



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

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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^{15} g) as CH₄, which constitutes the largest pool of readily exchangeable carbon on Earth's surface (Archer, 2007; Hester and Brewer, 2009; Kvenvolden, 1999). 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 $\delta^{13}\text{C}$ of foraminiferal shell carbonates (Kennett et al., 2000). However, this study was met with criticism primarily concentrated on the usage of carbonate carbon isotope 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.

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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):



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 geomicrobiological 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).

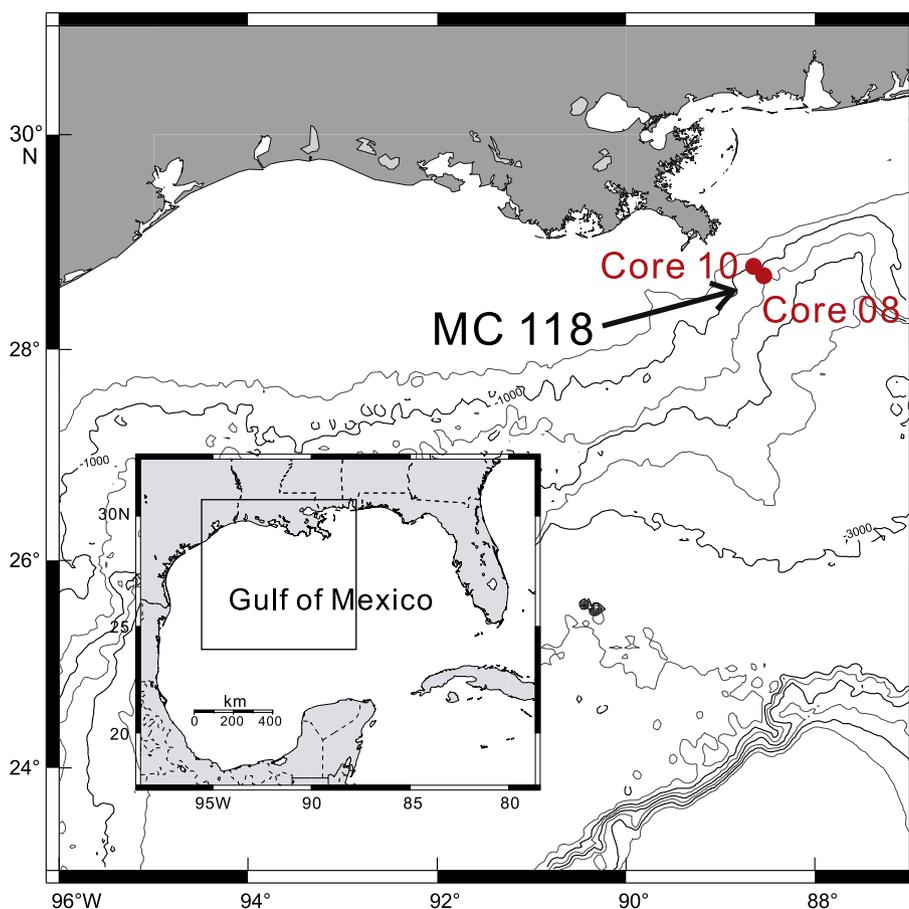


Fig. 1. Map shows the locations of Core 08 and Core 10, as collected from hydrate site MC 118, NE Gulf of Mexico.

The MC 118 gas hydrate site was discovered by the Johnson Sea-Link research submersible in 2002. Free gases (C_1 – C_5 hydrocarbons and minor CO_2) 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 crater-like 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 CH_4 ($\delta^{13}C = -45.7\%$, $\delta D = -163\%$) are consistent with a deep source, suggesting thermogenic origin of those gases. Ethane, propane, butanes and pentanes are the minor components. The $\delta^{13}C$ of CO_2 from vent gas is strongly enriched in ^{13}C (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 $\delta^{18}O$ and $\delta^{13}C$ 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 $\leq 0.04\%$ for $\delta^{13}C$ and $\leq 0.07\%$ for $\delta^{18}O$.

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 N_2 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/ethyl acetate, 1:1 acetonitrile/ethyl acetate, 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 N_2 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 Econosphere NH_2 column (250 × 4.6 mm, Alltech, Germany) heated to 30 °C in a Thermo Finnigan Surveyor 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 ramped 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 – isotope 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 BBr_3 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 CO_2 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 CO_2 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

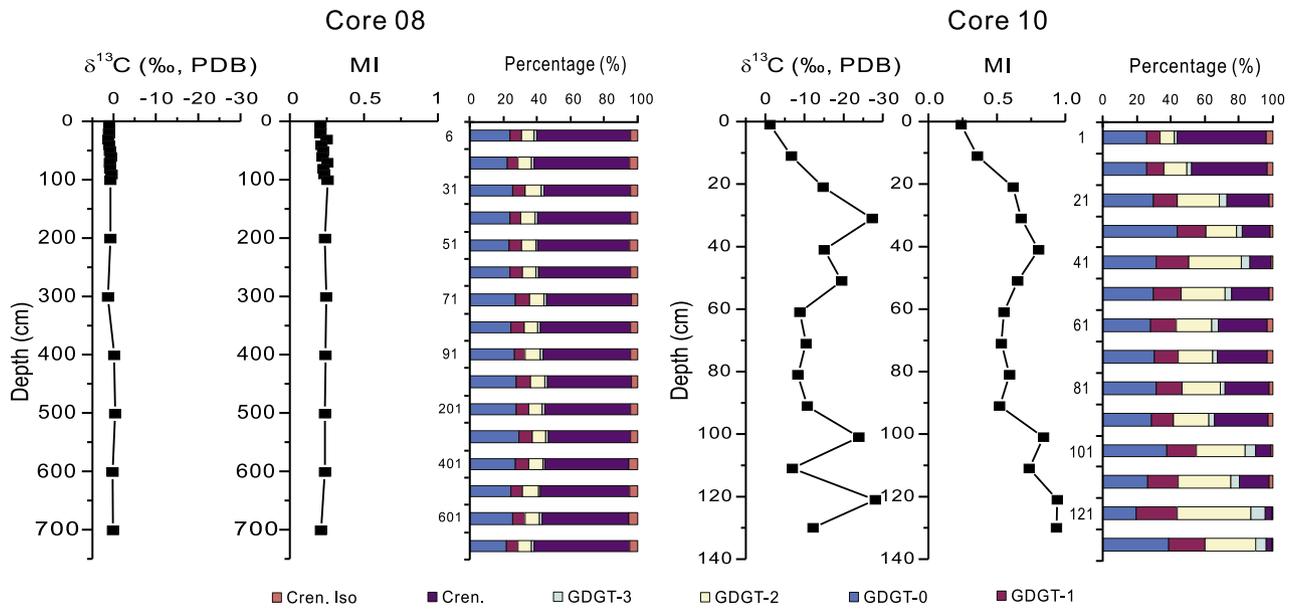


Fig. 2. Depth profile of stable carbon isotopes from bulk carbonates, calculated MI, and the distribution (relative abundance in percentage) of different GDGTs for Core 08 and Core 10.

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 $\delta^{13}\text{C}$ of carbonates varies from -0.4 to 1.3‰ in Core 08 and varies from -28.1 to -1.2‰ in Core 10. The $\delta^{18}\text{O}$ 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 ^{13}C , a signature inherited from the ^{13}C -depleted CH_4 . Limited variation in $\delta^{13}\text{C}$ of carbonate with an average close to 0‰ in Core 08 implies normal marine conditions, whereas the deviations of $\delta^{13}\text{C}$ to much lower values in Core 10 can be attributed to the contribution of ^{13}C -depleted, methane-derived carbon to the dissolved inorganic carbon (DIC) pool (Fig. 2). In addition, an inverse linear correlation between $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ has been found in Core 10, but not Core 08 (Fig. 3). Methane-derived carbon would be ^{13}C -depleted. Meanwhile, water released from the decomposition of gas hydrate would be ^{18}O -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

$$\text{MI} = \frac{[\text{GDGT-1}] + [\text{GDGT-2}] + [\text{GDGT-3}]}{[\text{GDGT-1}] + [\text{GDGT-2}] + [\text{GDGT-3}] + [\text{Crenarchaeol}] + [\text{Cren.isomer}]} \quad (2)$$

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

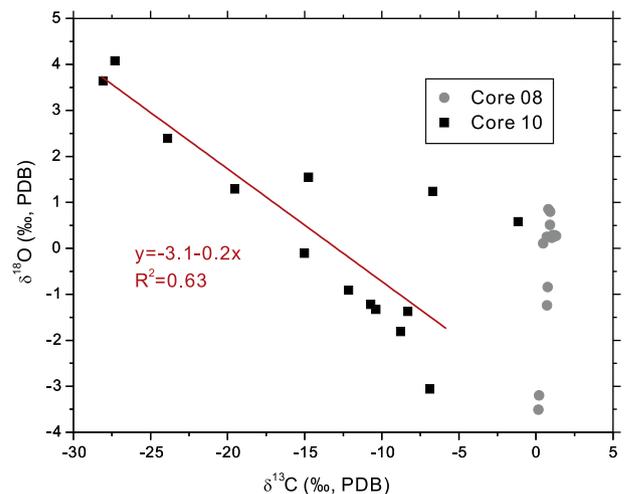


Fig. 3. Correlations between carbonate $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ for Core 08 and Core 10. A reverse correlation between $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ has been found in Core 10 as indicated by a linear regression. The regression function is $y = -3.1 - 0.2x$ where R^2 equals 0.63. The p -value (two-tailed) of this correlation coefficient < 0.001 .

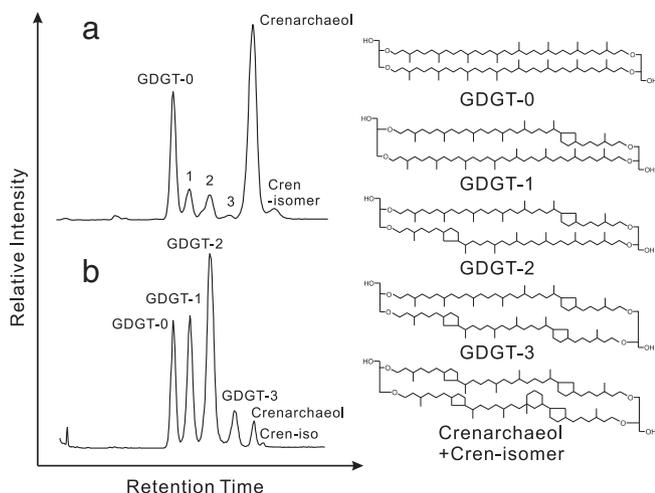


Fig. 4. HPLC/APCI-MS base peak chromatogram of two typical samples (a) and (b), from Core 08 (61cbsf) and Core 10 (121cbsf), respectively. Both records show the presence of GDGT-0 to -3, as well as crenarchaeol and its isomer, with the structures sketched in the right panel. Note that GDGT-1, -2 and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

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 *Euryarchaeota*. 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 cbsf. 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ of biphytane b, which acts as an indicator for ANME-1 *Euryarchaeota*, is compared with the $\delta^{13}\text{C}$ of biphytane d which

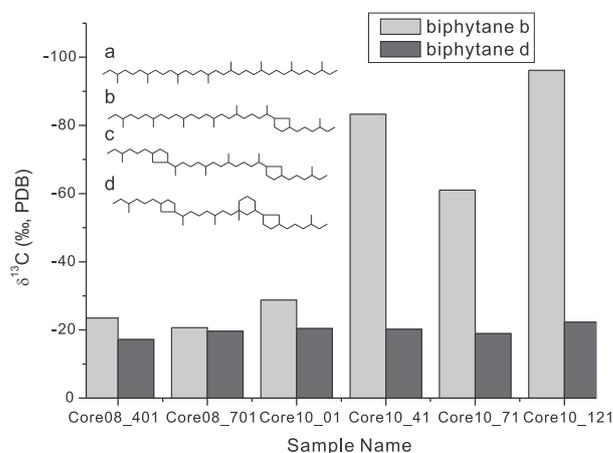


Fig. 5. Compound-specific carbon isotopes of GDGT-derived biphytanes in six samples from Core 08 and Core 10. Sample names are given by core number and sample depth in cbsf. The structures of biphytane a, b, c, d are also shown. Biphytane b would be most sensitive to methanotrophic ANME *Euryarchaeota*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

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 $\delta^{13}\text{C}$ of carbonate indicates strong AOM activity. The $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ from -22‰ in hydrate-free samples to -96‰ in hydrate-impacted samples, indicating enhanced AOM activity in the latter case. The extremely depleted $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ of biphytane d from exactly the same samples demonstrate contributions from planktonic *Crenarchaeota*. The disparate isotopic signature strongly

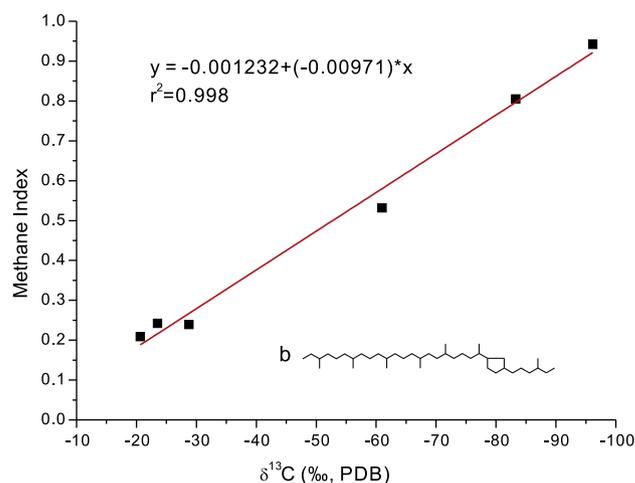


Fig. 6. Correlation between Methane Index and $\delta^{13}\text{C}$ from biphytane b in six samples from both Core 08 and Core 10, MC 118, Gulf of Mexico. Linear regression gives $y = -0.001232 + (-0.00971)*x$ with $R^2 = 0.998$. The p -value (two-tailed) of this correlation coefficient is <0.0001 .

suggests binary sources of GDGTs presented in the same sample, which 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 $\delta^{13}\text{C}$ 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 TEX_{86}

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 TEX_{86} 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 TEX_{86} .

To better calibrate the SST proxy of TEX_{86} , 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 TEX_{86} with different temperatures to assess potential temperature influences on MI (Fig. 8). Changing TEX_{86} 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 ($\sim 30^\circ\text{C}$) the MI never exceeds 0.3, whereas TEX_{86} 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 TEX_{86} , 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, TEX_{86} 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

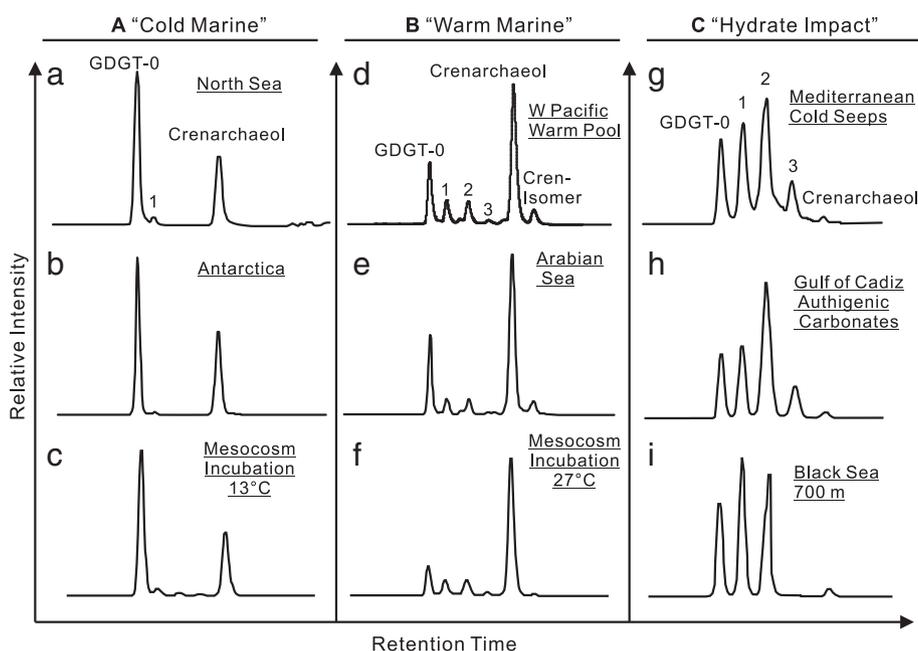


Fig. 7. LC/MS peak chromatogram showing the GDGT distribution in different environments. A: “cold (normal) marine” environment. a) Skagerrak (North Sea, (Schouten et al., 2000), b) Halley Bay (Antarctica, (Schouten et al., 2002), c) mesocosm incubation at 13°C for three months (Wuchter et al., 2004). B: “warm (normal) marine” environments. d) Holocene sediments from ODP 806 at the heart of the Western Pacific Warm Pool (Liu et al., 2009), e) surface sediments from Arabian Sea (Schouten et al., 2002), f) mesocosm incubation at 13°C for three months (Wuchter et al., 2004). C: methane impacted environment. g) Mediterranean cold seeps (Pancost et al., 2001), h) Gulf of Cadiz authigenic carbonates (Stadnitskaia et al., 2008), i) Black Sea water depth at 700 m (Wakeham et al., 2003, 2004).

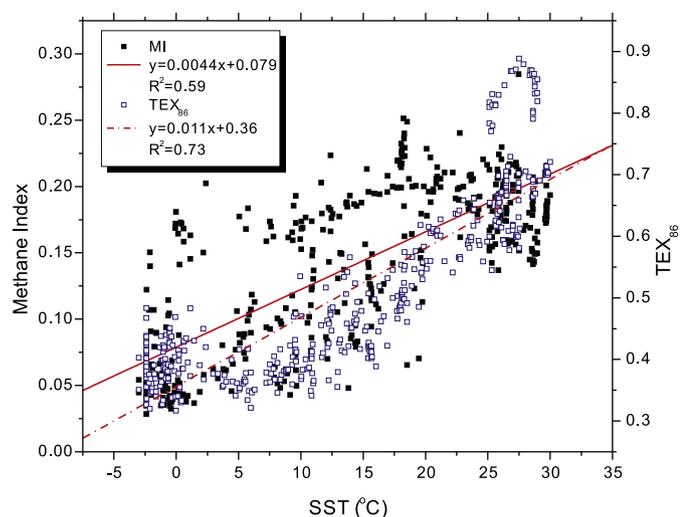


Fig. 8. Comparison of calculated MI and TEX_{86} under different sea surface temperature conditions of the Kim et al. (2010) global core-top dataset. The two regressions give $\text{TEX}_{86} = 0.011 \cdot \text{SST} + 0.36$ with $R^2 = 0.73$ and p -value of 0, and $\text{MI} = 0.0044 \cdot \text{SST} + 0.079$ with $R^2 = 0.59$ and p -value < 0.0001 . Different calibrations for the TEX_{86} thermometry are not discussed here because they are beyond the scope of this paper.

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 boarder 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

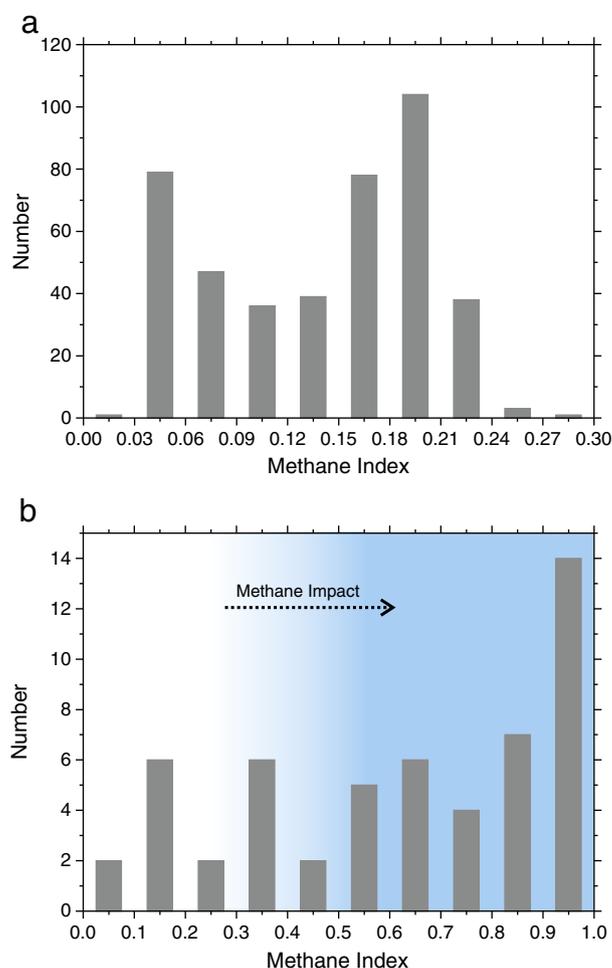


Fig. 9. Comparison of the MI calculated from the “normal marine” environment (a) and possible methane-rich environment (b). The Kim et al. (2010) global core-top dataset is used in (a, $n = 426$), and (b, $n = 57$) is from literature listed in Table 1 (Bouloubassi et al., 2006; Gontharet et al., 2009; Pancost et al., 2001, 2005; Pi et al., 2009; Schouten et al., 2003; Stadnitskaia et al., 2008; Wakeham et al., 2003, 2004). Note the difference between the x-axis scales of (a) and (b).

Table 1

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

Location	Nature of Sample	Evidence of AOM	Highest MI detected	Reference
Black Sea (Central)	Suspended particulate matter filtered from seawater at different depths	Chemistry; Biomarkers; Compound specific stable isotopes	0.96	(Wakeham et al., 2003, 2004)
Gulf of California (Guaymas Basin)	Sediment cores collected close to CH_4 -rich hydrothermal vents	Biomarkers; Molecular microbiology; Compound specific stable isotopes	0.88	(Schouten et al., 2003)
Mediterranean Sea (Central Province)	Surface sediments collected close to cold seeps	Biomarker; Compound specific stable isotopes	1 ^a	(Gontharet et al., 2009)
Mediterranean Sea (East, Dome Napoli)	Authigenic carbonates collected close to mud volcanoes	Biomarkers; Carbonate carbon isotopes;	1 ^a	(Bouloubassi et al., 2006)
Mediterranean Sea (East, Dome Napoli, Milano and Amsterdam)	Authigenic carbonates collected close to cold seeps	Biomarkers; Compound specific stable isotopes	0.97 ^a	(Pancost et al., 2001)
Gulf of Cadiz (El Araish Field)	Authigenic carbonates collected close to cold seeps	Biomarkers; Molecular microbiology; Carbonate carbon isotopes; Compound specific stable isotopes	0.99 ^a	(Stadnitskaia et al., 2008)
Gulf of Mexico (Green Canyon)	Authigenic carbonates collected close to cold seeps	Biomarkers; Carbonate carbon isotopes; Compound specific carbon isotopes	0.98 ^a	(Pancost et al., 2005)
Gulf of Mexico (Mississippi Canyon)	Sediment cores collected close to cold seeps	Chemistry; Molecular microbiology	0.85	(Pi et al., 2009)
Gulf of Mexico (Mississippi Canyon)	Sediment cores collected close to cold seeps	Chemistry; Carbonate carbon isotopes; Compound specific carbon isotopes	0.94	This study

^a Calculated from peak integration of published LC-MS chromatograms. Abbreviations: AOM, anaerobic oxidation of methane; MI, Methane Index.

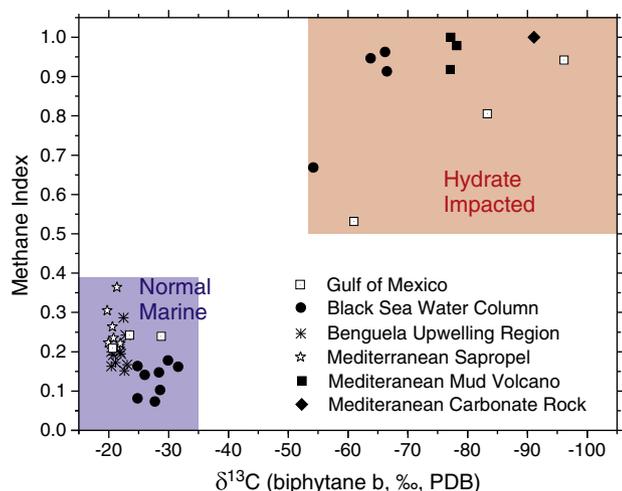


Fig. 10. Methane Index vs. $\delta^{13}\text{C}$ of biphytane b plot using data from a variety of sample locations as well as the nature of the sample. Data used include (1) Gulf of Mexico (this study), (2) Black Sea water column (Wakeham et al., 2003, 2004), (3) Benguela upwelling region (Pancost et al., 2008), (4) Mediterranean sapropel (Pancost et al., 2008), (5) Mediterranean mud volcano (Pancost et al., 2001), and Mediterranean carbonate rocks (Bouloubassi et al., 2006).

methane substrate. However, two distinct areas can be identified on the graph: (1) the “normal marine” block with MI smaller than ~ 0.3 and $\delta^{13}\text{C}$ greater than -35‰ and (2) “methane impacted” block with MI exceeding 0.5 and $\delta^{13}\text{C}$ 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 alterations. In addition, no preferential diagenesis 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, *sn*2-hydroxyarchaeol, isoprenoidal hydrocarbons like crocetane (2,6,11,15-tetramethylhexadecane), PMI (2,6,10,15,19-pentamethyleicosane) and their unsaturated homologues (PMIA) 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 (c.f. 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 TEX_{86} 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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