Summons RE, Hinrichs K-U, 2012a. Isoprenoid glycerol dialkanol diethers: A series of novel archaeal lipids in marine sediments. *Org. Geochem.* 43: 50-55.
- Liu X-L, Lipp JS, Simpson JH, Lin Y-S, Summons RE, Hinrichs K-U, 2012b. Mono- and dihydroxyl glycerol dibiphytanyl glycerol tetraethers in marine sediments: Identification of both core and intact polar lipid forms. *Geochim. Cosmochim. Acta* 89:102-115.
- Liu X-L, Summons RE, Hinrichs K-U, 2012c. Extending the known range of glycerol ether lipids in the environment: structural assignments based on tandem mass spectral fragmentation patterns. *Rapid Commun. Mass Spectrom.* 26:2295-2302.
- Loomis SE, Russell JM, Ladd B, Street-Perrott FA, Sinninghe Damsté JS, 2012. Calibration and application of the branched GDGT temperature proxy on East African lake sediments. *Earth. Planet. Sc. Lett.* 357-358:277-288.
- Loomis SE, Russell JM, Eggermont H, Verschuren D, Sinninghe Damsté, JS, 2014. Effects of temperature, pH and nutrient concentration on branched GDGT distributions in East African lakes: Implications for paleoenvironmental reconstruction. *Org. Geochem.* 66:25-37.
- Loomis SE, Russell JM, Sinninghe Damsté JS, 2011. Distributions of branched GDGTs in soils and lake sediments from western Uganda: Implications for a lacustrine paleothermometer. *Org. Geochem.* 42:739-751.
- Matsumoto GI, Akiyama M, Watanuki K, Torii T, 1990. Unusual distributions of long-chain *n*-alkanes and *n*-alkenes in Antarctic soil. *Org. Geochem.* 15:03-412.
- Mazurek MA, Simoneit BRT, 1984. Characterization of biogenic and petroleum-derived organic matter in aerosols over remote, rural and urban areas, p. 353-370. In: L.H. Keith (ed.), *Identification and analysis of organic pollutants in air*. Butterworth-Heinemann Publishers.
- Meyers P, 1997. Organic geochemical proxies of paleoceanographic, paleolimnologic and paleoclimate processes. *Org. Geochem.* 27:213-250.
- Naghbi F, Tabatabai Yazdi M, Sahebgharani M, Noori Dalooi MR, 2002. Microbial transformation of cholesterol by *Mycobacterium megmatris*. *J. Sci. I. R. Iran* 13:103-106.
- Nishimura M, Koyama T, 1977. The occurrence of stanols in various living organisms and the behavior of sterols in contemporary sediments. *Geochim. Cosmochim. Acta* 41:379-385.
- Ourisson G, Albrecht P, Rohmer M, 1979. The hopanoids: palaeochemistry and biochemistry of a group of natural products. *Pure Appl. Chem.* 51:709-729.

- Ourisson G, Rohmer M, Poralla K, 1987. Prokaryotic hopanoids and other polyterpenoid sterol surrogates. *Annu. Rev. Microbiol.* 41:301-333.
- Owen RW, Mason AN, Bilton RF, 1983. The degradation of cholesterol by *Pseudomonas* sp. NCIB10590 under aerobic conditions. *J. Lipid. Res.* 24:1500-1511.
- Paoletti C, Pushparaj B, Florenzano G, Capella P, Lercker G, 1976. Unsaponifiable matter of green and blue-green algal lipids as a factor of biochemical differentiation of their biomasses: I. Total unsaponifiable and hydrocarbon fraction. *Lipids* 11:258-265.
- Pearson A, Ingalls AE, 2013. Assessing the use of archaeal lipids as marine environmental proxies. *Annu. Rev. Earth Pl. Sc.* 41:359-384.
- Pearson EJ, Juggins S, Talbot HM, Weckström J, Rosén P, Ryves DB, Roberts SJ, Schmidt R, 2011. A lacustrine GDGT-temperature calibration from the Scandinavian Arctic to Antarctic: Renewed potential for the application of GDGT paleothermometry in lakes. *Geochim. Cosmochim. Acta* 75:6225-6238.
- Peterse F, van der Meer MJT, Schouten S, Weijers JWH, Fierer N, Jackson RB, Kim J-H, Sinninghe Damsté JS, 2012. Revised calibration of the MBT-CBT paleotemperature proxy based on branched tetraether membrane lipids in surface soils. *Geochim. Cosmochim. Acta* 96:215-229.
- Pitcher A, Hopmans EC, Mosier AC, Park SJ, Rhee S-K, Francis CA, Schouten S, Sinninghe Damsté JS, 2011. Core and intact polar glycerol dibiphytanyl glycerol tetraether lipids of ammonia-oxidizing Archaea enriched from marine and estuarine sediments. *Appl. Environ. Microbiol.* 77:3468-3477.
- Pond JL, Langworthy TA, Holzer G, 1986. Long-chain diols: a new class of membrane lipids from a thermophilic bacterium. *Science* 231:1134-1136.
- Pond JL, Langworthy TA, 1987. Effect of growth temperature on the long-chain diols and fatty acids of *Thermomicrobium roseum*. *J. Bacteriol.* 169:1328-1330.
- Powers L, Werne JP, Vanderwoude AJ, Sinninghe Damsté JS, Hopmans EC, Schouten S, 2010. Applicability and calibration of the TEX<sub>86</sub> paleothermometer in lakes. *Org. Geochem.* 41:404-413.
- Randlett M-E, Coolen MJL, Stockhecke M, Pickarski N, Litt T, Balkema C, Kwiecien O, Tomonaga Y, Wehrli B, Schubert CJ, 2014. Alkenone distribution in Lake Van sediment over the last 270 ka: influence of temperature and haptophyte species composition. *Quaternary Sci. Rev.* 104:53-62.
- Roberts N, Moreno A, Valero-Garcés BL, Corella JP, Jones M, Allcock S, Woodbridge J, Morellon M, Luterbacher J, Xoplaki E, Türkeş M, 2012. Palaeolimnological evidence for an east-west climate see-saw in the Mediterranean since AD 900. *Global Planet. Change* 84-85:23-34.
- Robinson N, Cranwell PA, Eglinton G, Brassell SC, Sharp CL, Gophen M, Pollinger U, 1985. Lipid geochemistry of Lake Kinneret. *Adv. Org. Geochem.* 10: 33-742.
- Rohmer M, Bouvier-Nave P, Ourisson G, 1984. Distribution of hopanoid triterpenes in prokaryotes. *J. Genet. Microbiol.* 130:1137-1150.
- Rontani JF, Beker B, Volkman JK, 2004. Long-chain alkenones and related compounds in the benthic haptophyte *Chryssotila lamellosa* Anand HAP 17. *Phytochemistry* 65:117-126.
- Rontani JF, Zabeti N, Wakeham SG, 2009. The fate of marine lipids: biotic vs. abiotic degradation of particulate sterols and alkenones in the Northwestern Mediterranean Sea. *Mar. Chem.* 113:9-18.
- Rowland SJ, Robson JN, 1990. The widespread occurrence of highly branched acyclic C<sub>20</sub>, C<sub>25</sub> and C<sub>30</sub> hydrocarbons in recent sediments and biota - A review. *Mar. Environ. Res.* 30:191-216.
- Rowland SJ, Yon DA, Lewis CA, Maxwell JR, 1985. Occurrence of 2,6,10-trimethyl-7-(3-methylbutyl)-dodecane and related hydrocarbons in the green alga *Enteromorpha prolifera* and sediments. *Org. Geochem.* 8:207-213.
- Russell MJ, Ingham JK, Zedef V, Maktav D, Sunar F, Hall AJ, Fallick AE, 1999. Search for signs of ancient life on Mars: expectations from hydromagnesite microbialites, Salda Lake, Turkey. *J. Geol. Soc. London* 156:869-888.
- Russell MJ, Ingham JK, Zedef V, Maktav D, Sunar F, Hall AJ, Fallick AE, 1999. Search for signs of ancient life on Mars: expectations from hydromagnesite microbialites, Salda Lake, Turkey. *J. Geol. Soc. London* 156: 869-888.
- Schoon PL, de Kluijver A, Middelburg JJ, Downing JA, Sinninghe Damsté JS, Schouten S, 2013. Influence of lake water pH and alkalinity on the distribution of core and intact polar branched glycerol dialkyl glycerol tetraethers (GDGTs) in lakes. *Org. Geochem.* 60:72-82.
- Schouten S, Forster A, Panoto FE, Sinninghe Damsté JS, 2007. Towards calibration of the TEX<sub>86</sub> palaeothermometer for tropical sea surface temperatures in ancient greenhouse worlds. *Org. Geochem.* 38:1537-1546.
- Schouten S, Hopmans EC, Schefuss E, Sinninghe Damsté JS, 2002. Distributional variations in marine crenarchaeotal membrane lipids: A new tool for reconstructing ancient sea water temperatures? *Earth Planet. Sc. Lett.* 204:265-274.
- Schouten S, Hopmans EC, Sinninghe Damsté JS, 2013. The organic geochemistry of glycerol dialkyl glycerol tetraether lipids: A review. *Org. Geochem.* 54:19-61.
- Serruya C, 1971. Lake Kinneret: the nutrient chemistry of the sediments. *Limnol. Oceanogr.* 16:510-521.
- Serruya C, Edelsteik M, Pollischgher U, Serruya S, 1974. Lake Kinneret sediments: nutrient composition of the pore water and mud-water exchanges. *Limnol. Oceanogr.* 19:489-508.
- Sessions AL, Zhang L, Welander PV, Doughty D, Summons RE, Newman DK, 2013. Identification and quantification of polyfunctionalized hopanoids by high temperature gas chromatography - mass spectrometry. *Org. Geochem.* 56:120-130.
- Shirokova LS, Mavromatis V, Bundeleva I, Pokrovsky OS, Bénézet P, Pearce C, Gérard E, Balor S, Oelkers EH, 2011. Can Mg isotopes be used to trace cyanobacteria-mediated magnesium carbonate precipitation in alkaline lakes? *Biogeosciences Discuss.* 8:6473-6517.
- Sinninghe Damsté JS, Kenig F, Koopmans MP, Köster J, Schouten S, Hayes JM, de Leeuw JW, 1995. Evidence for gammacerane as an indicator of water column stratification. *Geochim. Cosmochim. Acta* 59:895-1900.
- Sinninghe Damsté JS, Rijpstra WI, Hopmans EC, Foesel BU, Wüst PK, Overmann J, Tank M, Bryant DA, Dunfield PF, Houghton K, Stott MB, 2014. Ether- and ester-bound isodiabolic acid and other lipids in members of acidobacteria subdivision 4. *Appl. Environ. Microbiol.* 80:207-5218.
- Sinninghe Damsté JS, Rijpstra WIC, Hopmans EC, Weijers WH, Foesel BU, Overmann J, Dedysh SN, 2011. 13,16-dimethyl

- octacosanedioic acid (iso-diabolic acid), a common membrane-spanning lipid of Acidobacteria subdivisions 1 and 3. *Appl. Environ. Microbiol.* 77:4147-4154.
- Sorokin DY, Lückner S, Vejmelkova D, Kostrikina NA, Kleerbezem R, Rijpstra WAC, Sinninghe Damsté JS, Le Paslier D, Muyzer G, Wagner M, van Loosdrecht MCM, Daims H, 2012. Nitrification expanded: discovery, physiology, and genomics of a nitrite-oxidizing bacterium from the phylum Chloroflexi. *ISME J.* 6:2245-2256.
- Stadnitskaia A, Blinova V, Ivanov MK, Baas M, Hopmans E, van Weering TCE, Damsté JSS, 2007. Lipid biomarkers in sediments of mud volcanoes from the Sorokin Trough, NE Black Sea: probable source strata for the erupted material. *Org. Geochem.* 38:67-83.
- Sun Q, Chu G, Liu G, Li S, Wang X, 2007. Calibration of alkenone unsaturation index with growth temperature for a lacustrine species, *Chrysotila lamellosa* (Haptophyceae). *Org. Geochem.* 38:1226-1234.
- Sun Q, Chu G, Liu M, Xie M, Li S, Ling Y, Wang X, Shi L, Jia G, Lü H, 2011. Distributions and temperature dependence of branched glycerol dialkyl glycerol tetraethers in recent lacustrine sediments from China and Nepal. *J. Geophys. Res.* 116:G01008.
- Tayanç M, Im U, Doğruel M, Karaca M, 2009. Climate change in Turkey for the last half century. *Clim. Change* 94:483-502.
- Ten Haven HL, Rohmer M, Rullkötter J, Bissert P, 1989. Tetrahymanol, the most likely precursor of gammacerane, occurs ubiquitously in marine sediments. *Geochim. Cosmochim. Acta* 53:3073-3079.
- Thiel V, Jenisch A, Landmann G, Reimer A, Michaelis W, 1997. Unusual distributions of long-chain alkenones and tetrahymanol from the highly alkaline Lake Van, Turkey. *Geochim. Cosmochim. Acta* 61:2053-2064.
- Thompson JB, Ferris FG, 1990. Cyanobacterial precipitation of gypsum, calcite, and magnesite from natural alkaline lake water. *Geology* 18:995-998.
- Tierney JE, Russell JM, Eggermont H, Hopmans EC, Verschuren D, Sinninghe Damsté JS, 2010. Environmental controls on branched tetraether lipid distributions in tropical East African lake sediments. *Geochim. Cosmochim. Acta* 74:4902-4918.
- Tierney JE, Russell JM, 2009. Distributions of branched GDGTs in a tropical lake system: Implications for lacustrine application of the MBT/CBT paleoproxy. *Org. Geochem.* 40:1032-1036.
- Toney JL, Leavitt PR, Huang Y, 2011. Alkenones are common in prairie lakes of interior Canada. *Org. Geochem.* 42:707-712.
- Tudryn A, Tucholka P, Özgür N, Gibert E, Elitok O, Kamaci Z, Massault M, Poisson A, Platevoet B, 2013. A 2300-year record of environmental change from SW Anatolia, Lake Burdur, Turkey. *J. Paleolimnol.* 49:647-662.
- Versteegh GJM, Bosch H-J, de Leeuw JW, 1997. Potential palaeoenvironmental information of C24 to C36 mid-chain diols, keto-ols and mid-chain hydroxy fatty acids: a critical review. *Org. Geochem.* 27:1-13.
- Volkman JK, Farrington JW, Gagosian RB, Wakeham SG, 1983. Lipid composition of coastal marine sediments from the Peru upwelling region, p. 228-240. In: M. Bjorøy (eds), *Proc. 10th Int. Meet. on Organic Geochemistry*, University of Bergen. *Advances in organic geochemistry* 1981. J. Wiley & Sons, London.
- Volkman JK, 1986. A review of sterol markers for marine and terrigenous organic matter. *Org. Geochem.* 9:83-99.
- Volkman JK, Barrett SM, Blackburn SI, Mansour MP, Sikes EL, Gelin F, 1998. Microalgal biomarkers: a review of recent research developments. *Org. Geochem.* 29:1163-1179.
- Volkman JK, Barrett SM, Dunstan GA, 1994. C<sub>25</sub> and C<sub>30</sub> highly branched isoprenoid alkenes in laboratory cultures of two marine diatoms. *Org. Geochem.* 21:407-414.
- Volkman JK, Kearney P, Jeffrey SW, 1990. A new source of 4-methyl sterols and 5 $\alpha$ (H)-stanols in sediments: prymnesiophyte microalgae of the genus Pavlova. *Org. Geochem.* 15:489-497.
- Wait R, Carreto L, Nobre MF, Ferreira AM, da Costa MS, 1997. Characterization of novel long-chain 1,2-diols in *Thermus* species and demonstration that *Thermus* strains contain both glycerol-linked and diol-linked glycolipids. *J. Bacteriol.* 179:6154-6162.
- Walsh K, Jones GJ, Dunstan RH, 1998. Effect of high irradiance and iron on volatile odour compounds in the cyanobacterium *Microcystis aeruginosa*. *Phytochemistry* 49:1227-1239.
- Weijers JWH, Panoto E, van Bleijswijk J, Schouten S, Rijpstra WIC, Balk M, Stams AJM, Sinninghe Damsté JS, 2009. Constraints on the biological source(s) of the orphan branched tetraether membrane lipids. *Geomicrobiol. J.* 26:402-414.
- Weijers JWH, Schouten S, Hopmans EC, Geenevasen JAJ, David ORP, Coleman JM, Pancost RD, Sinninghe Damsté JS, 2006a. Membrane lipids of mesophilic anaerobic bacteria thriving in peats have typical archaeal traits. *Environ. Microbiol.* 8:648-657.
- Weijers JWH, Schouten S, Spaargaren OC, Sinninghe Damsté JS, 2006b. Occurrence and distribution of tetraether membrane lipids in soils: Implications for the use of the TEX<sub>86</sub> proxy and the BIT index. *Org. Geochem.* 37:1680-1693.
- Weijers JWH, Schouten S, van den Donker J, Hopmans EC, Sinninghe Damsté JS, 2007. Environmental controls on bacterial tetraether membrane lipid distribution in soils. *Geochim. Cosmochim. Acta* 71:703-713.
- Wu D, Raymond J, Wu M, Chatterji S, Ren Q, Graham JE, Bryant DA, Robb F, Colman A, Tallon LJ, Badger JH, Madupu R, Ward NL, Eisen JA, 2009. Complete Genome Sequence of the Aerobic CO-Oxidizing Thermophile *Thermomicrobium roseum*. *PLoS One* 4:e4207.
- Yang H, Pancost RD, Dang X, Zhou X, Evershed RP, Xiao G, Tang C, Gao L, Guo Z, Xie S, 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.
- Zink KG, Leythaeuser D, Melkonian M, Schwark L, 2001. Temperature dependency of long-chain alkenone distributions in Recent to fossil limnic sediments and in lake waters. *Geochim. Cosmochim. Acta* 65:253-265.
- Zink KG, Vandergoes MJ, Mangelsdorf K, Dieffenbacher-Krall AC, Schwark L, 2010. Application of bacterial glycerol dialkyl glycerol tetraethers (GDGTs) to develop modern and past temperature estimates from New Zealand lakes. *Org. Geochem.* 41:1060-1066.