The Anaerobic Oxidation of Methane: New Insights in Microbial Ecology and Biogeochemistry

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Abstract: As the major biological sink of methane in marine sediments, the microbially mediated anaerobic oxidation of methane (AOM) is crucial in its role of maintaining a sensitive balance of our atmosphere's greenhouse gas content. Although there is now sufficient geochemical evidence to exactly locate the "hot spots" of AOM, and to crudely estimate its contribution to the methane cycle, a fundamental understanding of the associated biology is still lacking, consequently preventing a thorough biogeochemical understanding of an integral process in the global carbon cycle. Earlier microbiological work trying to resolve the enigma of AOM mostly failed because it was largely focussed on the simulation of AOM under laboratory conditions using cultivable candidate organisms. Now again, understanding the biological and biochemical details of AOM is the declared goal of several international research groups, but this time in a combined effort of biogeochemists and microbiologists using novel analytical tools tailored for the study of unknown microbes and habitats. This review gives an overview on very recent progress in the study of AOM that dramatically advanced this ~ 30-yr-old field. New insights on the quantitative significance of AOM are combined to refine older estimates.

Introduction

Since pioneering reports by Reeburgh (1976) and Barnes and Goldberg (1976), subsequent studies employing stable isotopes, radiotracers, modeling, and inhibition techniques have established that methane in marine sediments is oxidized biologically under anoxic conditions (see review of Valentine and Reeburgh 2000, and references therein). Despite the compelling evidence for the anaerobic oxidation of methane (AOM), details of the related biochemical mechanisms and organisms are still unknown. Zehnder and Brock (1979) showed that incubation of active methanogenic cultures with $^{14}CH_4$ would result in formation of some $^{14}CO_2$, thus demonstrating the oxidation of a small fraction of methane under anaerobic conditions. Although no anaerobic methanotroph has ever been isolated, biogeochemical studies have shown that the overall process involves a transfer of electrons from methane to sulfate (Iversen and Jørgensen 1985; Hoehler et al. 1994). The isotopic and genetic signature of the microbial biomass in environments enriched with methane shows that this transfer is probably mediated by a microbial consortium that includes archaea and sulfate-reducing bacteria (Hinrichs et al. 1999; Boetius et al. 2000). The distributions of abundant lipid products apparently derived from members of AOM consortia indicate a substantial diversity among the microbial players in different methane-rich environments. However, the intermediate substrate (e.g. H_2 + CO_2, acetate, formate), which is exchanged between archaeal and bacterial members of the consortium and thereby coupling methane oxidation and sulfate reduction remains unidentified. In several sedimentary environments, AOM can be the dominant sulfate-consuming process, e.g. in sediments from Carolina Rise and Blake Ridge (Borowski et al. 1996) or at Hydrate Ridge (Boetius et al. 2000). Also, AOM may prove to be the oxidative process that penetrates most deeply

From WEFER G, BILLETT D, HEBBELN D, JØRGENSEN BB, SCHLÜTER M, VAN WEERING T (eds), 2002, *Ocean Margin Systems.* Springer-Verlag Berlin Heidelberg, pp 457-477

into anoxic environments wherever sulfate is available. In the methane budget proposed by Reeburgh (1996), more than 80% of the methane produced annually in marine sediments is consumed mostly in anoxic environments before it can reach the atmosphere. The previously estimated 75 Tg/yr methane consumption is nearly twice the annual increase in the atmospheric inventory of CH_4 (40 Tg/yr). However, our updated compilation of published AOM rates shows that the consumption of methane in anoxic sediments is probably several times higher than previously estimated, implying that methane production estimates are probably too low as well. In contrast to earlier assessments, AOM may have been an important biogeochemical process in earlier stages of Earth history.

The Process

The metabolic process of AOM is still unknown and all reactions discussed in the literature remain speculative as long as the elusive microorganisms involved in AOM are not available for physiological investigations. However, different lines of evidence, including pore water concentration profiles, radioisotope measurements, biomarker studies, phylogenetic analyses, and thermodynamic models serve as a basis to examine the likelihood of the various proposed pathways.

In the mid 70's, it was established that in certain horizons in anoxic sediments and waters at continental margins net consumption of methane occurs (Barnes and Goldberg 1976, Reeburgh 1976). It was observed that AOM peaks coincide with increased sulfate reduction. Thus, Barnes and Goldberg (1976) suggested sulfate as the terminal electron acceptor for this process according to reaction (1).

$$CH_4 + SO_4^{2-} \rightarrow HCO_3^- + HS^- + H_2O \qquad (1)$$

Thermodynamic models show that this reaction can become favorable at in situ conditions in marine sediments, however, only with a low free energy yield of –25 kJ per mol methane consumed (Hoehler et al. 1994). This is only approximately half the energy required for the formation of ATP. Until today it has been discussed controversially whether AOM can support microbial growth. However, recently, extremely high amounts of biomass of aggregated archaea and sulfate reducing bacteria involved in AOM were found in surface sediments above marine gas hydrate (Boetius et al. 2000). The biomass of the consortia in the zone of AOM (1-10 cm sediment depth) exceeded the total microbial biomass of surrounding sediments by an order of magnitude and even served as food to some members of the macrofauna community, as indicated by stable isotope analysis (Levin, unpubl. data). Furthermore, the cell-specific rates of methane based sulfate reduction were similar to those of cultivated SRB under optimal culture conditions. These field data indicate that the process of AOM can support significant cell growth and activity.

Counterintuitive to thermodynamic considerations predicting an extremely low energy gain of AOM, the involvement of two or more microorganisms as syntrophic partners was discussed already at the beginning of microbiological research on this process (see review of Valentine and Reeburgh 2000, and literature therein). It was proposed that a consortium of microorganisms involving both methanogens and bacteria may mediate AOM, with the latter oxidizing intermediate products derived from methane. In field experiments conducted by Hoehler et al. (1994), AOM was possible as long as hydrogen concentrations were kept at extremely low levels. The authors concluded that AOM is mediated by two syntrophic partners, which rely on interspecies hydrogen transfer: methanogenic archaea mediating the oxidation of methane with water (reaction 2), and sulfate reducing bacteria scavenging the intermediate hydrogen (reaction 3).

$$CH_4 + 2\ H_2O \rightarrow CO_2 + 4\ H_2 \qquad (2)$$
$$SO_4^{2-} + 4\ H_2 + H^+ \rightarrow HS^- + 4\ H_2O \qquad (3)$$

The free energy gain of the reversal of methanogenesis as shown in reaction (2) depends to a large degree on the concentration of dissolved hydrogen in pore waters. For example, under reactant/product concentrations and temperatures typical for Cape Lookout Bight sediments, AOM becomes

favorable at H_2 concentrations below 0.3 nM (Hoehler et al. 1994). The involvement of methanogenic archaea in the first part of the reaction is supported by the occurrence of strongly ^{13}C-depleted archaeal biomarkers in environments with evidence for high rates of AOM (Hinrichs et al. 1999, Elvert et al. 1999, Thiel et al. 2001). Extreme depletions of ^{13}C were also observed in bacterial biomarker lipids, consistent with a derivation from methane-C (Boetius et al. 2000, Hinrichs et al. 2000, Elvert et al. 2000). To explain the low $\delta^{13}C$ in the lipids of the SRB by reaction (3), one would have to assume a direct transfer and assimilation of the $\delta^{13}C$-depleted CO_2 produced from methane within the archaea/SRB-consortium. This mechanism requires formation of microenvironments with strong physicochemical gradients, so that utilization of CO_2 from reaction (2) would be much faster than diffusion of pore water CO_2 to the SRB partner. According to a diffusion model, this is only possible if the distance between both partners is smaller than 10 µm, which agrees with dimensions observed in aggregates of archaea/SRB in sediments at Hydrate Ridge (Boetius et al. 2000 and literature therein). However, uncertainty remains with respect to the fractionation factors for autotrophic consumption of CO_2 by the SRB, which could also cause significant depletion of ^{13}C in their lipids. In principle, biosynthesis from inorganic carbon in the SRB appears feasible and is consistent with recent observations at Hydrate Ridge, where many relatives of the sulfate reducers associated with archaea are facultative autotrophs.

According to the biomarker and 16S rRNA analyses, the dominating archaea in zones of AOM at methane seeps and above methane hydrate (Hinrichs et al. 1999; Boetius et al. 2000; Orphan et al. in press) are closely related to the *Methanosarcinales*. These methanogens include the known major producers of hydroxyarcheols and typically use acetate and reduced C_1-compounds for methanogenesis. Hoehler et al. (1994) discussed acetate as an intermediate in AOM. The reversal of acetoclastic methanogenesis would involve the formation of acetate according to reaction (4), which could serve as a carbon as well as an energy source to the sulfate reducing bacteria. Also, cultivated bacteria of the *Desulfosarcina/Desulfococcus* group of the deltaproteobacteria are known as complete acetate oxidizers, able to mediate reaction (5).

$$CH_4 + HCO_3^- \rightarrow CH_3COO^- + H_2O \quad (4)$$
$$SO_4^{2-} + CH_3COO^- \rightarrow HS^- + 2HCO_3^- \quad (5)$$

However, to make AOM with acetate as intermediate thermodynamically attractive, acetate concentrations would have to be lower than 2 nM to gain at least –10 kJ of free energy (Hoehler et al. 1994; Boetius et al. 2000). Such low concentrations are uncommon for most marine sediments and would be far below the *Michaelis Menten constant* for acetate uptake by known SRB.

Valentine and Reeburgh (2000) recently proposed an alternative pathway involving acetate that yields a higher change of free energy (-50 kJ) and could explain isotopic depletions in biosynthetic products of the SRB partner more satisfactorily than reactions (2) and (4). They suggest a formation of acetate solely from methane, and a transfer of both acetate and hydrogen to the SRB partners (reaction 6-8). However, the biochemical pathways of reaction (6) are without example in cultured organisms.

$$2CH_4 + 2H_2O \rightarrow CH_3COOH + 4H_2 \quad (6)$$
$$4\,H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4H_2O \quad (7)$$
$$CH_3COOH + SO_4^{2-} \rightarrow 2HCO_3^- + HS^- + H^+ \quad (8)$$
$$2(CH_4 + SO_4^{2-} \rightarrow HCO_3^- + HS^- + H_2O)$$
(net reaction)

The *Methanosarcinales* uniquely include the methylotrophic methanogens. These organisms are capable of disproportionating methanol, methylamines, or methyl sulfides to methane and carbon dioxide, indicative of an enzyme system to oxidize R–CH_3 to CO_2 anaerobically. Hence, such C_1 compounds could also be potential intermediates according to reactions (9/10) with methanol as an example. However, only very few known SRB are able to use methanol as their sole carbon substrate, and no SRB have been isolated yet which can grow on methylamine (Hansen pers. comm. 2000).

$$4CH_4 + 4H_2O \rightarrow 4CH_3OH + 4H_2 \quad (9)$$

$$3SO_4^{2-} + 4CH_3OH \rightarrow 3HS^- + 4HCO_3^- + 4H_2O + H^+ \quad (10)$$

A thermodynamic model considering the difference in concentrations between the producing and consuming partners by Sørensen et al. (in press) tests the likelihood of the different possible elec-tron shuttles hydrogen, acetate, methanol, and formate. Only transfer of formate according to reactions (11/12) resulted in free energy gain. Formate is used by members of the order *Methanobacteriales* and *Methanococcales* for methanogenesis but no member of the order *Methanosarcinales* is known to utilize formate.

$$CH_4 + 3\ HCO_3^- \rightarrow 4HCOO^- + H^+ + H_2O \quad (11)$$

$$SO_4^{2-} + 4HCOO^- + H^+ \rightarrow HS^- + 4HCO_3^- \quad (12)$$

In the attempt of predicting the metabolic pathways of AOM on the basis of our current knowledge, a reversal of methanogenesis on the basis of known enzymes appears attractive. In the case of methanogenesis, some of the enzymes of methanogens used for the reduction of CO_2 to CH_4 are operating in reverse in the oxidative pathway of the sulfate-reducing archaeon *Archaeoglobus*. However, the final enzymatic step in methane production involves the protonation of methyl-nickel by the enzyme methyl-CoM reductase and is considered irreversible (see review of Hoehler and Alperin 1996 and literature therein). Hence, it is likely that other, yet unknown enzymes are involved. The complete sequencing of the genomes of the methanogenic archaea *Methanococcus jannaschii* and *Methanobacterium thermoautotrophicum* detected 30% putative coding regions with no similarity to any sequence in other organisms (Gaasterland 1999) and most regions similar to other genomes code for proteins of unknown function. Notably, members of the order *Methanosarcinales* have a much larger genome than other methanogens. The sequencing of a first member of the *Methanosarcinales*, *Methanosarcina mazei*, is presently in progress.

Present Knowledge on Microbes Involved in AOM

Early experiments by Zehnder and Brock (1979) indicated that methanogenic archaea are capable of oxidizing $^{14}CH_4$ to $^{14}CO_2$ under anaerobic culture conditions, although only at a fraction <0.5% of the concurrent methane production. Additionally, a small amount of ^{14}C was incorporated into archaeal biomass. The most efficient CH_4 oxidizer was *Methanosarcina barkeri*, which also produced acetate and methanol during CH_4 oxidation. Early experiments using ^{14}C-labelled methane were likely biased by impurities of ^{14}CO, which is oxidized by several anaerobic microorganisms to $^{14}CO_2$ (Harder 1997). Using pure $^{14}CH_4$, Harder (1997) showed that *Methanosarcina acetivorans*, *Methanospirillum hungatei*, and *Methanolobus tindarius* oxidized methane at low rates. None of the sulfate reducing bacteria tested with pure $^{14}CH_4$ produced significant amounts of $^{14}CO_2$ from methane. So far, all pure culture experiments (including *Methanosarcina*, *Methanosaeta* and *Methanobacterium spp.* as test organisms) employing maintenance of low H_2 pressure via gas sparging, or co-culturing of SRB (*Desulfotomaculum*) failed to reverse methanogenesis to methane consumption (Valentine and Reeburgh 2000). However, at very high methane pressure (100 atm), Shilov et al. (1999) observed a reversal of acetoclastic methanogenesis in sludge granules that consisted of mixed cultures of microorganisms dominated by *Methanosarcina* and *Methanosaeta spp.* In any case, the direct association of archaea with SRB would allow for a highly efficient transfer of intermediates by molecular diffusion compared to free-living cells. This could be a prerequisite to render the process of AOM thermodynamically favorable (Sørensen et al. in press).

Hinrichs et al. (1999) combined evidence from biomarker studies and phylogenetic analyses and thereby detected a new group of archaea possibly involved in AOM in sediments from methane seeps of the Eel River Basin. The archaea-specific lipid biomarkers isolated from the seep sediments were strongly depleted in ^{13}C ($\delta < -100‰$ *vs*. PDB),

indicating that methane was the carbon source for the organisms that synthesized them. These biomarkers are known to occur in methanogenic archaea and have been isolated from members of the order *Methanosarcinales*. The ribosomal-RNA gene library yielded only two major archaeal phylogenetic groups from 176 clones analyzed. Most rDNA sequences, accounting for 148 of the 176 archaeal clones recovered, form a cluster of unique but highly related sequences (group ANME-1) (Fig. 1), each distinguishable by RFLP analysis and primary structure. Other clones were comprised of a sequence type closely related to cultivated members of the *Methanosarcinales* (ANME-2 group; Orphan et al. in press). The group ANME-1 is distinct from, but related to, methanogenic archaea of the orders *Methanomicrobiales* and *Methanosarcinales*. ANME-1 related sequences have been recovered from a variety of methane-rich locations but not from any other aquatic environments. In sediments of the Eel River Basin and Hydrate Ridge, no rRNA sequences of previously cultured methanogens like *Methanosaeta concilii* or *Methanosarcina spp.* - known producers of *sn*-2-hydroxyarchaeol - were detected, implying that the ^{13}C-depleted archaeal biomarkers are likely produced by organisms represented by sequences either within the

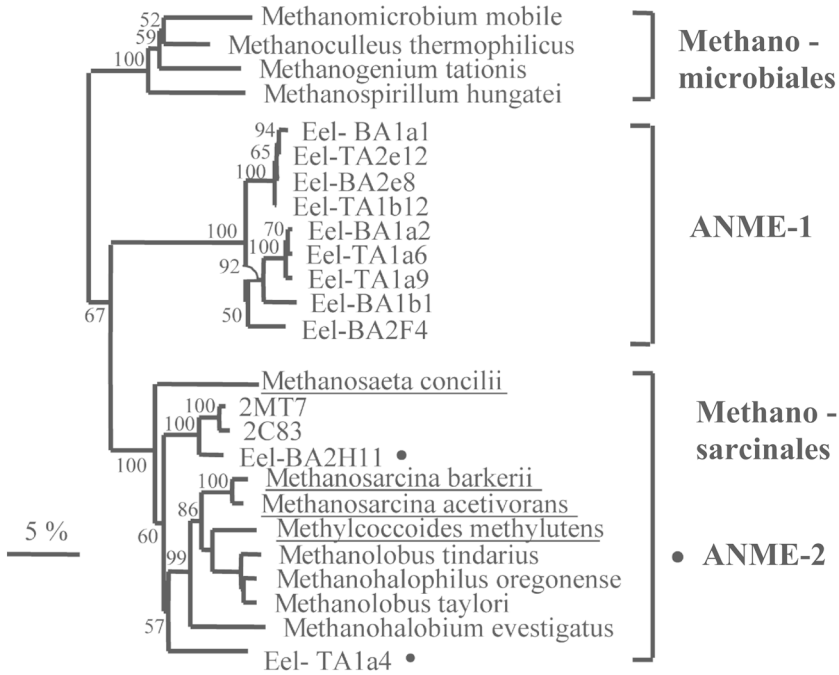

Fig. 1. Partial illustration of phylogenetic analysis of archaeal ribosomal rRNA sequences recovered from seep sediments in the Eel River Basin (modified after Hinrichs et al. 1999), showing the methanogenic archaeal orders *Methanomicrobiales*, *Methanosarcinales*, and the newly recovered groups ANME-1 and ANME-2 (anaerobic oxidation of methane). *Escherichia coli*, *Thermotoga maritima*, *Aquifex pyrophilus* and *Synechococcus* PCC6301 were used as outgroups. Scale bar represents 5 fixed mutations per 100 nucleotide sequence positions. Sequences obtained in this study have been submitted to GenBank under the accession numbers AF134380-AF134393. Sequences beginning with Eel- were obtained from samples that contain extremely ^{13}C-depleted archaeol and hydroxyarchaeol. Methanogenic archaea of the order *Methanosarcinales* that are known major producers of hydroxyarchaeols are indicated by underlined letters.

ANME-1 or ANME-2 cluster. Recent observations of conspicuous aggregates of archaea and sulfate reducing bacteria complement and strengthen earlier findings based on lipids and molecular phylogeny (Boetius et al. 2000). Fluorescence in situ hybridization (FISH) revealed that both archaea and SRB grow together in aggregates of approximately 3 µm diameter with an inner core consisting of archaeal cells and an outer shell of sulfate reducers (Fig. 2). The rRNA probes targeted specifically the ANME-2 group among the archaea and a new cluster of delta-proteobacterial SRB that is closely related to the *Desulfosarcina-Desulfococcus* group. In some sampling intervals, these aggregates comprised over 90% of the total microbial biomass and are most likely responsible for the extremely high rates of sulfate reduction (>5 µmol cm^{-3} d^{-1}) in the methane-saturated sediments.

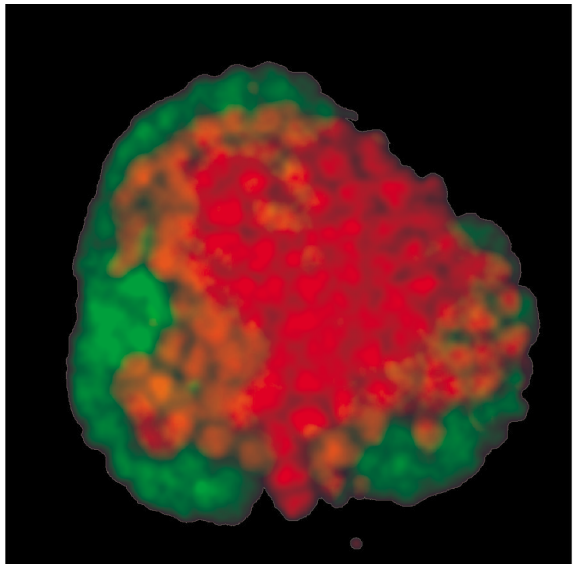

Fig. 2. An archaea/SRB consortium apparently mediating AOM. Aggregated archaea and sulfate reducing bacteria were found in sediments above gas hydrates (Hydrate Ridge, continental slope off Oregon, USA). The aggregated cells were exposed to a green-fluorescent RNA-probe targeting sulfate-reducing bacteria (*Desulfococcus-Desulfosarcina* group) and a red-fluorescent RNA-probe targeting archaea (ANME-2 group). The aggregate has a size of approxi-mately 10 µm. The image was taken by confocal laser scanning microscopy. Reprinted with permission from Nature (Boetius et al., Nature 407:623-626). Copyright (2000) Macmillan Magazines Limited.

Recent work showed that both ANME groups and the members of the *Desulfosarcina-Desulfococcus* group co-occur in other methane-rich sediments. They were found in surface sediments at deep-sea hydrothermal vents in the Guaymas Basin (Teske et al. 2001) and the Eel River Basin (Orphan et al. in press). In their study on microbial diversity in Cascadia margin deep subsurface sediments with abundant methane hydrate, Bidle et al. (1999) detected 16S rDNA sequences of SRB belonging to the *Desulfosarcina-Desulfococcus* group as well as sequences of the methyl coenzyme M reductase gene originating from members of the order *Methanosarcinales*. In sediments of the Aarhus Bay, Denmark, rDNA sequences of the ANME-1 group were detected in the AOM zone at 165 cm below the seafloor. Phylogenetic analysis of the gene of dissimilatory sulfite reductase (DSR) revealed that all retrieved sequences belonged to a novel deeply branching lineage unrelated to all previously described DSR genes (Thomsen et al. in press). Currently, it is not known whether the ANME group or any other methanogenic archaeon possesses the DSR gene and could account for the deep branching DSR.

Evidence derived from parallel biomarker chemotaxonomy and 16S rRNA or FISH probing in several environments supports the hypothesis that several phylotypes have to be considered as producers of ^{13}C-depleted archaeol and hydroxyarchaeol (for details, see chapter on lipid biomarkers). A series of studies report the predominant occurrence of phylotypes from the ANME-2 group (more closely related to *Methanosarcinales* than ANME-1) in sediments hosting active AOM communities (Boetius et al. 2000; Orphan et al. in press; Teske et al. 2001). On the other hand, we observed cases in which the ANME-1 group was predominant (Hinrichs et al. 1999) or even the exclusive archaeal group (Teske et al. 2001), but ^{13}C-depleted archaeol and hydroxyarchaeol were present as well. This indicates a considerable archaeal diversity in AOM communities. In this regard, interesting observations were made at surface sediments in the Guaymas Basin next to hydrothermal vents, where samples taken in close proximity with either ANME-1 or ANME-2 were

analyzed for AOM process markers. The presence of archaeol and hydroxyarchaeol was confirmed for both sample types, but their isotopic compositions displayed significant differences. Specifically, the archaeal lipids associated with the ANME-1 assemblage were enriched in ^{13}C compared to the sample with the ANME-2 assemblage. ANME-1 was associated with archaeol and *sn*-2-hydroxyarchaeol being –58 and –70‰, respectively, whereas $\delta^{13}C$ was –82‰ for both compounds in association with the ANME-2 assemblage. Interestingly, the relative contribution of ^{13}C-depleted ether lipids assigned to bacterial members of the AOM consortium (Hinrichs et al. 2000) was significantly lower in the ANME-2 dominated sample.

One important question for understanding the process of AOM is whether this process obligatorily requires the syntrophy of SRB with methanogenic archaea in the form of symbiotic associations as observed in the sediments of the Hydrate Ridge. Orphan et al. (in press) found a similar consortium in sediments of the Eel River Basin. A different archaea/bacteria consortium has been detected in surface sediments above a subsurface gas hydrate layer in the Congo basin (Ravenschlag et al. unpubl. data). It is likely that different forms of syntrophic associations are responsible for AOM in such methane-rich environments. No microscopic investigations have been carried out in low energy zones like in the subsurface sulfate/methane transition zone. Some of the data of Thomsen et al. (in press) point to the existence of a single microorganism capable of AOM in subsurface sediments from Aarhus Bay, Denmark. In subsurface sediments several 100 m below the sea-floor, active sulfate-reducing bacteria are found, and concentrations of methane vary at levels suggestive of dynamic microbial control. It is possible that populations of microorganisms mediating AOM are an integral part of the deep bacterial biosphere.

Lipid Biomarkers Associated with AOM

Lipid biomarkers in the study of microbial communities allow distinction on the level of kingdoms and sometimes orders. A biomarkers' stable carbon isotopic composition bears diagnostic information on the carbon source and/or metabolic carbon fixation pathway utilized by its producer. This property so far has remained inaccessible for gene fragments recovered in environmental studies of microbial ecology. On the other hand, modern, culture-independent techniques such as the de-termination of molecular phylogeny based on 16S rRNA are more specific than lipid biomarkers.

The study of microbial biomarkers in sedimentary environments with active AOM led 1994 to the first evidence that anaerobic microorganisms are capable of assimilating methane-derived carbon (Bian 1994; Bian et al. 2001). Lipid biomarker of methanotrophic archaea often display $\delta^{13}C$ values of –100‰ and lower. This is indicative of more or less exclusive utilization of methane-C as carbon source for biosynthesis, because the $\delta^{13}C$ of biogenic methane is commonly lower than -60‰. Consequently, nowadays in environmental studies of the microbial ecology associated with AOM, molecular bio-markers are a crucial test for the presence of anaerobic methanotrophs and allow a circumstantial connection of other microbiological evidence to AOM (Hinrichs et al. 1999; Boetius et al. 2000; Orphan et al. in press).

Archaeal biomarker

The first observation of a biosynthetic product of anaerobic microorganisms using methane as a carbon source was restricted to crocetane (Bian 1994; Bian et al. 2001), a C_{20} isoprenoid hydrocarbon with a tail-to-tail linkage of presumed archaeal origin (Fig. 3). It was found in anoxic sediments in the Kattegat in the sulfate reduction/methane production-transition zone that exhibited maximum rates of AOM. It took several years until similar findings were made, at that time more or less simultaneously by several groups who studied lipids in ancient and modern sediments deposited in direct vicinity to active methane seeps (Elvert et al. 1999; Hinrichs et al. 1999; Pancost et al. 2000; Thiel et al. 1999). Unlike other archaeal compounds discussed in the following, crocetane has been exclusively associated with AOM, and its biological producers have never been isolated. In between, even the occurrence of sev-

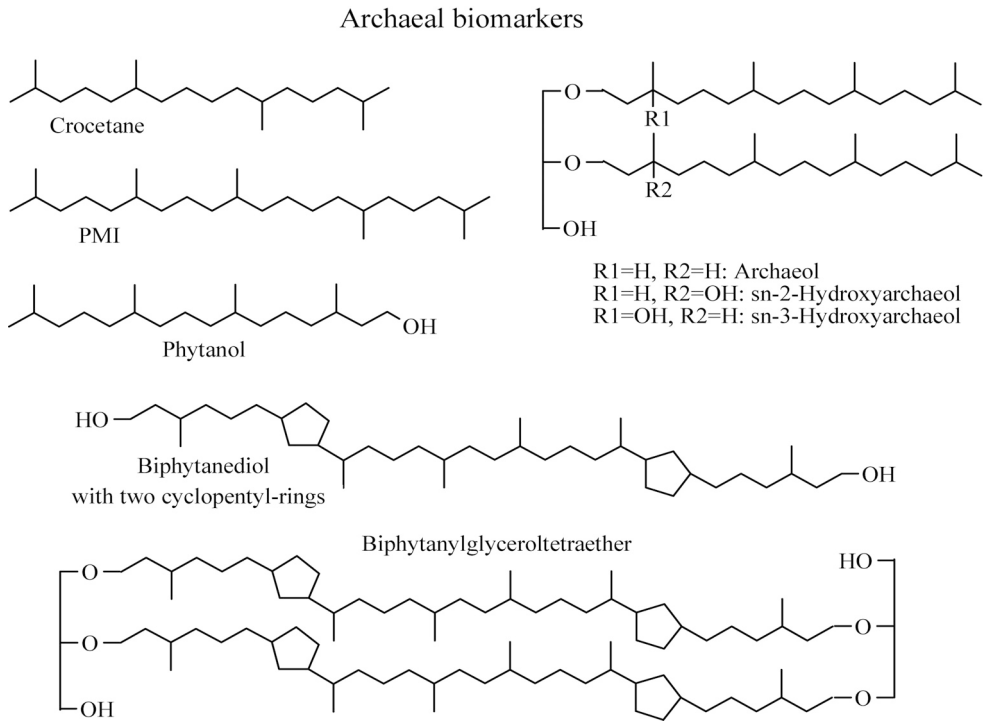

Fig. 3. The most prominent archaeal biomolecules found in AOM environments. Additional compounds are unsaturated analogs of crocetane and PMI.

eral ^{13}C-depleted, unsaturated analogs of crocetane was documented (Elvert et al. 2000; Pancost et al. 2000).

The first unequivocal evidence for an involvement of archaea in AOM came from the detection of ^{13}C-depleted archaea-specific molecules in methane seep environments (Hinrichs et al. 1999). In that study, the compounds archaeol and *sn*-2-hydroxyarchaeol (Fig. 3) with $\delta^{13}C$ values of –100‰ and lower clearly testified to an involvement of archaea in AOM. Based on the chemotaxonomy of cultured organisms, the latter compound pointed to an involvement of members of the *Methanosarcinales*, with certain strains synthesizing hydroxyarchaeol in particular high abundance (Koga et al. 1998 and literature therein). However, we note that *sn*-2- and *sn*-3-hydroxyarchaeol were also observed in low amounts in selected members of the order *Methanococcales*. Archaeol and hydroxyarchaeol were subsequently found at many other methane-rich sampling locations, including Mediterranean mud volcanoes, deep-sea sediments from the Aleutian subduction zone, and in surface sediments at Hydrate Ridge and in the Black Sea, respectively (Table 1). A representative reconstructed-ion-chromatogram of an alcohol fraction from a sedi-ment extract from a seep in the Eel River Basin is illustrated in Figure 4 (after Orphan et al. in press) and exemplifies the imprint of the AOM activity on the inventory of sedimentary lipids.

Additional compounds of archaeal origin that are frequently found in AOM environments are 2,6,10,15,19-pentamethylicosane (PMI) and unsaturated analogs (PMIΔ), phytanol, and biphytanediols and biphytanyltetraethers with both cyclic and acyclic isoprenoid moieties (Fig. 3). Table 1 lists the isotopic compositions of the molecules most commonly associated with AOM for different environments. In this compilation, the presence or absence of data for certain archaeal lipids – in particular archaeol and hydroxyarchaeol - does not necessarily imply that these were not present, because many surveys were limited to hydro-

Environment	Crocetane	PMI, PMIΔ	Archaeol	Hydroxyarchaeol	Phytanol	CH_4[1]
Hydrate Ridge [1,2]	-118 to –108 (n=3)	-124 to –102 (PMI, n=3),	-114	-133		-72 to -62
Eel River Basin [3-5]	-92	-92, –76 (PMIΔ, n=2)	-103 to –100 (n=6)	-106 to –101 (n=6)	-88 to –81 (n=2)	-50
Black Sea [6]	-107	-101	-112	Na	Na	-58
Kattegat, transition zone [7,8]	-100 to –67 (n=5)	-47 to –32 (n=5)	Nd	Nd	Nd	-72
Guaymas Basin [9]	Nd	Nd	-81 to -58 (n=3)	-85 to –70 (n=3)	Nd	-51 to –43
Mediterranean mud volcanoes [10]	~-64 (crocetene)	-91 to –52 (n=6)	-96 to –41 (n=6)	~ -77, –58 (n=2)	Nr	Na
Aleutian SDZ [11]	-130 to –125 (n=3)	-107 to –71, (PMI, n=3), -117 to –98 (PMIΔ, n=3)	-124 (n=2)	Nd	~-120 to -82	Na
Santa Barbara Basin [4]	-119	-129 (PMI: 2)	-119	-128	-120	Na

[1]: $\delta^{13}C$ of methane are data cited in referenced papers. Cited references: [1] Elvert et al. 1999; [2] Boetius et al. 2000; [3, 4] Hinrichs et al. 1999, 2000, respectively; [5] Orphan et al. in press; [6] Thiel et al. 2001; [7] Bian, 1994; [8] Bian et al. 2001; [9] Teske et al. in prep.; [10] Pancost et al. 2000; [11] Elvert et al. 2000. Na = not analyzed, Nd = not detected, Nr = not reported.

Table 1. Overview on isotopic compositions of selected archaeal lipids in different sedimentary environment hosting active AOM communities.

carbons. The distribution and isotopic signatures of archaeal lipids suggest that the archaeal AOM communities are diverse and may differ from site to site. Several studies indicate that crocetane and archaeol/hydroxyarchaeol have different archaeal producers. For example, cro-cetane was present in the transition zone in Kattegat, but the analysis of ether-bound lipids did not reveal any indication for the presence of archaeol and hydroxyarchaeol (Bian et al. 2001). PMI was detected and displayed very different isotopic compositions than crocetane, suggesting that in Kattegat sediments these two compounds originate from different archaea. At some seeps in the Eel River Basin, only archaeol and hydroxyarchaeol were abundant while crocetane and PMI were absent (Hinrichs et al. 1999, 2000). Other seeps contained all four compounds, but $\delta^{13}C$ values differed significantly from each other (Orphan et al. in press). Moreover, at some sites crocetane and/or PMI are more depleted in ^{13}C than archaeol and hydroxyarchaeol, while the opposite was observed at other sites. This lack of systematic isotopic relationships between compounds is consistent with at least a partially different origin rather than biosynthesis-related differences in fractionation in a single organism. Similarly, relative concentrations of these compounds at different sites do not follow any systematic rules. An isotopic difference between archaeol and hydroxyarchaeol is observed frequently and may also indicate an at least partially different origin. In most samples from several different environments with both compounds present, hydroxyarchaeol is more depleted in ^{13}C relative to archaeol by 4 to 19‰.

Additional chemotaxonomic evidence for the diversity of archaea involved in AOM is available when comparing biomarker data from different environments. For example, Pancost et al. (2000) and Thiel et al. (2001) report the presence of strongly ^{13}C-depleted biphytanediols and biphytanyltetraethers (Fig. 3) in methane-laden environments in the Mediterranean and the Black Sea (Table 1). In contrast, the biphytanediols found at methane seeps from the Californian continental margin display $\delta^{13}C$ values of around –20‰

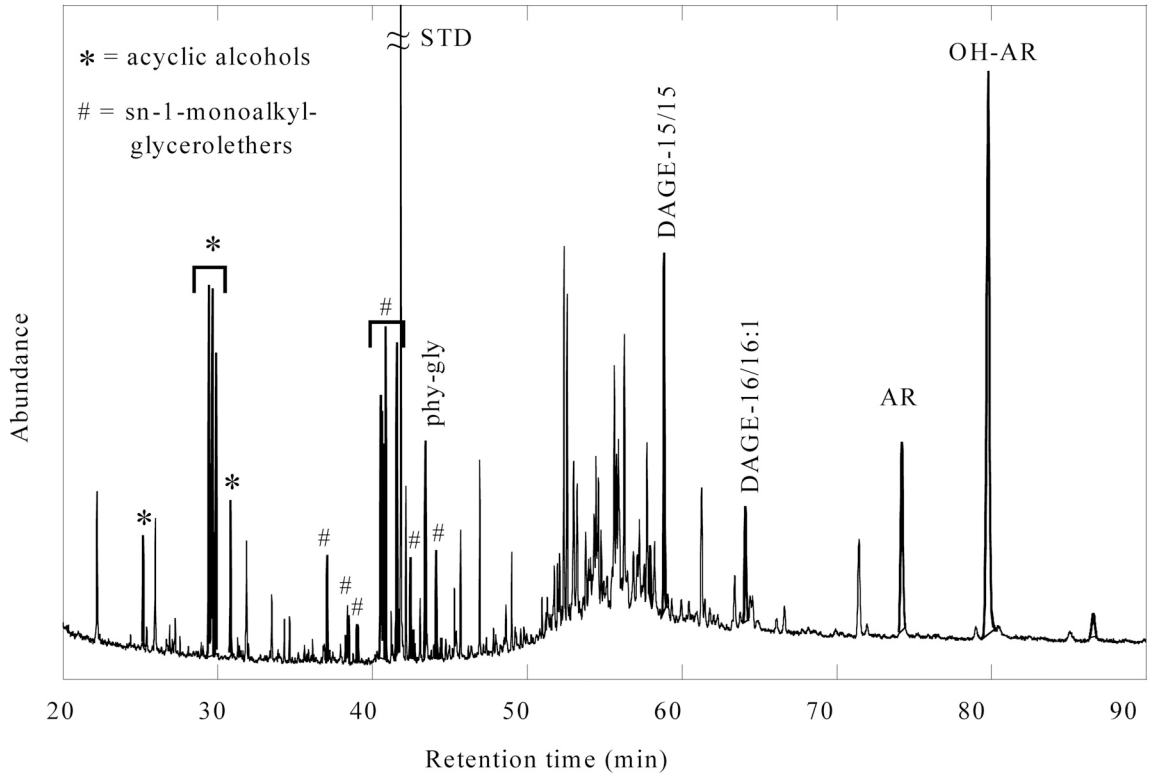

Fig. 4. Reconstructed-ion-chromatogram of alcohol fraction from a sediment sample taken at 6-9 cm depth at an active methane seep in the Eel River Basin (adapted from Orphan et al. in press). Labeled peaks designate compounds with isotopic compositions indicating a partial or exclusive derivation from anaerobic methanotrophic microbes. * = C_{14} to C_{17} acyclic alcohols from bacteria, # = sn-1-monoalkylglycerolethers with ether linked C_{14} to C_{18} acyclic alcohol moieties from bacteria. Text symbols: DAGE designates dialkylglycerolether with carbon numbers of ether-linked alkyl moieties expressed as numbers, e.g. DAGE-15/15 = diether with two C_{15}-alkyl moieties, phy-gly = sn-1-monophytanylglycerolether (archaea), AR = archaeol (archaea), OH-AR = sn-2-hydroxyarchaeol (archaea).

(K. Hinrichs, unpubl. data), which is consistent with their derivation from archaeal plankton (e.g. Schouten et al. 1998). This suggests that certain members of the archaeal methanotrophic community present in the Mediterranean and Black Sea do not participate in AOM communities at the Californian continental margin. An additional indication for the archaeal diversity is the detection of *sn*-3-hydroxyarchaol as a product of archaeal AOM members in Mediterranean Sea sediments (Pancost et al. 2000). This compound, originally found in *Methanosaeta concilii*, was not ob-served in other environments, where only the *Methanosarcina*-type *sn*-2-hydroxyarchaeol was found (Hinrichs et al. 1999; 2000; Orphan et al. in press; Teske et al. 2001).

Bacterial biomarker

In most of the previously discussed surveys of lipid inventories associated with AOM communities, ^{13}C-depleted archaeal biomarker co-occur with ^{13}C-depleted compounds of presumed bacterial origin. The bacterial compounds are usually slightly enriched in ^{13}C relative to their archaeal counterparts with typical $\delta^{13}C$ values between -100 and –50‰, depending on compound and environment. Similarly to archaeal products, this range of values indicates a partial to exclusive incorporation of methane-derived carbon. The structural distribution of these lipids is not specific for, but consistent with, their derivation from sulfate-reducing bacteria.

These lipids encompass fatty acids with 14 to 18 carbon atoms, glycerolether derivatives with one or two non-isoprenoidal alkyl chains that show strong structural resemblance to the concurrently occurring fatty acids (e.g. identical number of carbon atoms, position of double bonds, and methyl substitution), and alcohols with structural features very similar to those of the two aforementioned classes of compounds (Fig. 5). Figure 4 illustrates a high relative contribution of ^{13}C-depleted alcohols and ether lipids to an extract from a sediment at an active methane seep. The fatty acids associated with AOM communities display typical features of fatty acids from sulfate reducing bacteria, i.e., high relative amounts of methyl-branched and cyclopropyl isomers and double-bond positions typical for fatty acids from sulfate reducers (see Hinrichs et al. 2000, for a review of structural and isotopic features of these compounds in seep sediments).

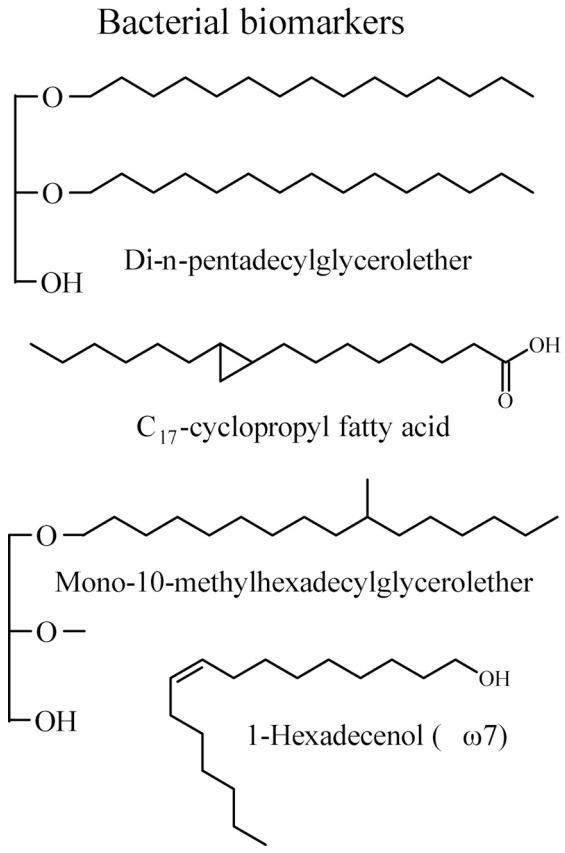

Fig. 5. Selected biomarkers assigned to bacterial syntrophic partners of methanotrophic archaea.

Little is known about the biological precursors of mono- and dialkyglycerolethers (MAGE and DAGE, respectively) and alcohols with similar alkyl structures (Fig. 5). Their structures are distinct from those of the ether lipids produced by archaea. The latter synthesize exclusively isoprenoidal ether lipids whereas these compounds utilize *n*-alkyl and methyl-alkyl substituents. Among bacteria, the only known producers of non-isoprenoidal mono- and dialkyl-ether lipids are the phylogenetically deeply branching autotrophic phyla *Aquifex spp.* and *Thermodesulfotobacterium commune* (Huber et al. 1992 and literature therein). However, these ether lipids have not been isolated from any mesophilic or psychrophilic sulfate reducer. Both are (hyper)thermophiles restricted to hydrothermally active environments. In seep sediments with abundant bacterial ether lipids, phylotypes associated with these known producers were not detected in bacterial 16S rRNA libraries generated from seep sediments (Orphan et al. in press; Teske et al. 2001). Nevertheless, the chemotaxonomic link suggests that psychrophilic or mesophilic bacterial members of the methanotrophic community have their closest relatives among thermophiles that occupy functionally well-defined niches in complex hydrothermal microbial mat systems. For instance, the only prior environmental occurrence of di-*n*-pentadecylglycerolether, a major compound in Eel River Basin seep sediments (Hinrichs et al. 2000) (see also Fig. 3) (DAGE 15/15), was noted in Yellowstone hot spring bacterial mats (Zeng et al. 1992). These compounds may be a key to further information on bacterial AOM members with unknown biochemical capabilities, e.g. as hypothesized by Thomsen et al. (in press).

The carbon isotopic compositions of individual lipids related to different AOM community members are consistent with existing hypotheses concerning the trophic structure. In general, maximum ^{13}C-depletion is observed in biolipids derived from methanotrophic archaea (Fig. 6), followed by slightly smaller depletions in the non-isoprenoidal ether lipids (Hinrichs et al. 2000). This isotopic

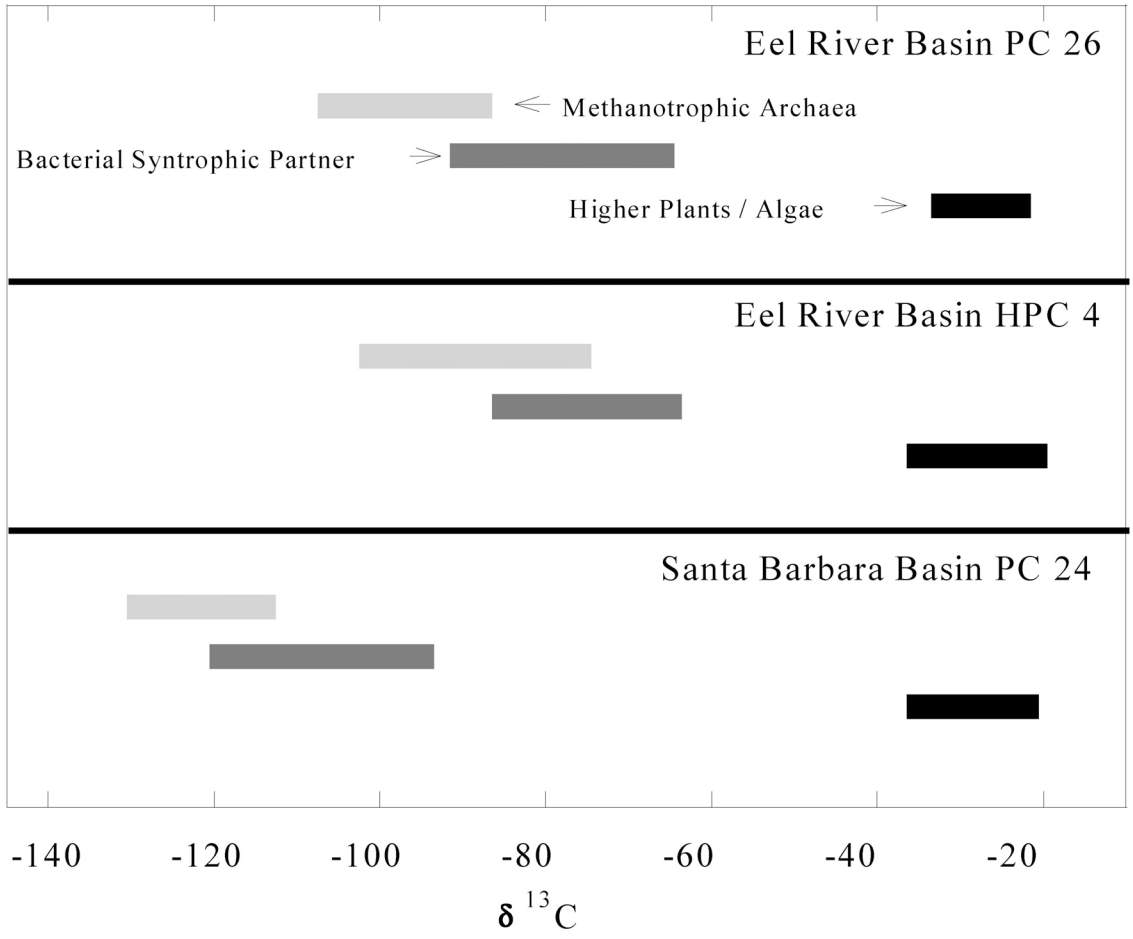

Fig. 6. Ranges of carbon isotopic compositions of sedimentary lipids assigned to different sources from three different methane seeps. Non-isoprenoidal ether- lipids were used for ranges for the bacterial syntrophic partner (adapted from Hinrichs et al. 2000).

stratification is consistent with, but not proof for, an initial oxidation of methane by methanotrophic archaea, which transfer their oxidation products (inorganic carbon plus H_2, or organic C_1 or C_2 compounds) to an intimately associated bacterial partner (e.g. Fig. 2). This partner utilizes pre-dominantly the carbon supplied by the archaeon, as indicated by the strong ^{13}C-depletion of the ether lipids. Under the assumption that the ether-lipid producers are autotrophs, this mechanism requires formation of microenvironments with strong physicochemical gradients so that diffusion of porewater CO_2 to the reaction center would be much slower than microbial utilization of CO_2 from the oxidation of methane.

Relationship between environmental factors and the $\delta^{13}C$ of biomarker lipids

In general, the carbon isotopic composition of the lipid products of methanotrophic microorganisms appears to be controlled by the following factors:

- The carbon isotopic composition of methane.
- The composition of the microbial community, i.e., different organisms fractionate differently during lipid biosynthesis.
- Environmental conditions such as temperature, substrate limitations and ^{13}C-enrichment in residual methane in predominantly diffusion-controlled sediment columns.

For data from methane seep environments, the general influence of $\delta^{13}C$ of methane on that of lipid biomarkers assigned to methanotrophic archaea is evident and is most consistently reflected in the isotopic compositions of archaeol, hydroxyarchaeol, and crocetane. In most sediments with available data on $\delta^{13}C$ of methane, archaeol is depleted in ^{13}C relative to methane by about –50‰. The relative depletions in other lipids associated with AOM appear to be more variable. For example, the difference in $\delta^{13}C$ of methane between the Eel River Basin and Hydrate Ridge is reflected in ^{13}C-depletions in archaeol of a similar magnitude. The large fractionation between substrate and biomass is consistent with findings from laboratory experiments of the methanogen *Methanosarcina barkeri* growing on methylamine (Summons et al. 1998). Notably, the difference between the isotopic composition of methanotrophic lipid products and the substrate is significantly lower at hydrothermal vent sites in the Guaymas Basin, where methane, derived from thermal and microbial decomposition of organic matter with $\delta^{13}C$ of –51 to –43‰ occurs in high concentrations in the upper sediment column. Here, an influence of the about 15°C higher temperatures in the uppermost centimeters of the sediment may reduce the carbon isotopic fractionation during lipid biosynthesis, resulting in archaeal products with $\delta^{13}C$ between –81 and –58‰.

The microbial oxidation of methane leads to an enrichment in ^{13}C in the residual methane due to a preferential utilization of ^{12}C-methane. This process can cause a "distillation" of ^{13}C-methane in upward sediment profiles and may affect the carbon isotopic composition of the microbial biomass formed upon methane oxidation, especially in sediment zones with limited, diffusion-controlled supply of methane. However, the relatively constant fractionation between methane and methanotrophy-based, biosynthetic products at most seep sites suggest that this effect is less important when the flux of methane is large compared to the fraction consumed. For example, down-core variations of $\delta^{13}C$ of archaeol and hydroxyarchaeol at an active seep are negligible and do not indicate any effect of ^{13}C-enrichment in methane on the lipid isotopic composition (Fig. 7). These depth profiles are consistent with a constant biological fractionation under conditions of large excess of the methane substrate. In contrast, the $\delta^{13}C$ of an ether lipid assigned to a bacterial member of syntrophic AOM community decreases with depth (Fig. 7). This trend is probably related to a parallel decrease of $\delta^{13}C$ in pore water CO_2, which may be partially utilized by the bacterial syntrophic partner in addition to intermediates and products of methane oxidation that are transferred directly to the bacterial syntrophic partner. This increasing ^{13}C-depletion with depth is supported by the isotopic compositions of authigenic carbonates in the same core, with $\delta^{13}C$ values decreasing from –18 to ~ -30‰ over the same depth interval (Orphan et al. in press).

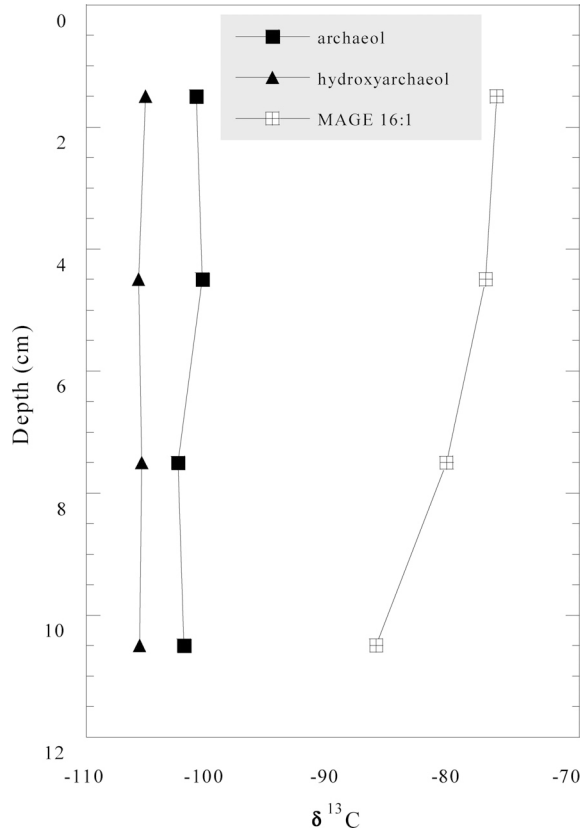

Fig. 7. Depth profile of carbon isotopic compositions of individual compounds assigned to members of the AOM consortium in a sediment core at an active seep in the Eel River Basin (data from Orphan et al. in press). MAGE 16:1 designates a monoalkylglycerolether with the alcohol moiety being $C_{16:1(\omega7)}$.

A very different situation was observed at the *Napoli mud breccia* in the Mediterranean (Pancost et al. 2000). At this site, the flux of methane was significantly lower than at other sites studied by the team. The "distillation" of ^{13}C-methane in the upward sediment profile by continuous preferential consumption of ^{12}C-methane is evident in $\delta^{13}C$ of PMI and archaeol, which increase by about 20‰ in the depth interval from 18 to 3 cm below the seafloor to values that are indistinguishable from those of products of primary production (e.g. archaeol = -20‰). The generally elevated $\delta^{13}C$ values here might be related to (a) a higher ^{13}C-content of the methane source compared to other environments, (b) a limitation of the methane substrate with possibly nearly quantitative consumption of the methane, thereby preventing the full expression of the biological fractionation during lipid biosynthesis, and (c) a continuous ^{13}C-enrichment in the residual methane, which may have been initiated already at sediment depths below those sampled by Pancost et al. (2000). A similar observation was made in Kattegat sediments, where the depth profiles of $\delta^{13}C$ of crocetane and PMI did not display any resemblance, although the isotopic composition indicated that both compounds contained at least partially methane-derived carbon (Bian et al. 2001).

The rates

First estimates of methane oxidation rates in anoxic marine sediments were obtained by numerical modeling of changes in pore water concentrations of methane and sulfate in shallow water as well as deep-sea sediments (Barnes and Goldberg 1976, Reeburgh 1976). Reeburgh (1980) and Iversen and Blackburn (1981) used ^{14}C-labeled methane for sediment incubations and subsequent measurement of $^{14}CO_2$ production in coastal sediments. Both methods are still used to estimate rates of AOM in marine sediments and in the water column. For the measurement of methane concentrations in sediments, a sample is mixed into a sodium hydroxide solution. After equilibration with the headspace of the sample vial, gas is sampled with a gas tight syringe and injected into a gas chromatograph with a flame ionization detector. The modeling approach assumes a steady state of pore water concentrations and a transport of methane by molecular diffusion only. Then, the consumption of methane (R) is balanced by the supply of methane due to molecular diffusion. Usually, the sediment zone of interest is divided into several depth intervals (z) and the simplest consumption profile is chosen which fits the concentration profile best, according to the formula

$$R = D_s \, d^2C/dz^2 \qquad (13)$$

At temperatures between 2-10°C, the diffusion coefficient (D_s) for methane is between 0.7-1 x 10^{-5} cm^2 s^{-1} (Li and Gregory 1974). One of the largest problems of this method is the accurate determination of methane concentrations (C) in zones of supersaturation of methane. In seawater, methane is soluble to a concentration of up to 1.5 mM at low temperatures and atmospheric pressure. At higher concentrations, gas bubbles form and methane is lost due to ebullition. The solubility of methane increases linearly with increasing pressure. Thus, methane can escape detection due to depressurization of deep-sea sediments during core recovery. Unfortunately, no methane sensors are yet available for *in situ* profiling in deep-sea sediments.

The radioisotope measurements are more sensitive and can detect very low methane oxidation rates. ^{14}C-methane is injected into undisturbed sediment samples and incubated for hours to days. Subsequent mixing of the samples into a solution of sodium hydroxide terminates the microbial activity. After degassing of methane from the sample, the $^{14}CO_2$ produced by oxidation of radiolabeled methane is stripped from the sample by acidification and captured in a CO_2-adsorbing agent for measurement in a scintillation counter. The AOM rate is calculated from the amount of $^{14}CO_2$ formed and the concentration and radioactivity of methane in the sample (Iversen and Blackburn 1981). Problems with this method may arise from the use of impure ^{14}C-methane (containing ^{14}CO or $^{14}CO_2$) or due to aeration, warming, and decompression of the samples during recovery and handling. The resulting ebullition of methane causes alteration of the sediment struc-

ture and of the pore water gradients, which can lead to substantial analytical bias. No instrument for the *in situ* measurement of methane oxidation is available yet.

Other approaches to estimate AOM include numerical modeling based on pore water carbon isotope data (Burns 1998) and measurements of sulfate reduction rates (SRR; Aharon and Fu 2000; Boetius et al. 2000). However, the percentage of sulfate consumed by sulfate reduction coupled to AOM is rather variable and thus impedes the accuracy of estimates based on consumption of sulfate. In surface sediments of non-seep environments, AOM generally accounts for around 10% of sulfate reduction, with increasing proportions towards the subsurface methane-sulfate transition zone. Here, several studies detected contributions of AOM to total SRR of 40-100% (Borowski et al. 2000; Fossing et al. 2000; Niewöhner et al. 1998). To date, only two studies determined the contribution of AOM rates to SRR at methane seeps. The proportion of sulfate reduction coupled to AOM was 8% at methane seeps in Eckernförde Bay (Bussmann et al. 1999) and around 30% at the gas hydrate bearing caldera of the Arctic Haakon-Mosby mud vulcano (Lein et al. 2000). However, the comparison of SRR at methane seeps with those at close-by control stations indicates that AOM is largely responsible for sulfate reduction at seeps. In the Gulf of Mexico, SRR was 600 times higher at methane seeps than at reference stations (Aharon and Fu 2000). At Hydrate Ridge, SRR were extremely high above gas hydrate (ca. 5 $\mu mol\ cm^{-3}\ d^{-1}$), but below detection limit at a nearby reference station.

Table 2 shows an overview on published rates of AOM measured in different areas of continental margins. In each depth zone, modelled AOM rates are lower than AOM rates determined with ^{14}C-methane. In a comparative study using both methods, the modelled rates were 4-fold lower than measured rates (Iversen and Jørgensen 1985). Despite all the uncertainties related to the comparability of methods and to their intrinsic errors, the range of rates (less than two orders of magnitude) is not different to that found in SRR or oxygen consumption rates. In contrast to O_2 consumption, there is only a slight decrease in AOM rates with water depth. The decrease in O_2 consumption with water depth in marine sediments is explained by the decrease of the input of POC to the seafloor via sedimentation. In the case of methane turnover, one would also assume a link to POC sedimentation rates, since methane is mostly derived from the microbial degradation of POC. However, this link is not obvious for the process of AOM. The calculation of the relative amounts of methane consumed via AOM in four different depth zones of continental margins indicates about equal contributions of each zone to the global budget of methane consumption in marine sediments (Table 3). In contrast, the distribution of aerobic POC degradation (equivalent to oxygen uptake) is an order of magnitude higher on the shelf than on the slope (Jørgensen 1983). According to our estimate, AOM in marine sediments sums up to 300 Tg a^{-1}, which is nearly four times higher than the previous estimate by Reeburgh (1996). The revised budget compares to 10% of the total O_2 consumption in marine sediments, i.e., to 12% of the aerobic POC oxidation (applying the Redfield ratio).

These calculations do not include AOM rates at methane seeps, which are at least an order of magnitude higher than AOM rates in non-seep sediments. Unfortunately, reliable estimates of the total area of sediments affected by methane seepage are not yet available. In addition, conditions similar to those at cold seeps are likely to prevail at hydrothermally active, marine sedimentary environments as indicated by observations of diagnostic biomarkers and phylotypes at hydrothermal vent sites in the Guaymas Basin (Teske et al. in 2001). A variety of different seep types are known from active and passive margins. Methane seeps may be caused by hydrocarbon deposits, brine fluids, sediment compaction, or land slides (Sibuet and Olu 1998). Also, methane seepage is often related to subsurface gas hydrate deposits which can continuously feed methane seeps at active and passive continental margins. Furthermore, the dissociation of surficial methane hydrates can support methane seepage in those zones of continental margins where the hydrates are at their stability limit (500-1000 m water depth). Areas with gas hydrates exposed at the

Area	Location	Water depth (m)	Sediment depth (cm)	AOM (nmol cm^{-3} d^{-1})	Average (nmol cm^{-3} d^{-1})	Integrated (mmol m^{-2} d^{-1})	Integrated (mmol m^{-2} a^{-1})	Method	Source
Inner	Kysing Fjord	1	12	0.01-0.27	0.07	0.01	3	tracer exp.	Iversen and Blackburn 1985
shelf	Norsminde Fjord	1	40	0-17	7	2.80	1022	tracer exp.	Hansen et al. 1998
0-50 m	Cape Lookout	10	35	0-19	5	1.75	639	tracer exp.	Hoehler et al. 1994
	Aarhus Bay	16	300	0-1		0.05	16	tracer exp.	Thomsen et al. 2001
	Eckernförde Bay	25	50	0-34	0.6	0.29	106	tracer exp.	Bussmann et al. 1999
Outer	Kattegat	65	170	0-6	0.5	0.83	303	tracer exp.	Iversen and Jørgensen 1985
shelf	Skan Bay	65	35	0-9	3	1.14	415	tracer exp.	Reeburgh 1980
50-200m	Skan Bay	65	40	0-10	2	0.88	321	tracer exp.	Alperin and Reeburgh 1985
	Black Sea	130	350			0.11	41	modelling	Jørgensen et al. 2001
		181	350			0.10	35		
Upper	Skagerak	200	110	0-12	1.0	1.16	423	tracer exp.	Iversen and Jørgensen 1985
margin	Saanich Inlet	225	27	0-0.75	5	1.26	460	tracer exp.	Devol 1983
200-	Black Sea	396	350			0.08	30	modelling	Jørgensen et al. 2001
1000m		1176	350			0.05	18		
Lower	Cariaco trench	1300	120			0.44	159	modelling	Reeburgh 1976
margin		1400	120			0.15	55	modelling	
1000-	Namibia slope	1312	70			0.08	30	modelling	Niewöhner et al. 1998
4000m		2060	70			0.14	52		
	Namibia slope	1373	400			0.22	80	modelling	Fossing et al. 2000
		2065	600			0.15	55		
	Amazon Fan	2700	5000	0-0.3				isotopes	Burns 1998
	Blake Ridge	3000	2300	0-0.015	0.004	0.01	3	modelling	Borowski et al. 2000
	Zaire fan	3950	1550			0.10	36	modelling	Zabel and Schulz 2001
Methane	Eckernförde Bay	25	50	0-50	10	5	1880	tracer exp.	Bussmann et al. 1999
seeps	Eel River Basin	520	10			32	11,500	isotopes	Hinrichs subm.
	Gulf of Mexico	590	30			4	1338	SRR	Aharon and Fu 2000
	Hydrate Ridge	750	15	0-3000	300	45	16,425	SRR	Boetius et al. 2000
	HMMV Caldera	1250	20	1-70	26	5	1898	tracer exp.	Lein et al. 2000

[a] For the calculation of AOM from SRR we used the average AOM/SRR ratio of all investigations (30%). Most likely, this leads to an underestimate of AOM for reasons discussed in the text.

Table 2. Rates of anaerobic oxidation of methane in sediments at continental margins and at methane seeps.

seafloor (e.g. Hydrate Ridge; Suess et al. 1999) are characterized by similar chemosynthetic communities and carbonate structures as other methane seeps. The data compiled in Table 3 indicate that, even if the area affected by methane seepage at continental margins is below 1%, this might have a significant impact on the total methane budget. Thus, a more accurate mapping of seepage areas is extremely important for realistic calculations of methane consumption in the sea. Recent investigations by Suess et al. (1999) show that the same might be true for oxygen consumption at seeps, which might significantly contribute to the total oxygen demand of continental margin sediments.

The Potential Role of AOM in the Evolution of Biogeochemical Cycles on Earth

Studies of carbon isotopic compositions of kerogens and organic carbon suggest that around 2.7 Gyr ago methanotrophy was an important pathway of carbon assimilation in earth's biogeochemical cycle (Hayes 1994). Until recently, it was accepted as common sense that this isotopic excursion indicates a period in Earth history when increases in the concentration of free oxygen in the ocean and atmosphere allowed aerobic methanotrophic bacteria to radiate. Due to the abundant methane in ocean and atmosphere, methanotrophs were able to produce significant amounts of microbial biomass that were subse-quently buried in the rock record and had de-creased the isotopic composition of the buried organic carbon. An anaerobic mechanism was not seriously considered because the anaerobic, sulfate-dependent oxidation of methane was believed to be very inefficient in biomass production, at least compared to the pathway involving methanotrophic bacteria. AOM was therefore thought to be inadequate to explain large contributions by methane assimilating microbes to buried organic carbon on the order of 30 to 40% (Hayes 1994).

New findings, however, of unexpectedly large quantities of methane-derived carbon from anaerobic, methanotrophic microbial consortia in recent marine sediments have motivated a reassessment of the paleoenvironmental conditions during the era of methanotrophy 2.7 Gyr ago (Hinrichs subm.). Comparisons of the accumulation rates of methane-derived carbon in anoxic modern sedimentary environments to those in the Late Archaean Tumbiana Formation served as the basis for this reassessment. The Tumbiana Formation is a member of the Fortescue Group with consistently ^{13}C depleted organic carbon. The comparison indicated that the rates of accumulation of methane-derived carbon in rocks from the Tumbiana Formation were significantly lower than rates at modern methane seeps. Instead, they compared well to rates observed in the methane/sulfate transition zone of modern anoxic sediments at Kattegat, where AOM occurs at moderate concen-

	AOM ($mmol\ m^{-2}\ d^{-1}$)	O_2 uptake ($mmol\ m^{-2}\ d^{-1}$)	Area ($10^{12}\ m^2$)	AOM ($10^{12}\ mol\ a^{-1}$)	O_2 uptake ($10^{12}\ mol\ a^{-1}$)
Inner shelf	1.0	20	13	4.6	95
Outer shelf	0.6	10	18	4.0	66
Upper margin	0.6	3	15	3.5	16
Lower margin	0.2	0.3	106	6.9	12
			Sum: 152	19 (304 Tg CH_4)	
Seepage areas	18	[a]471	*0.5%= 0.75 10^{12} m^2*	*4.9*	*129*

[a] Average of oxygen uptake rates measured at different seeps as reported by Suess et al. (1999).

Table 3. Anaerobic oxidation of methane in different depth zones of continental margins. The areas and O_2 uptake rates are adapted from Jørgensen (1983). The values represent averages of the data shown in Table 2. The calculation of the contribution of areas affected by methane seepage is purely speculative (indicated in italics). 10^{12} mol CH_4 is equivalent to 16 Tg CH_4.

trations of both methane and sulfate (Iversen and Jørgensen 1985; Bian et al. 2001). This finding indicates that, in principle, AOM could provide a mechanism for the formation of iso-topically light organic carbon 2.7 Gyr ago.

This alone does not explain the temporary nature (probably ~ 0.1 Gyr duration) of a hypothetical era of methanotrophy based on AOM. An aerobic mechanism as proposed (Hayes 1994) would be consistent with the evidence that oxygenic photosynthesis was already established at that time (see Summons et al. 1999 and literature therein). Accordingly, the termination of the "isotopic event" was related to the continuous increase of atmospheric oxygen concentrations to levels that drove methanogens into more restricted environments with poorer supplies of fermentable organic matter, thereby progressively decreasing the amounts of methane produced.

On the other hand, two points argue against the aerobic mechanism: (1) In the present marine environment under well aerated conditions, methane oxidation and in particular the accumulation of methane-derived carbon are clearly dominated by AOM, even at methane seeps with a large interface between methane-rich fluids and oxygenated waters. (2) Although oxygen was obviously produced, there is little consensus on the actual degree of oxygenation of the Earth's surface around that time. However, it appears well established that a significant accumulation of oxygen commenced not later than around 2.1 Gyr. Several recent studies suggest that concentrations of sulfate were significantly lower than those at present (Canfield et al. 2000; Farquhar et al. 2000) and that significant changes in the sulfur cycle occurred with the onset of the Proterozoic. In that light, an anaerobic pathway of methane-derived carbon fixation may well mark an important transition in the biogeochemical cycles of carbon and sulfur.

Accordingly, the sequence of possible events can be described in three stages (Fig. 8). (1) Prior to ~2.7 Gyr, accumulations of free oxygen and sulfate were probably minimal and the remineralization of organic carbon occurred in anoxic habitats, presumably by fermentative pathways with meth-ane and CO_2 being the terminal products. Due to low levels or absence of biologically utilizable electron acceptors, methane was probably stable at saturation levels in pore waters of sediments and in large parts of water bodies. (2) With increasing oxygenation of the Earth's surface as a result of oxygenic photosynthesis, sulfate concentrations increased locally and/or globally above certain thresholds that led to more favorable conditions for sulfate reducers and consequently for AOM. An excellent analog for this temporal transition in the sulfur-methane cycle can be found in a vertical sequence of a modern anoxic sediment (Fig. 8). Moving upwards, at the intersection of the zones of methanogenesis and sulfate-reduction, the an-aerobic consumption of methane becomes the dominant sulfate-consuming microbial process and leads to stoichiometric consumption of sulfate. Similarly, in certain modern environments, almost all sulfate flows to the anaerobic oxidation of methane (e.g. Boetius et al. 2000; Aharon and Fu 2000). In analogy to these modern examples, the 2.7 Gyr excursion possibly marks an geological era during which sulfate reduction is predominantly coupled to AOM. (3) The termination of the era of methanotrophy was caused by the continuous increase of oxygenation, leading to larger inven-tories of sulfate. For the microbial breakdown of organic carbon the consequences of such a tran-sition are an increasing importance of anaerobic respiratory processes and a decreasing importance of fermentative pathways. Simultaneously with this transition, the fraction of methane formed from each mole of organic carbon decreases. Again, the vertical sedimentary sequence displays model character for the characterization of this transition in the modes in carbon remineralization in Earth's history. For example, in sediments containing sulfate-rich pore waters, methanogens are not successful in the competition with sulfate reducers for H_2 or acetate, preventing significant formation of methane in this zone.

In conclusion, an anaerobic mechanism for the deposition of unusually ^{13}C-depleted organic carbon at 2.7 Gyr ago appears feasible. Accordingly, the isotopic anomaly marks a period of increasing importance of microbial sulfate reduction for Earth's carbon cycle.

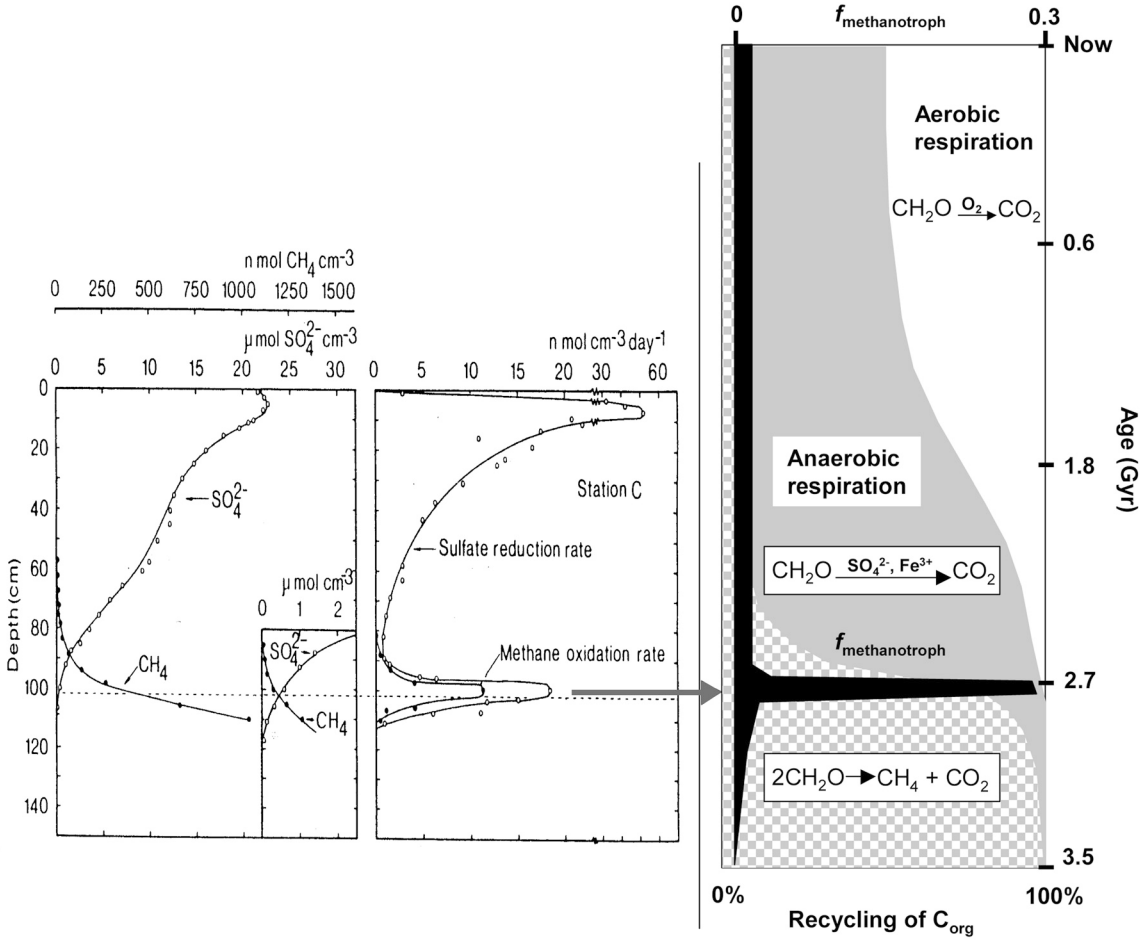

Fig. 8. Speculative comparison of a typical vertical distribution of biogeochemical zones in coastal marine sediments with a hypothetical, temporal evolution of biogeochemical cycles in Earth's history (Hinrichs in prep.).

Acknowledgements

This manuscript profited considerably from discussions with Marcus Elvert, Lisa Levin, Vicki Orphan, Andreas Teske, Tina Treude, and David Valentine. We thank Marcus Elvert, Bo Barker Jørgensen, and Rudi Amann for their helpful reviews. KH gratefully acknowledges the support provided by a fellowship at the Hanse Institute of Advanced Study in Delmenhorst. This is Woods Hole Oceanographic Institution contribution 10488.

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