**Biogeosciences
Discussions**

Limitations of microbial hydrocarbon degradation at the Amon Mud Volcano (Nile Deep Sea Fan)

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Abstract

The Amon mud volcano (MV), located at 1250 m water depth on the Nile Deep Sea Fan, is known for its active emission of methane and non-methane hydrocarbons into the hydrosphere. Previous investigations showed a low efficiency of hydrocarbon-degrading
5   anaerobic microbial communities inhabiting the Amon MV center in the presence of sulphate and hydrocarbons in the seeping subsurface fluids. By comparing spatial and temporal patterns of in situ biogeochemical fluxes, temperature gradients, pore water composition and microbial activities over three years, we investigated why the activity of anaerobic hydrocarbon degraders can be low despite high energy supplies. We found
10 that the central dome of the Amon MV, as well as a lateral mud flow at its base, showed signs of recent exposure of hot subsurface muds lacking active hydrocarbon degrading communities. In these highly disturbed areas, anaerobic degradation of methane was less than 2 % of the methane flux. Rather high oxygen consumption rates compared to low sulphide production suggest a faster development of more rapidly growing aerobic
15 hydrocarbon degraders in highly disturbed areas. In contrast, the more stabilized muds surrounding the central gas and fluid conduits hosted active anaerobic hydrocarbon-degrading microbial communities. Furthermore, within three years, cell numbers and hydrocarbon degrading activity increased at the gas-seeping sites. The low microbial activity in the hydrocarbon-vented areas of Amon mud volcano is thus a consequence
20 of kinetic limitations by heat and mud expulsion, whereas most of the outer mud volcano area is limited by hydrocarbon transport.

1   Introduction

Submarine mud volcanoes (MV) form where tectonic or gravitational forces induce vigorous discharge of muds, fluids and methane gas from deep subsurface strata (Milkov,
25 2000; Kopf, 2002). Long-term degassing after eruption events via subsurface conduits can promote the formation of cold seep ecosystems at the seafloor, attracting

diverse and rich chemosynthetic communities (Sibuet and Olu, 1998; Werne et al., 2004; Niemann et al., 2006). In most cold seep ecosystems, the anaerobic oxidation of methane (AOM) with sulphate is a key biogeochemical process, delivering sulphide to fuel chemosynthetic life (Boetius et al., 2000). Sulphide is used as energy source by thiotrophic bacteria that are either free living or symbiotically associated with chemosynthetic animals (Jørgensen and Boetius, 2007). Cold seeps emitting higher hydrocarbons and oil in addition to methane generally show the highest sulphate reduction rates and sulphide fluxes, often exceeding AOM (e.g., Joye et al., 2004; Omoregie et al., 2009). AOM is known to control methane emission to the hydrosphere across a wide range of environmental conditions, wherever methane and sulphate meet (Knittel and Boetius, 2009). Due to the activity of methanotrophic microbial communities, the ocean is a relatively minor source of the greenhouse gas methane (Reeburgh, 2007).

Recently, numerous studies investigated spatial distribution patterns of seep communities and biogeochemical processes, indicating that transport of gases and fluids to the surface seafloor are the main factors shaping cold seep ecosystems (Sibuet and Olu, 1998; Olu-Le Roy et al., 2004; Niemann et al., 2006; Huguen et al., 2009; Cordes et al., 2010; Vanreusel et al., 2010; Grünke et al., 2011; Ritt et al., 2011). Generally one may expect that higher hydrocarbon flux relates to higher microbial activity, as more electron donor becomes available. However, several recent studies have also indicated transport-related limitations to the microbial efficiency in anaerobic consumption of methane: for example, in active mud volcano systems with high upward flow rates of sulphate-depleted subsurface fluids microbial activity is limited by the lack of electron acceptors (De Beer et al., 2006; Felden et al., 2010). Furthermore, kinetic limitations affecting the function of microbial metabolism may apply for example in highly acidic environments (Inagaki et al., 2006; Yanagawa et al., 2012), briny (Joye et al., 2009) or hyperthermophilic environments (Brazelton et al., 2006; Holler et al., 2011).

Here we studied biogeochemical processes at the active Amon MV, which is situated on the border between the Central and Eastern province of the Nile Deep Sea Fan and characterized by high thermal gradients (Dupré et al., 2007). Deposition of

evaporites during the Messinian sealed an underlying petroleum system, which leaks through faults piercing the Messinian evaporites and enriching the ascending fluids in sulfate from gypsum dissolution (Foucher et al., 2009; De Lange and Krijgsman, 2010). The Amon MV has a dome-shaped structure formed by one main feeding channel for the upward transport of muds, fluids and gases from the deep subsurface. In addition, a lateral outflow of briny muds occurs at its base (Girnth et al., 2011). Earlier investigations showed conspicuously high methane and hydrocarbon emissions into the hydrosphere, and an apparent limitation of anaerobic microbial hydrocarbon degradation in the sediments of the Amon MV, despite the availability of sulphate excluding transport limitation (Mastalerz et al., 2009; Omoregie et al., 2009). Hence the aim of this study was to investigate potential kinetic limitations of hydrocarbon degradation, and the consequences for the structure and functioning for the cold seep ecosystem of Amon MV. We tested the following hypotheses: (I) Methanotrophic activity is repressed by the composition of subsurface fluids; (II) Methane oxidation is absent from hot subsurface mud advected rapidly from greater depth; (III) Methanotrophic communities develop too slowly to populate MV sediments disturbed from recent gas eruptions.

2   Materials and methods

2.1   Sampling site

The Nile Deep Sea Fan is one of the world's largest deep sea fans ($90\,000\,\text{km}^2$) and comprises large subsurface gas and oil reservoirs (Mascle et al., 2006 and references therein). The Amon MV (31°42.6′ E; 32°22.2′ N) is associated with an area of the Egyptian margin subject to rapid subsidence and abundant sedimentation, leading to the burial of thick accumulations of organic-rich sediments and the formation of hydrocarbons (Dolson et al., 2002). It is located at a water depth of about 1120 m, has a diameter of 2 km, and a maximal elevation of 90 m above the surrounding seafloor (Fig. 1). Previous studies of the Amon MV were based on bathymetric and visual investigations

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(Mascle et al., 2001; Dupré et al., 2007, 2008, 2010) or focused on the community composition in some selected habitats (Girnth et al., 2011; Grünke et al., 2011; Ritt et al., 2011). The slope of the Amon MV decreases gently from the central mound to the outer edges (Dupré et al., 2007). Sampling and in situ measurements (Supplement Table 1 and PANGEAEA database, http://www.pangaea.de) were performed at locations indicated in Fig. 1 during the M70-2 Bionil cruise with the R/V *Meteor* (2006) and the MSM13-3 with the R/V *M.S. Merian* (2009). Substantial gas flares were detected above Amon in the ship's; ROV's and AUV's hydro-acoustic sonars indicating free gas ebullition (Freiwald et al., 2011; Boetius, 2012). Precise positioning and operation of the in situ tools at the seep sites as well as the targeted sampling were achieved with the remotely operated vehicle (ROV) "Quest 4000" (Marum, Bremen, Germany). Based on visual observation and in consistence with micro-bathymetric measurements published previously (Dupré et al., 2007, 2008; Girnth et al., 2011), four habitats can be visually distinguished and were sampled at the Amon MV (Fig. 1): (I) central dome, (II) bacterial mats, (III) biogenic mounds, and (IV) a lateral mud flow at the foot of the Amon MV termed "sulfur band". Additionally, measurements and sampling were performed at a reference site outside of the Amon MV area. At all habitats, the upper 30 cm of the sediment were sampled with push cores (PCs) operated by the ROV. On board of the ship, the PCs were stored and processed at in situ bottom water temperature of 14 °C. Some samples contained high amounts of gas and their retrieval disturbed the sediment stratification by gas ebullition. To re-establish the geochemical gradients the cores were stored at in situ temperature until the mud settled and bacterial mats recovered. In addition to sampling with PCs, sediments were sampled by gravity coring down to 380 cm b.s.f. (below seafloor). The positioning system POSIDONIA (IXSEA SAS; Marly-le-Roi; France) was used for precise sampling of the different habitats. After recovery, all gravity cores (GCs) were immediately processed.

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2.2 Consumption rates of methane and sulphate

2.2.1 Ex situ turnover rates of methane and sulphate

Rates were determined according to Treude et al. (2003) and references therein, using radioisotope tracer methods for sulfate reduction and anaerobic methane oxidation. For each method and sampling station 3–4 replicate sub-cores were used. On board, either 25 µL $^{14}CH_4$ (dissolved in water, 2.5 kBq) or 5–10 µL carrier-free $^{35}SO_4^{2-}$ (dissolved in water, 50 kBq) were injected in 1 cm intervals through silicon-sealed holes into the sub-cores (whole core injection method, Jørgensen, 1978). The sediments were incubated in the dark for 8–12 h at in situ temperature and afterwards sliced in 1 cm sections. Sediments incubated for methane oxidation were fixed in 25 mL NaOH (2.5 %, w/v) in glass bottles that were immediately closed with rubber stoppers. Sediments for the sulphate reduction rate measurements were fixed in 20 mL zinc acetate solution (20 %, w/v). The turnover rates of methane and sulphate were then determined in the home laboratory by scintillation counting and turnover rates calculated as previously described (Treude et al., 2003; Kallmeyer et al., 2004; Felden et al., 2010). The substrate concentrations were measured by gas chromatography (5890A, Hewlett Packard) and anion exchange chromatography (see pore water section) for methane and sulphate, respectively. Sulphate consumption was determined in 2006 and 2009, but AOM was only measured in 2006.

2.2.2 Incubation experiment of center (I) and bacterial mat (II) sediments

To test the effect of the Amon MV seep fluids on methane- and hydrocarbon-fuelled SR, the upper 5 cm of sediments collected in 2009 from the bacterial mat area or the central area were gently mixed, divided into 9 replicates and diluted (1 : 3) with pore water originating either from their respective sampling site, or from the other area. As a third treatment, they were supplied with anaerobic growth medium for sulphate reducing bacteria (Widdel and Bak, 1992). Each treatment had three replicates. Subsequently, sediments

were incubated at in situ temperature with 10 µL carrier-free $^{35}SO_4^{2-}$ in headspace-free glass vials for 10.5 h. The reaction was terminated with ZnAc and samples were processed as described in Sect. 2.2.1.

2.3 Geochemistry

2.3.1 Methane concentration

Sediment methane concentrations were determined from subsamples obtained by slicing sediment cores in 2–3 cm intervals. 3 mL of each sediment horizon were quickly transferred with a cut-off syringe into a 25 mL glass bottle containing 5 mL NaOH (2.5 %, w/v). The glasses were immediately closed with butyl rubber stoppers and were shaken in order to release the dissolved pore water methane into the headspace. The methane concentrations were measured by injecting 100 µL of the glass bottle headspace into a gas chromatograph (5890A, Hewlett Packard).

2.3.2 Pore water extraction

Pore water from two replicate PCs per habitat in 2006 and one PC in 2009 was extracted with Rhizons (Rhizon CSS: length 5 cm, pore diameter 0.15 µm; Rhizosphere Research Products, Wageningen, Netherlands) in 1 cm-resolution and immediately fixed in 5 % ZnAc for sulphate, chloride, and sulphide analyses. Furthermore, samples for dissolved inorganic carbon (DIC) were preserved by adding $HgCl_2$ and stored headspace-free in gas tide glass vials. The extraction of pore water from GC sediments was either performed by centrifugation according to de Lange (1992) in 2006 or with Rhizons in 2009. All samples were stored at 4 °C until analyses in the home laboratory.

2.3.3 Pore water analyses

Sulphate and chloride concentrations in the PC pore water were measured by non-suppressed anion exchange chromatography (Waters IC-Pak anion exchange column,

waters 430 conductivity detector) after filtration and dilution. The total sulphide concentrations ($H_2S + HS^- + S^{2-}$) were determined with the diamine complexation method (Cline, 1969). DIC concentrations were measured with the flow injection method (conductivity detector: VWR scientific model 1054) according to Hall and Aller (1992). Inter-
[5] fering dissolved sulphide in the samples were eliminated before the DIC measurement by adding 0.5 M molybdate solution as described by Lustwerk and Burdiger (1995). In 2006, the reported sulphate and chloride data from GCs were analysed using inductively coupled plasma optical emission spectrometry (ICP-OES); these results were verified using on-board acidified and N_2-bubbled subsamples. The pore water salin-
[10] ity was determined with a conventional refractometer on board. In 2009, sulphate and chloride concentration in GC pore waters were determined by non-suppressed anion exchange chromatography as described above.

2.4 Acridine Orange Direct Counts (AODC)

To determine the total number of single cells with Acridine Orange Direct Counts, sed-
[15] iment sections were preserved in artificial seawater with 2 % formalin. The Acridine Orange staining was performed in the home laboratory based on previously described methods (Meyer-Reil, 1983; Boetius and Lochte, 1996). For each sample, two replicate filters and at least 30 grids per filter were randomly counted and only single cells were considered.

[20] ## 2.5 In situ measurements

2.5.1 Benthic chamber

Total benthic oxygen uptake (TOU) and total methane efflux (only measured in 2009) were determined with a benthic chamber module equipped with two Clark-type oxygen mini-electrodes or oxygen optodes (AADI, Norway) by the ROV QUEST to ensure

targeted measurements at selected spots. The method was carried out according to Felden et al. (2010) and details are provided in the supplements.

2.5.2 Microprofiler

To measure high-resolution gradients at visually targeted sites, a deep-sea micropro-filer module was operated by the ROV. During the autonomous measurements, the microsensors mounted to the microprofiler module were vertically driven in 250 μm steps from the water column to a sediment depth of up to 10 cm (De Beer et al., 2006). At each deployment, two pH, two sulphide, and two oxygen microelectrodes (Revsbech et al., 1983; Revsbech and Ward, 1983; Jeroschewski et al., 1996) were used. Temperature gradients in the top few cm (T gradient surface) were recorded with a temperature sensor (Pt100, UST Umweltsensorentechnik GmbH). All sensors were calibrated on board according to previously described methods (Wenzhöfer et al., 2000; De Beer et al., 2006). Total sulphide ($H_2S + HS^- + S^{2-}$) profiles were calculated from the H_2S and the pH profiles. All calculations were carried out according to Lichtschlag et al. (2010) and details are provided in the supplements. In 2009, the microsensor sulphide measurements at the Amon MV failed due to problems with the profiler module for sulphide microelectrodes.

2.5.3 In situ subsurface sediment temperature measurements

In situ sediment temperature measurements were carried out with gravity corers equipped with MICREL THP temperature loggers during both expeditions in 2006 and 2009 (for detail see Feseker et al., 2008). The loggers were mounted on outriggers welded on the corer barrel. The sensors were calibrated to a precision of 0.002 °C. The temperature loggers remained in the sediment for a period of seven to ten minutes, during which each logger recorded the sediment temperature at a time interval of 5 s. The equilibrium temperatures were calculated through extrapolation of the recorded equilibration curves (Villinger and Davis, 1987).

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3 Results

We distinguished four different habitats of the Amon MV (Fig. 2): (I) The central dome area is ca. 250 m in diameter and characterized by gas expulsions and mud breccia extrusions. Besides the detection of gas flares by the ship's acoustic sonar, we observed release of gas bubbles upon ROV touch down and sampling, indicating gas overpressure at 1250 m water depth. Blocks of greyish mud with sharp edges were separated by cracks and troughs of several meters in width and height indicating processes of seafloor displacement. At the northern boundary of the central dome we could not observe any indications of recent gas ebullition or bacterial mats at the seafloor ("transition zone" Ia). (II) Surrounding the central dome structure was an area of about 50 m width characterized by smooth ripples, marked by more stabilized sediments. The seafloor was covered by 10–20 % with patches of bacterial mats and black sediment spots with a diameter of a few decimetres to meters. No signs of recent eruptions were found in this area. The variable distribution and size of the mat patches in the central area suggest a spatially heterogeneous, but temporally persistent outflow of seep fluids in this habitat as indicated by constant surficial temperature gradients in 2006 and 2009 (Fig. 5b and e). (III) The largest part of the Amon MV was covered by biogenic mounds of 20–50 cm in height and width, formed by thalassinid mud shrimps. Fresh borrows of the shrimps were characterized by lumps of greyish reduced subsurface sediments deposited on the beige surface muds (Fig. 2c). (IV) A lateral outflow of briny mud, called "sulfur band", was detected at the foot of the MV (southwestern area) (Girnth et al., 2011).

3.1 Methane efflux and temperature gradients

High methane effluxes, defined as the amount of dissolved methane being emitted from the seafloor to the water column, were measured at the central dome (I), and associated with bacterial mats (II) (Table 1). No methane efflux was detected at the biogenic mounds, the bacterial mat of the lateral mud flow "sulfur band (IV)" or the

reference area. Hence, methane efflux was restricted to areas of high temperature gradients (Table 1). Surface and subsurface T gradient measurements showed similar trends of rapid cooling from the inner center of the Amon MV towards its outer zone, as well as a decrease in temperature between 2006 and 2009. The highest sediment temperature was observed in 2006 with 70 °C at several meters depth below surface. In 2009 the highest overall temperature was 50 °C.

3.2 Methane and other pore water constituents

The sediments from the central dome (I) were gas saturated at a water depth of 1120 m and an in situ temperature of 14 °C, which indicates an equilibrium methane concentration of at least 140 mM at the seafloor (Yamamoto et al., 1976). However, due to depressurization and degassing during core retrieval, ex situ measured methane concentrations were only about 2 mM throughout the entire cores (data not shown). Degassing of samples from the central dome may have affected also ex situ sulphide and DIC concentrations, which are shown in Figs. 3 and 4. The sulphide concentration profiles (Fig. 3) indicated substantial sulphide and DIC production in the top 15 cm of the bacterial mat (II) habitat, together with sulphate depletion below 15 cm sediment depth. In contrast, sulphide production was low at the central dome, despite the high methane and sulphate concentrations (I). Sulphate concentrations increased from 30 mM to up to 40 mM in the top 14 cm (Fig. 3), indicating an upflow of sulphate- and DIC-rich subsurface fluids at the central dome (Fig. 3 and 4, I). Upflow of sulphate-rich subsurface fluids was confirmed by additional pore water profiles obtained from gravity cores, which showed sulphate concentration of up to 100 mM (3-times seawater concentration) at about 350 cm b.s.f. (Fig. 4). In contrast, chloride concentrations decreased from close to seawater values at the sediment surface to less than 130 mM at 250 cm b.s.f. at the central dome (Fig. 4). Differences between 2006 and 2009 were difficult to detect because of the high heterogeneity of sediments and subsurface fluid flow in the inner dome and the surrounding bacterial mat zone. The subsurface pore

water data indicated a higher microbial activity in the bacterial mat zone in 2009 compared to 2006.

Only low (maximum 0.007 mM) ex situ methane concentration were found at the northern transition (Ia) indicating absence of seepage. Here, sulphate (average 34 mM), chloride (average 547 mM) and DIC (average 2.8 mM) were constant with depth, confirming absence of upward advecting fluids. In the surface sediment (0–15 cm) from the biogenic mounds (III) no methane or sulphide were detected. All analysed pore water constituents had values close to seawater concentrations and no sulphide was detected (Fig. 3).

In sediments of the "sulfur band" mudflow (IV) at the base of the Amon MV in 2006, methane concentration was slightly elevated in the topmost surface sediments (0.01 mM), but was not detected deeper than 3 cm b.s.f. Sulphide concentrations were also low and restricted to the top few cm, indicating lateral surface transport in accordance with visual observations of brine flow (in detail described in Girnth et al., 2011). Furthermore, no decrease in sulphate or increase in DIC (Fig. 3) was observed in the mud underlying the microbial mat. However, in 2009 a substantial increase in methane was recorded (0.3 mM), which co-occurred with a depletion of sulphate and an increase of sulfide. Also the chloride concentration in the sediment was elevated up to 990 mM (nearly 2-times seawater concentration) at 9 cm b.s.f., indicating the influence of temporally variable brine flow in this habitat of the Amon MV. This is in contrast to the chloride depletion of vertically rising subsurface fluids reaching the top of the central dome, indicating different processes of fluid formation, alteration and transport below the MV.

3.3 In situ oxygen and sulphide fluxes

Oxygen and sulphide fluxes measured in situ serve as indicators of microbial activity. The difference between oxygen and sulphide fluxes at background sites to those associated with hydrocarbon seepage can be related to aerobic and anaerobic hydrocarbon oxidation. At the bacterial mat (II) characterized by high methane fluxes,

the sulphide profiles indicated a substantial production of sulphide directly below the bacterial mat. The sulphide was entirely consumed with oxygen within the sediment before it reached the water column. This site showed a limited penetration of oxygen of < 1 mm (Fig. 5) following the sediment topography (Supplement Fig. 1). In contrast, at the highly methane vented and disturbed central dome (I), oxygen penetrated 3–4 mm into the sediment (Table 1). No sulphide production was detected in the upper 2 cm, matching the pore water data and microbial rate measurements, and pH showed a continuous decrease with depth, indicating no AOM. At the biogenic mounds (III) characterized by an absence of methane transport, oxygen penetration reached several cm into the seafloor due to bioturbation by mud shrimps, excluding AOM.

Total oxygen flux measured with the benthic chambers comprises the total benthic respiration rates including microorganisms and animals. The TOU was 1–2 orders of magnitude higher at the central dome (I) and bacterial mat (II) sites than at the biogenic mounds (III) (Table 1), hence correlated with methane transport. Furthermore, in 2009 total oxygen uptake (TOU) was slightly higher than in 2006 (Table 1).

3.4 Methane and sulphate consumption

A substantial difference in AOM and SR depth integrated rates (0–15 cm) was detected between the different habitats and with time at the Amon MV (Fig. 6; Table 1). The highest hydrocarbon turnover was detected in the upper 5 cm of sediment and associated with the patchy bacterial mats surrounding the central dome (II) (Fig. 6d). In the gas-oversaturated sediments of the central dome of Amon MV (I), SR values were considerably lower (Fig. 6a), despite the co-occurrence of methane and sulphate throughout the core. AOM was up to 5 times lower than SR (Fig. 6b), indicating that a substantial fraction of sulphate reduction was based on the anaerobic oxidation of higher hydrocarbons (see Mastalerz et al., 2009 for gas composition). At the biogenic mounds (III), SR and AOM rates (Fig. 6g, h) were mostly at the detection limit in the top 15 cm, due to the very low methane content of these sediments. At the sulfur band (IV), where the briny mud was covered by a whitish microbial mat (Fig. 6j, k), sulphate and

methane turnover was restricted to the upper 3 cm of the blackish sediment (SR maximum 26 nmol mL^{-1} d^{-1}) in 2006. In contrast, three years later much higher sulphate consumption rates of > 1000 nmol mL^{-1} d^{-1} were found over the entire investigated depth range.

To investigate the potential repression of hydrocarbon fuelled sulphate reduction by pore water fluids from the main gas conduit, we carried out a pore water transplantation experiment with sediments from the central dome and the surrounding active bacterial mat-covered sediments (Fig. 7). The sediments containing the natural microbial communities were mixed with pore waters of either site, or were replaced with sulphate reducer medium amended with methane, and the resulting sulphate reduction rates was measured in replicate slurries. The experiment showed that SR rates remained unaffected of the pore water composition. They were low in center sediments and high in sediments from a bacterial mat, even when pore waters of the central dome area were added. Thus we concluded that the low SR and AOM in the cone were not caused by an inhibiting substance in the fluids transporting sulphate and hydrocarbons.

3.5 Cell counts

In sediments of the Amon MV central dome (I), total cell numbers in the top 15 cm were surprisingly low, decreasing with increasing depth in 2006 (Fig. 6c). Three years later, cell abundances had substantially increased at sediment surface. In the sediment below the bacterial mats (II, IV), the abundance of microbial cells (Fig. 6f, I) was much higher than the inner dome sediments, and no significant change with time was detected. Both the Amon central dome and the biogenic mound sediments, showed lower cell abundance than a reference core taken outside the Amon MV at a similar water depth.

4   Discussion

The aim of this study was to test several hypotheses related to the limitation of microbial activity in sediments of active mud volcanoes. Previous investigations as to the efficiency of the benthic microbial filter for hydrocarbons have shown that hydrocarbon-fuelled sulphate reduction and methane consumption could be repressed by limitations in transport - e.g. low supply with electron acceptors (sulphate and oxygen, De Beer et al., 2006; Niemann et al., 2006), or by kinetic factors affecting microbial metabolism such as adverse chemical composition of subsurface fluids (Joye et al., 2009), by heat (Teske et al., 2002; Schouten et al., 2003; Brazelton et al., 2006, 2010), or by disturbances such as mud expulsion (Mastalerz et al., 2009). The Amon MV is an interesting natural laboratory to test these hypotheses, as previous investigations showed a wide range of geological and microbial activities associated with the different habitats of the Amon MV (Mastalerz et al., 2009; Omoregie et al., 2009; Dupré et al., 2010; Girnth et al., 2011; Grünke et al., 2011). We could confirm that despite the longevity of the Nile Deep Sea Fan gas seeps, the high supply of methane and sulphate, and the favourable bottom water temperatures of the deep Eastern Mediterranean of around 14 °C, the hydrocarbon-degrading communities of Amon MV showed a low efficiency compared to other mud volcanoes. Only 2 % of the total methane transported was consumed in the inner center, compared to 19 % at the surrounding bacterial mats (Table 2). We investigated and compared limitations of the biogeochemical activities of all four major habitats of Amon MV (Fig. 2): (I) central dome, (II) bacterial mat zone, (III) biogenic mound zone, and (IV) a lateral mudflow at the flank of the mud volcano named "sulfur band" considering both spatial and temporal variations.

4.1 Transport limitation

4.1.1 Biogenic mound zone

The largest area of the Amon MV comprised suboxic sediments covered by biogenic mounds (III). The mounds are built by thalassinid mud shrimps (Fig. 2f). Methane fluxes in the top 20 cm of seafloor were negligible, and concentrations too low to sustain active hydrocarbon-degrading microbial communities in the investigated surface sediments. However, as oxygen penetration was lower, and oxygen consumption ($5\,\mathrm{mmol\,m^{-2}\,d^{-1}}$) higher than background values of non-seep influenced seafloor at similar water depths outside of the Amon MV ($1.3\,\mathrm{mmol\,m^{-2}\,d^{-1}}$), we assume that this zone is still influenced by low rates of subsurface hydrocarbon transport, which may be efficiently utilized by subsurface microbial communities for which the mud shrimps may dwell.

A specific association of mound-forming mud shrimps with mud volcanism has been previously observed in the Eastern Meditrranen deep-sea (Dupré et al., 2008), and is also known from the fossil record (Amano and Kiel, 2007). The cause of their association with cold seep ecosystems remains unknown, but may be due to the effects of mud volcanism on the seafloor substrate (less consolidated muds), and probably also due to the grazing of bacterial communities getting enriched at the redox boundaries. A similar type of ecosystem engineering is known from the coastal mud shrimp *Callianassa*. These live on organic material, mainly sea-grass debris, and its associated microorganism (Ziebis et al., 1996; Dworschak, 2001; Abed-Navandi and Dworschak, 2005).

4.1.2 Sulphuric mud flow "sulfur band" at the base of Amon

The source area of the mud flow was littered with shells of thyasirid bivalves, indicating that the foot of the Amon MV is a highly dynamic environment (Ritt et al., 2011). In 2006, the mud flow appeared fresh, as the blackish reduced muds were exposed at the source of the flow, and partially covered by brine. With increasing distance from

the source, they were densely covered by a whitish mat of Thiomargarita (Fig. 2d) (Girnth et al., 2011). Benthic chamber measurements indicated a substantial efflux of sulphide with highest values at the source of the mud flow, decreasing with increasing distance along the sulfur band (Girnth et al., 2011). However, in 2006 methane concentrations were very low, rates of microbial sulphate reduction and AOM were close to detection limit, and no in situ sulphide production was detected in the sediments (Fig. 3 and 5). Accordingly, we found rather low cell numbers below the bacterial mat and concluded that most of this sulphide was transported laterally along with the briny outflow, and not produced in the sediments. In 2009, the situation had changed considerably, and methane transport, microbial sulphate reduction as well as total oxygen consumption and cell numbers had increased substantially (Table 1), indicating the development of active hydrocarbon degrading anaerobic communities. In the surrounding of the mud flow, active growing chemosynthetic communities characterized by Lamellibrachia tubeworms (Duperron et al., 2009) and various bivalves associated with carbonate crusts were found (Ritt et al., 2011). The finding of such lateral mud flows supporting chemosynthetic communities indicates that methane transport may not be restricted to the central areas of MVs and illustrates the need for systematic surveys of such seafloor structures (Omoregie et al., 2008).

4.2 Kinetic limitation by disturbance and heat in the central area

It has previously been shown that in MV centers with high seepage rates of $> 1 \, \mathrm{m \, yr^{-1}}$, the upward flow of sulphate-depleted fluids can restrict the downward diffusion of sulphate from the water column into deeper sediment strata, thus limiting SR and AOM to the topmost sulphate-penetrated horizons (De Beer et al., 2006; Lichtschlag et al., 2010). However, at Amon MV, subsurface fluids are enriched in sulphate, hence electron acceptor limitation cannot explain the low AOM and SR rates. Therefore, we searched for kinetic explanations.

To further test whether the uprising Amon MV fluids could contain substances repressing sulphate reduction, sediments from the center (I) and bacterial mat (II) sites

were incubated on board with gas-enriched pore water extracted from the central dome, the bacterial mat zone or with sulphate reducer medium amended with methane. Results showed that methane-fuelled SR activity in samples containing the microbial communities from either habitat remained the same, independent of the origin of the pore water amended (Fig. 7). Hence, we can exclude that anaerobic hydrocarbon degradation in the central dome zone is inhibited by potentially toxic compounds co-migrating with the fluids and gases.

The central dome area of Amon MV showed the steepest temperature gradient and was characterized by elevated mud breccia blocks indicate recent gas outbursts and disturbances of the seafloor. Subsurface seafloor temperatures in the central gas conduit reach to at least 70°C, as recorded in 2006. The currently known upper temperature limit for AOM and growth of anaerobic methanotrophic communities is around 65°C (Kallmeyer and Boetius, 2004; Holler et al., 2011). Hence, it is likely that anaerobic methanotrophs cannot populate subsurface muds and fluids at Amon MV, despite the high availability of methane and sulfate. Expulsions of hot fluids and subsurface muds to the seafloor of the central area are likely to repress microbial communities able to oxidize methane anaerobically. Only after cooling these gas- and sulphate-laden sediments can be repopulated by microbial communities. But given the very slow growth of anaerobic hydrocarbon degraders with generation times of 6–12 months (Nauhaus et al., 2007; Knittel and Boetius, 2009), it may take years before active microbial communities develop in such freshly exposed subsurface muds to control hydrocarbon emissions. Accordingly, the relatively high total oxygen consumption rates detected suggest a dominance of more rapidly growing aerobic hydrocarbon degraders in this area.

In contrast, at the boundary of the central dome, the surface of the seafloor changed from a highly disturbed landscape (Fig. 2a) to smoother eroded mud breccia covered by bacterial mats (Dupré et al., 2007). These sediments were cooled sufficiently long to allow colonization of AOM. This explains best why – despite the high availability of methane and sulphate in both habitats – anaerobic microbial activity (sulphide

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production, SR and AOM) was much lower on the center than in the bacterial mat covered areas (Fig. 6, 6a; Table 1). Supporting this hypothesis, we found rather low cell abundances in sediments of the inner Amon MV center (Fig. 6c), which were 1–2 orders of magnitude lower than cell numbers usually found in similar sediment horizons at active cold seeps (Knittel and Boetius, 2009). They were also an order of magnitude lower than cell numbers of surface sediments in the bacterial mat zone, but increased with time from 2006 to 2009 along with the general cooling of the Amon MV.

Also the temporal change observed in the bacterial mat zone indicates a further development of the hydrocarbon degrading community. Between 2006 and 2009 (Fig. 3), sulphate became depleted in the sediment below 10 cm, causing a lower integrated sulphate reduction rates, and indicating the onset of transport limitation. Accordingly, the DIC concentration profile had increased substantially between 2006 and 2009, indicating an overall increase in hydrocarbon conversion. This further supports the above hypothesis that the development of an active anaerobic hydrocarbon-degrading community may take years, before controlling efficiently hydrocarbon emission and before developing a strong signal in the pore water gradients.

5 Conclusions on limitations of microbial activity at cold seep ecosystems

The microbial and faunal activity at cold seeps is primarily linked to hydrocarbon transport and fluid flow (e.g. Dubilier et al., 2008; Felden et al., 2010; Lichtschlag et al., 2010). Although morphology, seepage intensity and composition of seep fluids vary considerably between known submarine mud volcanoes, most of them show a concentric pattern in the distribution of chemosynthetic habitats, explained by the presence of one major central chimney for gas, fluid and mud transport (Olu et al., 1997; Kopf, 2002; Niemann et al., 2006; Dupré et al., 2007, 2008; Huguen et al., 2009; Sommer et al., 2009). Here we found evidence that besides the known limitation of microbial hydrocarbon degradation by rapidly rising sulphate-depleted subsurface fluids (De Beer et al., 2006; Niemann et al., 2006; Joye et al., 2009), the disturbance caused by eruptions of

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Table 1. Overview of data from the four main Amon MV habitats. Surface and subsurface T-gradients were measured in situ with microsensor and T-lance measurements, respectively. Methane efflux and total oxygen uptake (TOU) was measured with the benthic chamber. The dissolved oxygen uptake (DOU), and upward diffusive sulphide flux were calculated based on in situ microsensor measurements. The averaged sulphate reduction (SR) and methane oxidation rates (AOM) were integrated over the first 15 cm. Single cells determined by AODC were depth integrated over the first 2 cm of the sediment. (n.d. = not determined; n = numbers of replicates if available)

		central dome (I)	bacterial mat zone (II)	biogenic mounds(III)	sulfur band (IV)	reference
Position		32°22.16′ N 31°42.64′ E	32°22.13′ N 31°42.66′ E	32°22.12′ N 31°42.35′ E	32°22.05′ N 31°42.27′ E	
Area (m²)		8000[a]	10 000[b]	1 900 000[a]	200[b]	n.d
T-gradients surface (°C m⁻¹)	2006	17.2 ($n = 1$)	2.7 ($n = 1$)	n.d.	0 ($n = 2$)	n.d.
	2009	2.3 ($n = 1$)	2.7 ($n = 1$)	n.d.	0 ($n = 3$)	n.d.
T-gradients subsurface (°C m⁻¹)	2006		7.39 min ($n = 6$; min. 5.65; max. 9.09)	0.62 ($n = 7$; min. 0.04; max. 2.37)		0.02[d]
	2009		5.58 ($n = 13$; min. 0.57; max. 5.65)	0.34 ($n = 33$; min. 0.04; max. 2.13)		
CH₄-efflux (mmol m⁻²d⁻¹)	2006	n.d.	n.d.	0 ($n = 3$)[5]	n.d.	0 ($n = 3$)[e]
	2009	1; 24; 70 ($n = 3$)	49; 83; 85 ($n = 3$)	n.d.	0 ($n = 1$)	n.d.
TOU (mmol m⁻²d⁻¹)	2006	n.d.	50 ($n = 1$)	5 ($n = 1$)	35; 71 ($n = 2$)	n.d.
	2009	19; 102 ($n = 2$)	69 ($n = 1$)	n.d.	119 ($n = 1$)	n.d.
DOU (mmol m⁻²d⁻¹)	2006	10 ($n = 1$)	40; 44 ($n = 2$)	n.d.	11; 12; 26; 46 ($n = 4$)	1.3[c] ($n = 1$)
	2009	11 ($n = 1$)	47 ($n = 1$)	n.d.	3; 6 ($n = 2$)	n.d.
J(upward diffusive sulphide-flux) (mmol m⁻²d⁻¹)	2006	0 ($n = 1$)	40 ($n = 1$)	n.d.	0; 1; 1; 20 ($n = 4$)	0[c] ($n = 1$)
	2009	n.d.	n.d.	n.d.	n.d.	n.d.
SR (mmol m⁻²d⁻¹)	2006	5.1 ± 1.6 ($n = 4$)	33.1 ± 15.9 ($n = 4$)*	0.2 ± 0.2 ($n = 4$)	0.5 ± 0.2 ($n = 3$)	0 ($n = 3$)
	2009	1.4 ± 0.6 ($n = 3$)	16 ± 11 ($n = 3$)	n.d.	30 ± 29 ($n = 9$)	n.d.
AOM (mmol m⁻²d⁻¹)	2006	0.6 ± 6.6 ($n = 4$)	17.2 ± 14.1 ($n = 6$)	0.1 ± 0.1 ($n = 4$)	0.1 ± 0.01 ($n = 3$)	0 ($n = 3$)
	2009	n.d.	n.d.	n.d.	n.d.	n.d .
Single cells (× 10¹³cells per m²)	2006	0.46 ± 0.08 ($n = 4$)	4.14 ± 1.96 ($n = 10$)	0.60 ± 0.30 ($n = 4$)	5.62 ± 1.36 ($n = 8$)	1.74 ± 0.60 ($n = 4$)
	2009	1.24 ± 0.60 ($n = 4$)	3.7 ± 0.76 ($n = 4$)	n.d.	6.60 ± 1.60 ($n = 16$)	n.d.

[a] Data by Dupré et al. (2007).
[b] Data by Grünke et al. (2011).
[c] Ex situ data by Ritt et al. (2011).
[d] Feseker et al. (2008).
[e] According to sediment methane concentration profiles.
* One outlier with extraordinary high sulphate consumption rates (138 mmol m⁻² d⁻¹) was not further considered as there was no replicate measurement available for confirmation.

Table 2. Comparison of total oxygen uptakes (TOU), methane effluxes, SR rates and temperature surface and subsurface gradients at the Amon MV, Håkon Mosby MV (HMMV) and Dvurechenskii (DMV). The efficiency of the anaerobic biological methane sink is calculated as the percent anaerobic oxidation of methane from the total methane flux (sum of CH_4 efflux and methane oxidation).

	T-gradients*** (°C m^{-1})	TOU* (mmol m^{2}d^{-1})	Total CH_4-efflux* (mmol m^{2}d^{-1})	AOM** (mmol m^{2}d^{-1})	SR** (mmol m^{2}d^{-1})	Biological methane sink (%)
Amon MV[1]						
central dome (I)	2–17	19–102 (n = 2)	69 (n = 1)	0.6 ± 6.6 (n = 4)	4 ± 2 (n = 6)	2
bacterial mat zone (II)	3	50–69 (n = 2)	71 ± 20 (n = 3)	17.2 ± 14.1 (n = 6)	54.1 ± 49 (n = 5)	19
biogenic mounds (III)	n.d.	5 (n = 1)	n.d.	0.1 ± 0.1 (n = 4)	0.2 ± 0.2 (n = 4)	n.d.
HMMV[2]						
center	7–27	15–72 (n = 4)	58–777 (n = 4)	4.1 ± 3.7 (n = 18)	0.4 ± 0.3 (n = 15)	4
bacterial mat zone	4–5	60–114 (n = 3)	25–78 (n = 2)	9.4 ± 5.6 (n = 18)	24.2 ± 23.8 (n = 46)	21
Siboglinid tubeworms	2	161 (n = 1)	2 (n = 1)	2.3 ± 3.0 (n = 6)	6.5 ± 7.0 (n = 6)	90
DMV[3] (anoxic system)						
center	10	–	458 (n = 1)	0.1 ± 0.1 (n = 3)	0.1 ± 0 (n = 3)	<1
western rim	0.2	–	n.d.	11.2 ± 9.6 (n = 3)	108 ± 38 (n = 3)	n.d.

* Measured by in situ benthic chamber incubations.
** Depth integrated rates (Amon MV, HMMV = 0–15 cm; DMV = 0–10 cm).
*** Calculated based on in situ microsensor (Amon MV, DMV) or T-probe measurements (HMMV).
[1] This study.
[2] From Felden et al., 2010.
[3] From Lichtschlag et al., 2010. Note that the DMV lies in anoxic waters.
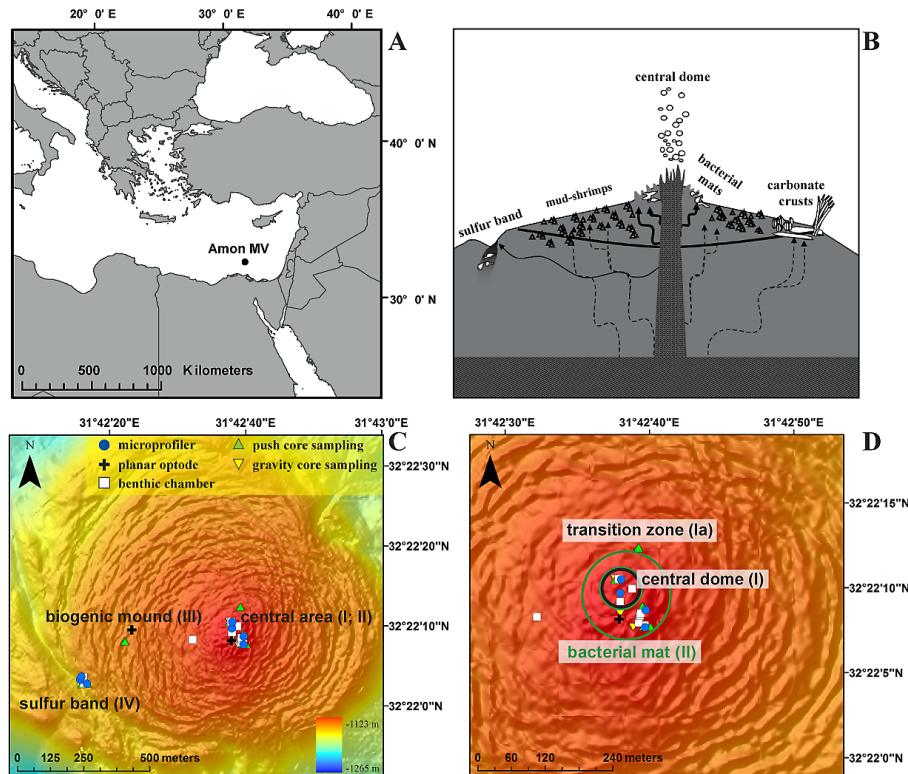
Fig. 1. Sampling sites. **(A)** The Amon MV is located on the Nile Deep Sea Fan of the Eastern Mediterranean. **(B)** Schematic drawing of habitats at the Amon MV. The habitats are not scaled to the same dimensions. **(C, D)** Positions of all in situ instrument deployments and push core sampling at the Amon MV in 2006 and 2009. In the central area bacterial mats (II) surround the central dome (I). The different tools are color coded: Microprofiler as blue circles, planar optode as black crosses, benthic chamber as red squares, push cores as green triangles (up) and gravity cores as yellow triangles (down) (map modified from Dupré et al., 2008).
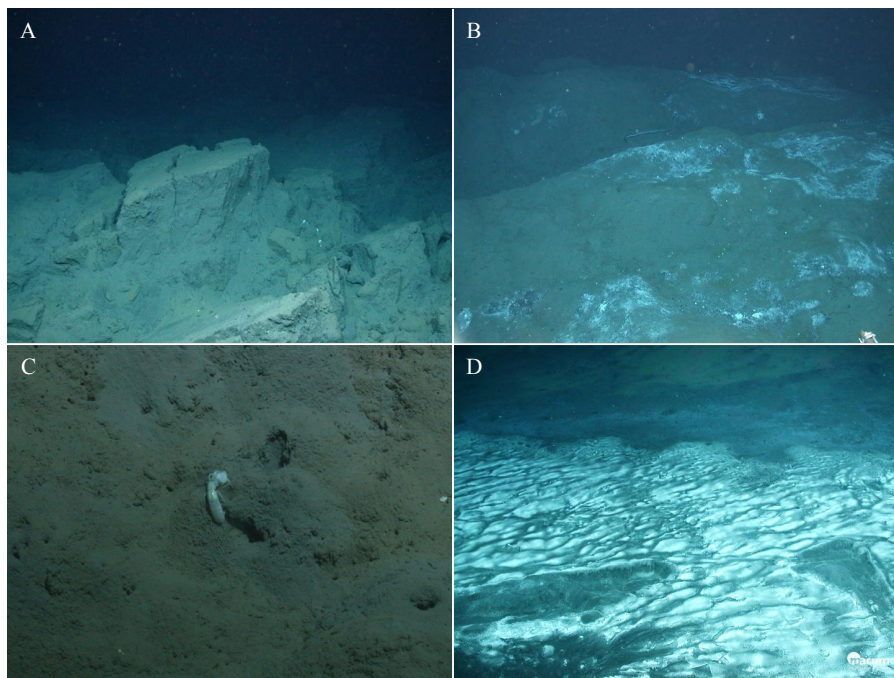
Fig. 2. Different cold seep habitats of the Amon MV. At the central dome (I), degassing of the seafloor created bathymetric anomalies of several meter in height in 2006 **(A)**. Around the central dome, the seafloor becomes smoother and bacterial mats (II) are found covering the hummocks **(B)**. Biogenic mounds of up to half a meter in height (III) comprise the largest habitat of the Amon MV and are formed by mud shrimps **(C)**. A unique feature at the outer rim of the Amon MV is the lateral outflow of sulphidic muds ("sulfur band") **(D)**. All pictures are copyright MARUM, Bremen.
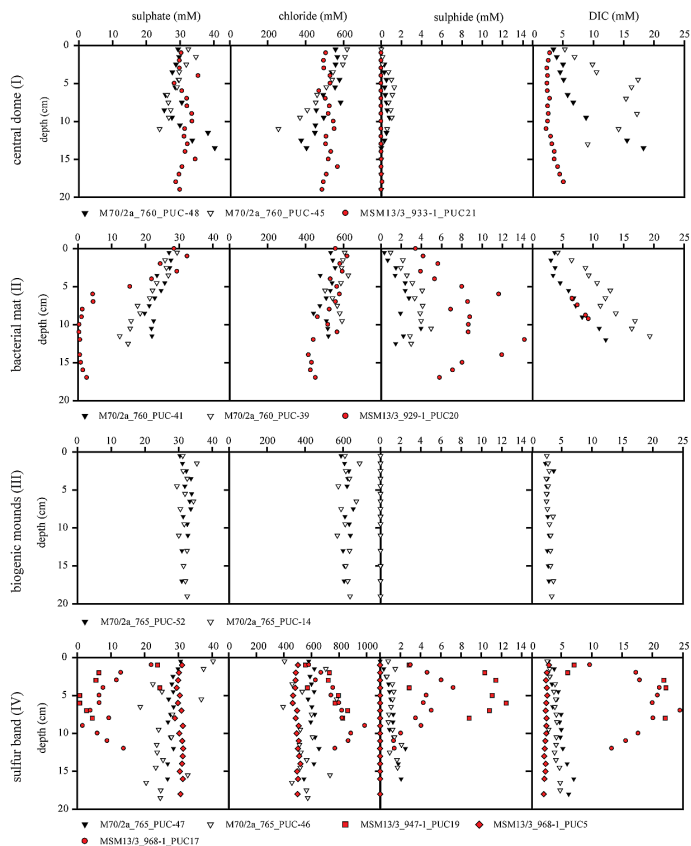
Fig. 3. Pore water data of sulphate, chloride, sulphide and DIC (Columns) from the four Amon MV habitats (Rows): central dome (I), bacterial mat (II), biogenic mounds (III) and sulfur band (IV). The black and white symbols indicate replicate cores of the same station in 2006. Red symbols indicate pore water samples collected in 2009. Please note the different X-scale of chloride in the lowest panel.

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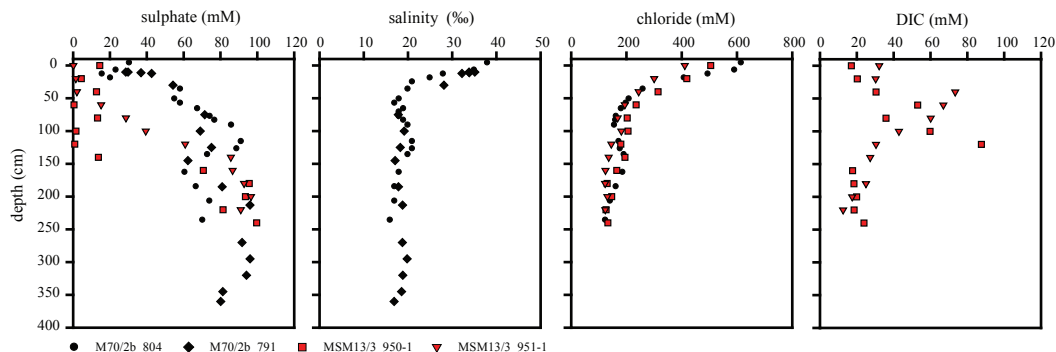
Fig. 4. Sulphate, chloride concentrations and salinity from deeper coring of the central dome of the Amon MV. 2006 and 2009 are indicated by black and red symbols, respectively.
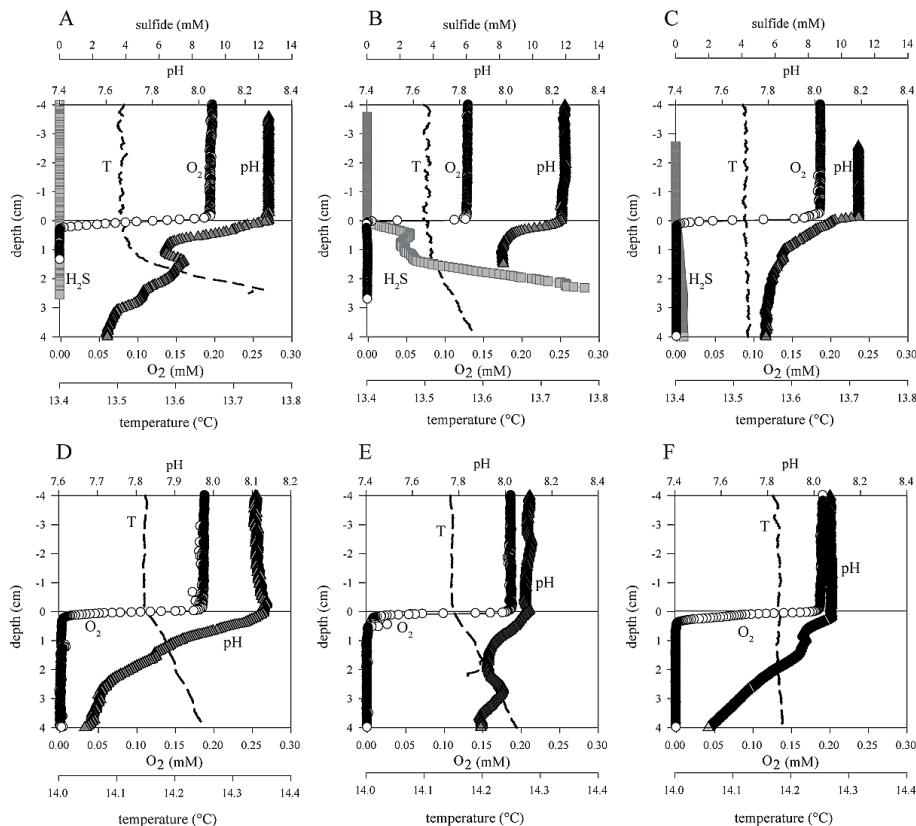
Fig. 5. Microsensor measurements from the **(A, D)** central dome (I), **(B, E)** the bacterial mat (II) site and **(C,F)** the bacterial mats at the sulfur band (IV). The profiles show oxygen (O$_2$) (spheres), total sulphide (H$_2$S) (grey line) concentrations, pH (triangles) and temperature (T, dashed line). **(A–C)** and **(D–F)** are profiles measured in 2006 and 2009, respectively. In 2009, no sulphide was measured due to failure of the respective microsensor units.

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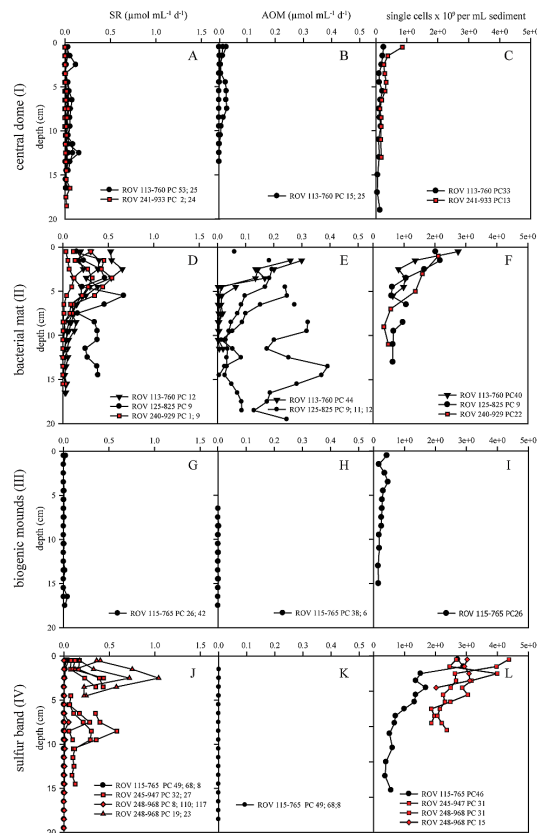
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Fig. 6. Biogeochemistry of the different habitats at Amon MV. Sulphate reduction (left panel); anaerobic oxidation of methane (middle panel), and single cell counts (right panel) at (I) the central dome **(A, B, C)**, (II) bacterial mats **(D, E, F)**, (III) biogenic mounds **(G, H, I)** and (IV) sulphur band **(J, K, L)**. Replicates of one station are represented with the same symbol and the same year with the same colour (black = 2006, red = 2009). Note: sulphate reduction and anaerobic oxidation of methane are on different scales.
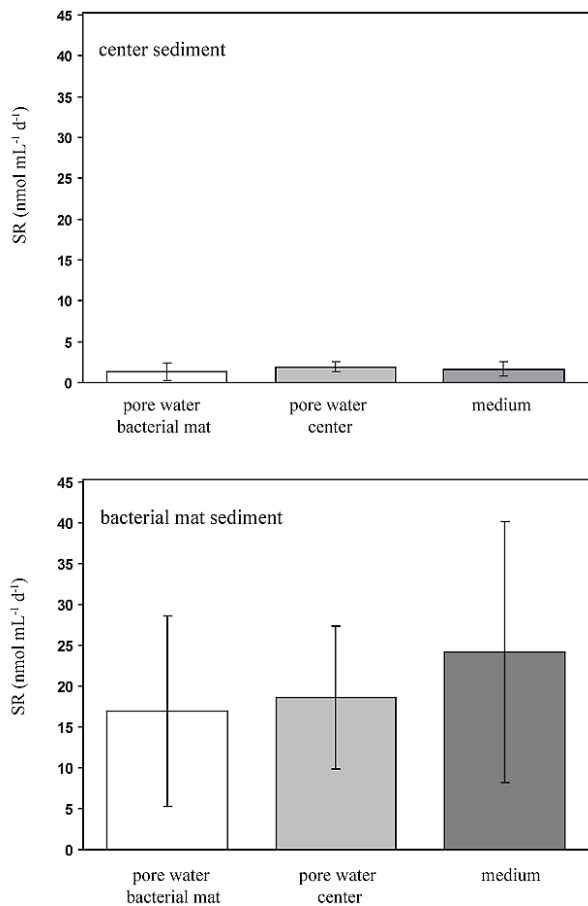
Fig. 7. SR inhibition experiment. Error bars indicated standard deviation ($N = 6$).