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Mixed sources contribute to the molecular isotopic signature of methane-rich mud breccia sediments of Kazan mud volcano (eastern Mediterranean)

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Abstract

We have investigated the molecular carbon isotopic signature of mud breccia sediments from a methane-seep environment on Kazan mud volcano in the eastern Mediterranean. Many different classes of lipids have been identified and attributed to methane-oxidizing archaea, sulfate-reducing bacteria, methane-oxidizing bacteria, and sulfide-oxidizing bacteria, as well as older organic matter associated with the ascending mud diapirs in the region. Of particular interest is the record of glycerol dialkyl glycerol tetraethers (GDGTs) derived from various types of archaea. A geochemical depth profile of the upper 30 cm of sediment allows the assessment of vertical variability in the microbial community, which proves to be diverse based on molecular isotopic analyses, and the importance of present-day microbes relative to paleo-organic matter. In this environment, it seems that anaerobic oxidation of methane (AOM) has progressed at relatively low rates or for a shorter time compared with other seep sites, based on the high relative abundance of organic matter associated with the ascending mud matrix rather than with AOM, and the carbon isotopic composition of GDGT-derived biphytanes. The presence of many different biomarkers of AOM-related microbes with varying depth trends in both concentration and carbon isotope composition suggests substantial variability in the microbial community on a small vertical scale (~30 cm).

1. Introduction

Mud volcanoes in the eastern Mediterranean form as a result of tectonic compression and associated strike-slip faulting, which leads to the extrusion of fluid-rich mud flows (Cita et al., 1996; Woodside et al., 1998). Methane and other hydrocarbons are often associated with mud volcanism. Indeed, active methane seeps have been identified on a number of mud volcanoes in the Anaximander Mountains mud dome field in the eastern Mediterranean (Emeis et al., 1996; Limonov et al., 1996; Woodside et al., 1998; MEDINAUT/MEDINETH Shipboard Scientific Parties, 2000). This methane, if it reached the atmosphere, could contribute to global warming; however, much of the methane associated with

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mud volcanoes in the eastern Mediterranean and elsewhere is scavenged through a variety of processes, and therefore never reaches the atmosphere.

In marine sediments, the dominant pathway of methane consumption is microbially mediated anaerobic oxidation of methane (AOM) (Reeburgh, 1980; Iversen and Jørgensen, 1985; Blair and Aller, 1995; Borowski et al., 1996), which is generally believed to be carried out by a consortium of methane-oxidizing archaea and sulfate reducing bacteria (SRB) (Hoehler et al., 1994; Boetius et al., 2000; Valentine and Reeburgh, 2000; Valentine, 2002). In fact, as much as 90% of methane in oceanic sediments is scavenged in the anaerobic portion of the sediments (Reeburgh et al., 1993) and methane oxidation has been identified in the anaerobic water column of various environments such as the Black Sea (Schouten et al., 2001; Wakeham et al., 2003) and Cariaco Basin (Reeburgh, 1976).

Molecular isotopic studies have identified a variety of microbial biomarkers in environments associated with AOM, including biomarkers typically associated with methanogenic archaea and SRB, as well as other less well-constrained biomarkers (Thiel et al., 1999, 2001; Hinrichs et al., 1999, 2000; Elvert et al., 1999, 2000, 2001; Pancost et al., 2000, 2001a,b; Bian et al., 2001; Werne et al., 2002; Schouten et al., 2003a). The biomarkers in all of these studies were depleted in ^{13}C relative to photoautotrophic biomarkers. This carbon-isotopic depletion is a result of: (1) the fact that methane is ^{13}C depleted relative to CO_2 in the natural environment by 30–60‰ (Whiticar, 1999) and (2) biosynthetic carbon isotope fractionation processes associated with methane consumption (Alperin et al., 1988; Whiticar, 1999; Jahnke et al., 1999), providing significant geochemical support for the microbial consortium hypothesis. Microbiological studies are increasingly identifying multiple lineages of similar methane-oxidizing archaea and SRB that may be implicated in AOM (Hinrichs et al., 1999; Boetius et al., 2000; Orphan et al., 2001a,b; Thomsen et al., 2001; Michaelis et al., 2002; Teske et al., 2002).

The present study is a molecular carbon isotopic investigation of the sediments of Kazan mud volcano, a methane-seep site in the eastern Mediterranean. AOM has been identified in eastern Mediterranean mud volcano environments based on pore water profiles of sulfate and methane (De Lange and Brumsack, 1998; Haese et al., 2003). In a previous study, we tracked the flow of methane-derived carbon through a microbial community and into higher trophic levels (Werne et al., 2002) based on the carbon isotope composition of a selected suite of diagnostic biomarkers. In this study, the concentrations and carbon isotope compositions of various biomarkers were determined for six samples from a 30-cm box core taken from Kazan mud volcano. These biomarkers are derived from a variety of sources, including marine and terrestrial photoautotrophic sources associated with ascending mudflows as well as microbes (bacteria and archaea) involved in AOM in the sediments. While the full range of biomarkers identified is discussed briefly, the primary focus is on the distribution of intact isoprenoid glycerol dialkyl glycerol tetraethers (GDGTs), and its variability with depth. Biomarker depth profiles and carbon isotope compositions are utilized to determine stratigraphic variability in the contribution of AOM and other processes to the sedimentary organic matter, and the vertical heterogeneity of these biomarkers and their $\delta^{13}C$ values is discussed in terms of its implications for the methane-oxidizing microbial community, and comparisons are made with findings from other methane-seep environments.

2. Methods

2.1. Sampling

Samples were taken by box core during the MEDINETH cruise of the *R/V Professor Logachev* to the eastern Mediterranean in August 1999. Core MNLBC19 covers the upper 30 cm of sediments from Kazan mud volcano in the Anaximander Mountains area in the eastern Mediterranean Sea (35°25.950′N, 30°33.679′E, water depth 1673 m, Fig. 1). The core was sub-sampled on board ship, and samples were frozen until analyzed. This study is carried out using the same samples as described in Werne et al. (2002). Analytical procedures are as described in Werne et al. (2002), with the following additions.

2.2. Extraction and separation

After ultrasonic extraction and alumina column chromatography as described in Werne et al. (2002), aliquots of the polar fractions were treated to cleave ether bonds to facilitate carbon isotopic measurement of biphytanes released from GDGTs (glycerol dialkyl glycerol tetraethers, see Appendix A for structures). Samples were refluxed in 57% HI (in H_2O by weight) for 1 h and the generated alkyliodides were reduced to hydrocarbons with $LiAlH_4$ (for details see Schouten et al., 1998).

2.3. Biomarker analysis

Apolar and polar fractions of the total lipid extract (TLE) were analyzed by gas chromatography and gas chromatography/mass spectrometry to identify and quantify most biomarkers as described in Werne et al. (2002). Aliquots of the TLE were analyzed by high-performance liquid chromatography–mass spectrometry (HPLC–MS) to determine the concentrations of GDGTs following the method of Hopmans et al. (2000). Compounds were identified based on protonated molecular ions in their mass spectra and comparison with retention times of GDGTs from *Sulfolobus solfataricus* (De Rosa

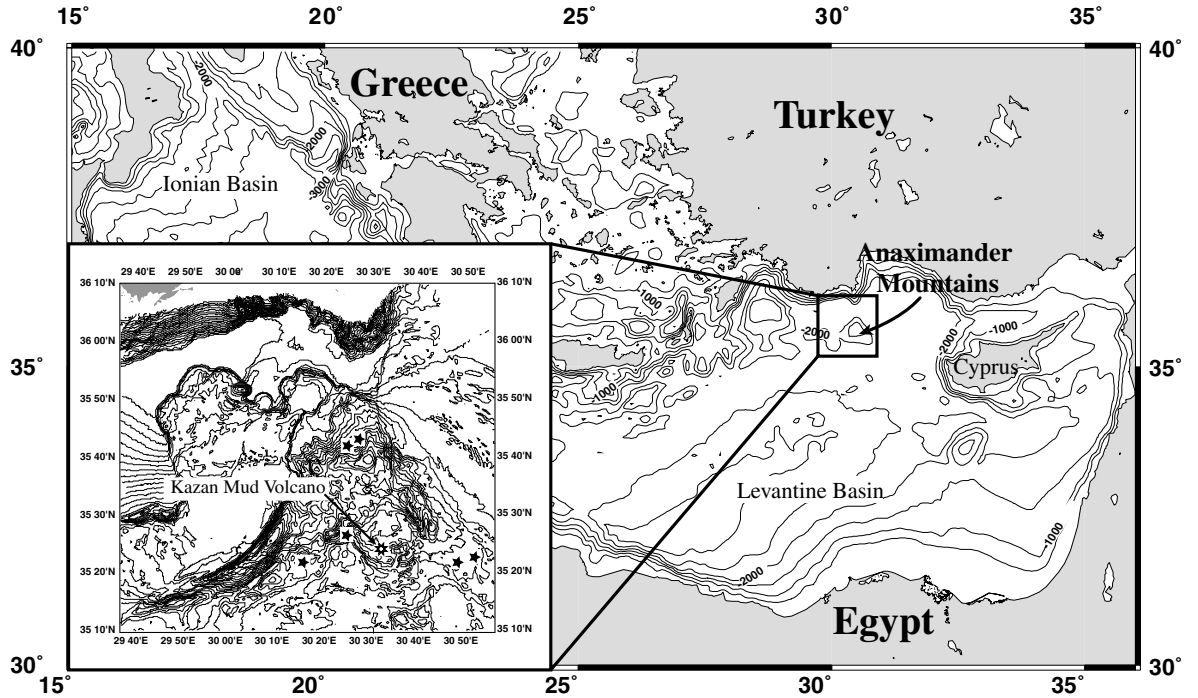

Fig. 1. Map showing location of Kazan mud volcano in the Anaximander Mountains area of the eastern Mediterranean. Site MNLBC19 is indicated.

et al., 1986; Hopmans et al., 2000) and *Cenarchaeum symbiosum* (Sinninghe Damsté et al., 2002a).

2.4. Carbon isotope analysis

Isotope ratios are reported relative to the VPDB (Vienna Pee Dee Belemnite) standard in conventional per mil (‰) notation. $\delta^{13}C$ values have been corrected for carbon added during derivatization, and have an error of less than ±1‰ unless otherwise noted (based on analytical accuracy and precision of measurements of co-injected standards).

3. Results and discussion

3.1. Geochemical environment

Mud breccia sediments of Kazan mud volcano are characterized by elevated concentrations of pore water methane associated with steep gradients of pore water sulfate (Haese et al., 2003), which is typically indicative of AOM (Martens and Berner, 1977; Reeburgh, 1980; Devol et al., 1984; Iversen and Jørgensen, 1985). Other pore water constituents, such as the concentration and carbon isotope composition of dissolved inorganic carbon also indicate AOM (Haese et al., 2003). The extent of AOM is not fully known; however, it has been ongoing long enough in Kazan mud volcano sediments to produce ^{13}C-depleted authigenic carbonates as a result of the microbially mediated AOM process (Aloisi et al., 2000, 2002; Werne et al., 2004).

3.2. Terrestrial and marine photoautotrophic (eukaryotic) biomarkers: indicators of OM associated with mud flows

The mud-flows at the study site are believed to be fairly recent based on a lack of overlying pelagic sedimentation (though as discussed below, some pelagic sediment may have been mixed down in the upper few centimeters by bioturbation). Any biomarkers of terrestrial or marine planktonic origin are therefore expected to be associated with the ascending mud-flows rather than with methane-based microbial activity. Apolar fractions of the extractable OM are composed largely of a homologous series of *n*-alkanes ranging from C_{17} to C_{35} (Fig. 2(a)). The concentrations of individual *n*-alkanes do not vary substantially with depth, ranging from 0.01 to ~0.3 μg/g sediment (Table 1). The *n*-alkanes display a bimodal distribution centered around C_{31} and C_{19} (Fig. 3). A moderate odd-over-even predominance is exhibited in the upper chain lengths (C_{25}–C_{35}), characteristic of terrestrial higher plant inputs (Eglinton and Hamilton, 1967). The carbon isotope compositions of the *n*-al-kanes generally range from −28‰ to −32‰, with average values between −29‰

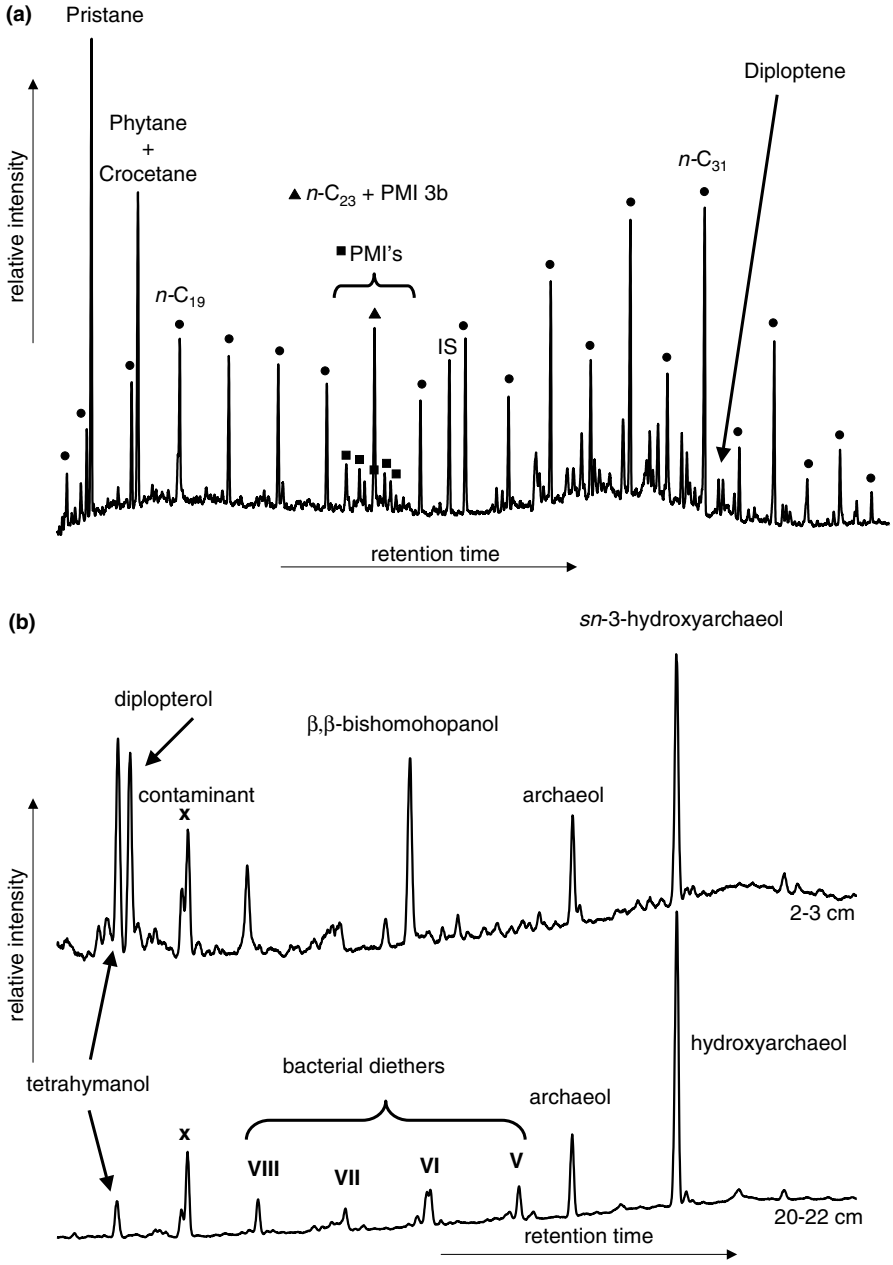

Fig. 2. Partial chromatogram of: (a) the apolar fraction of extractable organic matter from 20 to 22 cm sediment depth and (b) the polar fraction of extractable organic matter from two depths (2–3 and 20–22 cm) in surface sediments of Kazan mud volcano. Note difference in relative abundance of archaeal diethers and bacterial hopanoids with depth. "IS" is the internal standard.

and −30‰ (Table 1), clearly indicative of a photoautotrophic source for all *n*-alkanes. Exceptions include *n*-C_{30}, *n*-C_{32}, and *n*-C_{34}, which are all very low abundance and may be co-eluting with minor hopanoid compounds, and *n*-C_{23}, which co-elutes with ^{13}C-depleted pentamethylicosanes (PMIs, see discussion below for relevant PMI data). ^{13}C-depleted *n*-C_{23} alkenes have previously been identified in Black Sea seeps and microbial mats, and *n*-C_{23} was also reported in high abundance in ancient seep carbonates (Thiel et al., 2001) as well as other localities (Elvert et al., 1999). The present study did not identify ^{13}C depletion in *n*-C_{23} or the presence of *n*-tricosenes, but *n*-C_{23} was present in higher abundance than other adjacent *n*-alkanes.

Concentrations of pristane and phytane (ca. 0.2–0.5 μg/g sediment) in sediments from Kazan mud volcano

Table 1
Concentrations and carbon isotope compositions of biomarkers associated with the ascending mud matrix

Depth (cm)	Concentration (μg/g sed)						$\delta^{13}C$ (‰ VPDB)					
	0–2	2–3	10–12	16–18	20–22	27–29	0–2	2–3	10–12	16–18	20–22	27–29
Pristane[a]	0.23	0.36	0.38	0.48	0.36	0.42	−29.1	−28.8	−27.8	−27.9	−28.3	−31.0
Phytane[c]	0.20	0.22	0.28	0.32	0.24	0.25	−29.0	−30.6	−32.0	−34.7	−39.5	−30.3
n-C_{17}[b]	0.05	0.06	0.06	0.10	0.08	0.07	nd	−29.7	nd	−28.5	−27.6	nd
n-C_{18}	0.09	0.08	0.10	0.15	0.11	0.08	−29.0	−29.9	−29.3	−31.1	−29.9	−29.9
n-C_{19}[b]	0.12	0.11	0.16	0.22	0.13	0.13	−29.5	−28.5	−28.4	nd	−29.5	−29.0
n-C_{20}	0.10	0.08	0.12	0.18	0.12	0.10	−29.9	−29.3	−30.5	−29.6	−29.0	−29.6
n-C_{21}	0.11	0.08	0.11	0.18	0.11	0.10	−28.2	−30.6	−29.8	−29.5	−29.0	−29.6
n-C_{22}	0.11	0.07	0.10	0.17	0.10	0.10	−29.3	−30.9	−31.0	−29.7	−30.0	−30.8
n-C_{23}	0.15	0.11	0.17	0.26	0.19	0.14	−30.6	−35.0	−49.6	−47.0	−59.3	−38.4
n-C_{24}	0.11	0.07	0.09	0.16	0.10	0.09	−30.0	−28.7	−29.1	−28.9	−29.1	−30.2
n-C_{25}	0.19	0.13	0.15	0.22	0.14	0.15	−29.1	−28.4	−29.1	−29.1	−29.0	−29.5
n-C_{26}	0.12	0.08	0.09	0.16	0.10	0.09	−28.5	−30.5	−29.5	−28.9	−29.0	−29.6
n-C_{27}	0.22	0.16	0.18	0.24	0.14	0.16	−29.0	−30.6	−29.7	−29.2	−29.7	−29.8
n-C_{28}	0.15	0.10	0.12	0.17	0.11	0.11	−30.2	−30.6	−31.8	−30.8	−30.4	−28.8
n-C_{29}[b]	0.31	0.23	0.24	0.29	0.17	0.20	−29.7	−29.8	−30.0	−29.4	−30.3	−30.5
n-C_{30}	0.15	0.12	0.14	0.15	0.09	0.12	−28.9	−23.1	−32.9	−30.6	−34.4	−32.1
n-C_{31}[a]	0.30	0.27	0.30	0.30	0.16	0.21	−31.3	−30.7	−30.9	−30.5	−31.0	−32.7
n-C_{32}	0.07	0.06	0.06	0.08	0.05	0.05	nd	−35.2	nd	−30.8	−31.8	−32.5
n-C_{33}	0.21	0.17	0.16	0.19	0.10	0.14	−31.8	−31.7	−31.5	−31.6	−31.8	−31.6
n-C_{34}	0.05	0.04	0.04	0.04	0.03	0.04	nd	nd	nd	nd	−32.0	−38.6
n-C_{35}	0.06	0.06	0.07	0.08	0.04	0.06	nd	−30.0	nd	−29.2	−28.0	−29.0
n-C_{36}	0.02	0.02	0.02	0.03	0.01	0.02	nd	nd	nd	nd	nd	nd
n-C_{37}	0.03	0.03	0.03	0.04	0.02	0.03	nd	nd	nd	nd	nd	nd
n-C_{38}	0.02	0.02	0.03	0.03	0.01	0.02	nd	nd	nd	nd	nd	nd
n-C_{39}	0.01	0.02	0.02	0.02	0.01	0.02	nd	nd	nd	nd	nd	nd

nd indicates not determined.
[a] Denotes data published in Werne et al. (2002).
[b] Denotes data published in Werne et al. (2004).
[c] sometimes co-eluting with small amounts of crocetane (see text for details).

are slightly higher than those of *n*-alkanes, but display a similar lack of variation (Fig. 3). The carbon isotope composition of pristane is ~−30‰ (Table 1), clearly indicating a photoautotrophic source. Phytane, however, is −30‰ in surface sediments and in the deepest sample (29 cm below sea floor), but reaches a value of nearly −40‰ at 22 cm sediment depth (Table 1). This negative isotopic off-set could be due to two possible factors: addition of phytane derived from archaeal sources, or co-elution of phytane with the C_{20} irregular isoprenoid crocetane (2,6,11,15-tetramethylhexadecane), which is believed to be derived from methane consuming archaea (e.g., Elvert et al., 1999; Pancost et al., 2000; Bian et al., 2001). Close examination of the mass spectral data indicates that the phytane peak does contain smaller amounts of crocetane in the 22-cm sample.

3.3. Markers of anaerobic methane-oxidizing archaea

As a result of the extremely ^{13}C-depleted isotopic signature of methane-derived organic matter (Whiticar, 1999; Alperin et al., 1988), biomarkers can be implicated in the net consumption of methane without having identified these compounds unambiguously in cultures. It was in fact the identification of compounds typically found in methanogenic archaea, rather than bacteria, in environments characterized by AOM that gave the first major support to the hypothesis that the process was being carried out by archaea (i.e., methanogens operating in reverse) (Hinrichs et al., 1999; Elvert et al., 1999; Thiel et al., 1999; Pancost et al., 2000).

The biomarker compositions of many sediments where AOM plays an important role are dominated by glycerol diethers such as archaeol (bis-*O*-phytanyl glycerol diether, compound **I**, see Appendix A for structure) and hydroxyarchaeol (*sn*-3- and *sn*-2-hydroxyarchaeol; **IIa** and **IIb**, respectively) (Hinrichs et al., 1999, 2000; Pancost et al., 2000, 2001a,b; Elvert et al., 2000; Werne et al., 2002). The occurrence of these compounds in archaea has been demonstrated previously (Koga et al., 1993, 1998; Sprott et al., 1997). In sediments of Kazan mud volcano, ^{13}C-depleted archaeol and *sn*-3-hydroxyarchaeol are present in high relative abundance (Werne et al., 2002; Fig. 2b, Table 2).

Dominance of the organic matter by *sn*-3-hydroxyarchaeol is noteworthy, as most other systems characterized by AOM are dominated by *sn*-2-hydroxyarchaeol instead, including other Mediterranean mud volcano

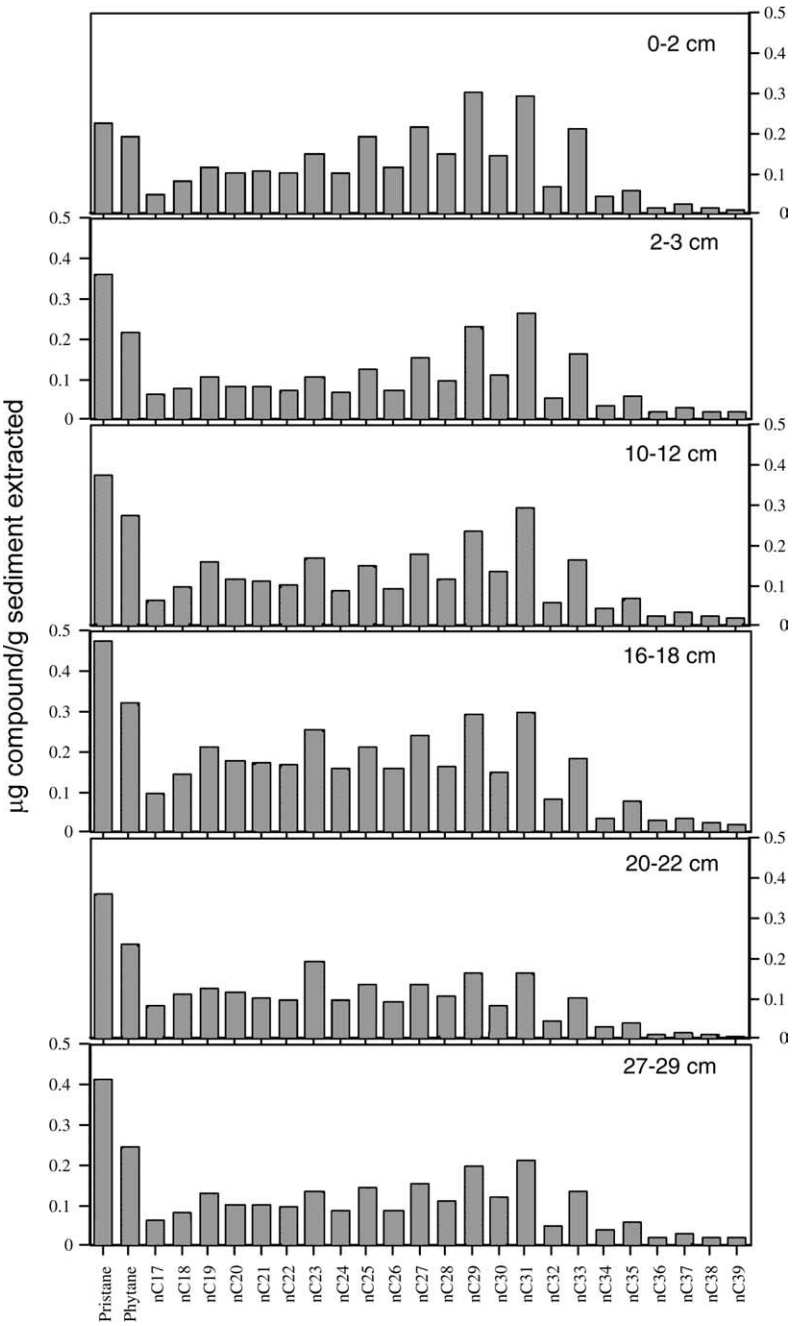

Fig. 3. Distributions of *n*-alkanes, pristane, and phytane in mud breccia from different depths, in µg/g sediment extracted. The concentration of phytane includes a small contribution from crocetane, especially at greater depths, and *n*-C_{23} co-elutes with a PMI.

sites (cf. Hinrichs et al., 1999, 2000; Pancost et al., 2000, 2001a,b; Elvert et al., 2000). This observation suggests either that the archaea performing AOM synthesize compounds in different relative abundance under different conditions, or that there are different but very similar archaea capable of carrying out the AOM process under different conditions. Given the data presently obtained, either explanation (or both) is equally likely to be valid, but certainly points to variability in the AOM-related microbial community that is worthy of further investigation. Other compounds that have been associated with anaerobic methane-oxidizing archaea in numerous environments are C_{20} and C_{25} irregular isoprenoids such as crocetane (2,6,11,15-tetra-

Table 2
Concentrations and carbon isotope compositions of archaeal and bacterial biomarkers

Depth (cm)	Concentration (μg/g sed)							δ^{13}C (‰ VPDB)					
	0–2	2–3	10–12	16–18	20–22	27–29		0–2	2–3	10–12	16–18	20–22	27–29
sn-3-Hydroxyarchaeol[a]	nd	0.19	0.20	0.93	0.61	0.29		nd	−103	−108	−111	−111	−101
Archaeol[a]	nd	0.07	0.07	0.32	0.16	0.10		nd	−94	−95	−103	−101	−81
PMI:4a	0.03	0.05	0.04	0.04	0.02	0.01		−50	−69	−68	−69	−70	−43
PMI:2	nd	0.02	0.02	0.03	0.04	nd		nd	−83	−83	−88	−115	nd
PMI:3a	nd	nd	0.02	0.01	nd	nd		nd	nd	−98	nd	nd	nd
PMI:3c[b]	nd	nd	0.03	0.04	0.02	nd		nd	nd	−87	−102	−124	nd
PMI:4b	nd	nd	0.03	0.02	0.01	nd		nd	nd	−112	−110	−105	nd
Diether 1[a]	nd	nd	0.01	0.06	0.06	0.03		nd	nd	nd	−91	−89	−70
Diether 2[b]	nd	nd	0.02	0.08	0.12	0.10		nd	nd	nd	−90	−97	−82
Diether 3[a]	nd	nd	0.04	0.07	0.10	0.03		nd	nd	−85	−91	−73	−62
Diether 4[b]	nd	nd	0.01	0.06	0.06	0.05		nd	nd	nd	−90	−95	−73
Diplopterol[a]	0.20	0.14	nd	nd	nd	nd		−56	−62	nd	nd	nd	nd
Diploptene[a]	0.08	0.06	0.02	0.01	nd	nd		−54	−60	nd	nd	nd	nd
bishomohopanol[b]	0.22	0.16	nd	nd	nd	nd		−46	−53	nd	nd	nd	nd
GDGT-0[b]	0.82	0.45	0.28	0.12	0.20	0.18							
GDGT-1[b]	0.17	0.09	0.06	0.02	0.05	0.05	**bp-0**	−27	−30	nd	nd	−35	−32
GDGT-2[b]	0.17	0.17	0.13	0.08	0.14	0.10	**bp-1**	nd	−27	nd	nd	−32	nd
GDGT-3[b]	0.06	0.07	0.08	0.04	0.07	0.05	**bp-2**	−23	−25	nd	nd	−29	nd
GDGT-4[b]	0.00	0.00	0.06	0.04	0.06	0.04	**bp-3**	−22	−22	nd	nd	nd	nd
Crenarchaeol	0.99	0.73	0.05	0.02	0.02	0.02							

nd indicates not determined.
[a] Denotes data published in Werne et al. (2002).
[b] Denotes data published in Werne et al. (2004). Diether 3 here is the same as diether 2 in Werne et al. (2000).

methylhexadecane, **III**) and PMIs (2,6,10,15,19-pentamethylicosane with various unsaturations **IV**) (Thiel et al., 1999, 2001; Elvert et al., 1999, 2000, 2001; Pancost et al., 2000, 2001a). Unsaturated PMIs have also been identified in cultures of methanogenic archaea (Schouten et al., 1997). Both crocetane and a suite of saturated and unsaturated PMIs have been identified in sediments of Kazan mud volcano (Fig. 2(a)). The concentrations of PMIs (and crocetane) are all quite low, particularly compared to *sn*-3-hydroxyarchaeol, but the extreme ^{13}C depletion (as much as −124‰, PMIs are the most ^{13}C-depleted compounds in sediments of Kazan mud volcano, Table 2) unambiguously indicates an origin from archaea involved in AOM. Indeed, the isotopic compositions of PMIs and crocetane attributed to anaerobic methane-oxidizing archaea in other systems such as Hydrate Ridge (Elvert et al., 2001) were found to be quite similar to those in the present study.

It should also be noted that the carbon isotope compositions of the different PMI isomers range from −43‰ to −124‰, varying by as much as 50‰ in a single sample (−70‰ to −124‰, Table 2) and the C-isotope composition of individual PMIs varies by as much as 70‰ over the interval studied (PMI:3c, Table 2). Some of this variation could be attributable to the small concentrations of these compounds that were at the detection limits of the available instrumentation for both concentration and C-isotopic determinations, and consequently have larger analytical uncertainties associated with the measurements. It is possible that the heterogeneity in PMI distributions and δ^{13}C values result from variability in the AOM microbial community with depth, however, given the low concentrations, we are only able to speculate.

3.4. Archaea-derived GDGTs

Isoprenoid glycerol dialkyl glycerol tetraethers (GDGTs) have been identified as widespread in marine sediments (Schouten et al., 2000). These compounds occur widespread in archaea and their structural variability provides an opportunity to utilize the distribution of GDGTs in varying environments as markers of the particular archaea present (Hopmans et al., 2000). Recent studies have identified a series of GDGTs containing 0–3 cyclopentane rings in methane cold-seep sediments, which was proposed to be derived predominantly from anaerobic methane-oxidizing archaea (Pancost et al., 2001a; Aloisi et al., 2002; Schouten et al., 2003a; Stadnitskaia et al., 2003). A similar suite of GDGTs was recently found in the deep water masses of the Black Sea and also attributed to archaea involved in AOM (Wakeham et al., 2003). GDGTs identified in Kazan mud volcano sediments include crenarchaeol (**IX**), which has been attributed to pelagic, non-thermophilic, likely chemoautotrophic archaea (Crenarchaeota) (Schouten et al., 2000; Sinninghe Damsté et al., 2002a,b; Wuchter et al., 2003; Powers et al., 2004) and GDGT-0 (a GDGT with no cyclopentyl moieties, **X**), which

occurs much more widespread in archaea (Schouten et al., 2000; Sinninghe Damsté et al., 2002a). We have identified four other cyclopentane-containing GDGTs (GDGT-1, **XI**; GDGT-2, **XII**; GDGT-3, **XIII**; and GDGT-4, **XIV**, number indicates number of cyclopentyl moities), which may originate from thermophilic archaea (De Rosa and Gambacorta, 1988; Sprott et al., 1997), archaea involved in AOM (Pancost et al., 2001a; Wakeham et al., 2003), and/or non-thermophilic crenarchaeota (Sinninghe Damsté et al., 2002a,b). These GDGTs are generally in low abundance in non-thermophilic crenarchaeota (Sinninghe Damsté et al., 2002a,b) compared to crenarchaeol and GDGT-0, whereas in archaea involved in AOM the GDGTs with cyclopentyl moieties (especially GDGT-1 and GDGT-2) occur in approximately the same abundance as GDGT-0 (Pancost et al., 2001a,b; Wakeham et al., 2003).

Crenarchaeol and GDGT-0 are the most abundant biomarkers in surface sediments of Kazan mud volcano with concentration of almost 1 $\mu g\,g^{-1}$ sediment, and both exhibit down-core trends of decreasing concentration (Fig. 4(a)). Their concentrations are substantially lower than that of *sn*-3-hydroxyarchaeol in the zone of AOM. In fact, crenarchaeol goes from the most abundant compound in the extractable OM in the surface layer to the least abundant GDGT in deeper layers. The other GDGTs identified appear to display no clear trends in concentration with depth (Table 2); however, when summed, their cumulative concentration shows a trend of decreasing with depth (Fig. 4(a)). The fact that all GDGTs identified have trends of decreasing concentration with depth suggests that a major source of these compounds is the overlying water column, rather than sedimentary processes such as AOM. Given the lack of pelagic sediments overlying the mud breccia, this seems contradictory, however, it is possible that bioturbation has mixed the upper centimeters of mud breccia with any pelagic sediments deposited in the past ~100 years (thought to be the time of emplacement, Werne et al., 2004), thereby mixing in water column-derived GDGTs.

We can also use the fractional abundance (i.e., individual GDGT as a fraction of the cumulative abundance of the 6 discussed) of these compounds to determine whether they are likely to be derived from the same source. Based on the depth trends of relative abundance (Fig. 4(b)), crenarchaeol appears to have primary contributions from the surface sediments or pelagic marine environment only, though as mentioned previously, this interpretation is difficult to reconcile with the lack of pelagic sedimentation above the mud breccia flows unless we invoke bioturbation. Given the stability of GDGTs (e.g. Schouten et al., 2003a,b) we cannot rule out the possibility that crenarchaeol is simply transported from deeper down in the sediment with the extruding mud breccia, however, the expected profile would not have a surface maximum (cf. *n*-alkane profiles).

GDGT-0 exhibits no systematic variation in its fractional abundance with depth (Fig. 4(b)). Given that this biomarker occurs widespread in archaea, it is likely that it has contributions from multiple sources, including pelagic archaea, AOM-related archaea, and mud-matrix associated organic matter. GDGTs 1–4 all increase in

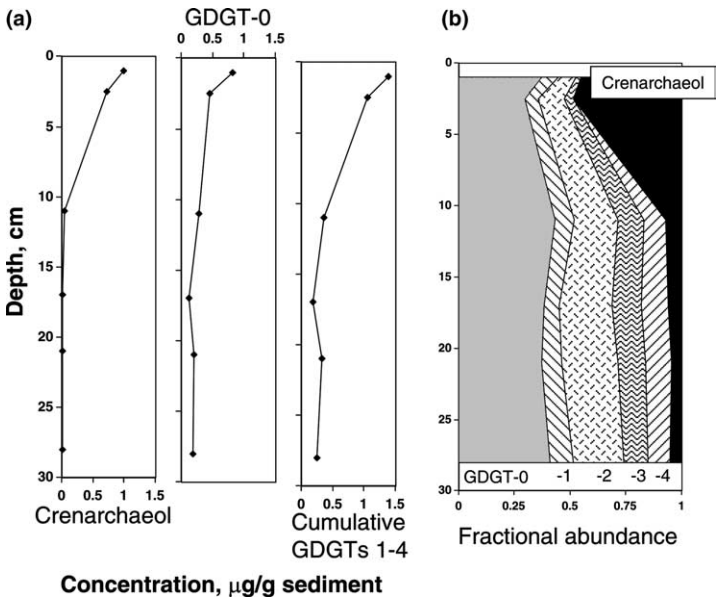

Fig. 4. (a) Depth profiles of concentrations of crenarchaeol, GDGT-0, and the sum of GDGTs 1–4, showing down core decrease in concentrations. (b) Relative abundance of all GDGTs measured in Kazan mud volcano sediments, showing an increase in relative importance of cyclopentane-containing GDGTs with depth.

their relative abundance with depth (Fig. 4(b)), suggesting that their contributions may be related, at least in part, to methane-oxidizing archaea. We cannot rule out the possibility that the GDGTs are associated with the ascending mud matrix, and the observed trend in relative abundance is simply an artifact of the trend in crenarchaeol (which has a much larger abundance, though that did not seem to be the dominant control on GDGT-0); however, GDGT distributions below 10 cm are inconsistent with a dominant origin from non-thermophilic crenarchaeota because crenarchaeol typically constitutes 30–70% of all GDGTs in these organisms (Schouten et al., 2002), and in these samples it is <10% (Fig. 5).

The carbon isotope compositions of the C_{40} isoprenoids (biphytanes) released from the GDGTs by ether cleavage should indicate whether these compounds are derived from archaea consuming methane. Biphytanes with 0, 1, and 2 cyclopentane rings were identified, which can be derived from GDGTs of both methane-oxidizing archaea as well as planktonic archaea (crenarchaea, cf. Schouten et al., 1998), as well as a biphytane with 2 cyclopentanes and a cyclohexane ring (i.e., derived from crenarchaeol, biphytane-3). This suite of biphytanes is expected given the distribution of GDGTs. The carbon isotope composition of biphytane-3 is −22‰, which is in fact the most ^{13}C enriched value of

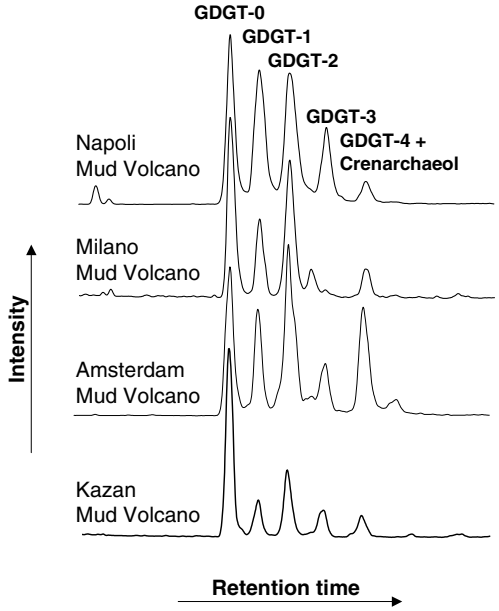

Fig. 5. HPLC/MS base peak chromatograms of GDGTs from multiple cold seep sites in the eastern Mediterranean Sea. Kazan mud volcano sample from 20 to 22 cm sediment depth. Note variations in relative abundances between different mud volcanoes. GDGT-4 and crenarchaeol co-elute, but relative inputs were determined via partial ion chromatograms. Data from Napoli, Milano, and Amsterdam mud volcanos from Pancost et al., 2001a.

any compound analyzed, indicating an origin from marine, chemooautotrophic crenarchaeota (Hoefs et al., 1997; Wuchter et al., 2003). None of the biphytanes released have a $\delta^{13}C$ more negative than −35‰ (Table 2), despite the fact that the acyclic, monocyclic, and bicyclic biphytanes (biphytane 0, 1, and 2, respectively) were found to be ^{13}C depleted in studies in other eastern Mediterranean cold-seep sites (−78‰, Pancost et al., 2001a; below −90‰, Aloisi et al., 2002) as well as in Black Sea water-column particulates (up to −58‰; Schouten et al., 2001; Wakeham et al., 2003) and carbonates (up to −100‰, Aloisi et al., 2002; up to −97‰, Thiel et al., 2001). These biphytanes do trend slightly more negative in deeper samples of Kazan Mud Volcano, but the variation is only ~5–10‰ (Table 2).

The most ^{13}C-depleted biphytane, at −35‰, is the acyclic moiety that is derived from GDGT-0 and GDGT-1, both of which could be derived in part from AOM-related archaea. Isotopic mass balance calculations suggest that at most ~20% of acyclic biphytane, 15% of monocyclic biphytane, and 10% of the bicyclic biphytane could be derived from AOM-related archaea. It should be noted that these data were difficult to obtain (biphytanes released were at the limits of detection), and therefore have larger uncertainties associated with them, so the mass balance approach is only approximately valid. It is perhaps more important that the GDGT distribution is neither consistent with a predominant origin from non-thermophilic crenarchaeota (Schouten et al., 2002; Sinninghe Damsté et al., 2002a,b; Powers et al., 2004) nor with AOM-related Archaea (e.g., Pancost et al., 2001a). Thus, the mixing of smaller amounts of AOM-derived GDGTs with larger amounts of "background" GDGTs (e.g., associated with both the ascending mud matrix and possibly pelagic deposition from the overlying water column) effectively masks the isotopic signal of AOM in this suite of biomarkers.

These results indicate a reduced contribution of AOM-derived biphytanes to the sediments compared to other environments. The sediments investigated by Pancost et al. (2001a), Thiel et al. (2001) and Aloisi et al. (2002) that had very ^{13}C-depleted biphytanes were primarily taken from actively seeping sites and carbonate crusts (believed to be formed as a by-product of extensive AOM, Aloisi et al., 2000, 2002). One hydrothermal vent site characterized by AOM also contained ^{13}C-depleted biphytanes (Schouten et al., 2003a). Conversely, biphytanes released from sediments of the Kattegat Strait, an environment of low but measurable AOM, showed $\delta^{13}C$ values similar to those of the present study (~−27‰; Bian et al., 2001). Thus, it is likely that the mud breccia sampled in the present study did not have sufficient methane oxidation rates to produce GDGTs in quantities that could "overwhelm" the mud matrix associated signal. This interpretation is consistent with findings of Pancost et al. (2001a) who

found GDGTs on Napoli mud volcano (eastern Mediterranean) in varying distribution, suggesting dilution from non-AOM sources (though the study did not report $\delta^{13}C$ values for biphytanes).

It is also possible that there are different groups of archaea carrying out AOM in these different cold-seep environments, and that only some of them synthesize these GDGTs. Indeed, the relative distributions of GDGTs differ substantially between different eastern Mediterranean mud volcano cold-seep sites (cf. Fig. 5; Pancost et al., 2001a, this study), suggesting that site-specific variability has some effect, if not on the specific archaeal methane-oxidizers present then at least on their lipid distributions. Given the data, the former interpretation seems more likely, but we cannot rule out this second possible explanation.

3.5. Markers of syntrophic sulfate reducing bacteria

A number of molecular isotopic studies have identified various isotopically depleted biomarkers that were attributed to a syntrophic bacterial partner (likely a sulfate reducer), including certain *n*-alcohols (Elvert et al., 2000, 2001), fatty acids (Hinrichs et al., 2000; Pancost et al., 2000; Thiel et al., 2001; Zhang et al., 2002, 2003; Elvert et al., 2003), non-isoprenoidal monoethers (Hinrichs et al., 2000), and non-isoprenoidal dialkyl glycerol diethers (Hinrichs et al., 2000; Pancost et al., 2001b; Werne et al., 2002). In each of these studies, the SRB biomarkers were found to be ^{13}C depleted, though not to the extent of the archaeal methane-oxidizer biomarkers.

Of particular interest are the non-isoprenoidal dialkyl glycerol diethers that were first identified by Hinrichs et al. (2000) and attributed to syntrophic SRB by Pancost et al. (2001b). These novel compounds were identified as one of two suites of compounds (the other suite was archaeal isoprenoid diethers such as archaeol and hydroxyarchaeol) identified in an authigenic carbonate crust associated with a methane cold-seep (Pancost et al., 2001b). Parallel 16S rRNA gene surveys on the same carbonate samples revealed only two major groups of microbes, both previously unknown, one related to known methanogens and suspected methane-oxidizing archaea and one related to known SRB (S.K. Heijs, unpublished data; Pancost et al., 2001b; Aloisi et al., 2002). Sediments from Kazan mud volcano contain compounds similar to series II compounds of Pancost et al. (2001b) (compounds **V**, **VI**, **VII**, and **VIII**,), some of which were previously described in Werne et al. (2002). The additional compounds included here show very similar depth trends, with C-isotope compositions ranging from −61.5‰ to −94.5‰ (Table 2). It is interesting to note that at one specific depth (16–18 cm) the isotope compositions of the different diethers are not distinguishable (Table 2). This horizon is the same one as the maximum concentration of *sn*-3-hydroxyarchaeol.

3.6. Hopanoids

Hopanoid compounds occur ubiquitously in sediments, and have been well documented as biomarkers for bacteria (Ourisson and Rohmer, 1992). Hopanoids of various types have been identified in cold-seep deposits, includng the C_{30} compounds hop-17(21)-ene (Thiel et al., 1999) and hop-22(29)-ene (diploptene, **XV**; Elvert et al., 2000; Thiel et al., 2001; Werne et al., 2002). The occurrence and carbon isotope composition of diploptene and its precursor biosynthetic hopanol, diplopterol (**XVI**), in Kazan mud volcano sediments have been discussed by Werne et al. (2002), where they were attributed to aerobic methanotrophic bacteria.

A C_{32} hopanoid compound, 17β, 21β(H)-bishomohopanol (**XVII**), has also been identified in sediments of Kazan mud volcano, as well as in other seep environments (Pancost et al., 2000; Pancost and Sinninghe Damsté, 2003; Elvert et al., 2001). Its concentration profile closely matches that of diplopterol in Kazan mud breccia sediments, decreasing from 0.22 to 0.16 μg/gsediment in the upper 3 cm of sediment, below which it is not detectable (Table 2). This profile suggests some dependence on oxygen diffusing from the overlying water column, however, hopanoid compounds were also identified in samples from cold seeps in the anoxic zone of the Black Sea (Thiel et al., 2003), so a source of hopanoids from anaerobic bacteria cannot be ruled out (Pancost et al., 2000). Indeed, Sinninghe Damsté et al. (2004) recently reported hopanoids in strictly anaerobic planctomycetes, bacteria capable of anaerobic ammonium oxidation. The carbon isotope composition of β,β-bishomohopanol is offset from diplopterol by 10‰, ranging from −45.7‰ to −52.7‰, clearly indicating a relationship to methane-derived carbon such as would be expected from chemoautotrophic bacteria utilizing methane-derived CO_2.

Pancost and Sinninghe Damsté (2003) speculated that this compound in such environments could be attributed to aerobic sulfide-oxidizing bacteria such as *Beggiatoa*. Most-probable number counts suggest the presence of colorless sulfur bacteria that could be sulfide-oxidizers in sediments from Kazan mud volcano (Werne et al., 2004) and "white patches" of bacteria were observed that could be *Beggiatoa* (Werne et al., 2004). Though pore water H_2S concentrations remain below detection limits until ~8 cm below the sea floor (Haese et al., 2003), bioirrigation is believed to provide oxygen to similar depths (~7 cm, Haese et al., 2003). Furthermore, sulfide-oxidizing bacteria were identified in sediments from other Mediterranean mud volcanoes (Pancost et al., 2000). Thus, a sulfide-oxidizing bacterial source is one possible interpretation; however, hopanoids have not been proven to derive from *Beggiatoa*, and given the diversity of bacterial species present in methane-rich environments (Lanoil et al., 2001) other sources of β,β-bishomohopanol in such environments certainly are possible.

3.7. Implications for cold-seep microbial community variability

The biomarkers identified in this study and their carbon isotope compositions provide evidence of a diverse microbial community carrying out the net process of methane oxidation in mud breccia sediments of Kazan mud volcano. This community contains at least anaerobic methane-oxidizing archaea and sulfate-reducing bacteria, and possibly aerobic methane-oxidizing bacteria and sulfide-oxidizing bacteria. The depth profiles indicate significant variations in the distribution of this microbial community on the centimeter scale. Comparison of this system with other methane-seep environments reveals substantial differences in the biomarker distributions and carbon isotope compositions in different systems.

In these active cold-seep systems, the biomarker distributions appear to be dominated by certain facets of the total distribution identified in Kazan sediments. For example, Hydrate Ridge and the Aleutian Trench systems are dominated by PMIs and crocetanes (Elvert et al., 1999, 2000, 2001), as are Black Sea carbonates (Thiel et al., 2001) and an ancient methane vent associated carbonate (Thiel et al., 1999). The Eel River basin (Hinrichs et al., 1999, 2000) and other more active mud volcanoes from the eastern Mediterranean (Pancost et al., 2000, 2001a) were dominated by diethers such as archaeol and hydroxyarchaeol. In contrast, the molecular isotopic signals from the mud breccia sediments of Kazan mud volcano reveal dominance by mud-matrix-associated organic matter, and secondarily signals from methane-oxidation related processes, including archaeal and various bacterial signals.

Such significant variation in the biomarker distributions and the relative carbon isotopic signatures of these different environments suggests one of two things. Either the specific communities involved in carrying out the net oxidation of methane in the anaerobic environment vary from system to system based on geochemical conditions, or the microbial communities in each system are the same, but change their cellular composition in different systems based on local geochemical conditions. Various studies have demonstrated that growth conditions do influence the specific cellular compound distributions in archaea (Morth and Tindall, 1985), so this explanation is certainly a possibility. Support for the alternative hypothesis, microbial community variability, comes from recent studies utilizing molecular phylogenetic surveys that have increasingly been identifying multiple archaeal lineages (e.g., the ANME-1 and –2 clusters) associated with AOM (e.g. Orphan et al., 2001a,b; Thomsen et al., 2001). Furthermore, associated with the diverse archaeal phylotypes being identified, genetic sequences of a number of different SRB believed to be the syntrophic partner are being identified in seep systems.

4. Conclusions

The molecular isotopic signature of mud breccia sediments on Kazan mud volcano indicates diverse sources of organic matter, including both the present-day microbial community (associated with AOM) and "fossil" organic matter associated with the ascending mud matrix. The microbial community related with AOM on Kazan mud volcano is shown to be diverse based on biomarkers, showing a great diversity in both distribution and carbon isotopic composition even within a few centimeters. Several overlapping microbial communities are evident from the concentration and carbon isotopic profiles of the various biomarkers studied. The variability within certain compound classes, such as the PMIs, suggests that there are many processes and organisms related to AOM that remain poorly constrained.

The distribution of GDGTs, and the carbon isotopic composition of GDGT-derived biphytanes indicate that AOM in this environment is not strongly expressed in the organic geochemical record. This observation is likely related to both lowered activity of AOM related microbes (associated with a low methane flux compared with other seep sites). It is also possible that the lack of a distinct AOM signal in the GDGT-derived biphytanes reflects the presence of AOM-related archaea that do not produce this suite of biomarkers in high abundance, but this is speculation.

The differences between the molecular isotopic signature of this site and other seep sites indicate either that there are a number of different archaeal species that are capable of oxidizing methane anaerobically, or that these archaea synthesize different lipids depending on their geochemical environment.

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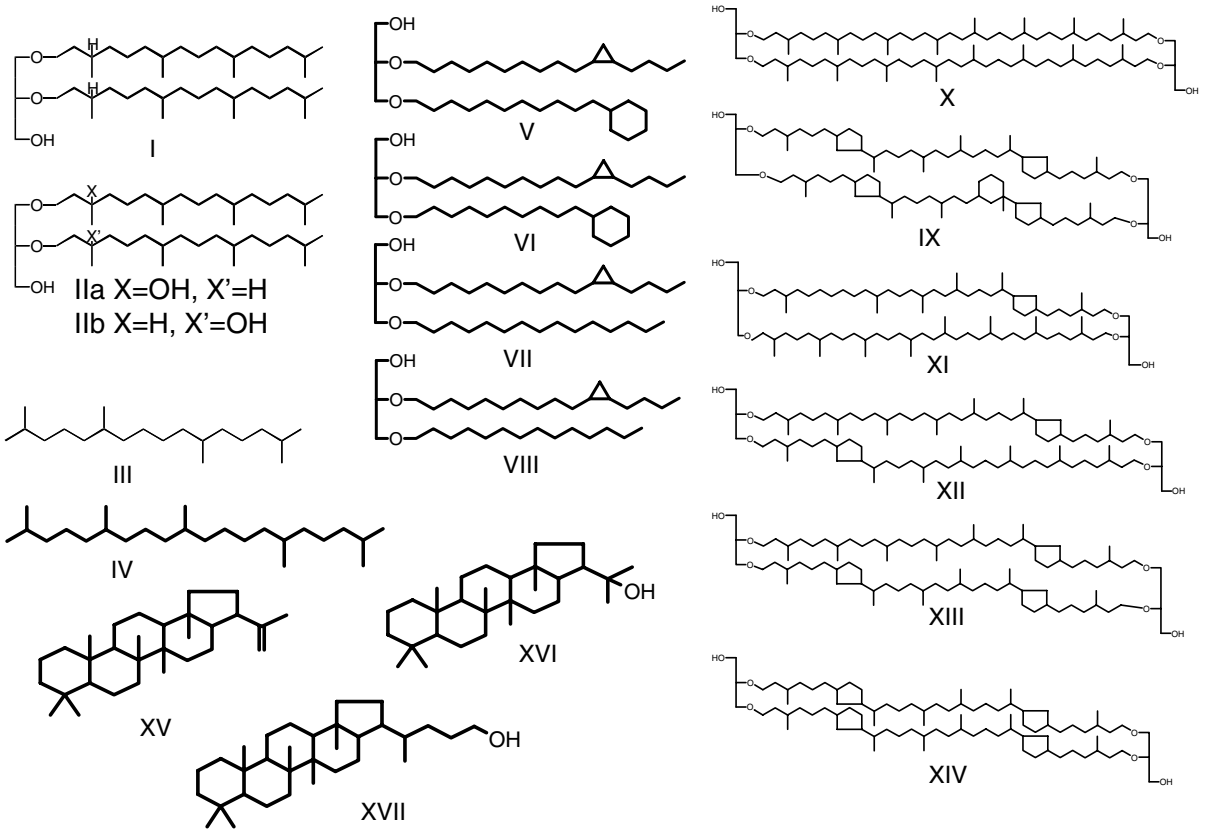
Appendix A

Appendix: Biomarker Structures



Associate Editor—**Lorenz Schwark**

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