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Biomarker evidence for nitrogen-fixing cyanobacterial blooms in a brackish surface layer in the Nile River plume during sapropel deposition

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ABSTRACT

Sapropels are organic-rich sediment layers deposited in the eastern Mediterranean Sea during precession minima, resulting from an increase in export productivity and/or preservation. Increased freshwater delivery from the African continent resulted in stratification, causing deep-water anoxia, while nutrient input stimulated productivity, presumably at the deep chlorophyll maximum. Previous studies have suggested that during sapropel deposition, nitrogen fixation was widespread in the highly stratified surface waters, and that cyanobacteria symbiotic with diatoms (diatom-diazotroph associations, DDAs) were responsible. Here we analyzed sapropel S5 sediments for heterocyst glycolipids (HGs) from three locations in the eastern Mediterranean. HG biomarkers can differentiate between those heterocystous cyanobacteria that are free living (found predominately in freshwater or brackish environments) and those that are from DDAs (found in marine settings). In our primary core, from a location which would have been influenced by the Nile River outflow, we detected a HG with a pentose (C_5) head group specific for DDAs. However, HGs with a hexose (C_6) head group, specific to free-living cyanobacteria, were present in substantially (up to 60×) higher concentration. These data suggest that at our study location, free-living cyanobacteria were the dominant diazotrophs, rather than DDAs. The C_6 HGs increased substantially at the onset of sapropel S5 deposition, suggesting that substantial seasonal cyanobacterial blooms were associated with a brackish surface layer flowing from the Nile into the eastern Mediterranean. Two additional S5 sapropels were analyzed, one also from the Nile delta region and one from the region between Libya and southwestern Crete. Overall, comparison of the HG distribution in the three S5 sapropels provides evidence that all three locations were initially influenced by surface salinities that were sufficiently low to support free-living heterocystous cyanobacteria. While free-living heterocystous cyanobacteria continued to outnumber DDAs during sapropel deposition at the two Nile-influenced sites, DDAs, indicators of persistent marine salinities, were the dominant diazotrophs in the upper part of the sapropel at the more westerly site. These results indicate that N_2 fixation by free-living cyanobacteria offers an important additional mechanism to stimulate productivity in regions with strong river discharge during sapropel deposition.

INTRODUCTION

The dark-colored, organic-rich sapropels found in the eastern Mediterranean Sea are associated with a stratification-linked reduction in ventilation resulting from freshwater outflow during precession minima, concurrent with increased organic-matter export from the photic zone. The increase in productivity is, for sapropel S5 and probably most other sapropels, linked to a highly productive deep chlorophyll maximum (DCM) (Rohling et al., 2015). Increased organic-matter flux into the poorly ventilated bottom waters and subsequent respiration resulted in anoxia development and sapropel formation (Rossignol-Strick et al., 1982; Rossignol-Strick, 1985; Rohling et al., 2015). Isotope, biomarker, and microfossil studies have suggested that the nutrient-sparse surface waters would have been favorable for cyanobacterial nitrogen fixation and, in particular, diatom-cyanobacteria symbioses (Kemp et al., 1999; Sachs and Repeta, 1999; Bauersachs et al., 2010) termed diatom-diazotroph associations (DDAs).

Heterocyst glycolipids (HGs) are biomarkers for cyanobacteria that use heterocysts to carry-out N_2 fixation (Nichols and Wood, 1968; Gambacorta et al., 1995; Bauersachs et al., 2009a). The sugar moiety of HGs found in free-living heterocystous cyanobacteria is typically hexose (hereafter C_6), while the HGs associated with endosymbiotic heterocystous cyanobacteria in DDAs contain a pentose moiety (hereafter C_5) (Schouten et al., 2013; Bale et al., 2015). C_6 HGs have been applied as specific paleo-biomarkers for the presence of N_2-fixing cyanobacteria in marine and lacustrine geological records (Bauersachs et al., 2010; Sollai et al., 2017). Previously, Bauersachs et al. (2010) found that C_6 HGs were abundant in the S5 sapropel from a Nile-influenced location in the eastern Mediterranean Sea and suggested that they were likely derived from DDAs. However, the subsequent discovery that all DDAs studied to date produce C_5 HGs (Schouten et al., 2013; Bale et al., 2015), not C_6 HGs, casts uncertainty on this explanation. Furthermore, the analytical method of Bauersachs et al. (2010) did not cover the detection of C_5 HGs. Here we comprehensively analyzed

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the C_5 and C_6 HGs in a sediment core from the eastern Mediterranean Sea containing a well-preserved sapropel S5 (ca. 128.3–121.5 ka; Grant et al., 2016), as well as two other S5 sapropels also located in the eastern Mediterranean Sea, in order to unravel the primary mechanism for N_2 fixation by cyanobacteria during this interval.

METHODS

A piston core and suspended particulate matter (SPM) were obtained during Netherlands Earth System Science Centre (NESSC) research cruise 64PE406 onboard the R/V *Pelagia* in the eastern Mediterranean in January 2016. A 920.5-cm-long piston core was collected at cruise station 1 (core 64PE406-E1, 33°18.14898′N, 33°23.71998′E, water depth 1760 m; larger red filled circle in Fig. 1) in the southeastern Levantine Sea. McLane *in situ* pumps (McLane Laboratories Inc., Falmouth, UK) were used to collect SPM from the water column for lipid analysis (see the GSA Data Repository[1] for details). Two other S5 sapropels from core MS66PC (2004 MIMES [Multiscale Investigations of Eastern Mediterranean Seep Systems] cruise; Bauersachs et al., 2010) and Ocean Drilling Program (ODP) core 160-971C-2H-3 (Pearce et al., 1998; Kemp et al., 1999) were also analyzed for their HG lipid content. For details of sample extraction and lipid analysis, see the Data Repository. The core chronology of the sapropel S5 interval (for details, see the Data Repository) was obtained by correlating the Ba variability to that of published sapropel boundaries (Grant et al., 2016) and, for intervals prior to S5, to other Ba excursions (Ziegler et al., 2010). Sedimentary bulk elemental composition measurements by X-ray fluorescence (XRF) core scanning were performed with trace-metal settings (Hennekam et al., 2019); for details, see the Data Repository.

RESULTS AND DISCUSSION

We examined the HG distribution in 30 × 1 cm sediment slices from a depth interval including sapropel S5 (as evidenced from total organic carbon [TOC] and XRF elemental ratios; Fig. 2). We detected (1) a single C_5 HG, 1-(O-ribose)-3,27,29-triacontanetriol (C_5 HG_{30} triol); and (2) three C_6 HGs, 1-(O-hexose)-3,25-hexacosanediol (C_6 HG_{26} diol), 1-(O-hexose)-3,27-octacosanediol (C_6 HG_{28} diol), and 1-(O-hexose)-3,27,29-tricontanetriol (C_6 HG_{30} triol) (see Fig. 2 for structures). We found a notably higher concentration of C_6 HGs (maxima between 20 and 31 µg [g TOC]$^{-1}$) relative to the C_5 HG (maximum 1.8 µg [g TOC]$^{-1}$). The concentrations of all HGs (normalized to TOC) were higher in the sapropel (Figs. 2B–2E).

The occurrence of C_5 HG_{30} triol prior to and during the onset of S5 (Fig. 2B) suggests that cyanobacteria belonging to DDAs, specifically *Richelia intracellularis* (Schouten et al., 2013; Bale et al., 2015, 2018), were present in the euphotic zone at our study site before the deposition of the S5 sapropel, and then either they increased in number or their HG was more efficiently preserved during S5 deposition. The presence of C_5 HG_{30} triol agrees with the high numbers of the DDA host diatoms, in particular *Hemiaulus hauckii*, that have been reported in sapropel S5 (Kemp et al., 1999) at ODP site 971 south of Crete. Kemp et al. (1999) concluded that increased freshwater-induced stratification of surface waters, although still highly marine, led to conditions that were ideally suited for DDAs. In the modern-day eastern Mediterranean, DDAs containing *R. intracellularis* have been detected in low levels throughout the year with a maximum in autumn (Zeev et al., 2008). We also detected C_5 HG_{30} triol in modern-day SPM collected in the winter at the site where piston core 64PE406-E1 was recovered (Table DR1 in the Data Repository).

A quite different trend was observed for the C_6 HGs than for the C_5 HG: all three C_6 HGs increased substantially at the onset of S5 deposition (Figs. 2C–2E). The three C_6 HGs detected have been found in varying proportions both in cultures of the order Nostocales (Bauersachs et al., 2009a; Wörmer et al., 2012) and in freshwater and brackish environments (Bauersachs et al., 2009b, 2010, 2011, 2013; Wörmer et al., 2012; Bühring et al., 2014; Bale et al., 2015, 2016). Based on comparison of the sapropel S5 C_6 HG distribution with literature data (cf. Table DR1), we suggest that during sapropel deposition there was an initial peak in the genus *Nodularia* (C_6 HG_{26} diol; Fig. 2C), followed by dominance in the genus *Aphanizomenon* (C_6 HG_{28} diol and C_6 HG_{30} triol; Figs. 2D and 2E). To date, the C_6 HGs detected in this study have not been detected in any DDAs (see the Data Repository for further discussion).

The presence of free-living Nostocales in eastern Mediterranean sapropel layers is unexpected, as these freshwater and/or brackish cyanobacteria have not been reported in the modern eastern Mediterranean, where the surface salinity is >35 PSU and no C_6 HGs were detected in the recovered SPM (Table DR1). It is not likely that the HGs detected were delivered to the sediment by river as the contributions of terrestrial biomarker lipids into sapropels are very low (Bosch et al., 1998; Menzel et al., 2003). Moreover, the Ti/Al ratio, indicative of the relative contribution of aeolian (Ti) versus Nile-derived fluvial (Al) sediment input (Lourens et al., 2001), shows the highest Nile discharge well after the peak in C_6 HGs (Fig. 2I). Hence, we find no support for the hypothesis that C_6 HGs were produced on land, in lakes or rivers, and delivered to the sediment record via rivers. Reports of free-living heterocystous cyanobacteria in present-day marine settings are very limited: planktonic *Nostoc* has been described in the Indian Ocean and the Red and Mediterranean Seas (Taylor, 1966), and *Anabaena gerdii* has been reported to occur in the western Pacific Ocean, Arabian Sea, and Arafura Sea (Carpenter and Janson, 2001). Tuo et al. (2017) reported free-living *Richelia* in the western North Pacific. In contrast, blooms of free-living cyanobacteria are common in brackish environments. For example, there are extensive summer blooms of the free-living Nostocales species *Nodularia*, *Aphanizomenon*, and *Dolichospermum* in the

[1]GSA Data Repository item 2019375, details of experimental methods used, additional discussion of diatom-diazotroph associations, Table DR1 (heterocyst glycolipid compositions cited in the text and data from literature), Table DR2 relative abundance of C5 heterocyst glycolipids and C6 heterocyst glycolipids as cited in the literature), and Figure DR1 (records used for age model construction)., is available online at http://www.geosociety.org/datarepository/2019/, or on request from editing@geosociety.org.

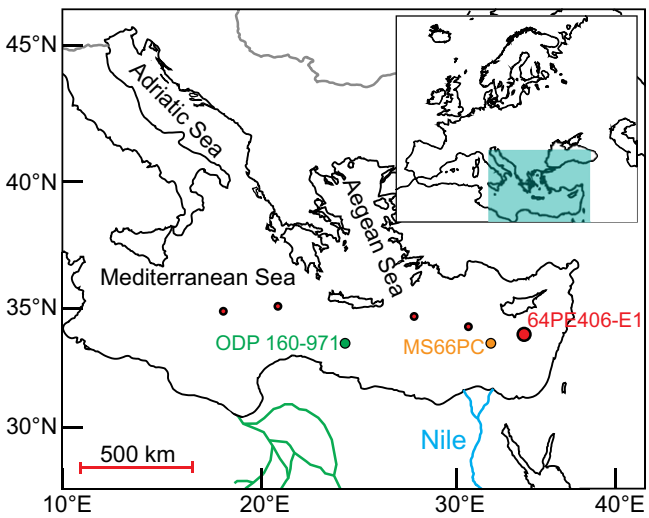

Figure 1. Map of area where core 64PE406-E1 was recovered, eastern Mediterranean Sea. Recovery position of other cores referenced in text are also indicated: Ocean Drilling Program (ODP) Site 160-971. (Kemp et al., 1999; Sachs and Repeta, 1999) and core site MS66PC (Bauersachs et al., 2010). Stations for suspended particulate matter collection are marked with red circles (including 64PE406-E1 recovery station). Present-day location of river Nile is shown in blue, while approximation of extant river-wadi system of Libyan Basin is shown in green (adapted from Rohling et al., 2002).

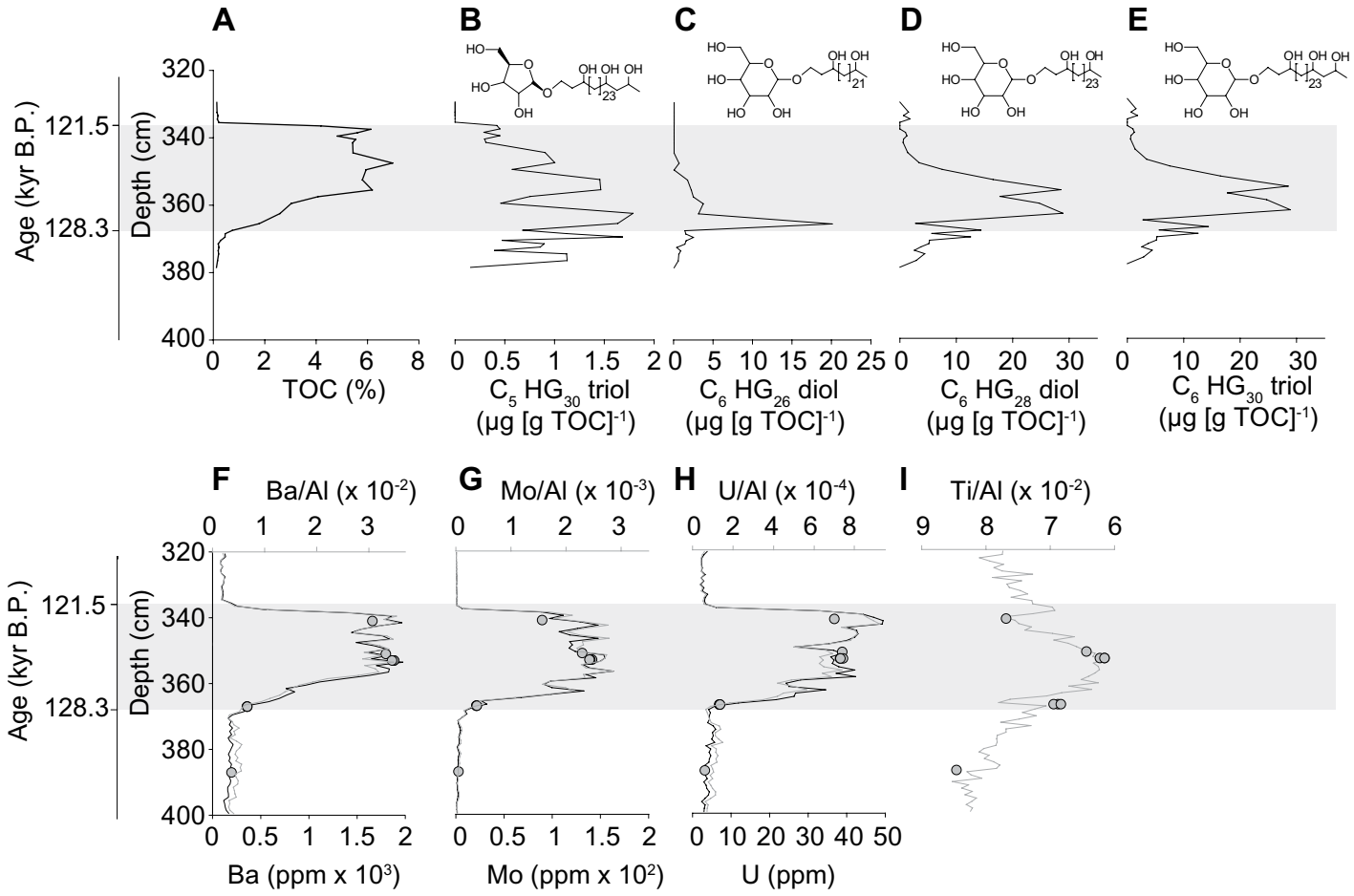

Figure 2. Depth profile of eastern Mediterranean piston core 64PE406-E1 (33°18.14898′N, 33°23.71998′E, water depth 1760 m; NESSC research cruise 64PE406 onboard the R/V *Pelagia*). Horizontal gray bar shows deposition of sapropel. A–E: Organic analyses of total organic carbon (TOC) (A) and concentration (µg [g TOC]$^{-1}$) of heterocyst glycolipids (HGs) C_5 HG_{30} triol (B), C_6 HG_{26} diol (C), C_6 HG_{28} diol (D), and C_6 HG_{30} triol (E). Structures of HGs are shown on respective graphs. Stereochemistry of C_5 HG_{30} triol is as determined by Schouten et al. (2013). F–I: Multivariate log-ratio calibrated X-ray fluorescence scan data in concentration (ppm, black lines) and as ratios (gray lines) for Ba and Ba/Al (F), Mo and Mo/Al (G), U and U/Al (H), and Ti/Al (I). Concentrations derived by inductively coupled plasma–mass spectrometry data are overlain (gray circles).

upper 20 m of the Baltic Sea (Hajdu et al., 2007; Ploug, 2008; Celepli et al., 2017), where surface salinity is on average 6–8 PSU (Gustafsson and Westman, 2002). Hence, we propose that the abundant C_6 HGs in the S5 sapropel at this location indicate the presence of free-living heterocystous cyanobacteria in a brackish surface layer, likely resulting from the freshwater Nile river plume in the eastern Mediterranean at the onset of S5 deposition.

The presence of a surface brackish layer is seemingly inconsistent with the simultaneous presence of DDAs, albeit their HGs occur in much lower concentrations (Fig. 2B). DDAs would also be expected to inhabit the uppermost 40 m (as seen in the analogous Amazon River plume; Foster et al., 2007). DDAs are unimportant at salinities <30 PSU (Foster et al., 2007, 2009; Subramaniam et al., 2008; Bombar et al., 2011). We postulate that the presence of low levels of the C_5 HG alongside the dominant C_6 HGs is due to variation in monsoonal river flow and/or plume location causing seasonal and/or yearly oscillations between marine and brackish water at the surface and thus an alternation between Nostocales and DDA-dominated cyanobacterial populations. Furthermore, in the modern-day Baltic Sea, cyanobacterial blooms are not observed as a pervasive cover, but in surface patches (Stal et al., 2003; Ploug, 2008). This can explain how, despite the cyanobacterial bloom, sufficient light penetrated the surface to support the high level of productivity that occurred at the DCM during sapropel formation (Rohling and Gieskes, 1989). Our results indicate that the low-salinity surface cyanobacterial blooms started to establish at the onset of suboxic to euxinic conditions (indicated by U and Mo concentrations and Mo/Al and U/Al ratios [Figs. 2G and 2H]; Tribovillard et al., 2006) and were occurring periodically for ~4.5 k.y. The occurrence of these blooms peaked and then diminished well before the end of the sapropel deposition (Figs. 2C–2E), possibly due to a change in the nutrient availability in the surface waters or a reduced input of freshwater, leading to a system which did not support N_2 fixation. Indeed, the TOC of the sediments was still increasing toward a maximum (of ~9% at 357–339 cm depth; ca. 126–122 kyr B.P.; Fig. 2A), in tune with increasing export of productivity (high Ba/Al ratios; Fig. 2F) from a potential DCM, while the concentration of all HGs was already decreasing (Figs. 2C–2E).

The presence of a lowered-salinity surface layer in the eastern Mediterranean due to enhanced freshwater influx is well established (Rossignol-Strick et al., 1982; Rossignol-Strick, 1985; Rohling et al., 2004; Rohling, 2007; van der Meer et al., 2007; Rodríguez-Sanz et al., 2017), although the extent and intensity of this surface freshening is uncertain. In order to constrain the geographical spread of the bloom, we also examined the HG distribution in the S5 sapropels recovered in cores MS66PC (Bauersachs et al., 2010) and ODP 160-971C-2H-3 (Pearce et al., 1998; Kemp et al., 1999) (see Fig. 1). Cores 64PE406-E1 and MS66PC are both from the Nile delta region, and hence their sites were likely influenced by freshwater delivery from the Nile. Conversely, the ODP 160-971 core site is in the region between Libya

and southwest Crete, which currently is out of reach of any modern-day river systems but has been shown to have been influenced by monsoon flooding during the previous interglacial maximum, thought to have been delivered via extant river systems of northern Libya (Rohling et al., 2002). These additional locations also revealed a high C_6 HG concentration in the lower part of the sapropel (Table DR1), consistent with the expected high input of freshwater at this time. At the site closest to the mouth of the Nile (MS66PC), the relative abundance of C_6 HG was high (97% ± 3% of total HGs), as it was at our site (64PE406-E1) slightly further from the Nile plume (97% ± 2%). The ODP 160-971C-2H-3 core had a lower relative abundance of C_6 HG (78% ± 10%) consistent with a lower freshwater input. The upper half of the sapropel was also characterized by a moderate or high relative abundance of C_6 HGs in the MS66PC and 64PE406-E1 cores (on average 54% and 87%, respectively). Conversely, the upper part of S5 in the ODP 160-971C-2H-3 core contained a high percent of C_5 HG (96%). Interestingly, the percent of the DDA-forming diatom *H. hauckii* has also been reported to be significantly higher in the upper half of S5 in the ODP 160-971C-2H-3 core (Pearce et al., 1998; Kemp et al., 1999), further confirming the C_5 HG_{30} triol is a marker for DDAs.

Overall, comparison of the HG distribution in the three S5 sapropels provides evidence that all three locations were initially influenced by surface salinities that were sufficiently low to support free-living heterocystous cyanobacteria. While free-living heterocystous cyanobacteria continued to outnumber DDAs during sapropel deposition at the two Nile-influenced sites, DDAs, indicators of persistent marine salinities, were the dominant diazotrophs in the upper part of the sapropel at the ODP 160-971 site.

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