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Archaea mediate anaerobic oxidation of methane in deep euxinic waters of the Black Sea

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Abstract—We evaluate anaerobic oxidation of methane (AOM) in the Black Sea water column by determining distributions of archaea-specific glyceryl dialkyl glyceryl tetraethers (GDGTs) and ^{13}C isotopic compositions of their constituent biphytanes in suspended particulate matter (SPM), sinking particulate matter collected in sediment traps, and surface sediments. We also determined isotopic compositions of fatty acids specific to sulfate-reducing bacteria to test for biomarker and isotopic evidence of a syntrophic relationship between archaea and sulfate-reducing bacteria in carrying out AOM. Bicyclic and tricyclic GDGTs and their constituent ^{13}C-depleted monocyclic and bicyclic biphytanes (down to −67‰) indicative of archaea involved in AOM were present in SPM in the anoxic zone below 700 m depth. In contrast, GDGT-0 and crenarchaeol derived from planktonic crenarchaeota dominated the GDGT distributions in the oxic surface and shallow anoxic waters. Fatty acids indicative of sulfate-reducing bacteria (i.e., *iso*- and *anteiso*-C_{15}) were not strongly isotopically depleted (e.g., −32 to −25‰), although *anteiso*-C_{15} was 5‰ more depleted in ^{13}C than *iso*-C_{15}. Our results suggest that either AOM is carried out by archaea independent of sulfate-reducing bacteria or those sulfate-reducing bacteria involved in a syntrophy with methane-oxidizing archaea constitute a small enough fraction of the total sulfate-reducing bacterial community that an isotope depletion in their fatty acids is not readily detected. Sinking particulate material collected in sediment traps and the underlying sediments in the anoxic zone contained the biomarker and isotope signature of upper–water column archaea. AOM-specific GDGTs and ^{13}C-depleted biphytanes characteristic of the SPM in the deep anoxic zone are not incorporated into sinking particles and are not efficiently transported to the sediments. This observation suggests that sediments may not always record AOM in overlying euxinic water columns and helps explain the absence of AOM-derived biomarkers in sediments deposited during past periods of elevated levels of methane in the ocean.

1. INTRODUCTION

Methane has played a major role in the biogeochemical carbon cycle through Earth's history. As a radiatively active trace gas, it is important in present-day global climate (Hansen et al., 2000) and has been implicated in past global warming events (Dickens et al., 1995; Hesselbo et al., 2000; Kennett et al., 2000; Hinrichs, 2001; but see also Stott et al., 2002). Consequently, identification of past and present sources and sinks of methane is essential for understanding the impact of methane on global climate. Anoxic marine sediments are a major source of methane, 90% of which is recycled through anaerobic oxidation processes to the extent that marine environments contribute only 2% of the annual methane flux to the atmosphere (Reeburgh, 1996). Novel methanotrophic archaea may be responsible for anaerobic oxidation of methane (AOM) (Hinrichs et al., 1999; Orphan et al., 2002; Hinrichs and Boetius, 2002). There is also evidence that a consortium of methane-oxidizing archaea and sulfate-reducing bacteria can carry out AOM (Hoehler et al., 1994; Valentine and Reeburgh, 2000; Valentine et al., 2000; Hinrichs and Boetius, 2002). Support for such a syntrophy follows various lines. Several studies found a correspondence between sulfate reduction and methane oxidation profiles in sediments (Iversen and Jørgensen, 1985; Alperin et al., 1988; Jørgensen et al., 2001; Thomsen et al., 2001). Molecular gene sequencing shows a co-occurence of sulfate-reducing bacteria and methanotrophic archaea in anoxic sediments (Hinrichs et al., 1999; Orphan et al., 2001a, 2001b; Teske et al., 2002). There is a physical association of archaea and sulfate-reducing bacteria in aggregates from sediments in which AOM occurs (Boetius et al., 2000; Orphan et al., 2001a). Molecular carbon isotope measurements of archaeal biomarkers (e.g., archaeol, hydroxyarchaeol, crocetane, 2,6,10,15,19-pentamethylicosane (PMI), and acyclic and cyclic biphytanes) and bacterial biomarkers (fatty acids and alkyl ethers) show ^{13}C isotopic depletions consistent with methanotrophy (Hinrichs et al., 2000; Elvert et al., 2000; Pancost et al., 2001a, 2001b, 2001c; Thiel et al., 2001; Bian et al., 2001; Schouten et al., 2001), and Orphan et al. (2001a, 2001b, 2002) showed the biomass of aggregated archaea and sulfate-reducing bacteria to be depleted in ^{13}C. To date, sediments have been the focus of most investigations of AOM, but euxinic water columns are also important sites where this process occurs (Reeburgh, 1976; Ward et al., 1987, 1989; Reeburgh et al., 1991).

The Black Sea is the world's largest surface water reservoir of dissolved methane, with concentrations of up to 11 μmol/L and an inventory of 96 Tg (Scranton, 1988; Reeburgh et al., 1991). Anoxic sediments and methane seeps are important sources of methane to the water column of the Black Sea (Reeburgh et al., 1991; Luth et al., 1999). AOM in the anoxic water column consumes ~99% of the 2.9×10^{11} mol of CH_4 released annually from Black Sea sediments (Reeburgh et al., 1991). Ether-bound acyclic and cyclic biphytanes derived from

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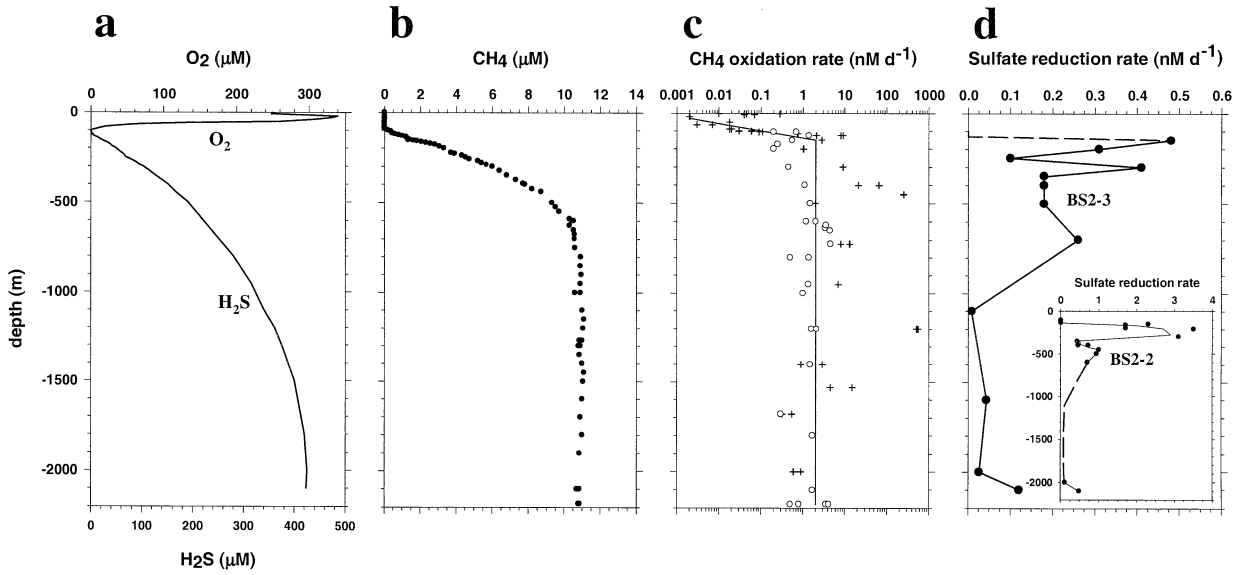


Fig. 1. Bulk parameters in the Black Sea water column. (a) Dissolved oxygen (μmol/L) and hydrogen sulfide (μmol/L), (b) methane concentrations (μmol/L), (c) methane oxidation rates (+ = $^{14}CH_4$; o = C^3H_4; note the logarithmic scale) at station BSK-2 (43°05'N, 34°00'E) during leg 5 (July 1988) of the 1988 Black Sea expedition, and (d) sulfate reduction rates measured on leg 2 (May 1988) in the central basin at station BS2-3 (43°05'N, 34°00'E) and at station BS2-2 (insert: 42°50'N, 32°00'E). O_2 and H_2S data are replotted from White et al. (1980) and Friederich et al. (1990); CH_4 and CH_4 oxidation rate data are replotted from Reeburgh et al. (1991) (see Reeburgh et al., 1991, for a discussion of the differences between rates of anaerobic oxidation of methane obtained from $^{14}CH_4$ and C^3H_4 experiments); sulfate reduction rates are from Albert et al. (1995).

archaea are present in the anoxic zone (Hoefs et al., 1997; King et al., 1998). At least some of these archaea may be anaerobic methanotrophs because several cyclic biphytanes from the anoxic zone are strongly depleted in ^{13}C (as low as −58‰; Schouten et al., 2001). The water column of the Black Sea below ~100 m depth is also characterized by intense sulfate reduction (Jørgensen et al., 1991; Albert et al., 1995). Parallel depth profiles of methane oxidation and sulfate reduction can be interpreted as indirect evidence for a syntrophic relationship between methane-oxidizing archaea and sulfate-reducing bacteria. Depth distributions of archaea involved in anaerobic methanotrophy in the euxinic water column of the Black Sea are unknown because only a single composite sample was analyzed in our previous study (Schouten et al., 2001), nor are there complementary isotopic data for biomarkers of sulfate-reducing bacteria to test a syntrophy.

We now report measurements from a detailed depth profile of suspended particulate matter (SPM) over the entire 2200-m water column in the central Black Sea, along with sediment trap samples from the anoxic zone and underlying surface sediments. Distributions of intact glyceryl dialkyl glyceryl tetraethers (GDGTs), which are diagnostic for identifying archaea involved in AOM, were determined using high-pressure liquid chromatography with positive ion atmospheric pressure chemical ionization mass spectrometry (HPLC-APCI-MS). Distributions of ether-bound biphytanes were also determined by HI/$LiAlH_4$ treatment, and their isotopic compositions have been measured. We now show that biomarkers diagnostic for archaea associated with AOM are most abundant below 700 m in the anoxic zone and have isotope compositions as low as −67‰. Branched-chain fatty acids derived from sulfate-reducing bacteria are abundant throughout the anoxic zone but are not particularly depleted in ^{13}C. A comparison of SPM, sediment trap and surface sediment GDGT distributions, and biphytane isotope compositions shows that AOM-derived biomarkers are not incorporated into sinking particulate matter and thus do not accumulate in the underlying sediments.

2. Setting and Biogeochemical Background

The water column of the central Black Sea site (station BSK-2; 43°05'N, 34°00'E) studied during leg 5 of the 1988 Black Sea expedition was characterized by dissolved oxygen concentrations that decreased to undetectable levels (<0.1 μM/L) by ~120 m (Fig. 1a; White et al., 1989; Friederich et al., 1990). Hydrogen sulfide was first detected (1 μM/L) at 110 m and increased in concentration to ~400 μM/L near the seafloor. Nitrate and chlorophyll-*a* maxima were centered at ~70 m, and the transmission minimum was between 125 and 130 m. Suspended particulate organic carbon (POC) concentrations (Fig. 2a) decreased from high values in surface waters, where particulate organic matter production was greatest, to low values in the suboxic zone between 85 and 100 m, where aerobic decomposition of organic matter was high. A secondary POC maximum occurred at ~130 m at the top of the anoxic zone, where new particles are produced by phototrophic sulfur bacteria (Repeta and Simpson, 1991) and where particulate Mn and Fe are alternately produced by their redox chemistries (Lewis and Landing, 1991). Our sampling scheme bracketed the chemocline, with SPM collected at 100 m coming from the bottom of suboxic zone (the filter was light green), whereas

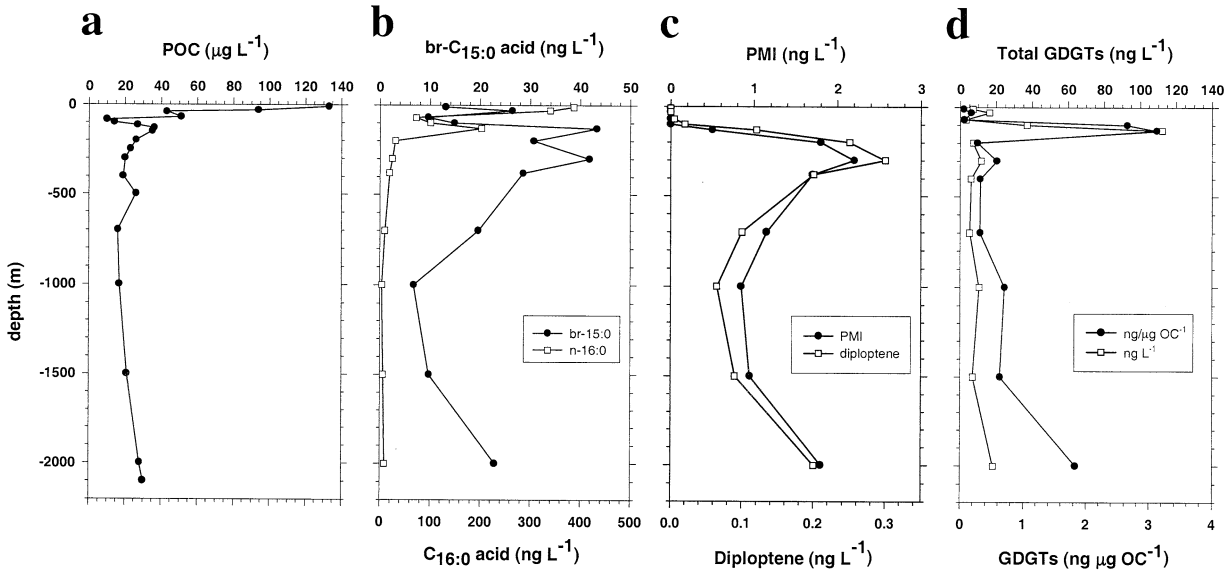


Fig. 2. Particulate organic carbon (POC) and biomarker profiles in the Black Sea water column during leg 5 (July 1988) of the 1988 Black Sea expedition. (a) POC (μg L^{-1}), (b) branched C_{15} fatty acids and *n*-$C_{16:0}$ (ng L^{-1}), (c) 2,6,10,15,19-pentamethylicosane (PMI) and diploptene (ng L^{-1}), and (d) total glyceryl dialkyl glyceryl tetraethers (GDGTs) (ng L^{-1} and ng μg OC^{-1}). POC, fatty acid, and hydrocarbon data are from Wakeham and Beier (1991), Wakeham et al. (1991), and Wakeham (1995); GDGT concentrations are from this study. OC = organic carbon.

SPM from 130 m came from the top of the anoxic zone (the filter was black).

Reeburgh et al. (1991, 1996) investigated the biogeochemistry of methane at station BSK-2 during leg 5 of the 1988 cruise. Methane concentrations were 10 nM/L in the upper 100 m, increased to 11 μM/L by 550 m, and remained uniform to the bottom (Fig. 1b). The profile for $\delta^{13}CH_4$ was smooth and concave downward, changing from -57 to -51‰ from 225 to 1200 m, and uniform at -48‰ to 2200 m. Methane oxidation rates (Fig. 1c) were low in the surface 100 m and increased several orders of magnitude in the anoxic zone. Rates of AOM were highly variable (note the logarithmic scale), ranging over several orders of magnitude, but averaged $\sim 2\ \mu$ M d^{-1} below 500 m. Reeburgh et al. (1991) constructed a methane budget for the Black Sea. Shelf and slope sediments were determined to be methane sources, whereas sediments in the deep basin were methane sinks. AOM in the anoxic water column was determined to be the major sink of methane, consuming nearly all (99%) of the methane released from sediments and yielding an AOM rate of $\sim$0.7 mol C m^{-2} yr^{-1} integrated for the entire anoxic basin (>100 m depth) of the Black Sea. Methane may become isotopically enriched as it is oxidized at depth, with the uniform $\delta^{13}CH_4$ below 1200 m possibly reflecting smaller isotope fractionation as closed-system end-member concentrations and isotope compositions are approached (Reeburgh et al., 1996).

Sulfate reduction rates were also measured in the water column and sediments at two locations in the Black Sea on an earlier leg of the 1988 expedition (leg 2, May 1988) (Jørgensen et al., 1991; Albert et al., 1995). Water column profiles of sulfate reduction rate varied depending on location but in general were negligible above 130 m, high in the upper anoxic zone, and decreased in the deep anoxic zone (Fig. 1d). Sulfate reduction rates at station BS2-3 (43°05'N, 34°00'E) located in the central basin near BSK-2 reached a maximum rate of 0.48 μM/L d^{-1} at 150 m (the shallowest depth sampled), whereas at station BS2-2 (Fig. 1d insert) in the western gyre (42°50'N, 32°00'E), rates of ~ 3 μM/L d^{-1} were measured between 200 and 300 m depth. Albert et al. (1995) suggested that the difference between stations 2 and 3 might be that station 2, within the western gyre, experiences a different mixing regime than station 3, located between the eastern and western gyres. Basinwide, the sulfate reduction rate corresponds to oxidation of $\sim$1.5 mol C m^{-2} yr^{-1} (Albert et al., 1995).

Bacterial biomarkers measured previously on particulate matter at station BSK-2 (Wakeham and Beier, 1991, Wakeham et al., 1991; Wakeham, 1995) provide ample evidence of active bacterial alteration of organic matter in the water column, especially in the upper anoxic zone, and provide a counterpoint for distributions of archaeal lipids discussed below. Fatty acids characteristic of sulfate-reducing bacteria (e.g., *iso*- and *anteiso*-$C_{15:0}$ in Fig. 2b) (Parkes and Taylor, 1983; Taylor and Parkes, 1985; Kaneda, 1991) dominated the fatty acid distribution throughout the anoxic zone, with a broad concentration peak in the upper part of the anoxic zone (130 to 700 m), contrasting with eukaryote-derived hexadecanoic acid (*n*-$C_{16:0}$ in Fig. 2b). Diploptene (hop-22[29]-ene), derived from diverse aerobic prokaryotes (Rohmer et al., 1984; Ourisson et al., 1987), and PMI, indicative of archaea (Holzer et al., 1979; Tornabene and Langworthy, 1979; Risatti et al., 1984; Schouten et al., 1997), peaked between 100 and 400 m (Fig. 2c). In addition, these earlier observations demonstrated a distinction between fine, suspended particles and coarse, sinking particles that again becomes important in the biogeochemistry of archaeal lipids described below.

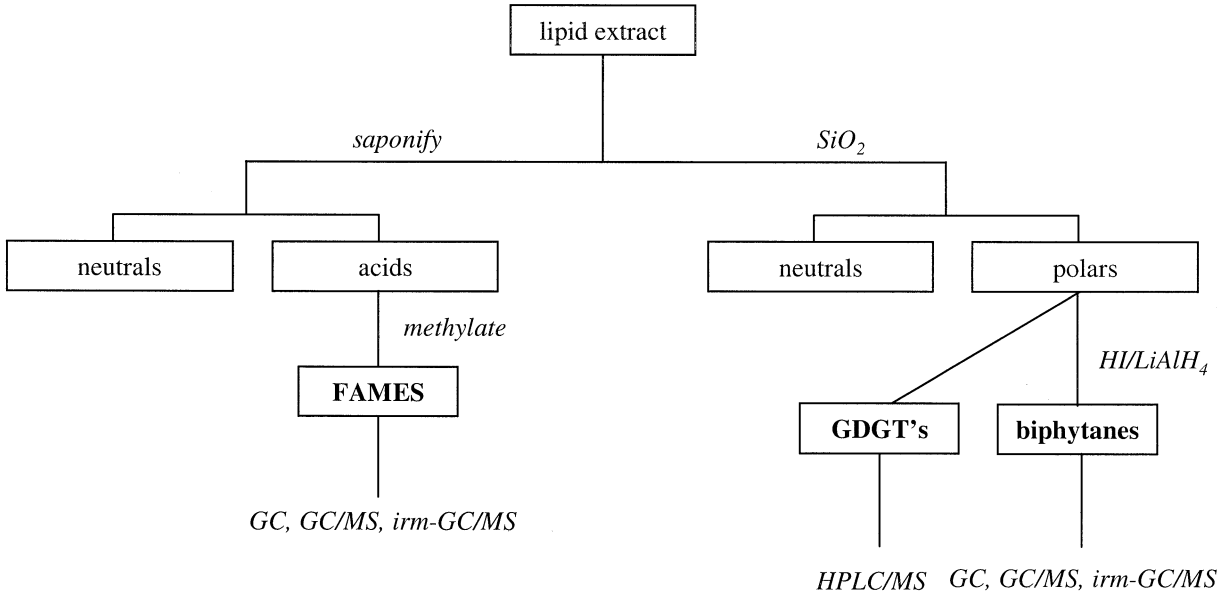


Fig. 3. Analytical scheme used for sample processing. FAMES = fatty acid methyl esters; GDGT = glyceryl dialkyl glyceryl tetraether; GC = gas chromatography; GC/MS = gas chromatography mass spectrometry; irm-GC/MS = isotope ratio monitoring gas chromatography mass spectrometry; HPLC/MS = high-pressure liquid chromatography mass spectrometry.

3. MATERIALS AND METHODS

3.1. Samples

SPM, sediment trap, and surface sediment samples were collected at station BSK-2 (43°05'N, 34°00'E, water depth 2200 m) on leg 5 (July 13 to 29) of the 1988 Black Sea expedition of *R/V Knorr* (Murray, 1991; Izdar and Murray, 1991; Wakeham and Beier, 1991; Wakeham et al., 1991). SPM was collected by in situ filtration using Woods Hole in situ pumps (WHISPs) deployed at depths between 10 and 2000 m. The WHISPs filtered between 1000 and 5000 L of seawater through a filter sandwich consisting of a 53-μm solvent-extracted Nitex screen and a 293-mm Gelman A/E glass fiber filter with a nominal pore size of 0.7 μm. This procedure gave nominal $<$53-μm (glass fiber filter) and $>$53-μm (Nitex screen) fractions for each WHISP deployment. Only the glass fiber filter fractions ($<$53 μm or SPM) were analyzed for this study, and we recognize that concentrations reported here could be lower limits if the filters we used undersampled picoplankton-sized material (Lee et al., 1995). Archaea may be of the order of 0.5 μm in diameter, although aggregates of archaea and sulfate-reducing bacteria may be much larger (e.g., 1- to 11-μm aggregates have been observed in sediments; Boetius et al., 2000; Orphan et al., 2001a, 2001b).

Sinking particles were collected by S. Honjo (Woods Hole) in time-series Mark-5 sediment traps deployed at 470 and 1384 m deployed for the year (July 1988 to July 1989) following the 1988 cruise. Trap material was preserved in situ with buffered formalin and was split in Honjo's laboratory, giving a 1/16 split fraction for organic geochemical studies. For this study, the time-series samples at each depth were composited. The 470-m trap functioned properly for the entire year, whereas the 1384-m trap failed after 3 months. Surface sediments (0 to 1 cm) were collected with a Soutar MK-4 box corer during the 1988 cruise. Subcores were sectioned onboard the ship and stored frozen until analysis.

3.2. Lipid Analyses

Samples were extracted wet with chloroform-methanol (2:1, v/v) in a Soxhlet extractor. Solvent-extractable lipids were partitioned into chloroform by washing with 5% NaCl solution and dried over Na_2SO_4. A flow diagram of the subsequent analytical procedure is given in Figure 3.

Fatty acids were determined on an aliquot of the extract that was saponified with aqueous 0.5 N KOH in methanol. Nonsaponifiable (neutral lipids) and acid fractions were sequentially extracted with hexane at pH 14 and pH 2, respectively. The acid fraction was methylated with BF_3-MeOH, and the methyl esters were extracted into hexane. Fatty acid methyl esters (FAMES) were analyzed by gas chromatography (GC), GC mass spectrometry (GC-MS), and isotope ratio monitoring GC-MS (irm-GC-MS), as described below. Detailed compositions of fatty acids and of the neutral lipids were reported elsewhere (Wakeham and Beier, 1991; Wakeham et al., 1991; Freeman and Wakeham, 1992; Freeman et al., 1994; Wakeham, 1995).

GDGTs and ether-linked biphytanes were determined on a second aliquot of the extract that was partitioned into "neutral" and "polar" fractions by silica gel adsorption chromatography. Neutral lipids were eluted with solvents ranging in polarity from hexane to 20% ethyl acetate in hexane, whereas polar lipids were eluted with solvents ranging in polarity from 25% ethyl acetate in hexane to methanol. An aliquot of the polar lipid fraction was analyzed for intact GDGTs by HPLC-APCI-MS (see below). A second aliquot of the polar lipid fraction was saponified using aqueous 0.5 N KOH in methanol, from which neutral and acid fractions were sequentially extracted at pH 14 and pH 2, respectively, using hexane. The neutral fractions of the polar lipids were treated with 57% HI for 4 h at 110°C to cleave ethers (King et al., 1998), the solution was diluted with 5% NaCl, and iodide derivatives were extracted into hexane and dried over Na_2SO_4. The iodides were reduced to the corresponding saturated hydrocarbons using $LiAlH_4$ in tetrahydrofuran by heating for 2 h at 70°C. Five hundred microliters of ethylacetate were added to decompose the remaining $LiAlH_4$. Hydrocarbons were extracted into ethylacetate, dried over Na_2SO_4, and analyzed by GC, GC-MS, and irm-GC-MS.

3.3. GC and GC-MS

FAMES and hydrocarbons released by $HI/LiAlH_4$ treatment were analyzed by GC on a Carlo Erba 4160 equipped with an on-column injector and using hydrogen as carrier gas. FAMES were separated using a Restek Rtx-1 capillary (60 m $\times$ 0.25 mm inside diameter, 0.1-μm film thickness), whereas hydrocarbons were analyzed with a DB-5 capillary (J & W Scientific; 30 m $\times$ 0.25 mm inside diameter, 0.1-μm film thickness). Samples were dissolved in isooctane and

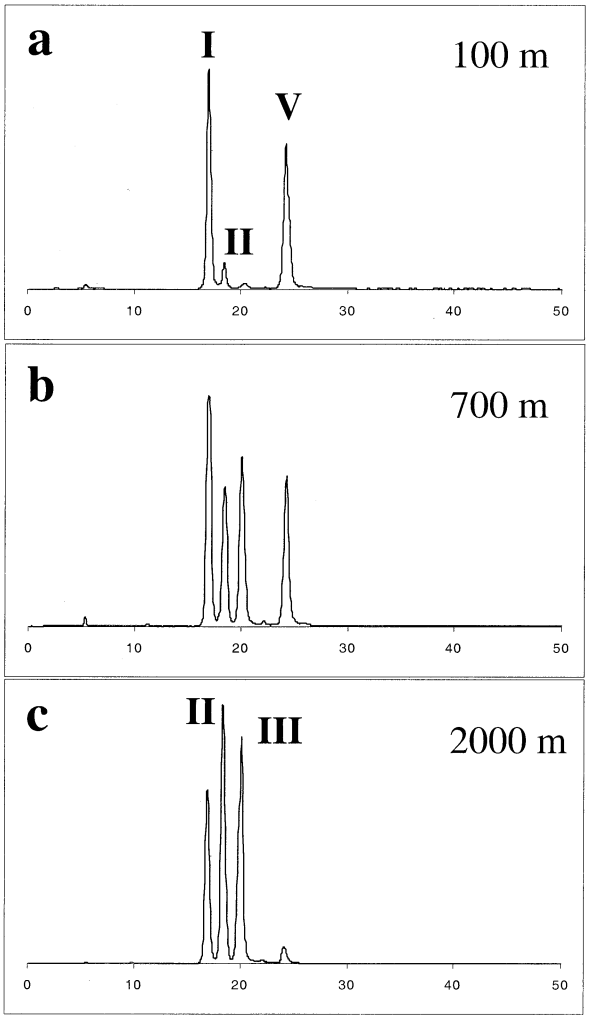


Fig. 4. Base peak ion current chromatograms of glyceryl dialkyl glyceryl tetraethers (GDGTs) in 100-, 700-, and 2000-m suspended particulate matter (SPM). Roman numerals refer to GDGT structures shown in Figure 5. Sediment trap material from 470 and 1384 m depth and surface sediments had chromatograms similar to that of the 100 m SPM.

injected at 100°C column temperature and held for 1 min, after which the column oven was programmed to 320°C at 3°C/min and held at 320°C for 20 min. Data were collected and processed on a PC using ChromPerfect software (Justice Laboratories). GC-MS analyses were performed on a Hewlett-Packard 5890II gas chromatograph with on-column injection and helium as carrier gas coupled to a Finnigan Incos 50 mass spectrometer. Separations were achieved using a DB-5 capillary (30 m × 0.25 mm inside diameter, 0.1-μm film thickness). Samples were injected at 80°C, the temperature was ramped to 120°C at 20°C/min, and then to 320°C at 4°C/min and held for 30 min. Spectra were obtained by operating the mass spectrometer at 70 eV and scanning from *m*/*z* 60 to 700 in 0.8 s.

3.4. HPLC-APCI-MS

Analyses of intact GDGTs in polar lipids were made using a Hewlett-Packard high-precision 1100 series liquid chromatograph mass spectrometer following the method described by Hopmans et al. (2000). Aliquots of neutral fractions obtained following alkaline hydrolysis of polar lipids were dissolved in hexane/propanol (99:1, v/v), and the resulting suspension was filtered through a 0.45-μm, 4 mm-diameter FPTE filter. Liquid chromatography (LC) separations were achieved on an NH_2 column (Econosphere, 4.6 × 250 mm, 5 μm; Alltech Associates) maintained at 30°C. Tetraethers were eluted isocratically with 99% hexane to 1% propanol for 5 min, followed by a linear gradient to 1.8% propanol in 45 min, with flow of 1 mL/min. Detection of GDGTs was achieved by HPLC-APCI-MS under the following conditions: nebulizer pressure 420 kPa, vaporizer temperature 300°C, drying gas (N_2) flow 6 mL/min and temperature 250°C, capillary voltage –3 kV, and corona 5 μA (−3.2 kV). Positive ion spectra were generated by scanning *m*/*z* 1225 to 1325 in 1.9 s. Concentrations of individual GDGTs were estimated by integrating peaks in summed mass chromatograms of $[M + H]^+$ and $[M + H + 1]^+$ ions and comparing with a standard curve obtained by serial dilutions of GDGT-0[1] reference standard.

3.5. irm-GC-MS

Compound-specific stable carbon isotope ratios were determined on biphytanes and FAMES using a DELTA-plus XL irm-GC-MS system. The gas chromatograph was equipped with a fused silica capillary column (25 m × 0.32 mm) coated with CP Sil-5 (0.12-μm film thickness) and used helium as carrier gas. Samples dissolved in hexane were injected at 70°C, and the oven was programmed to 130°C at 20°C/min and then to 320°C at 4°C/min, followed by an isothermal hold for 10 min. Most values reported were determined by duplicate analyses and were averaged, with errors of ~0.5‰. Isotopic compositions are reported in standard delta notation relative to the Vienna Peedee belemnite standard. $\delta^{13}C$ values of fatty acids were obtained by correcting the measured $\delta^{13}C$ values of FAMES for the isotopic composition of carbon added during the derivatization step.

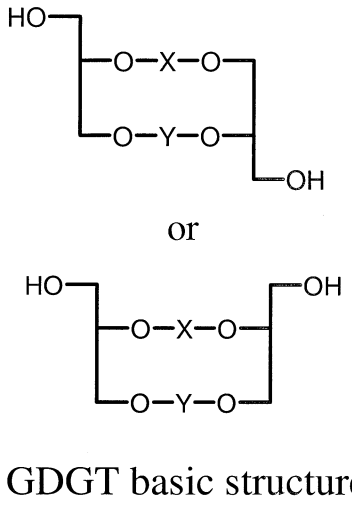
4. RESULTS

4.1. GDGTs and Constituent Biphytanes

All particulate matter samples contained a series of up to five GDGTs, as determined by HPLC-APCI-MS analyses. GDGTs were identified on the basis of (1) the molecular mass derived from the protonated molecule ($[M + H]^+$), (2) comparison of LC retention times with those of GDGTs in the hyperthermophilic archaeon *Sulfolobus sulfotaricus* (De Rosa et al., 1986; De Rosa and Gambacorta, 1988) and the psychrophilic archaeon *Cenarchaeum symbosium* (Sinninghe Damsté et al., 2002), and (3) distributions of C_{40} biphytanes released by $HI/LiAlH_4$ treatment. Total GDGT concentrations were highest in the transition zone between suboxic and anoxic conditions (100 and 130 m; Fig. 2d), in contrast to POC (Fig. 2a), which had its highest concentration at the surface, and branched fatty acids, PMI, and diploptene (Fig. 2b and 2c), with maximum concentrations in the upper part of the anoxic zone (200 to 400 m). GDGT concentrations were lower throughout the rest of the anoxic zone. When normalized to organic carbon (OC) (Fig. 2d), particles at 100 and 130 m displayed a nearly 10-fold higher concentration in GDGTs over particles immediately above and below. The redox transition zone thus appears to be an important zone of GDGT production. There may be some accumulation of particulate matter at the pycnocline, but this was not evident in the POC concentration profile. In the deep anoxic zone, OC-normalized GDGT concentrations increased below 700 m, reaching nearly fourfold higher levels than shallower in the anoxic zone.

Distributions of GDGTs in the oxic zone, at the chemocline,

[1] GDGTs are named according to their number of rings, so that GDGT-0 contains no rings (structure **I**), GDGT-1 contains 1 ring (structure **II**), and so on.



GDGT structure	GDGT no.	X	Y
I	**0**	**a**	**a**
II	**1**	**a**	**b**
III	**2**	**b**	**b**
IV	**3**	**b**	**c**
V	**cren**	**c**	**d**

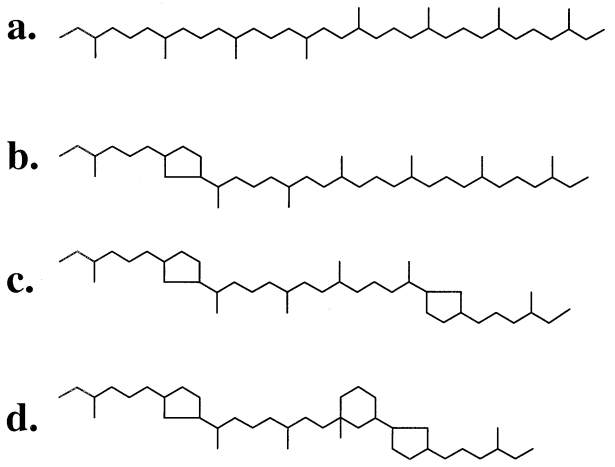


Figure 5. Structures of archaeal tetraether membrane lipids (glyceryl dialkyl glyceryl tetraethers [GDGTs]) composed of two glyceryl head moieties containing two linked isoprenoid chains (biphytanes **a** to **d**). cren = crenarchaeol.

and in the upper anoxic zone down to 400 m were dominated (Fig. 4) by GDGT-0 (structure **I** in Fig. 5) and crenarchaeol (**V**), along with a number of less abundant GDGTs with 1 to 3 cyclopentane rings (**II** to **IV**). GDGT-0 (I) and crenarchaeol (**V**) were roughly similar in abundance, each constituting ~45 to 55% of total GDGTs (Fig. 6). Concentrations of individual tetraethers are given in Table 1 and plotted in Figure 7 both as volume-normalized concentrations (Fig. 7a) and OC-normalized concentrations (Fig. 7b). Sediment trap material collected at 470 and 1384 m displayed GDGT patterns similar to upper-water SPM samples. Particles at and deeper than 1000 m, however, showed a significantly different GDGT composition (Figs. 4c and 6). The abundance of crenarchaeol (**V**) decreased substantially (to < 5% of total GDGTs), whereas that of GDGT-0 decreased by about half (to ~25% of GDGTs). In their place, monocyclic GDGT-1 (**II**) and bicyclic GDGT-2 (**III**) tetraethers increased to 30 to 40% of GDGTs, up from < 5% in particles from 400 m and shallower. Particles at 700 m contained an intermediate distribution of GDGTs (Figs. 4b and 6). Volume-normalized concentrations of GDGT-0, GDGT-1 (**II**), and GDGT-2 (**III**) were slightly elevated in the deep anoxic zone (Fig. 7b), but concentrations of crenarchaeol (**V**) and tricyclic GDGT-3 (**IV**) were not. OC-normalized concentrations of GDGT-0, GDGT-1, and GDGT-2 were significantly higher at the bottom of the anoxic zone. Treatment of the GDGTs with HI/$LiAlH_4$ that cleaves ether bonds yielded a series of hydrocarbons comprised of phytane and four biphytanes with 0 to 3 rings, consistent with distributions of the intact GDGTs. Phytane may derive from any of a number of phytanyl glyceryl ethers (e.g., archaeol and hydroxyarchaeol) that are present in archaea (De Rosa and Gambacorta, 1988; Koga et al., 1993; Koga et al., 1998). Identification of acyclic (**a** in Fig. 5), monocyclic (**b**), and bicyclic (**c**) biphytanes was based on their presence in *S. sulfotaricus* (De Rosa et al., 1986; De Rosa and Gambacorta, 1988; Schouten et al., 1998). The structure of tricyclic biphytane **d** has now been elucidated by two-dimensional, high-resolution nuclear magnetic resonance studies (Sinninghe Damsté et al., 2002) and contains two cy-

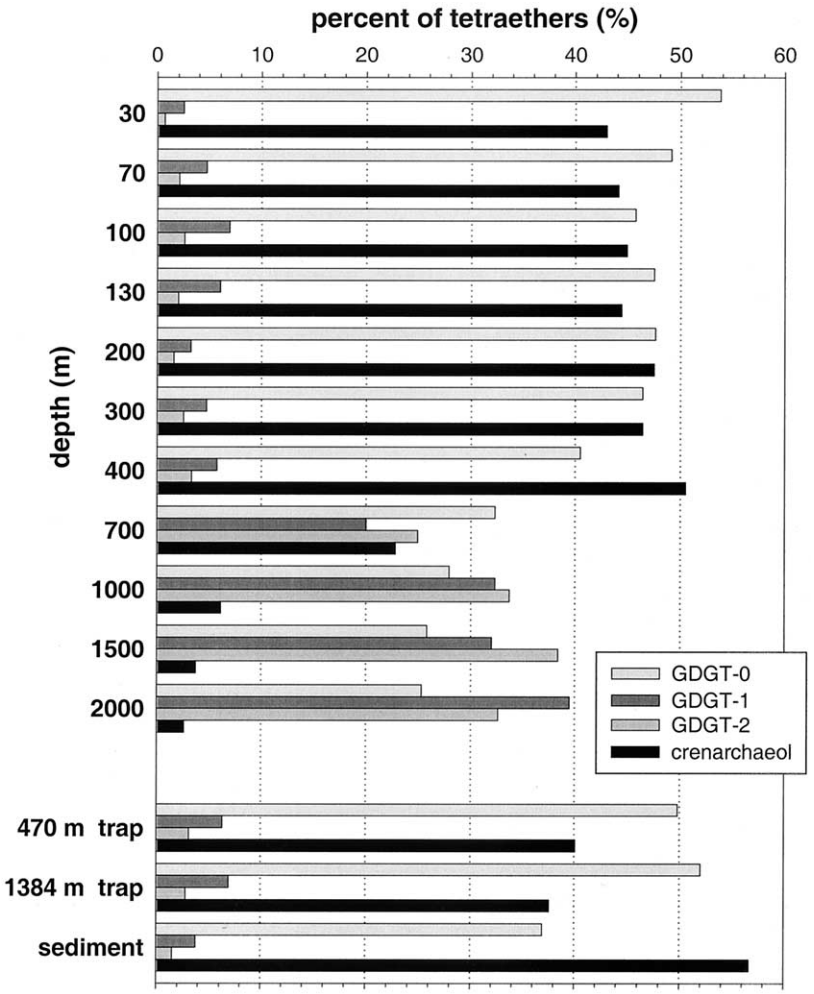


Fig. 6. Relative distributions of the four major glyceryl dialkyl glyceryl tetraethers (GDGTs) in particulate matter, sediment trap material, and surface sediments.

clopentyl rings and a cyclohexyl ring (**d** in Fig. 5) rather than three cyclopentyl rings, as reported previously for Black Sea SPM (Hoefs et al., 1997; King et al., 1998).

Distributions of biphytanes associated with suspended particles through 400 m depth in the Black Sea (Fig. 8), in 470-m trap material (there was insufficient sample from the 1384-m trap for biphytane analysis) and surface sediments were dominated by acyclic biphytane **a** that is mostly, but not entirely, derived from GDGT-0 (**I**). Bicyclic biphytane **c** and tricyclic biphytane **d** each constituted 15 to 25% of the biphytane composition down to 400 m and would derive mainly and only, respectively, from crenarchaeol (**V**). Consistent with the changing GDGT composition, the biphytane pattern started to change at 700 m. Whereas acyclic biphytane **a** continued to dominate, tricyclic biphytane **d** decreased in abundance (from 20 to 25% of total biphytanes to 5 to 10%), and monocyclic biphytane **b** increased in abundance (from ~5% to 30 to 40%). The increased proportion of biphytane **b** follows from the increased abundance of GDGT-1 and GDGT-2 (**II** and **III**, respectively) in the deep particles, and the decreased abundance of tricyclic biphytane d results from the decreased abundance of crenarchaeol (**V**).

4.2. Stable Carbon Isotopes of Biphytanes and Fatty Acids

Isotopic analysis of phytane and acyclic through tricyclic biphytanes (**a** to **d**) showed considerable variability in stable carbon isotope ratios as a function of compound, particle type (suspended, sinking, or sediment), and depth. Notably, several compounds were markedly depleted in ^{13}C in SPM from 700 m and below (Table 2). $\delta^{13}C$ values for biphytanes in the oxic zone ranged from -30 to -22‰. Phytane was depleted slightly in ^{13}C relative to the biphytanes ($\delta^{13}C$ of -35 to -30‰). Below 200 m, isotope signatures began to diverge (Fig. 9). Tricyclic biphytane **d** was isotopically invariant down the water column, remaining at ~ -22‰. At the other extreme, monocyclic biphytane **b** was strongly depleted in ^{13}C, reaching values down to -67‰ in the deep anoxic zone. This represents a depletion of > 40‰ for bicyclic biphytane **b** relative to both its isotope value in the upper 300 m (~ -30 to -26‰) and to the constant isotopic value of tricyclic biphytane **d** (-22‰). Phytane, acyclic biphytane **a**, and bicyclic biphytane **c** were also significantly depleted in ^{13}C, but to intermediate degrees. Isotope compositions of biphytanes from the 470-m sediment

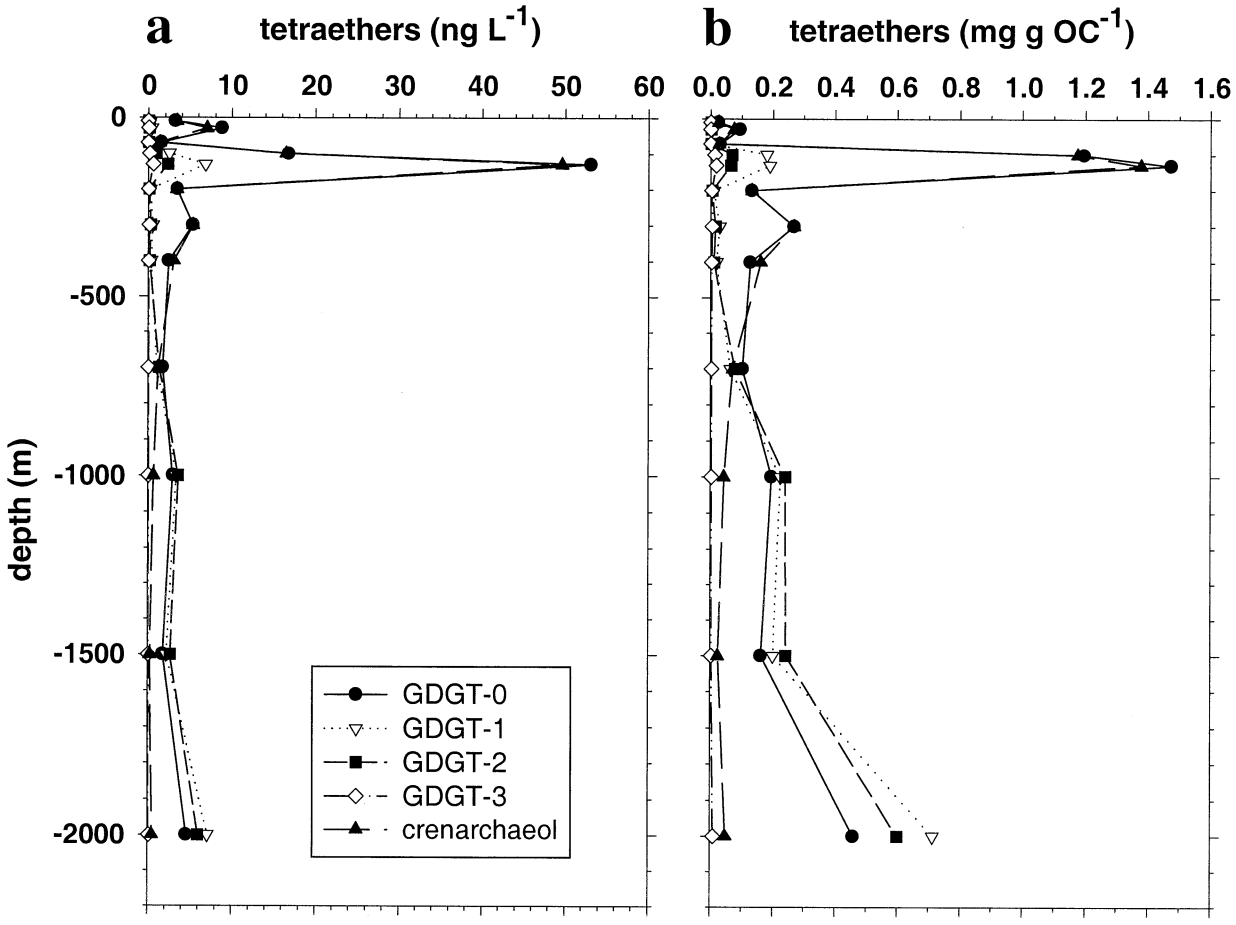


Fig. 7. (a) Volume-normalized concentrations (ng L^{-1}) and (b) organic carbon-normalized concentrations (mg tetraether g OC^{-1}) for the five major tetraethers in particulate matter, sediment trap material, and surface sediments.

trap material (there are no isotope data for the 1384-m trap) and surface sediments were not different from upper–water column SPM and consequently the compounds were not isotopically depleted.

Fatty acids, especially those that derive from sulfate-reducing bacteria (e.g., *iso*- and *anteiso*-C_{15}) and that dominate the fatty acid composition in the anoxic zone (Wakeham and Beier, 1991), did not show a parallel depletion in ^{13}C similar to several of the biphytanes. $\delta^{13}C$ values of fatty acids ranged between −31 and −25‰ (Table 2). $\delta^{13}C$ values for the *iso*-C_{15} fatty acid were remarkably uniform down the water column, in sediment trap material and in sediments, ranging from −28 to

Table 1. POC and GDGT concentrations in Black Sea suspended particulate matter.

		Tetraethers				
Depths (m)	POC ($\mu g/L^{-1}$)	GDGT-0	GDGT-1	GDGT-2 (ng/L^{-1})	GDGT-3	Cren
10	133	3.2	0.2	0.07	0.02	3.4
30	94	8.8	0.4	0.11	0.04	7.0
70	51	1.5	0.1	0.07	0.02	1.4
100	14	16.7	2.5	0.95	0.16	16.5
130	36	53.1	6.8	2.30	0.61	49.6
200	26	3.4	0.2	0.12	0.04	3.4
300	20	5.3	0.6	0.29	0.11	5.3
400	19	2.4	0.3	0.20	0.05	3.0
700	17	1.7	1.1	1.33	0.04	1.2
1000	15	2.9	3.4	3.59	0.05	0.6
1500	11	1.8	2.2	2.66	0.03	0.3
2000	10	4.6	7.1	5.99	0.09	0.5

POC = particulate organic carbon; GDGT = glyceryl dialkyl glyceryl tetraether; Cren = crenarchaeol.

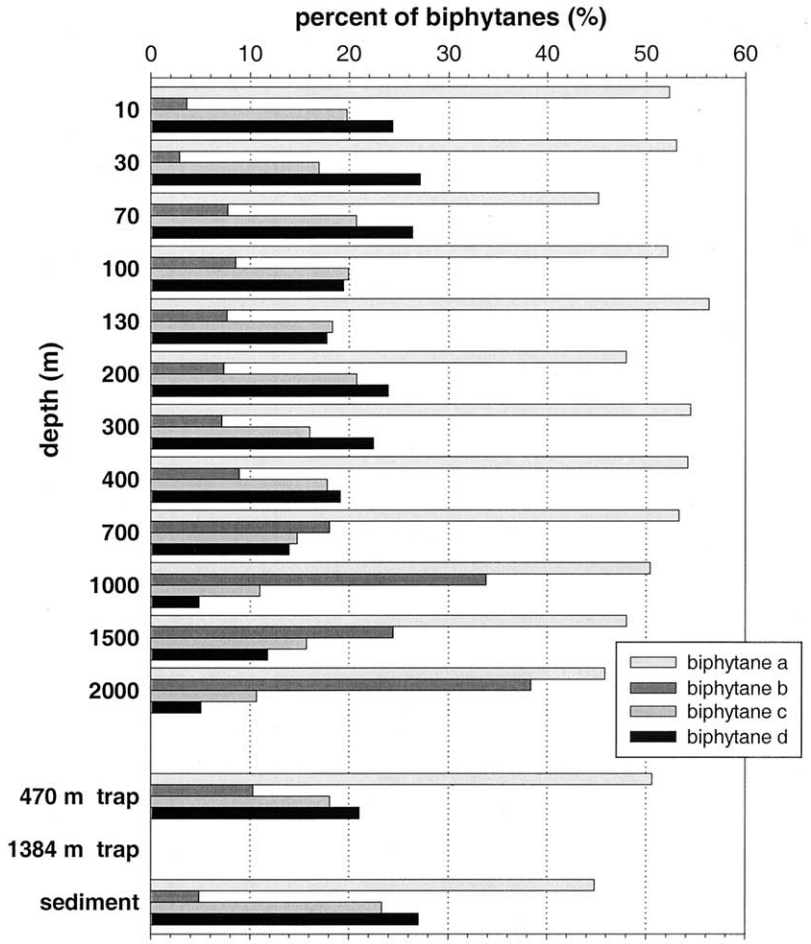


Fig. 8. Relative distributions of the four major biphytanes in particulate matter, 470-m sediment trap material, and surface sediments. There was insufficient material for determining biphytane distributions in the 1384-m trap sample.

−25‰. The *anteiso*-C_{15} fatty acid became slightly more depleted in ^{13}C with increasing depth, from −26‰ at 30 m to −31‰ at 2000 m. Monounsaturated $C_{16:1\omega7}$ and $C_{16:1\omega5}$ were the most depleted fatty acids, with $\delta^{13}C$ values of ~–36 to −33‰ at 30 m and −39 and −36‰, respectively, at 130 m. Overall, then, there was no strong connection between isotope values for archaeal ether-derived biphytanes and sulfate reducer-derived fatty acids.

5. DISCUSSION

5.1. Distributions of Pelagic Crenarchaeota in the Water Column of the Black Sea

The prominent GDGTs in Black Sea SPM from depths shallower than 700 m are GDGT-0 (**I**) and crenarchaeol (**V**) (Fig. 7), in common with the only other two water columns for which GDGT distributions are available (Arabian Sea, Sinninghe Damsté et al., 2002a; Cariaco Basin, Wakeham et al., in press) and numerous marine surface sediments (Schouten et al., 2000, in press; Sinninghe Damsté et al., 2002b). The dominant biphytane is thus acyclic biphytane **a** derived from GDGT-0, followed by bicyclic and tricyclic biphytanes **c** and **d** from crenarchaeol.

Crenarchaeol (**V**), with its distinctive cyclohexyl-containing tricyclic biphytane **d**, has been proposed as a biomarker for nonthermophilic planktonic crenarchaeota (Schouten et al., 2000; Sinninghe Damsté et al., 2002a) because it occurs widely in marine sediments and particulate matter from low-temperature environments (Schouten et al., 2000; Pancost et al., 2001a, 2001b, 2001c; Sinninghe Damsté et al., 2002a, 2002b; Wuchter et al., unpublished data; this report). Nonthermophilic crenarchaeota are widely distributed and ecologically diverse (DeLong, 1992; Fuhrman et al., 1992, 1993; Fuhrman and Davis, 1997; Massana et al., 2000; Karner et al., 2001; Sinninghe Damsté et al., 2002a). The only marine crenarchaeote that has been cultured, the sponge-associated symbiotic psychrophilic archaeon *Cenarchaeum symbiosum*, contains crenarchaeol as its dominant GDGT (Sinninghe Damsté et al., 2002). The concentration profile for crenarchaeol in the Black Sea suggests that most of its production (or accumulation if particles are physically concentrated) occurs at the chemocline.

Uniform $\delta^{13}C$ values of crenarchaeol's tricyclic biphytane **d** throughout the water column, in trap material, and in sediments of the Black Sea (~−22‰; Fig. 9) suggest that a single metabolic pathway contributes to all three particle pools: suspended, sinking, and sediments. Biphytane **d** is in fact isotopi-

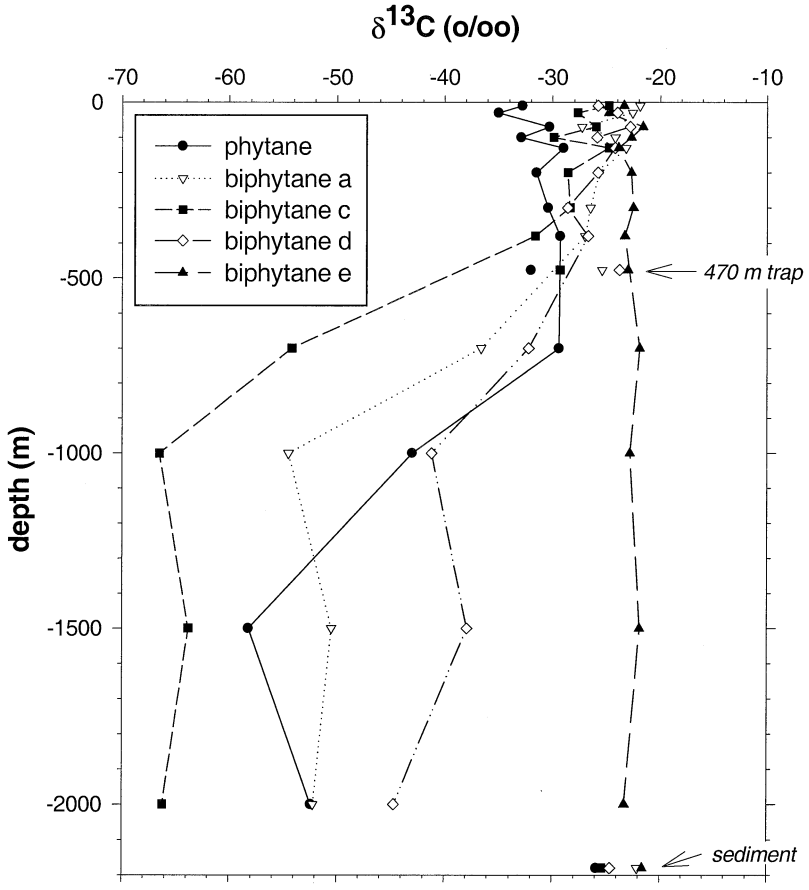


Fig. 9. $\delta^{13}C$ values of biphytanes **a** to **d** in SPM, 470-m sediment trap, and surface sediments. There was insufficient material for isotope analysis of the 1384-m trap sample.

cally enriched in ^{13}C by ~2 to 4‰ compared to POC (−25 to −24‰), to biomarkers of eukaryotic photoautotrophs (phytosterols, alkenones, highly branched C_{25} isoprenoid alkenes, and lycopane with $\delta^{13}C$ values of −30 to −26‰) and to microbial lipids (e.g., diploptene, −41 to −37‰) in the Black Sea water column (Freeman and Wakeham, 1992; Wakeham et al., 1993; Freeman et al., 1994). $\delta^{13}C$ values of dissolved inorganic carbon in the Black Sea range from −8.6‰ at the sea surface

Table 2. $\delta^{13}C$ values (‰ vs. Vienna Peede belemmite) for phytane, biphytanes, and fatty acids in Black Sea suspended particulate matter, trap material, and surface sediments.

Depth (m)	Phytane	Biphytane **a**	Biphytane **b**	Biphytane **c**	Biphytane **d**	$C_{14:0}$	*iso*-$C_{15:0}$	*anteiso*–$C_{15:0}$	$C_{16:1\omega7}$	$C_{16:1\omega5}$	$C_{16:0}$	$C_{18:1\omega(9+7)}$	$C_{18:0}$
10	–32.8	–21.9	–24.8	–25.8	–23.4	–31.5	–27.6	–26.6	–31.4	nd	–30.1	–29.7	–29.2
30	–35.0	–22.6	–27.7	–24.0	–24.8	nd	–25.4	–26.4	–33.7	–32.8	nd	–27.9	–27.6
70	–30.3	–27.3	–26	–22.8	–21.6	–23.8	–23.1	–22.8	–25.5	nd	–24.2	–24.9	nd
100	–32.9	–24.2	–29.9	–25.9	–22.7	–27.4	–30.2	–26.3	–34.2	–37.4	–28.0	–26.2	nd
130	–29.0	–23.2	–24.8	–24.2	–23.9	nd	–28.4	–27.7	–36.1	–39.3	–26.5	–22.5	–25.8
200	–31.5	–25.7	–28.6	–25.8	–22.7	–28.0	–28.3	–28.6	–28.8	nd	–28.5	–28.4	nd
300	–30.4	–26.5	–28.4	–28.6	–22.5	–29.6	–27.8	–30.0	nd	–27.2	nd	nd	nd
400	–29.3	–27.0	–31.6	–26.7	–23.3	nd	–27.6	–30.6	–27.8	–32.7	–29.1	–28.4	–31.4
700	–29.4	–36.6	–54.2	–32.2	–21.9	nd	nd	–28.1	nd	nd	–27.8	nd	nd
1000	–43.0	–54.5	–66.5	–41.2	–22.8	nd	nd	nd	nd	nd	nd	nd	nd
1500	–58.2	–50.5	–63.8	–37.9	–21.9	–28.7	–29.2	–32.3	nd	nd	–31.4	nd	nd
2000	–52.3	–52.4	–66.2	–44.7	–23.3	–28.6	–27.3	–31.3	–28.6	–30.9	–28.7	–27.7	–24.8
470-m trap	–32.0	–25.4	–29.3	–23.8	–23.0	–29.3	–25.4	–26.1	–29.8	–36.5	–29.5	–27.2	–25.9
Sediment	–25.9	–22.1	–25.4	–24.6	–21.6	–29.1	–26.5	–29.9	–25.4	–26.8	–28.8	–27.1	–26.5

nd = not determined.

to −12.6‰ at 130 m, and Calvin-cycle (C_3) autotrophs would produce biomass with ^{13}C values ranging from −38 to −24‰ over the same depth interval (Freeman et al., 1994). To account for the isotopic enrichment of the ether lipids in the Black Sea (and elsewhere), Hoefs et al. (1997) and, more recently, Kuypers et al. (2001) postulated that these archaea could be assimilating dissolved inorganic carbon as their carbon source but employing a biosynthetic pathway that fractionates less than the more common photosynthetic RUBISCO pathway. Alternatively, planktonic archaea obtain their carbon and energy as heterotrophs from organic substrates (Ouverney and Fuhrmann, 2001) that are enriched in ^{13}C, such as low–molecular weight decomposition products of carbohydrates or protein as opposed to lipids.

The other major GDGT at depths shallower than 700 m was GDGT-0. GDGT-0 is present in a wide variety of archaea (Kates, 1993; Koga et al., 1993) and has recently been shown to be a major archaeal biomarker in diverse low-temperature oceanic environments (Hoefs et al., 1997; DeLong et al., 1998; King et al., 1998; Schouten et al., 2000; Pancost et al., 2001a, 2001b, 2001c; Sinninghe Damsté et al., 2002a, 2002b). Given that concentrations of GDGT-0 are low in surface waters and in the upper anoxic zone of the Black Sea, peak at the chemocline, and then increase again in the deep anoxic zone, and that the isotope composition of GDGT-0's acyclic biphytane **a** is markedly depleted in ^{13}C in the deep anoxic zone (−50 compared to −25‰ in surface waters), multiple archaeal sources in different depth intervals are implicated.

5.2. Archaea Involved in AOM in the Anoxic Water Column of the Black Sea

Suspended particles in the deep anoxic zone of the Black Sea are enriched in GDGT-1 (**II**) and GDGT-2 (**III**) and contain isotopically depleted phytane and biphytane **b**, and to a lesser extent **a**, which are interpreted as derived from archaea involved in AOM. This conclusion is based in part on a study by Pancost et al. (2001b), who analyzed intact GDGTs and their constituent biphytanes in cold methane seep sediments (mud domes) in the eastern Mediterranean Sea, which are known to harbor methane-oxidizing archaea. GDGT-1 and GDGT-2 were particularly prevalent, and biphytanes **a**, **b**, and **c** were strongly depleted in ^{13}C (−78 to −50‰). Other biomarkers associated with AOM (archaeol [2,3-di-*O*-phytanyl-*sn*-glyceryl], hydroxyarchaeol [2-O-3-hydroxyphytanyl-phytanylglyceryl], crocetane [2,6,11,15-tetramethylhexadecane], and PMI) were also abundant and isotopically-depleted in the Mediterranean Sea sediments.

In our Black Sea SPM samples, isotopic compositions of biphytane **b** derived from GDGTs **II** and **III** are the most compelling evidence for methanotrophy in the deep anoxic water column. At ∼−67‰ at 1000, 1500, and 2000 m depth, biphytane **b** is the most isotopically depleted biphytane, being depleted in ^{13}C by 42‰ compared to biphytane **d**, which is thought to derive primarily from pelagic crenarchaeotes, and 20‰ relative to methane at the same depth (−48‰; Reeburgh et al., 1996). Biphytanes **a** and **c** and phytane had intermediate $\delta^{13}C$ values (−56 to −54‰, −55 to −40‰, and −52 to −42‰, respectively) consistent with archaea using multiple carbon sources or biosynthetic pathways. Biphytane **a**, with a $\delta^{13}C$ value only 2 to 4‰ depleted relative to methane, may derive from GDGT-0 (**I**) produced by upper–water column crenarchaeotes and methanogenic archaea in the anoxic zone, as well as from GDGT-1 (**II**) generated predominately by methane-consuming archaea in the deep anoxic zone. Biphytane **c**, with $\delta^{13}C$-values enriched relative to methane, derives from isotopically enriched crenarchaeol and, to a lesser extent, from isotopically depleted GDGT-3 (**IV**) from archaea involved in AOM.

The depth profiles of concentrations of GDGT-1 and GDGT-2 show AOM-mediating archaea to be most abundant at 1000 m and deeper, slightly less abundant at 700 m, and significantly less abundant still at shallower depths (Fig. 7a). This trend is consistent with the general pattern of methane oxidation rates in the anoxic zone (Fig. 1c) which, although scattered (note the log scale), shows a trend toward increasing AOM rate down to ∼700 m followed by relatively constant AOM rate to the bottom. We can make a rough estimate of cell densities of methane-oxidizing archaea below 1000 m. Archaea may contain 1.0×10^{-3} pg of GDGT $cell^{-1}$, assuming that archaeal cells are $\sim 1.0 \times 0.5$ μm (Boetius et al., 2000; Sinninghe Damsté et al., 2002a) and 1 μm^2 of archaeal cell membrane contains $\sim 1.7 \times 10^5$ GDGT molecules (Gabriel and Gong, 2000). SPM concentrations for GDGT-1 and GDGT-2 totaling 10 ng L^{-1} below 1000 m would thus correspond to 1.0×10^7 methane-oxidizing archaeal cells L^{-1}. For comparison, calculations of cell densities of planktonic archaea based on concentrations of GDGT-0 and crenarchaeol at 30 and 130 m (a total of 15 and 100 ng L^{-1}, respectively) would yield 1.5×10^7 and 1.0×10^8 planktonic-archaeal cells L^{-1}, respectively. Thus, cell densities of AOM-mediating archaea in the deep anoxic zone are similar to densities of planktonic archaea in surface waters of the Black Sea but an order of magnitude lower than at the top of the anoxic zone. They also fall within the ranges of archaeal cell densities in the water columns of the Arabian Sea estimated from crenarchaeol concentrations (6.7×10^6 to 1.5×10^7 cells L^{-1}; Sinninghe Damsté et al., 2002a) and of the central Pacific obtained from molecular biological data (0.3×10^7 to 4×10^7; Karner et al., 2001). We can further estimate, using Reeburgh et al.'s (1991) AOM rate of 2 nM/L d^{-1}, that the average AOM-mediating archaeal cell below 1000 m of the Black Sea could be oxidizing an upper limit of $\sim 2 \times 10^{-7}$ nmol of CH_4 d^{-1}, if indeed all of the AOM was carried out by these archaea.

5.3. Isotope Fractionation During AOM

Although molecular carbon isotope evidence supports the transfer of isotopically depleted methane-derived carbon into lipids of archaea in the deep anoxic zone of the Black Sea, the ^{13}C values we measured (−67‰ for the most depleted biphytane) are substantially higher than have been observed in most other environments that have been studied. Isotopic compositions of the irregular tail-to-tail C_{20} and C_{25} isoprenoids crocetane, PMI and unsaturated PMIs, isopranyl diethers archaeol and hydroxyarchaeol, and GDGT-derived biphytanes can be remarkably variable. They can be extremely depleted in ^{13}C, as low as −124‰, in a number of methane-rich sedimentary environments, including the Aleutian deep-sea trench (Elvert et al., 2000), the northwest Black Sea shelf (Thiel et al.,

2001), Hydrate Ridge (Cascadia continental margin; Elvert et al., 1999, 2001), the Eel River Basin (California margin; Hinrichs et al., 1999, 2000; Orphan et al., 2001a, 2001b, 2002) and Mediterranean mud volcanoes (Pancost et al., 2001b, 2001c). Intermediate $\delta^{13}C$ values (−89 to −58‰) were observed in hydrothermally active sediments of Guaymas Basin (e.g., archaeol, hydroxyarchaeol: Teske et al., 2002; biphytanes and biphytane diacids: Schouten et al., in press) and may be as high as −40‰ (crocetane) at the transition between sulfate reduction and methanogenesis in "normal" reducing coastal marine sediments (Bian et al., 2001).

Of these widely studied archaeal lipids, we have data only for PMI in the Black Sea. PMI is the predominant hydrocarbon in SPM from the anoxic zone (Wakeham et al., 1991), but its $\delta^{13}C$ values were invariant (−28‰ for an oxic composite [pooled SPM from 10 to 100 m], −27‰ for an anoxic composite [130 to 2000 m], and –27‰ for surface sediment [0 to 3 cm]) (Freeman et al., 1994). PMI is in fact only ~2 to 4‰ depleted in ^{13}C compared to crenarchaeol's biphytane **d** and significantly enriched in ^{13}C relative to methane and the biphytanes that we attribute to AOM. At first glance, PMI in our samples would appear not to be derived from organisms involved in AOM. However, if PMI were ^{13}C depleted in the deep anoxic zone, Freeman et al. (1994) could easily have missed it by the compositing carried out to ensure sufficient sample for analysis.

The isotope fractionation associated with production of archaeal biomarkers via AOM can be calculated from the $\delta^{13}C$ values of the methane substrate, if methane data are available. Uptake of carbon from isotopically light methane ($\delta^{13}C$ values of −90 to −50‰ are not uncommon; Whiticar, 1999) via its oxidation may involve a considerable, up to 95‰, isotope fractionation (Summons et al., 1994, 1998; Whiticar, 1999). Our Black Sea results here indicate a maximum fractionation of ~20‰ (for biphytane **b**) in the deep anoxic zone (methane being −48‰), assuming that biphytane **b** is produced only by methanotrophy. If, however, biphytane **b** is derived from mixed sources, as apparently are phytane and biphytanes **a** and **c**, then the methanotrophic end-member could actually be more ^{13}C depleted, and the fractionation would be greater. In the Guaymas Basin, Teske et al. (2002) observed isotopic fractionations of the order of 40 to 20‰ for archaeol, hydroxyarchaeol, and a biphytane diol (biomarker $\delta^{13}C$ values of −89 to −58‰ compared to methane $\delta^{13}C$ values of −51 to −43‰). At Hydrate Ridge, Elvert et al. (2001) reported carbon isotope fractionations of 65 to 30‰ for crocetane, PMI and unsaturated PMIs, archaeol, and alcohols in aragonites precipitated by AOM (methane $\delta^{13}C$ values of −72 to −62‰). The difference in fractionation between Hydrate Ridge sediments on one hand and the Black Sea water column on the other is intriguing. If biphytane **b** is the methanotrophic end-member, then isotopic fractionation during AOM in the Black Sea water column might be minimal because nearly all (>99%) of the methane released into the water column from sediments is oxidized. The uniform concentrations and $\delta^{13}C$ values of methane in the deep anoxic zone (−48‰; Reeburgh et al., 1996) have been interpreted (Reeburgh et al., 1996) in terms of a "closed system" in which end-member concentrations and isotope compositions are approached (Reeburgh et al., 1996). If true, then there might be little isotopic difference between methane and the archaeal biomass that is produced from this methane, and our data would suggest a 20‰ difference between $\delta^{13}C$ values of archaeal biomass (~48% on the basis of methane) and archaeal biphytanes (e.g., −67% for biphytane **b**). In the other sedimentary settings, in which AOM is not limited by methane availability, oxidation of a smaller fraction of the available methane would lead to greater isotopic fractionation.

5.4. A Link Between Archaeal AOM and Sulfate Reduction in the Black Sea?

Hoehler et al. (1994) (see also Hoehler and Alperin, 1996) proposed that a consortium of methanogenic archaea operating in reverse (oxidizing methane rather than producing it) and sulfate-reducing bacteria carry out AOM. H_2 generated from methane is consumed by sulfate-reducers, allowing methane oxidation to be energetically feasible. In fact, the rate of methane oxidation is highest in a narrow band at the base of the sulfate reduction zone in sediments (Iversen and Jørgensen, 1985; Alperin et al., 1988; Thomsen et al., 2001) or at the top of anoxic water columns (Reeburgh et al., 1991; Albert et al., 1995; this study). When molecular gene surveys of ribsomal RNA (16S RNA) were carried out in parallel with biomarker studies (Hinrichs et al., 1999; Orphan et al., 2001a, 2001b; Teske et al., 2002), both bacteria, mainly sulfate-reducers, and novel archaea were found. Boetius et al. (2000) and Orphan et al. (2001a, 2001b) used 16S ribosomal RNA–targeted probes and fluorescence in situ hybridization to visualize clusters of archaea surrounded by sulfate-reducing bacteria. Although apparently quite diverse, archaea involved in AOM generally are related to the *Methanosarcinales*, and sulfate-reducing bacteria are related to *Desulfosarcina*/*Desulfococcus*.

If anaerobic methane oxidation involves a consortium of methanogens and sulfate-reducing bacteria, then the biomarkers for the sulfate-reducers might also carry some isotopic signature of ^{13}C-depleted methane as carbon source. Indeed, straight-chain and methyl-branched fatty acids such as *iso*- and *anteiso*-C_{15} and alkyl ethers derived from sulfate-reducing bacteria are depleted in ^{13}C in methane seep sediments (Hinrichs et al., 2000) and Mediterranean mud volcano sediments (Pancost et al., 2001b, 2001c) having ^{13}C-depleted archaeal lipids. The $\delta^{13}C$ values of bacterial lipids (e.g., ~−110 to −40‰; Hinrichs et al., 1999, 2000) are intermediate between the source methane (−72 to −62‰) and the archaeal lipids (−112 to −119‰). Intermediate $\delta^{13}C$ values would arise if there were a second source of bacterial lipids from organisms not involved in AOM.

In the anoxic water column of the Black Sea, both AOM and sulfate reduction occur (Fig. 1). Isotopic compositions of certain archaeal biomarkers in the deep anoxic zone are depleted in ^{13}C, as would be expected for AOM, but fatty acids derived from sulfate-reducing bacteria are apparently not so depleted. $\delta^{13}C$ values for most fatty acids are surprisingly invariant in SPM down the water column, in trap material, and in sediments, ranging from –25 to −32‰. *Iso*-C_{15} and *anteiso*-C_{15} fatty acids displayed slightly different trends with depth: *anteiso*-C_{15} (−30 to −32‰) was depleted by ~5‰ in anoxic zone SPM and sediments relative to *iso*-C_{15} (−27‰), but both were essentially the same (−26‰) in the 470-m trap sample. It may be that the slight isotopic depletion of *anteiso*-C_{15} in the

anoxic zone reflects a small signal related to an archaeal-sulfate-reducing bacterial consortium, but clearly, the signal is not large. The only significantly ^{13}C-depleted fatty acids were two isomers of hexadecenoic acid, 16:1ω5 ($-$40‰) and 16:1ω7 ($-$36‰), and these only at 130 m. These two fatty acids may (partly) be derived from type I methanotrophic bacteria in which saturated and monounsaturated 16-carbon fatty acids dominate (Makula, 1978; Urakami and Komagata, 1984; Nichols et al., 1987).

The absence of a strong signal of ^{13}C-depleted fatty acids from sulfate-reducing bacteria of course does not negate a synergy between methanotrophic archaea and sulfate-reducing bacteria in the Black Sea. It is possible that the proportion of sulfate-reducing bacteria involved in AOM is small relative to the total population of sulfate-reducing bacteria, such that an isotopic signal is simply not as apparent as in the sedimentary environments noted above. Or a different carbon source or metabolic pathway is involved. However, independent measurements show that carbon cycling through AOM is comparable in the Black Sea to carbon cycling via sulfate reduction ($\sim$0.7 mol C m^{-2} yr^{-1} for AOM compared with $\sim$1.5 mol C m^{-2} yr^{-1} for sulfate reduction; Reeburgh et al., 1991; Albert et al., 1995). In this case, the absence of ^{13}C-depleted fatty acids could indicate that sulfate-reducing bacteria are not major players in AOM. Clearly, this point needs further investigation and would involve concurrent measurements of biomarkers, isotopes, and molecular biology.

5.5. Sediments Poorly Record Water Column AOM

The absence of a signal for AOM in the sediments is intriguing, because Reeburgh et al. (1991) showed AOM occurring throughout the anoxic water column and in the underlying sediments, and we see an unequivocal molecular signal in the deep anoxic zone. Our preliminary analyses of GDGT-derived biphytanes in the Black Sea (Hoefs et al., 1997; King et al., 1998) revealed compositional and isotopic distinctions between biphytane distributions in the water column and sediments, which our more detailed analyses now confirm. At the time of Hoefs et al.'s (1997) and King et al.'s (1998) papers, it was uncertain as to the relative contributions of planktonic archaea and sedimentary archaea for biphytane distributions found in the sediments. King et al. (1998) showed the biphytane distribution in the deep anoxic zone to be different from that of the oxic zone, upper anoxic zone, and sediments (Fig. 3 in King et al., 1998), suggesting a varying archaeal community structure at different depth intervals, but data were too sparse to draw a solid conclusion. The report by Schouten et al. (2001) subsequently pointed out that on the basis of only three composite samples, there are strong isotopic depletions for several biphytanes in the deep anoxic zone, but sediment biphytane distributions and isotope compositions resembled SPM from the oxic zone rather than SPM from the anoxic zone.

Our present analyses of sediment trap material explain why. Sinking material collected in traps deployed at both 470 and 1384 m depth showed GDGT compositions derived from the upper water column but not from the deep anoxic zone. Such a marked compositional distinction between suspended and sinking particles has been observed for other lipid classes in the Black Sea (Wakeham and Beier, 1991; Wakeham et al., 1991) and elsewhere (e.g., Wakeham and Canuel, 1988; Wakeham and Lee, 1993). Because sediments are derived largely from fast-sinking large particles (McCave, 1984), which are produced by biologically-mediated aggregation, including grazing, the absence of grazing in the anoxic zone may minimize incorporation of AOM-derived biomarkers into sinking particles as they transit the deep anoxic zone. This reinforces the proposition put forward by Schouten et al. (2001) that sediments may poorly record AOM in deep, overlying euxinic water columns, and caution is required in interpreting sedimentary records. This is especially of relevance for global events in the geological past, which are characterized by a sudden negative isotope excursion of carbonate. Such events have been proposed to be caused by episodic release of large volumes of carbon isotopically-depleted methane (Dickens et al., 1995) but so far, chemical fossils derived from methane-oxidizing bacteria or archaea have not been found despite the fact that such organisms are expected to substantially increase in abundance as a response to such an event. Our Black Sea data now show that such environmental signals may not be transported to the sedimentary record, perhaps explaining this apparent conundrum.

6. SUMMARY

Distributions of GDGTs and strongly ^{13}C-depleted isotopic compositions of their constituent phytane and biphytanes reveal that archaea mediate methane oxidation in the deep anoxic portion of the Black Sea. The most striking AOM archaeal biomarker signal was observed in the deep anoxic zone below 700 m depth, although other evidence clearly shows that high rates of AOM occur in the upper anoxic zone as well. Isotopic analyses of fatty acids were used to test whether there is a syntrophic relationship between archaea involved in AOM and sulfate-reducing bacteria. Fatty acids indicative of sulfate-reducing bacteria were not depleted in ^{13}C, suggesting a decoupling between the biomarker signal derived from AOM and that derived from sulfate reduction. This decoupling could indicate that archaea carry out AOM alone in the Black Sea rather than as part of a prokaryotic consortium of archaea and sulfate-reducing bacteria. Finally, it is clear that AOM in the deep anoxic zone is preferentially associated with the slowly sinking (suspended) particle pool, and this signal is not effectively transferred to the underlying sediments by sinking particles. This observation indicates that vigilance is needed in inferring the extent of AOM in past euxinic water columns on the basis of abundances and isotopic compositions of sedimentary archaeal biomarkers that may have water column sources.

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