Methane Index: A tetraether archaeal lipid biomarker indicator for detecting the instability of marine gas hydrates

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\textbf{ABSTRACT}

Gas hydrates represent one of the largest pools of readily exchangeable carbon on Earth’s surface. Releases of the greenhouse gas methane from hydrates are proposed to be responsible for climate change at numerous events in geological history. Many of these inferred events, however, were based on carbonate carbon isotopes which are susceptible to diagenetic alterations. Here we propose a molecular fossil proxy, i.e., the “Methane Index (MI)”, to detect and document the destabilization and dissociation of marine gas hydrates. MI consists of the relative distribution of glycerol dibiphytanyl glycerol tetraethers (GDGTs), the core membrane lipids of archaea. The rational behind MI is that in hydrate-impacted environments, the pool of archaeal tetraether lipids is dominated by GDGT-1, -2 and -3 due to the large contribution of signals from the methanotrophic archaeal community. Our study in the Gulf of Mexico cold-seep sediments demonstrates a correlation between MI and the compound-specific carbon isotope of GDGTs, which is strong evidence supporting the MI-methane consumption relationship. Preliminary applications of MI in a number of hydrate-impacted and/or methane-rich environments show diagnostic MI values, corroborating the idea that MI may serve as a robust indicator for hydrate dissociation that is useful for studies of global carbon cycling and paleoclimate change.

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1. Introduction

In marine sediments, under a delicate balance of temperature and hydrostatic pressure, methane can be stored within rigid cages of water molecules, forming the ice-like crystalline deposits called gas hydrates (Hester and Brewer, 2009; Kvenvolden, 1993). The estimated carbon stored in marine gas hydrate reservoirs is 5000 to 10,000 Gt (1 Gt = 10\textsuperscript{15} g) as CH\textsubscript{4}, which constitutes the largest pool of readily exchangeable carbon on Earth’s surface (Archer, 2007; Hester and Brewer, 2009; Kvenvolden, 1993). Even a small instability in the boundary conditions affecting this reservoir could potentially add a significant amount of methane into the oceans and atmosphere, resulting in profound global carbon cycle perturbations and climate change (Dickens, 2003). For instance, Dickens et al. (1995) suggested the Paleocene–Eocene thermal maximum event might have been a consequence of more than two thousand Gt methane catastrophically released from marine gas hydrates. After this pioneering work, the importance of gas hydrates has been increasingly recognized. For example, the “Clathrate Gun Hypothesis” has been proposed for the millennial-scale climate changes of the late Quaternary, based on the \textdelta^{13}C of foraminiferal shell carbonates (Kennett et al., 2000). However, this study was met with criticism primarily concentrated on the usage of carbonate carbon isotopes as an indicator of methane release: vital effects and diagenesis may distort the isotopic signals preserved in marine carbonates (Cannariato and Stott, 2004; Stott et al., 2002). The majority of currently inferred hydrate-dissociation events are either based on carbonate carbon isotopes (e.g. Jiang et al., 2003; Kennett et al., 2000) or conceptual models (e.g. Dickens et al., 1995). More direct evidence for methane-hydrate dissociation events was provided by a few biomarker-based studies that largely focused on aerobic methanotrophs (Hinrichs, 2001; Hinrichs et al., 2003; Ménot and Bard, 2010; Uchida et al., 2004). Since the respective aerobic methanotrophy presumably took place in an oxygenated, methane-laden water column, the respective signals are only preserved in the sedimentary record under exceptional conditions (e.g. those involving rapid sedimentation and/or low oxygen concentrations). Therefore, a more widely applicable, complementary proxy is needed to unambiguously evaluate the stability of gas hydrate reservoirs and to test the relationships between “hydrate-forcing” and climate change in the geological past.
Up to 90% of the methane produced in marine sediments and waters is being oxidized anaerobically (Barnes and Goldberg, 1976; Reeburgh, 1976; Valentine and Reeburgh, 2000). The anaerobic oxidation of methane (AOM) is mediated by a consortia of methanotrophic archaea (ANME groups) and sulfate-reducing bacteria (Boetius et al., 2000; Hinrichs et al., 1999) through the reaction first proposed by Reeburgh in 1976 (Reeburgh, 1976):

$$\text{CH}_4 + \text{SO}_4^{2-} \rightarrow \text{HCO}_3^- + \text{HS}^- + \text{H}_2\text{O}$$ (1)

This implies a close linkage between methane release from gas hydrates and the microorganisms (e.g. archaea) that are directly involved in the consumption of methane.

A large number of methane-metabolizing archaea synthesize glycerol dibiphytanyl glycerol tetraethers (GDGTs) as their core membrane lipids (Gambacorta et al., 1995; Koga et al., 1993; Koga and Nakano, 2008; Rossel et al., 2008). The GDGTs are quite stable and resistant to oxidation or degradation in geological formations (c.f. Kuypers et al., 2001). Therefore, GDGTs preserved in sediments could serve as biomarkers archiving abundant paleo-biology, ecology and environmental information (Brocks and Pearson, 2005; Eglinton and Eglinton, 2008). Gas hydrate and/or methane-rich deep sea environments exhibit distinct GDGT distribution profiles, namely by the predominance of GDGT-1, -2 and -3 (GDGTs with 1–3 cyclopentane rings, respectively), which are contributed by methane-oxidizing archaea associated with the methane hydrates (Blumenberg et al., 2004; Pancost et al., 2001). Analyses of polar lipid distributions in taxonomically-characterized natural archaeal methanotrophic communities suggest that the ubiquitous GDGT-producing ANME-1 clade is a primary player in AOM systems (Rossel et al., 2008). This implies that these compounds could potentially be used as biomarkers for detecting periods of instability of marine gas hydrates.

The Gulf of Mexico (GOM, Fig. 1) represents a unique system for biogeochemical and microbiological studies of gas hydrates because of the wide occurrence of subsurface- and seafloor-breaching gas hydrates and cold seeps, as well as the presence of free gas venting, diverse microbial communities and authigenic carbonate platforms within the ~1.6 million km² gulf basin (Sassen et al., 2004; Zhang and Lanoil, 2004). Previous studies of lipids, stable isotopes and molecular biology have shown that the consortia of methanotrophic archaea and sulfate-reducing bacteria mediated-AOM in the GOM (Zhang and Lanoil, 2004). Because of the difficulties in locating and monitoring present-day gas hydrate destabilization and catastrophic release of methane events, we believe that cold-seep environments like the GOM where CH₄ is actively degassing from hydrate dissociation might be the best analogs for ancient hydrate instability, particularly in terms of the microbial community and their lipid profiles.

Here we report new geochemical and lipid biomarker studies in the sediment cores from the Gulf of Mexico and discuss them in the context of previously published results to explore the feasibility of using archaeal lipids as an indicator for the instability of marine gas hydrates in the geological past.

2. Materials and methods

2.1. Site description and sample collection

Sediment core samples were recovered from Mississippi Canyon Block 118 (MC 118), located on the northeastern gulf slope (Fig. 1).
The MC 118 gas hydrate site was discovered by the Johnson Sea-Link research submersible in 2002. Free gases (C1–C3 hydrocarbons and minor CO2) are released from the sea floor to the water column at ~890 m depth where temperature is ~5.7 °C. This site is characterized by cratere depressions and mounds of authigenic carbonate rocks over an area of ~1 km². Abundant chemosynthetic communities like microbial mats (Beggiatoa), tube worms, mussels, and bivalves have developed around the gas vents (Sassen et al., 2006). A preliminary study by Sassen et al. (2006) showed that methane (94.4–96.5%) is the main component of the venting gas. Isotopic properties of CH4 (δ13C = −45.7%, δδD = −163‰) are consistent with a deep source, suggesting thermogenic origin of those gases. Ethane, propane, butanes and pentanes are the minor components. The δ13C of CO2 from vent gas is strongly enriched in 13C (as much as +25%), as is typical of a deeply buried source (Sassen et al., 2006).

The two gravity sediment cores used in this study, Core 08 (28°50′ 42.7943″N, 88°27′ 53.3108″W, 971 m water depth) and Core 10 (28°51′ 19.0349″N, 88°29′ 44.9651″W, 878 m water depth), were collected from MC 118 during a 2008 cruise by the R/V Pelican (Fig. 1). Core 08 is about 7 m in length and Core 10 is ~1.3 m in length. 16 samples and 14 samples were taken from Core 08 and Core 10, respectively. Core 08 is located off the hydrate mound and gas hydrate impact is minimal. Core 10 is on the southwest side of the hydrate mound, which is thought to be impacted by gas hydrate directly. The lithology is similar to previous samples collected from MC 118. In general, all cores are dominated by mud (particle size <0.063 mm) and contained less than 10% sand (particle size ranging from 2 to 0.063 mm) in most intervals (Pi et al., 2009). Samples were stored in a −20 °C freezer onboard and −80 °C in the laboratory until analysis.

2.2. Inorganic geochemistry

Sediment porewaters were extracted and analyzed for sulfate and sulfide concentrations by a portable spectrophotometer called Hach Kits according to manufacturer's instructions. After extraction for porewater, solid samples were freeze-dried and ~2 g of sediment samples were used for analysis of stable carbon and oxygen isotopes of the carbonate minerals. Samples were subject to δ18O and δ13C measurements on a MAT 252 isotope ratio mass spectrometer (IRMS) with a Kiel automated carbonate device at Tongji University, Shanghai. Standard isotopic corrections were made and the oxygen and carbon isotopes were reported relative to the VPDB scale. The precision (1σ) is ≤0.04‰ for δ13C and ≤0.07‰ for δ18O.

2.3. Archaeal tetraether lipid extraction and analysis

About 5 g lyophilized samples were used for lipid analysis. Total lipid extraction was performed following a modified Bligh/Dyer procedure (e.g. Zhang et al., 2006), which uses a single-phase organic solvent system comprised of methanol, chloroform and aqueous 50 mM phosphate buffer (pH = 7.4) in the ratio of 1:1:0.8 (v:v:v). After about 12 h, equal volumes of chloroform and nano-pure water were added, forming a double-phase system. The organic phase was collected and reduced in volume under pure N2 gas.

Total lipids were transesterified in 2 ml of methanol and hydrochloric acid (95:5, v/v) in a heating block at 70 °C for 2 h to hydrolyze polar side-chains of GDGTs. The transesterified lipids were passed through a Varian Bond Elut C-18 solid phase extraction column using different solvents. Four fractions were eluted: Polar I, Polar II, GDGT and neutral lipids by 2 ml of 3:1 acetonitrile/ethanol, 1:1 acetonitrile/ethanol, 1:3 ethyl acetate/hexane, and 1:10 ethyl acetate/hexane, respectively. The GDGT fraction was eluted with 1:3 ethyl acetate/hexane and then evaporated under an N2 gas stream. Extracted GDGT fraction was separated into two aliquots, one aliquot was used for liquid chromatography-mass spectrometry (LC-MS) analysis and the other one was used for ether cleavage experiments and compound specific isotope analysis.

High performance liquid chromatography-atmospheric pressure chemical ionization – mass spectrometry (HPLC/APCI-MS) analyses were performed at the University of Bremen. Samples were dissolved in hexane/isopropanol (99:1, v/v) for injection. Analysis of GDGT core lipids followed a slightly modified method according to Hopmans et al. (2000). Separation of GDGTs was achieved on an Ecosphere NH2 column (250 × 4.6 mm, Alltech, Germany) heated to 30 °C in a Thermo Finnigan System HPLC system. The following gradient was used at a 1 mL/min flow rate: held isocratically at 99:1 hexane/isopropanol (v/v) for 5 min, then ramp to 98:2:1.8 hexane/isopropanol (v/v) at 45 min, followed by flushing with 95:5 hexane/isopropanol (v/v) for 10 min and equilibrating with 99:1 hexane/isopropanol (v/v) for 10 min to prepare the system for the next injection. Mass spectrometric identification and quantification were applied on a Thermo Finnigan LCQ Deca XP Plus ion trap mass spectrometer coupled to the HPLC by an APCI interface. APCI settings were as follows: capillary temperature 200 °C, source heater temperature 400 °C, sheath gas flow 30 arbitrary units, source current 5 μA, while other parameters were optimized by manual tuning during infusion of a hydrolyzed commercially available intact GDGT standard (Matreya, USA). Samples were analyzed using selected ion scans, from 0 to 5.5 min at m/z 600–700 and from 5.5 to 45 min at m/z 1200–1500. Calculation of relative ring distribution was based on peak intensities in mass chromatograms of the [M+H]+ ions (m/z 1302, 1300, 1298, 1296, 1292).

2.4. Ether cleavage and compound-specific carbon isotopes

Samples from the gas hydrate-impacted core (Core 10) and hydrate-free core (Core 08) were selected for ether cleavage experiments so that the released isoprenoidal hydrocarbons can be analyzed for compound-specific carbon isotopes on gas chromatography – isotopic ratio mass spectrometry (GC-IRMS). The biphytane moieties of tetraether lipids were prepared for GC-based analysis according to a published protocol (Jahn et al., 2004) with slight modification. Samples were treated with 1 ml of 1 M BBF3 in dry DCM at 60 °C for 2 h under an atmosphere of argon. Then, after drying under a stream of argon, the samples were amended with 1 ml of 1 M lithium triethylborohydride in tetrahydrofuran and kept at 60 °C for another 2 h. The reaction was quenched by adding a few drops of Milli-Q water, and the products were extracted into hexane. The hydrocarbons were purified using a self-packed silica gel column with hexane as eluent.

For gas chromatography the oven temperature was set at 60 °C upon injection, held for 1 min, raised to 150 °C at 10 °C for 1 min, further increased to 320 °C at 4 °C for 1 min, and held isothermal for 22.5 min. The CO2 from the combustion of the isoprenoidal hydrocarbons was introduced to a Delta Plus XP IRMS via a combustion interface (Thermo Finnigan GmbH, Germany). The injector temperature was set at 310 °C. Separation of biphytanes was achieved using an Rxi-5 ms column (30 m × 0.25 mm, 0.25 μm film thickness; Restek GmbH, Germany). Helium was used as the carrier gas with a flow rate of 1.1 mL/min. Standardization of carbon isotope analyses is based on multiple injections of reference CO2 at the beginning and the end of each analytical run. Duplicate analysis was applied for all reported samples with a precision of <1‰.

3. Results

3.1. Geochemistry Profile: Core 08 vs. Core 10

Both porewater chemistry and stable carbon isotopes of bulk carbonate reveal distinct sedimentary conditions for Core 08 and Core 10 (Fig. 2). In Core 08, the sulfate concentration in porewater exhibits a normal seawater signature, with hydrogen sulfide close to 0 in most...
of the samples. In Core 10, sulfate became fully depleted at 130 cm below seafloor (cmbsf, data not shown here). With the exception of a few samples close to the surface, hydrogen sulfide is high in Core 10, especially at depth greater than 20 cmbsf. The porewater chemistry characterizes Core 08 as a normal marine sedimentation and Core 10 as significantly enhanced in sulfate reduction and hydrogen sulfide generation. Additional verification of contrasting environments for Core 08 and Core 10 comes from carbon and oxygen isotopes from bulk carbonates. The δ¹³C of carbonates varies from −0.4 to 1.3‰ in Core 08 and varies from −28.1 to −1.2‰ in Core 10. The δ¹⁸O varies from −4.8 to 0.9‰ in Core 08 and −3.1 to 4.1‰ in Core 10 (Figs. 2 and 3). Previous studies show that in methane-rich environments both foraminiferal carbonate shells (e.g. Kennett et al., 2000) and authigenic carbonates (e.g. Peckmann and Thiel, 2004) are depleted in ¹³C, a signature inherited from the ¹³C-depleted CH₄. Limited variation in δ¹³C of carbonate with an average close to 0‰ in Core 08 implies normal marine conditions, whereas the deviations of δ¹³C to much lower values in Core 10 can be attributed to the contribution of ¹³C-depleted, methane-derived carbon to the dissolved inorganic carbon (DIC) pool (Fig. 2). In addition, an inverse linear correlation between δ¹⁸O and δ¹³C has been found in Core 10, but not Core 08 (Fig. 3). Methane-derived carbon would be ¹³C-depleted. Meanwhile, water released from the decomposition of gas hydrate would be ¹⁸O-enriched because just like ice, heavy isotopes of water will be more concentrated in the lattice of gas hydrates than in the liquid water from which they formed (Davidson et al., 1983).

### 3.2. GDGT distribution at MC 118

HPLC/APCI-MS analyses reveal distinct GDGT distributional patterns in Core 08 and Core 10 (see GDGT structures and typical LC-MS chromatogram in Fig. 4). In Core 08, crenarchaeol and GDGT-0 are the dominant tetraether lipids and experience smaller variance with depth, which is similar to the pattern reported in other marine environments (e.g. Schouten et al., 2000, 2002). Crenarchaeol, which is the most abundant GDGT in Core 08, varies from 49 to 57%. In Core 10, however, significant increase in the relative abundance of GDGT-1, -2 and -3 is observed, especially at greater depths in the core such that they become the predominant GDGTs (Fig. 2). Correspondingly, crenarchaeol decreases from 53% at the surface to 4% at the bottom.

### 3.3. Methane Index

In order to quantify the relative contribution of methanotrophic Euryarchaeota (presumably represented by GDGT-1, -2 and -3) vs. planktonic and possibly benthic Crenarchaeota (represented by crenarchaeol and its regioisomer which have been suggested to be the biomarker for Crenarchaeota, Sinnighe Damste et al., 2002) into the sediment GDGT pool, we define the Methane Index (MI) as

$$MI = \frac{[GDGT-1] + [GDGT-2] + [GDGT-3]}{[GDGT-1] + [GDGT-2] + [GDGT-3] + [Crenarchaeol] + [Cren_isomer]}$$

(2)

The value of MI ranges from 0 to 1. High MI values correspond to large production of GDGT-1, -2 and -3, which may indicate strong impact of AOM microbial communities. Low MI values show the predominance of non-methanotrophic marine Crenarchaeota, which
would characterize normal marine sedimentary conditions. It is important to notice that the cyclized GDGT-1, -2, -3 are of course not exclusively produced by methanotrophic Planktonic Crenarchaeota. Pure cultures of marine cosmopolitan Crenarchaeota “Candidatus Nitrosopumilus maritimus” clearly exhibit those GDGTs in their lipid profile (Schouten et al., 2008). However, this index emphasizes cases in which the presence of substantial methanotrophic Euryarchaeota in the marine environment is suggested by GDGT-1, -2 and -3 dominance over crenarchaeol in the GDGT pool. Situations like this cannot be explained exclusively by Crenarchaeota production, even in warm environments (see discussions below).

There is distinct difference between the calculated MI of normal marine Core 08 and hydrate-impacted Core 10 (Fig. 2). Core 08 has low MI of −0.2 and shows little variation with depth. However, Core 10 shows relatively higher MI values with a maximum of 0.94 at 121 cmbsf. The geochemical and lipid profiles suggest that in Core 10, the shallow samples have little AOM activity and an insignificant contribution from ANME groups, whereas vigorous AOM influences the deeper sediment layers.

3.4. Compound-specific stable isotopes: origin of MI changes

Compound-specific stable carbon isotopes of lipid biomarkers provide information relevant to carbon metabolism at the molecular level. Therefore, the δ13C analyses of selected samples from Core 08 and Core 10 help us to trace the carbon source, as well as to determine the origin of different GDGTs.

Cleavage of the ether bonds in GDGTs results in four biphytane molecules: 1) acyclic biphytane a, 2) monocyclic biphytane b, 3) bicyclic biphytane c, and 4) tricyclic biphytane d (Fig. 5). Biphytane a can be released from GDGT-0 and GDGT-1. Biphytane b has one cyclopentane ring and may originate from GDGT-1, GDGT-2 or GDGT-3. Assuming methanotrophic ANME Euryarchaeota preferentially synthesize those three GDGT compounds (Blumenberg et al., 2004; Pancost et al., 2001; Wakeham et al., 2003), the isotopic signature from biphytane b would be most sensitive to the presence of AOM. The two-ring structure in biphytane c is most likely associated with GDGT-3 and crenarchaeol. Finally, biphytane d can only be produced by crenarchaeol and its isomer, hence bearing the planktonic Crenarchaeota signature.

The δ13C of biphytane b, which acts as an indicator for ANME-1 Euryarchaeota, is compared with the δ13C of biphytane d which represents Crenarchaeota (Fig. 5). Three of the six samples were selected from Core 08 and the surface of Core 10, where gas hydrate impact is minimal; the other three samples are from greater depth in Core 10, where δ13C of carbonate indicates strong AOM activity. The δ13C of biphytane d varies from −17.2‰ to −22.2‰. Those values are comparable to previously reported values from normal marine environments. For example, the surface sediments collected from the Indian Ocean (Hoefs et al., 1997) suggest an origin from planktonic Crenarchaeota using DIC as carbon source (c.f. Hoefs et al., 1997; Konneke et al., 2005; Wuchter et al., 2006). In contrast, biphytane b varies in δ13C from -22‰ in hydrate-free samples to -96‰ in hydrate-impacted samples, indicating enhanced AOM activity in the latter case. The extremely depleted δ13C values from biphytane b confirm our assertion that the majority of GDGT-1, -2 and -3 are produced by methanotrophic Euryarchaeota with the presence of methane released from gas hydrates. Much higher δ13C of biphytane d from exactly the same samples demonstrate contributions from planktonic Crenarchaeota. The disparate isotopic signature strongly

![Fig. 4. HPLC/APCI-MS base peak chromatogram of two typical samples (a) and (b), from Core 08 (61 cmbsf) and Core 10 (121 cmbsf), respectively. Both records show the presence of GDGT-0 to -3, as well as crenarchaeol and its isomer, with the structures sketched in the right panel. Note that GDGT-1, -2 and -3 increase dramatically in (b) and become the predominant GDGTs.](image)

![Fig. 5. Compound-specific carbon isotopes of GDGT-derived biphytanes in six samples from Core 08 and Core 10. Sample names are given by core number and sample depth in cmbsf. The structures of biphytane a, b, c, d are also shown. Biphytane b would be most sensitive to methanotrophic ANME Euryarchaeota input, while biphytane d could only be derived from Crenarchaeota. Note that biphytane d is enriched in 13C and less variable in δ13C, whereas biphytane b is extremely depleted in 13C in the deeper depth of Core 10, suggesting binary sources of GDGTs in the AOM environments.](image)

![Fig. 6. Correlation between Methane Index and δ13C from biphytane b in six samples from both Core 08 and Core 10, MC 118, Gulf of Mexico. Linear regression gives y = −0.001232 + (−0.00971)x with R² = 0.998. The p-value (two-tailed) of this correlation coefficient is <0.0001.](image)
suggests binary sources of GDGTs presented in the same sample, confirms that MI may be a valid measure of planktonic Crenarchaeota vs. methanotrophic Euryarchaeota contributions (Fig. 5). In addition, for the analyzed set of Gulf of Mexico samples, a linear relationship between their MI and the δ13C value of biphytane b is apparent (Fig. 6), indicating that both variables can be used to evaluate the relative contributions of methanotrophic vs. planktonic archaeal biomasses.

4. Discussions

4.1. Rationale of MI and comparison with TEX86

To explain elevated MI, we invoke abundant methane released from gas hydrates enhancing the activity of methanotrophic archaea, which provide the majority of GDGT-1, -2 and 3. Other environmental factors like temperature, however, have also been shown to impact GDGT distributions in marine environments. For example, Schouten et al. (2000, 2002) report elevated abundance of cyclized GDGTs in warm seawater like the Arabian Sea (Fig. 7B) relative to cold waters like the North Sea and Antarctica (Fig. 7A). Observations like this gave rise to the proposal of the TEX86 index, which is a sea surface temperature (SST) proxy shown to be a powerful tool in paleoclimate studies (Schouten et al., 2002). The temperature-dependence of GDGT distributions has been explained primarily as a physiological property of planktonic Crenarchaeota (Schouten et al., 2002). Nonetheless, in a gas-hydrate-impacted environment like one shown in Fig. 7C, GDGT-1, -2 and -3 are so abundant that the GDGT distribution pattern can no longer be explained by temperature-induced physiological responses. Rather, it suggests ecological shifts from planktonic Crenarchaeota dominance to a much higher portion of methanotrophic Euryarchaeota in terms of contribution to sedimentary tetraether lipid pools. In fact, as outlined by Schouten et al. (2002), high rates of AOM have already been marked as restrictions for the usage of TEX86.

To better calibrate the SST proxy of TEX86, Kim et al. (2010) recently reported a large dataset comprised of surface sediment archaeal lipid measurements and satellite temperature from all major ocean basins (n = 426). This “normal marine” dataset facilitates a detailed comparison of MI and TEX86 with different temperatures to assess potential temperature influences on MI (Fig. 8). Changing TEX86 readily correlates with SST. MI, to a much lesser extent, also increases with higher SSTs, consistent with the observations of Schouten et al. (2000, 2002) that more cyclized GDGTs exist in warmer waters. However, even in the warmest SST site of the dataset (30 °C) the MI never exceeds 0.3, whereas TEX86 increases to 0.9 at this temperature. Extrapolating the MI – temperature relationship derived here to a super greenhouse climate in Earth’s geological history like the Cretaceous, a hypothetical 40 °C SST would only yield a MI value of 0.26. One possible explanation for this observation is that the slight temperature dependence of MI may be induced by planktonic Crenarchaeota, the same archaea group used for TEX86, without involving methanotrophic Euryarchaeota. In Core 10 of MC118, GOM we find MI values as high as 0.94 (Fig. 2), which suggests that temperature influence is secondary and the presence of methanotrophic communities should largely be responsible for high MI values. In other words, TEX86 is an index that arguably measures the responses to environmental factors of the same group of archaea (Marine Group I) without involving major shifts in archaeal community composition; MI is an index that shows excess lipid contributions from different groups of archaea responding to the presence of methane.

4.2. Applicability of MI

To see how this Methane Index could be applied globally we first compiled GDGT data (Table 1) for marine gas-hydrate-impacted/methane-rich sites where molecular distributions are reported in literatures and available for calculation of the MI. At all sites, hydrate/methane impacts are identified by high MI values (>0.85), with their highest values of MI listed in Table 1. MI values from those places with possible AOM based on multiple lines of evidence given in Table 1, are also plotted in the histogram of Fig. 9b, which is...
compared to the “normal marine” sites using the Kim et al. (2010) global core-top dataset. The distribution of 426 samples shows that MI values vary within the range from 0 to 0.3 in normal marine conditions, with the majority between 0.03-0.24 (Fig. 9a). In contrast, samples that are possibly impacted by AOM have their MI value covering a much broader range, from close to 0 to near 1 (Fig. 9b). The lower values in Fig. 9b are likely from the oxic surface of the sediment/water column, whereas the higher values clearly show the presence of AOM.

The currently available data for both GDGT distribution and compound-specific carbon isotopes are also combined with our MC 118 data to further test the MI with isotopic insights (Fig. 10). Data used are from a variety of sample locations that include suspended particulate organic matter from seawater (Wakeham et al., 2003, 2004), sediments (Pancost et al., 2001, 2008) and authigenic carbonates (Bouloubassi et al., 2006). The data points are more scattered than at MC 118 since different habitats have different archaeal communities with different carbon isotope values of the

Table 1
Available archaeal lipid biomarkers studies in gas-hydrate-impacted and/or methane-rich marine environments and calculated Methane Index values.

<table>
<thead>
<tr>
<th>Location</th>
<th>Nature of Sample</th>
<th>Evidence of AOM</th>
<th>Highest MI detected</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black Sea (Central)</td>
<td>Suspended particulate matter filtered from seawater at different depths</td>
<td>Chemistry; Biomarkers; Compound specific stable isotopes</td>
<td>0.96</td>
<td>(Wakeham et al., 2003, 2004)</td>
</tr>
<tr>
<td>Gulf of California (Guaymas Basin)</td>
<td>Sediment cores collected close to CH4-rich hydrothermal vents</td>
<td>Biomarkers; Molecular microbiology; Compound specific stable isotopes</td>
<td>0.88</td>
<td>(Schouten et al., 2003)</td>
</tr>
<tr>
<td>Mediterranean Sea (Central Province)</td>
<td>Surface sediments collected close to cold seeps</td>
<td>Biomarker; Compound specific stable isotopes</td>
<td>1.0</td>
<td>(Gontharet et al., 2009)</td>
</tr>
<tr>
<td>Mediterranean Sea (East, Dome Napoli, Milano and Amsterdam)</td>
<td>Authigenic carbonates collected close to mud volcanoes</td>
<td>Biomarkers; Carbonate carbon isotopes; Compound specific carbon isotopes</td>
<td>0.97</td>
<td>(Bouloubassi et al., 2006)</td>
</tr>
<tr>
<td>Gulf of Cadiz (El Arish Field)</td>
<td>Authigenic carbonates collected close to cold seeps</td>
<td>Biomarkers; Molecular microbiology; Carbonate carbon isotopes; Compound specific carbon isotopes</td>
<td>0.99</td>
<td>(Stadnitskaia et al., 2008)</td>
</tr>
<tr>
<td>Gulf of Mexico (Green Canyon)</td>
<td>Authigenic carbonates collected close to cold seeps</td>
<td>Biomarkers; Carbonate carbon isotopes; Compound specific carbon isotopes</td>
<td>0.98</td>
<td>(Pancost et al., 2005)</td>
</tr>
<tr>
<td>Gulf of Mexico (Mississippi Canyon)</td>
<td>Sediment cores collected close to cold seeps</td>
<td>Chemistry; Molecular microbiology</td>
<td>0.85</td>
<td>(Pi et al., 2009)</td>
</tr>
</tbody>
</table>

* Calculated from peak integration of published LC-MS chromatograms. Abbreviations: AOM, anaerobic oxidation of methane; MI, Methane Index.
methylene substrate. However, two distinct areas can be identified on the graph: (1) the “normal marine” block with MI smaller than –0.3 and \(\delta^{13}C\) greater than –35\% and (2) “methane impacted” block with MI exceeding 0.5 and \(\delta^{13}C\) smaller than –55\% (Fig. 10). Combined GDGT distribution (Fig. 9) and isotopic evidence (Fig. 10) suggest that 0.3 to 0.5 of the MI might be a reasonable threshold for distinguishing gas hydrate impacted and/or methane-rich environments from normal marine realm.

The highly diverse settings shown above further support that “Methane Index” might be a globally applicable indicator well suited to distinguish AOM environments from normal marine conditions. MI would be particularly useful for detecting fossil gas hydrates where the molecular fossils might be the only reliable evidence available. Of course, more work needs to be done to explore the full scope of its applicability.

4.3. Strengths and limitations of MI

Compared to carbonates, tetraether lipid-based MI is much more directly associated with AOM and less susceptible to diagenesis or other alternations. In addition, no preferential diagnosis of GDGTs has been reported (e.g. Huguet et al., 2009; Kim et al., 2009). However, besides GDGTs, other archaeal lipid biomarkers have also been used in the literature for tracing past AOM and gas hydrates (Birgel et al., 2008a, 2008b; Niemann and Elvert, 2008; Peckmann et al., 2009). These biomarkers include diethers like archaeol, sn2-hydroxyarchaeol, isoprenoidal hydrocarbons like crocetane, PMI (2,6,10,15,19-pentamethylloicosane) and their unsaturated homologues (PMID) which are often related to the ANME group II and III. To compare and contrast MI with these biomarkers, although ANME-I and their tetraether lipids signatures (MI) are ubiquitous as exemplified by Table 1, we realize that there is not a single biomarker that can be used exclusively and diagnostically for all ANME archaea (cf. Niemann and Elvert, 2008). Consequently, we suggest tetraether-based MI and GC-amendable diethers and other hydrocarbons are integrated to obtain a better understanding of enhanced methane oxidation. In practice, since GDGT measurements are being carried out for an increasing number of sediment cores, primarily for the interest of time-series reconstruction of past ocean temperatures, MI can be calculated simultaneously and the benefit may be twofold: (1) it might be useful in flagging the samples with substantial ANME archaeal input that indicate the use of proxies like TEX86 are unlikely to give results with high accuracy; (2) it might be useful in studying the temporal behavior of the marine gas hydrate reservoir and exploring possible relationships between hydrate destabilization and important climate changes. Once MI has been used to identify intervals with evidence of possible gas hydrate dissociation events, additional biomarkers like biphytane b, archaeol, crocetane, PMI and their carbon isotopes could be carried out to verify the fidelity, and provide additional insights into the extent and magnitude of such events.

Our proposal of the MI as an indicator for gas hydrate instability cannot rule out the possibility that in methane-rich environments like the euxinic Black Sea, active microbial-mediated AOM in the anoxic water column or sediments could also lead to an increase in the lipid abundance of methanotrophs, even without the presence of gas hydrates. This raises the important question: can MI be used to differentiate gas hydrate-related AOM from other types of AOM that occur without the presence of gas hydrates in the geological past? We realize that biomarker alone might be difficult to address this question efficiently. Nevertheless, we expect that AOM without gas hydrates like the euxinic environments can be easily identified by using additional environmental indicators like C/S, C/P ratio, cerium anomaly and degree of pyritization (see reviews by Lyons et al., 2009; Meyer and Kump, 2008). By applying those simple measurements one might be able to determine if the AOM registered in tetraether lipids are related to gas hydrates or euxinia.

It is important to notice that the MI is premised on the current model of a binary source of GDGTs: Crenarchaeota from the water column and methanotrophic Euryarchaeota from the sediments. The MI may not work in environments where there are additional sources of GDGTs, such as coastal sediments where soil-derived GDGTs are prominent. Similarly, MI might be valid in large lakes, but could be problematic in places with large amounts of terrigenous input.

5. Conclusions

In this study we propose the molecular indicator “Methane Index (MI)” to better evaluate the role of methane in paleoenvironments and in the global carbon cycle. In properly designed studies, the MI may be useful for detecting past events involving the destabilization of marine gas hydrates. The rationale of MI is that in methane-laden environments conducive to AOM of when the pool of archaeal tetraether lipids is being affected by significant contributions of GDGT-1, -2 and -3 relative to normal marine environments. MI can range between 0 and 1 and a value of 0.3–0.5 appears to mark the boundary between normal marine sediments and hydrate-impacted sediments. Our study in the Gulf of Mexico sediments demonstrates the correlation between porewater chemistry, carbon isotopes of authigenic carbonates, and MI. In addition, compound-specific carbon isotope studies reveal contributions from both planktonic Crenarchaeota (represented by crenarchaeol and its isomer) and methanotrophic Euryarchaeota (represented by GDGT-1, -2 and -3) to the tetraether lipid pool which forms the basics for MI. Preliminary applications of MI in a number of both methane-rich and methane-free environments verify the robustness of this indicator.

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