**Supplementary Information**

**Linking diatom-diazotroph symbioses to nitrogen cycle perturbations and deep-water anoxia: Insights from Mediterranean Sapropel events**

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**Supplementary text:**

*Defining sapropel boundaries and assessing post-depositional burn-down and*  *organic matter provenance*

Age models and—where available—sapropel boundaries (initiation and termination depths) were taken from revised composite depth scales reported previously (**Table S1**; Emeis et al., 2000; Grant et al., 2017; Lourens, 2004; Sakamoto et al., 1998). When only midpoint depths were reported (i.e., S7, S74, and i-282), sapropel boundaries were taken from Emeis et al. (1996) and are based on TOC content. Previous work has suggested that defining the boundary of sapropel termination using this metric may be biased by progressive remineralization after deep-water reoxygenation (known as burn-down; Möbius et al., 2010). However, evidence for major burn-down effects is largely limited to records from sapropel S1. In contrast, trace-metal ratios argue against significant burn-down for the events studied here, particularly for sapropel S5 (Dirksen et al., 2019; Grant et al., 2017). This is supported by our bulk C/N and TOC δ13C data (**Fig. 2a-c**), which either decrease or remain constant after sapropel termination. In contrast, if burn-down were significantly biasing apparent sapropel termination depths, then we would expect both C/N and TOC δ13C to increase above each sapropel event due to the preferential remineralization of N-rich and 13C-poor OM. This is not observed. Nonetheless, if burn-down has occurred, then some samples currently designated as external to each sapropel should be reclassified as internal. The statistically significant *p* values of differences between sapropel and non-sapropel data reported here (**Fig. 3**) should thus be taken as maximum values since improper classification is expected to blur differences and artificially increase *p* values. Sapropel S5 boundaries assigned to previously reported biomarker data were taken as presented in the original publications (Bale et al., 2019; Rush et al., 2019).

In general, 13C-depletion and higher C/N ratios are considered indicative of terrestrial OM. Thus, the increasing C/N trend observed here during sapropel events could be interpreted to reflect increased terrestrial OM input at this time if this trend is accompanied by concomitant decreases in TOC δ13C values. However, a plot of TOC δ13C vs. C/N indicates no correlation between these variables—either within sapropel or marl sediments—with samples from site 964 in particular showing invariant δ13C values (**Fig. S** **4**). We thus conclude that terrestrial input of organic matter was not the primary driver of organic matter ratios and isotopic composition in the studied sediments.

*Influence of*  *physical, chemical, and biological parameters on thaumarchaeal and anammox lipid 13C compositions*

Changes in biological and chemical parameters can change carbon isotopic fractionation in bacteria and archaea and thus influence their biomarker carbon isotopic signatures (Freeman and Hayes, 1992; Wilkes et al., 2017). Specifically, higher availability of dissolved inorganic carbon (DIC) and/or CO2 concentrations can lead to larger 13C fractionation in ammonia-oxidizing archaea under specific conditions (Elling et al., 2019; Hurley et al., 2019; Pearson et al., 2019).

Additionally, due to the location of the thaumarchaeal habitat in the chemocline, crenarchaeol δ13C could record a mixed signature of DIC derived from both the oxic and anoxic zones. However, invariable crenarchaeol δ13C values across marl-sapropel intercalations indicate no significant change of in situ CO2 concentration at the habitat depth of ammonia-oxidizing archaea and no influence of anoxic zone DIC on crenarchaeol δ13C (Polik et al., 2018), despite the fact that some DIC must be diffusing up from below the chemocline. We interpret this using a simple diffusive flux calculated assuming a vertical separation of ~30 m between ammonia-oxidizing archaea just above the chemocline and anammox bacteria just below the chemocline (consistent with modern Black Sea observations; Lam et al., 2007) and DIC concentrations of 2200 µM above and 2600 µM below the chemocline just before sapropel termination (see main text). Following Fick’s first law and using a diffusivity of 7.7×10-10 m2 s-1 (Zeebe, 2011), this leads to an upward diffusive flux of DIC of 0.88 µmol m-2 day-1. If ammonia-oxidizing archaea are limited to a 1 m thick layer above the chemocline, this results in a DIC residence time of ~7000 years with respect to diffusion (longer if this habitat is thicker). Thus, DIC δ13C compositions within the habitat of ammonia-oxidizing archaea should be insensitive to any upward diffusion of 13C-depleted DIC from below.

In contrast, the factors influencing carbon isotope fractionation in anammox bacteria have not been studied. In other bacteria using the same carbon fixation pathway (Wood-Ljungdahl pathway), increased inorganic carbon availability can lead to larger 13C fractionation (Blaser et al., 2015). It is possible that DIC accumulation in deep waters during sapropel events leads to an increase in the expressed fractionation by anammox bacteria. If so, this would cause us to overestimate changes in chemocline δ13C gradients; further studies using anammox cultures are needed to distinguish the relative magnitude of the two effects. We therefore suggest that the change in chemocline δ13C of DIC represents a maximum estimate.

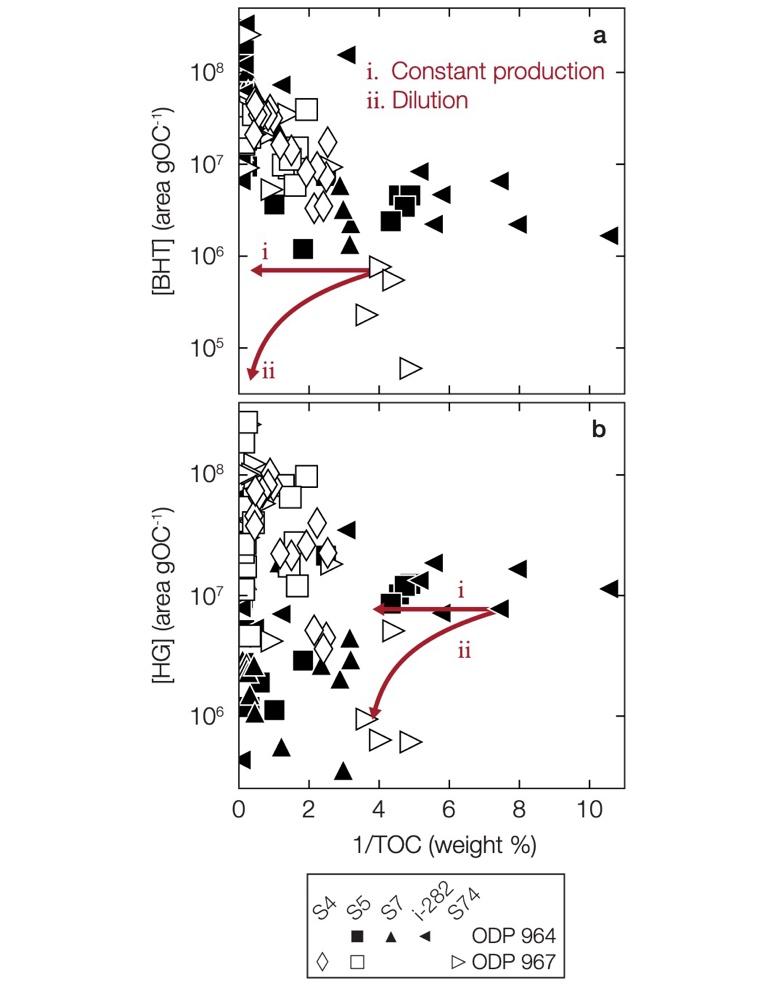
Habitat further may have influenced BHT-II δ13C, as benthic or planktonic anammox bacteria may have recorded different DIC pools. In euxinic basins, anammox occurs at the chemocline interface (Lam et al., 2007) but may not occur within sediments due to the lack of nitrite. Biomarker records suggest less persistent euxinic conditions at site 967 compared to site 964 (Menzel et al., 2002). Less persistent anoxic conditions or deep-water ventilation could have supported benthic anammox at site 967, similar to mid-water oxygen minimum zones (Dalsgaard et al., 2003), which may have contributed to the observed muted trends in δ13C gradients. In contrast, persistent euxinic conditions and the consistent δ13C gradients at site 964 provide evidence against a contribution of benthic anammox at this location.

**Supplementary figures:**

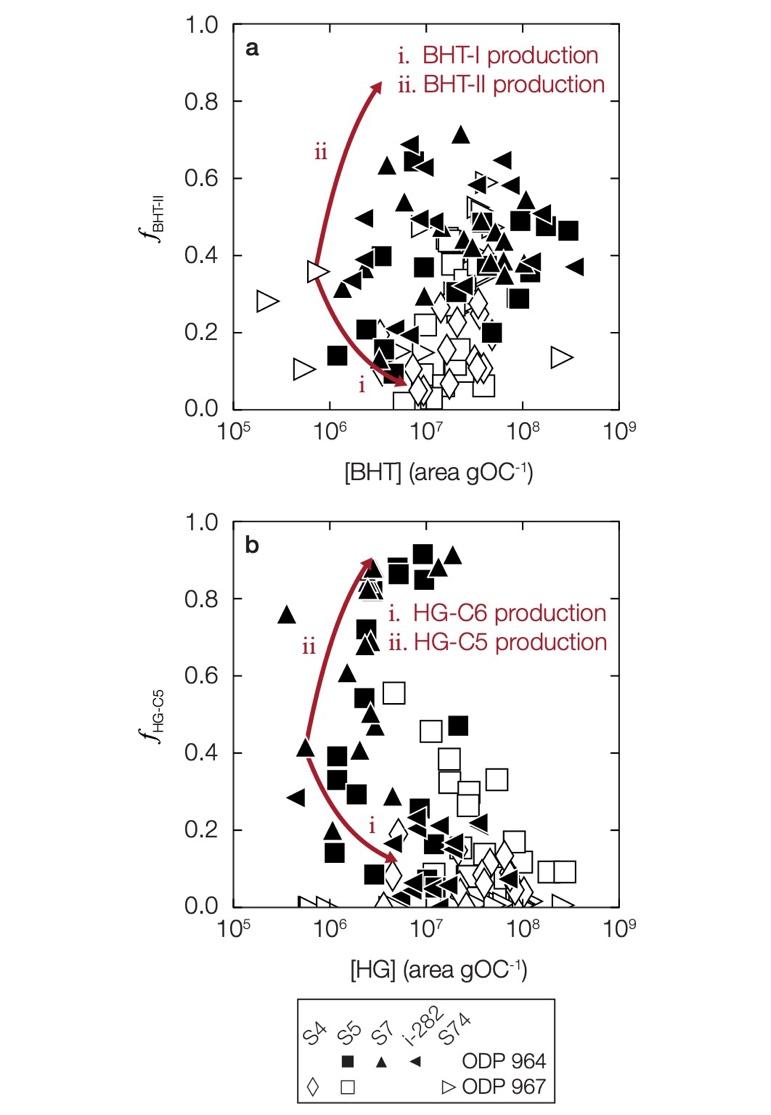
A close up of a map

Description automatically generated

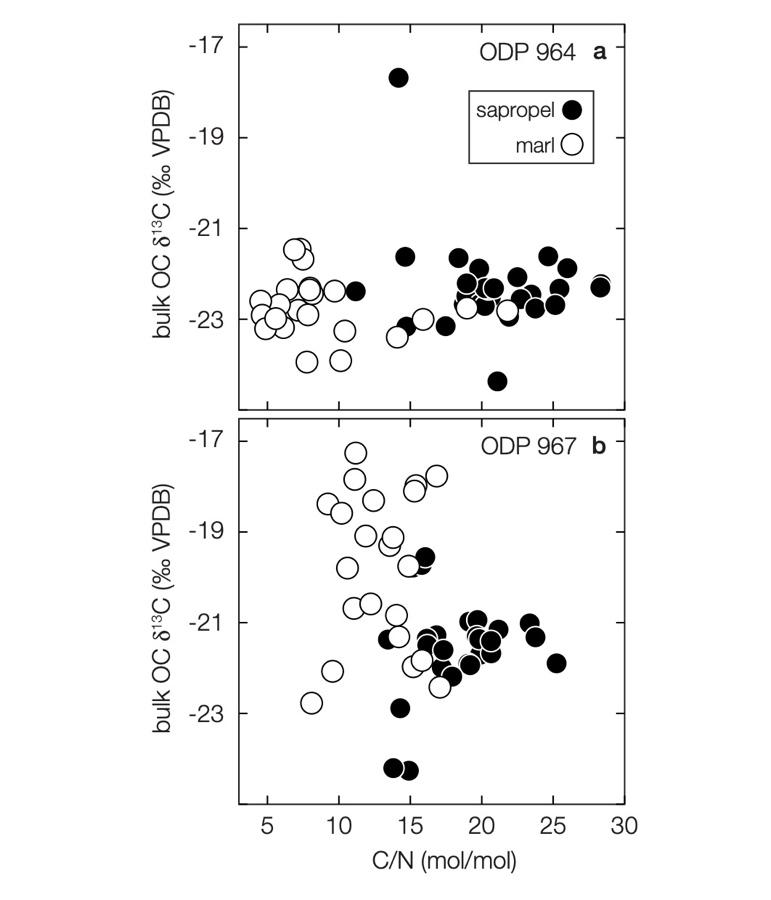
**Figure S1. Biomarker instrument response.** Dilution series of **a**, BHT-I and BHT-II instrument response during HPLC-APCI-MS and **c**, C5 and C6 heterocyst glycolipid instrument response during HPLC-ESI-MS. Change in fractional abundance of **b**, BHT-II and **d**, C5 heterocyst glycolipids with increasing dilution. Analyses were performed on a 0 to 100-fold (for BHT) or 0 to 500-fold (for HGs) diluted sapropel sample (ODP 969 E1H2 121-123 cm) and indicate that BHT-II fractional abundance shows no strong response with sample dilution whereas the relative amount of C5 heterocyst glycolipids is underestimated at high abundances such as in sapropel samples. To minimize this effect, all samples were diluted such that similar peak areas were obtained during HPLC-ESI-MS analysis.

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**Figure S2. Biomarker abundance mixing plots. a**, OC-normalized total BHT peak areas (BHT-I + BHT-II) and **b**, OC-normalized total HG peak areas (HG-C5 + HG-C6) as a function of the inverse of TOC content (1/TOC). Data are separated by event (S4: 108 ka onset, diamonds; S5: 128 ka onset, squares; S7: 197 ka onset, upward-pointing triangles; i-282: 2.947 Ma onset, left-pointing triangles; S74: 3.004 Ma onset, right-pointing triangles) and by site (964: black; 967: white). Red lines in panel **a** indicate expected trajectories starting from a random point if (i) OC-normalized BHT concentrations were constant and independent of TOC content (indicating enhanced preservation but no source change) or (ii) added “excess” TOC did not contain BHT (indicating dilution by a BHT-free source). Similar trajectories could be drawn in panel **b**. Neither trajectory explains the majority of observed concentration data, which clearly increase with increasing TOC and indicate a shift toward a BHT- and HG-rich source during sapropel events. Note the *y* axes of both panels are presented on a logarithmic scale.



**Figure**  **S3. Biomarker fractional abundance mixing plots. a**, fractional abundance of BHT-II (*f*BHT-II) as a function of OC-normalized total BHT peak areas (BHT-I + BHT-II) and **b**, fractional abundance of HG-C5 (*f*HG-C5) as a function of OC-normalized total HG peak areas (HG-C5 + HG-C6). Data are separated by event (S4: 108 ka onset, diamonds; S5: 128 ka onset, squares; S7: 197 ka onset, upward-pointing triangles; i-282: 2.947 Ma onset, left-pointing triangles; S74: 3.004 Ma onset, right-pointing triangles) and by site (964: black; 967: white). Red lines in panel **a** indicate expected trajectories starting from a random point if all new BHT production were as (i) BHT-I or (ii) BHT-II. Similar trajectories could be drawn in panel **b**. In general, data suggest a positive relationship between *f*BHT-II and total BHT concentrations, whereas the relationship between *f*HG-C5 and total HG concentrations differs by site (964: positive, particularly for events S5 and S7; 967: negative, particularly for event S5). Note the *x* axes of both panels are presented on a logarithmic scale.



**Figure S4. Bulk OC δ13C vs. C/N mixing plots** for **a,** ODP site 964 and **b,** ODP site 967. At both sites, there exists no statistically significant correlation between these two variables, either within sapropel (black circles) or marl (white circles) sediments, indicating that terrestrial OM input is not the primary driver of observed trends.

**Supplementary table and code captions:**

**Table S1.** Composite depths and age models for both coring sites. mbsf = meters below sea floor; rmcd = revised composite depth; kyr = kiloyears before present.

**Table S2.** Mass-to-charge ratios (*m*/*z*) used for selective reaction monitoring of BHPs at Quadrupole 1 (Q1) and 3 (Q3).

**Table S3.** Mass-to-charge ratios (*m*/*z*) of precursors and product ions as well as collision energies (CE) of diagnostic transitions during multiple reaction monitoring of heterocyst glycolipids at Quadrupole 1 (Q1) and 3 (Q3). Fragmentor voltage was 85 and accelerator voltage was 7 for all experiments.

**Table S4.** All bulk measurements presented in this study. Abundances and stable isotopic compositions of total organic carbon (TOC) and total nitrogen (TN), including uncertainty. n.d. = not determined.

**Table S5.** All biomarker abundance data presented in this study. OC-normalized abundances of crenarchaeol, bacteriohopanetetrol I (BHT-I), bacteriohopanetetrol II (BHT-II), pentose heterocyst glycolipids (HG-C5), and hexose heterocyst glycolipids (HG-C6). Fractional abundances of BHT-II [*f*BHT-II = BHT-I/(BHT-I + BHT-II)] and C5 HGs [*f*HG-C5 = HG-C5/(HG-C5 + HG-C6)] are also given. n.d. = not determined

**Table S6.** All biomarker isotopic data presented in this study. Carbon stable isotopic composition of crenarchaeol (δ13Ccren.), bacteriohopanetetrol I (δ13CBHT-I), and bacteriohopanetetrol II (δ13CBHT-II). F2/F1 size ratio describes the crenarchaeol-carbon to background-carbon ratio as an indicator of potential bias of δ13Ccren. by high background. Values above 2 indicate unbiased samples (Polik et al., 2018). Differences between δ13Ccren., δ13CBHT-I, and δ13CBHT-II are also given. n.d. = not determined.

**Table S7.** Compilation of Eastern Mediterranean Sea records that have been shown to contain preserved diatom frustules during event S5, including information on preservation quality and assemblage composition where available. n.r. = not reported.

**Supplementary Code 1.** All code used to analyze data and generate plots presented in this study. Zip file contains ipython notebook script (‘analysis.ipynb’), all sample data (‘all\_data.csv’), biomarker dilution-series data (‘biomarker\_dilution.csv’), and Mediterranean Sea bathymetry file for making **Fig. 1** (‘med\_bath.nc’).

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