The picture on the cover page shows a benthic chamber deployed on sediment inhabited by siboglinid tubeworms at the Håkon Mosby Mud Volcano during the expedition ARK XXIV-2. The seafloor in the back is covered by bacterial mats of sulfide oxidizing bacteria (copyright MARUM; Bremen).
Methane fluxes and associated biogeochemical processes in cold seep ecosystems

by

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Doctor of Philosophy in Biology

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DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from published or unpublished scientific work has been cited in the text and listed in the references.

Signature                  Date
This work is dedicated to my grandmother
Gerda Vetterlein.
Summary

Methane is an important greenhouse gas that originates not only from anthropogenic processes but is also released from natural sources such as volcanoes or wetlands. In the oceans, cold seeps are important methane emission sites. These geostructures have different seafloor surface appearances like mud volcanoes or pockmarks. By using the chemical energy of hydrocarbons rising up with mud, gas or fluids from the deep subsurface, diverse seep communities with high biomasses develop at these structures. Therefore, cold seeps are fascinating hot-spot ecosystems as they link the deep geosphere with the biosphere at the seafloor surface.

During this PhD study, methane efflux and consumption as well as related processes such as sulfate reduction were investigated at four different deep-sea cold seeps. The main focus was on in situ quantification of methane emission and oxygen consumption. The results showed that cold seeps are spatially heterogeneous ecosystems, which are controlled by variations of fluid flow intensity influencing benthic biogeochemical processes. The highest fluid flow velocities are found at the central outflow of mud volcanoes in combination with high methane emission but low methane consumption rates. Outside of these main emission sites, chemosynthetic organisms such as mat-forming thiotrophic bacteria or siboglinid tubeworms are observed. Here, medium to low fluid flow velocities with high methane oxidation and high sulfate reduction rates were measured. Within one seep ecosystem there are spatial variations in methane emission and consumption, but the benthic biological methane filter of the different seep habitats removes a significant fraction of the total methane flow (up to 90%). For the methane budgets of geostructures and ocean basins, diffusive methane effluxes were previously not considered. However, based on the obtained data during this PhD study, diffusive methane discharge contributes significantly to the total methane emission. Considering the diffusive methane release of the investigated deep-sea mud volcanoes, only mud volcanoes would release up to $15 \times 10^{12}$ g methane per year to the water column, which is a significant fraction of the total annual methane flux from the ocean to the atmosphere.

Cold seeps do not always release large amounts, as shown by the investigation of fault-related Calyptogena colonies in the Japan Trench. Here, the locally restricted methane flow is very low, allowing for methane to be entirely consumed within the sediment by the microbial community. However, the methane supply is sufficient enough to support high clam biomasses at the Calyptogena patches.

The approach of using state-of-the-art in situ technologies in combination with detailed exploration improved our knowledge about cold seep ecosystem functioning. Spatial heterogeneities of these impressive seafloor phenomena were identified and in situ quantification of fluxes significantly improved our estimates on the ocean methane budget. In the future, not only spatial variations of seep processes should be further investigated, but also temporal changes of methane emission and consumption rates as well as related biogeochemical processes require detailed studies.
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1. Introduction

1.1. Objectives and key questions of the PhD thesis

Methane is not only the most abundant hydrocarbon on Earth, it is also an important greenhouse gas. According to global atmospheric budget estimations, only small amounts of methane are released from the oceans, although high amounts are produced in marine environments (Reeburgh 2007). In order to balance the methane production and consumption in the ocean, a better understanding of the driving forces and related processes is required. This gets even more important taking global warming into account, which will affect not only the relevant biogeochemical processes but also the methane inventory (e.g. gas hydrates).

This study was performed within the framework of the DFG Project MUMM² (Methane in the Geo/Bio-System - Turnover, Metabolism and Microbes²) and the 6th FP EU project HERMES (Hot Spot Ecosystem Research on the Margin of European Seas) to target methane turnover and the role of involved organisms in methane-rich systems. The project focused on in situ quantification of microbial turnover and transport processes in cold seep ecosystems. In situ measurements are difficult to achieve because, often, expensive technology needs to be developed for deep sea approaches. The effort is worthwhile as depressurizing and degassing during recovery of methane-enriched sediments causes significant errors in geochemical analyses.

In my PhD thesis, I used state-of-art and innovative in situ technology for high resolution investigations of different habitats at cold seep ecosystems in order to compare their benthic biogeochemical processes and fluxes to each other and to other seep locations.
1.2. Outline of the PhD thesis

This PhD thesis is subdivided into four chapters in which studies of the methane cycle and related processes at different seep structures are discussed. Chapter 1 gives a general outline of the current knowledge of biogeochemical cycles in marine sediments at cold seeps. Chapter 2 provides the results of the main studies during my PhD time. These studies are presented in subchapters that are manuscripts submitted or in preparation for submission. Subchapter 2.1 describes the contribution of each co-author to the studies.

Subchapter 2.2 discusses methane discharge to the hydrosphere in comparison to benthic methane and oxygen consumption at the Håkon Mosby Mud Volcano (HMMV). Data from six different cruises from 2001-2009 were combined to investigate spatial variations of biogeochemical processes within and between the different habitats. The gained geostructure methane budget shows that the diffusive discharge of methane (3.7 x 10⁴ mol d⁻¹) is as important as gaseous methane release. Furthermore, a reverse relationship was detected between the temperature gradient, total oxygen uptake and methane consumption rates. The highest temperature gradient was found in the central area, accompanied by low oxygen uptake and methane turnover rates. The microbial sink for methane was limited, accounting for >22% of the dissolved methane emission, because all benthic activity at the HMVMV is strongly controlled by high fluid flow rates.

Subchapter 2.3 characterizes biogeochemical key processes at the Amon mud volcano (Eastern Mediterranean Sea). Four different habitats were distinguished at the Amon MV: (I) the central dome with high fluid flow velocities; (II) the bacterial mats covering sediments with very high sulfate reduction rates; (III) the biogenic mounds area; (IV) lateral outflow of sulfidic mud at the outer rim of the geostructure. The Amon MV seep fluids are enriched not only in methane but also in higher hydrocarbons (e.g. propane, ethane) and sulfate, which causes very high sulfate reduction (SR) rates in sediments below the bacterial mats. Furthermore, SR was measured even in central dome sediments despite the occurring high upward fluid flow velocity which usually prevents sulfate diffusion into the sediment. Most of the Amon MV seafloor is covered by biogenic mounds which seem to be formed by mud shrimps. This is the first time that this family (Thalassinid) is described from a cold seep ecosystem. However, in the Eastern Mediterranean Sea mud shrimps might often be associated with cold seeps because biogenic mound-like structures were also observed from another MV in this area. The exploration of the Amon MV resulted also in the discovery of a horizontal outflow of reduced mud that was associated with chemosynthetic organisms such as sulfide oxidizing bacterial mats. The so named “sulfur band” illustrates that biogeochemically active sediments are not exclusively associated with the central outflow of MVs.
Chapter 1

Subchapter 2.4 describes the microbial community and biogeochemical processes in sediments of a *Calyptogena* colony in the Japan Deep Sea Trench in 5346 m water depth. This fault-related ecosystem is characterized by low fluid flow rates, and hence a reduced methane supply compared to the active MVs investigated in the other chapters. Nevertheless, the focused outflow provides sufficient chemical energy to support locally restricted high numbers of *Calyptogena* clams. The microbial diversity analyses of the sediment found evidence for the, to our knowledge, deepest anaerobic methane oxidizing microbial community. There are also chemosynthetic bivalve communities found at greater depths especially in the Japan Trench, but so far the benthic microbial community was not explored at these sites.

Subchapter 2.5 discusses also biogeochemical processes at the HMMV, but focuses on the comparison of geochemically controlled redox reactions and biological turnover of sulfide in three different habitats which are associated with various fluid flow intensities. The aim was to quantify the amount of sulfide that is oxidized geochemically, and is hence not available for chemosynthetic biomass production. The HMMV center is characterized by high fluid flow rates, and the produced small amounts of sulfide are oxidized geochemically with oxygen or precipitated with dissolved iron. The *Beggiatoa* mat habitat has only medium fluid flow rates and the thiotrophic bacteria use oxygen and nitrate to oxidize the sulfide completely. Chemical oxidation or precipitation is not important at *Beggiatoa* mat sites. In the third investigated habitat, gray thiotrophic bacterial mats covered perturbed sediments with heterogeneous sulfate reduction and thus sulfide production rates. However, the high sulfide fluxes were still balanced by biological oxidation with nitrate and oxygen.

Subchapter 2.6 explores biogeochemical processes of the Dvurechneskii mud volcano (DMV), located in the permanently anoxic waters of the Black Sea. The study focused on fluxes of methane and sulfide, and the factors controlling transport, consumption and production of both compounds. In the DMV active center, temperature anomalies as well as solute and gas transport indicated high fluid flow rates which limited the diffusion to the sediment. Therefore, methane could not be consumed biologically and escaped to the water column with a rate of 440 mmol m$^{-2}$ d$^{-1}$. Outside of the active DMV center, the biological methane filter is more efficient and removes 50-70% of the methane flux within the upper 10 cm of the sediment at medium fluid flow rates. However, the DMV released still significant amounts of dissolved methane ($3.7 \times 10^4$ mol d$^{-1}$).

Chapters 3 summarizes and discusses the results of the different studies and provides a general conclusion of the PhD thesis. At the end of chapter 3, an outlook is given, based particularly on the findings of this PhD thesis.

Chapter 4 describes other activities during my PhD thesis, including contributions to further publications and oral presentations, as well as participation in cruises.
1.3. Cold seep ecosystems

Cold seeps can be roughly defined as emission sites at the seafloor, where fluids (sometimes e.g. petroleum, oil) and gases from the deep subsurface are released. In contrast to hydrothermal vents, the emitted fluids have nearly ambient bottom water temperature at the sediment surface (Judd and Hovland 2007). Cold seeps can have different geological origins and appearances at the seafloor (Fig. 1), and are typically associated with divers and biogeochemical active benthic communities when compared to the common deep sea seafloor (Sibuet and Olu 1998). They are found worldwide on active and passive continental margins, and in various water depths from 20 to 7326 meters (Fujikura et al. 1999; Treude et al. 2005b). More intensively studied cold seeps are located in the Black Sea (Treude et al. 2005a), at Hydrate Ridge (Hinrichs et al. 1999a; Treude et al. 2003), in the Gulf of Mexico (Joye et al. 2004), at the Nordic Margin (Niemann et al. 2006) and in the Mediterranean Sea (Dupré et al. 2007; Huguen et al. 2009).

Fig. 1: (A) Bacterial mats of sulfide oxidizing bacteria can cover large seafloor areas at cold seeps, like shown here from the Håkon Mosby mud volcano. (B) In the anoxic waters of the Black Sea, microbes can build up high amounts of biomass and form reef-like structures through which gas bubble streams escape (white arrows). (C) There can be massive outbursts of gases, fluid, and mud at mud volcanoes that form meter high sediment structures like, for instance, at the central dome of the Amon mud volcano. (D) In the Gulf of Mexico, higher hydrocarbons, often oil, are transported from the deep subsurface, and sometimes asphalt-like structures form on the seafloor. These higher hydrocarbons are the sole energy source for chemosynthetic organisms, including tubeworms and crabs. (Photos courtesy of MARUM, University Bremen)
At seeps, gases (mainly methane) rise, either dissolved, or in case of over-saturation as gas bubbles. Under special temperature and pressure conditions, methane can be temporarily stored in the form of gas hydrates. These ice-like structures are mainly composed of methane and water (Reeburgh 2007). Gas hydrate can dissociate due to e.g. uprising warm fluids releasing the methane to the surrounding.

Cold seeps are frequently associated with geological structures such as diapirs or faults. Diapirs form by forceful movement of more or less dense material from areas of higher pressure, e.g. great depth, to areas of less pressure (Kopf 2002). The transport of compounds from the subsurface is mainly initiated by two processes: high sedimentation rates in the past forming several kilometer-thick sedimentary sequences and tectonic compression in the deep subsurface (Milkov 2000). At the seafloor, the rising material forms either depressions (pockmarks) or mounds (mud volcanoes), depending on the density of the extruded material.

Fluids (including water, gas, brine and oil) and mud are emitted by flow or eruptive events at mud volcanoes. The activity of these cold seeps can change over time and ranges from gigantic outbursts to dormancy time periods with reduced fluid, gas and mud flow. The number of known or supposed offshore mud volcanoes is 500, but it is assumed that the total number of deep sea mud volcanoes is in the range of $10^3$-$10^5$ worldwide (Milkov 2000; Judd and Hovland 2007).

In contrast to mud volcanoes, pockmarks are formed by fluid escape events that removed the upper sediment layers. They are also associated with fluid and gas venting, and enhanced methane concentrations in the bottom water above pockmarks are reported. There is a wide range of sizes for pockmarks that are typically several tens of meters across and few meters deep. Furthermore, they can be highly abundant in some areas, for instance pockmarks occupy 30% of the seafloor in the North Sea (Judd and Hovland 2007).

Within the framework of this PhD, mud volcanoes were intensively studied (Fig. 2), namely the Håkon Mosby Mud Volcano at the Nordic Margin (chapter 2.2; 2.6), the Amon mud volcano in the Eastern Mediterranean Sea (chapter 2.3) and the Dvurechenskii mud volcano in the Black Sea (chapter 2.5).

Gases and fluids formed by geothermal processes in the deep subsurface can also rise along fracture zones, which are generated by tectonic plate movements. Similar to the mud volcanoes, accumulations of chemosynthetic organisms are found, indicating fluid flow. Patches of clams were found to be aligned along fracture zones in the Nankai Trough (Kobayashi 2002) or Peruvian margin (Olu et al. 1996). Such a seep community (Fig. 2) has been biogeochemically investigated in the subduction zone of the Japan Trench; chapter 2.4.
1.4. **Biogeochemical processes in cold seep sediments**

Cold seep sediments are biogeochemically highly active. Although seeps can still release high amounts of methane, a large fraction of it is aerobically and anaerobically consumed by methane oxidation, the latter being more important. Anaerobic methane oxidation is closely coupled to sulfate reduction producing sulfide in the top meter of the sediment (Fig. 3). Sulfide is an important electron donor for the chemosynthetic community members. It is re-oxidized either directly with oxygen or indirectly with other oxidized compounds like nitrate or manganese dioxide. Hence, aerobic and anaerobic processes are closely linked with each other in seep sediments, and the compounds are biogeochemically cycled (Fig. 3). In the following paragraphs, methane and sulfate geochemistry at seeps is shortly summarized. Furthermore, the meaning of benthic oxygen consumption and its in situ quantification is discussed.
1.4.1. Methane biogeochemistry

Methane is the most common hydrocarbon gas in marine sediments and is produced abiogenically, thermogenically or biogenically (Judd 2003). Abiogenic methane is formed by purely chemical processes without participation of biological organisms. It is a side product of ultramafic rock serpentinization, of carbon dioxide reduction during magma cooling or of water-rock interactions in hydrothermal systems (cf. Lollar et al. 2002; McCollom and Seewald 2007). The majority of methane is produced either by thermochemical breakdown of buried organic matter or by biological processes in sediments. Thermogenic methane is generated when complex, long chain organic molecules are broken down under high temperature and high-pressure conditions at depths typically deeper than 1 km below the seafloor (Judd 2003). Under anoxic conditions, biogenic methane is produced by methanogens, which might convert about 10% of the total organic carbon to methane (Judd 2004). This process is mediated by archaea and occurs as soon as other energetically more favorable electron acceptors (e.g. sulfate, nitrate) are depleted in the sediment. During methanogenesis, either carbon dioxide is reduced or acetate is fermented to form methane (Whiticar 1999; Reeburgh 2007), whereof the former (via carbon dioxide) seems to be more important in marine environments (Canfield et al. 2005).

How methane was formed can be discovered by identifying the isotopic composition of the gas. Thermogenic methane is generally more enriched in $^{13}$C than bacterial methane and has values in the
range of -50‰ to -20‰. In comparison, δ13C values of biogenic methane vary between -110‰ and -50‰ (Whiticar 1999).

Whether methane is microbially produced or originated from the deep subsurface, it can be oxidized aerobically and anaerobically in marine sediments. Aerobic methane oxidation is mediated by α- and γ-proteobacteria and occurs where methane and oxygen meet (Hanson and Hanson 1996). In seep sediments, oxygen diffusion into the sediment is often influenced by upward fluid flow, resulting in oxygen penetration depths of often less than one centimeter (de Beer et al. 2006; Niemann et al. 2006b). Therefore, anaerobic methane turnover is the dominant process in seep sediments (Treude et al. 2003; Boetius and Suess 2004; Orphan et al. 2004; Niemann et al. 2006b).

Anaerobic oxidation of methane (AOM) is mediated by consortia of methanotrophic archaea and sulfate reducing bacteria (Boetius et al. 2000). The first geochemical evidence of AOM taking place in marine sediments was found in the seventies of the last century (Martens and Berner 1974; 1977; Barnes and Goldberg 1976; Reeburgh 1976). However, the identification of those anaerobic methanotrophs (ANME) by membrane lipid and 16S rRNA gene analyses, and their visualization by fluorescence in situ hybridization (FISH) took another 20 years (Hinrichs et al. 1999b; Boetius et al. 2000). Nowadays, it is known that ANMEs are found wherever an overlap of methane and sulfate occurs over a wide range of abiotic conditions (Knittel et al. 2005). In marine environments, three different clades are distinguished. Two belong to the order Methanosarcinales (ANME-2; ANME-3), while the third one is more distantly related to the orders Methanosarcinales and Methanomicrobiales (ANME-1) (Knittel et al. 2005). The sulfate reducing bacteria of ANME-1 and ANME-2 are affiliated with the Desulfosarcina/Desulfovococcus group (Knittel et al. 2003). In contrast, ANME-3 is associated with SRB of the Desulfobulbus group (Lösekann et al. 2007; Omoregie et al. 2009).

The intermediates exchanged between the syntrophic partners and the biochemical pathways are still under investigation (cf. Reeburgh 2007), but the overall AOM net reaction (1) is widely accepted (Knittel and Boetius 2009)

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

In sediments, one of the end products, bicarbonate, leads to a precipitation of carbonate cement that binds sediment particles and forms methane-derived autogenic carbonate (Gontharet et al. 2007). The other product, hydrogen sulfide, is one of the most important electron donors at seeps and is re-oxidized by either free living (e.g. Beggiatoa) or symbiotically associated sulfide oxidizing bacteria (Schulz and Jørgensen 2001; Dubilier et al. 2008).
Fig. 4: Methane concentration in the cold seep sediment changes depending on fluid flow intensity, and subsequently the amount of produced sulfide from the anaerobic methane oxidation changes as well. The different chemosynthetic organisms are adapted to these geochemical gradients. Bacterial mats are often associated with high methane and sulfide concentrations close to the sediment surface, in contrast to tubeworms, where enhanced concentrations are found several decimeter below the seafloor (Boetius and Suess 2004; de Beer et al. 2006; Niemann et al. 2006b). (Photos are courtesy of MARUM, University Bremen)

The biological methane filter (Fig. 4) in marine sediments is very efficient and consumes most of the methane before it reaches the water column (Niemann et al. 2006b; Wallmann et al. 2006; Jørgensen and Boetius 2007). Nevertheless, seeps are also the main methane emission sites in the oceans, because the high flow velocity of fluids or gas bubble streams reduces the residual time of methane in the sediment and thus reduces the potential time for methane consumption (Reeburgh 2007).

The contribution of seeps to the ocean methane budget is difficult to define, because exact numbers for methane release from seep structures on continental shelves or even in the deep sea are missing, and direct quantifications of methane effluxes are rare (Torres et al. 2002; Linke et al. 2005; Mau et al. 2006; Sommer et al. 2006; Sommer et al. 2008; Sommer et al. 2009a; Sommer et al. 2009b). Nevertheless, the contribution of seabed gas seeps to the atmospheric methane pool was estimated to be 20 Tg per year. Compared to the total annual flux (535 Tg) of other methane sources (anthropogenic, fossil, and natural) to the atmosphere (Judd et al. 2002), this appears not significant. However, there are also annual flux estimations considering only submarine mud volcanoes, which are in the range of 6 to 45 Tg methane (Dimitrov 2002; Judd et al. 2002; Etiope and Milkov 2004; Kvenvolden and Rogers 2005). These two examples illustrate clearly that more methane flux measurements are necessary to improve budget estimations. Indeed, significant contributions of seeps for water column methane budgets were recently shown by a study in the Black Sea (Kessler et al. 2006).

1.4.2. Sulfur biogeochemistry

In marine sediments, sulfate reduction (SR) is associated with organic matter mineralization or with anaerobic methane oxidation (AOM). During these processes, sulfate is either incorporated into
organic matter (assimilatory SR) or used as an electron acceptor to gain energy (dissimilatory SR). In the following chapters, only dissimilatory SR is considered, because it is the more dominant pathway in marine ecosystems. SR is mainly performed by sulfate reducing bacteria, but also by some thermophilic archaea (Canfield et al. 2005; Kasten and Jørgensen 2005).

In ocean waters, sulfate is the most abundant anion next to chloride with an average concentration of 28 mM. Sulfate is transported mainly by diffusion and bioirrigation before it is used in anoxic sediment horizons by sulfate reducing microbes. Globally, SR is the most important pathway for organic matter degradation in marine sediments. Aerobic degradation of organic compounds yields more energy and is thus more efficient than SR. However, oxygen is often depleted within the upper centimeter of the sediment. After oxygen, nitrate (NO$_3^-$), manganese (Mn$^{2+}$) and iron (Fe$^{2+}$) are the preferred electron acceptors for oxidation processes in marine ecosystems, but their concentrations are usually low and they are subsequently rapidly consumed in the sediment.

A detailed description of the sulfur cycle in marine sediments was performed by Kasten and Jørgensen (2005). A simplified sulfur cycle is presented here (Fig. 3). Briefly, sulfate is reduced in the sediment during the degradation of organic matter, but also during AOM. Of the produced sulfide, 80-95% are gradually oxidized back to sulfate via a redox cascade and only a minor fraction is buried in the sediment, dominantly as pyrite. Sulfide is often completely re-oxidized before it reaches the oxic sediment horizon, or even the sediment surface.

At seeps, SR is often closely coupled to the anaerobic oxidation of hydrocarbons, mainly methane, resulting in high sulfide fluxes and sulfide oxidation close to the sediment surface. This process can be catalyzed by thiotrophic free living bacteria, such as Thioploca or Beggiatoa, using oxygen or nitrate as electron acceptors (Fig. 3) (Schulz and Jørgensen 2001; Preisler et al. 2007). These bacteria can reach high abundances and form dense bacterial mats at sites with high sulfide fluxes, and, vice versa bacterial mats indicate high SR rates in sediments underneath (e.g. Treude et al. 2003; Niemann et al. 2006b). Furthermore, sulfide is one of the main electron donors for symbiotic bacteria that are associated with benthic seep fauna, such as bivalves and tubeworms (cf. Dubilier et al. 2008). These symbioses are advantageous for the bacteria because animals can often access deeper sediment horizons with high sulfide concentrations and seawater oxygen at the same time. Hence, the host provides optimal growth conditions for its symbiont, and subsequently benefits from the energy and carbon supplied by the microbes. Many invertebrates show distinct physiological adaptations during their evolution and some are even able to enrich sulfide in their body fluids above ambient concentration, for example some Calyptogena species (Childress and Fisher 1992; Barry and Kochevar 1998; Sibuet and Olu 1998).

Biogeochemical studies of shelves and slopes showed a positive correlation of SR to organic carbon content at the sediment surface (Tab. 1). In other words, in the deep sea below 1000 m water depth, SR becomes less important compared to aerobic mineralization or other suboxic processes. The
small amount of organic matter reaching the deep sea sediment surface is mainly degraded before it can even be buried in suboxic sediment horizons (Ferdelman et al. 1999).

Tab. 1: Averaged sulfate reduction and oxygen consumption in different marine environments (modified from Canfield et al. 2005)

<table>
<thead>
<tr>
<th>Region</th>
<th>Area (km²)</th>
<th>SR rate (mmol m⁻² d⁻¹)</th>
<th>O₂ uptake (mmol m⁻² d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shallow, high deposition</td>
<td>1 x 10⁴</td>
<td>36</td>
<td>71</td>
</tr>
<tr>
<td>Salt marsh</td>
<td>4 x 10⁵</td>
<td>77</td>
<td>233</td>
</tr>
<tr>
<td>Mangroves</td>
<td>1 x 10⁵</td>
<td>20</td>
<td>167</td>
</tr>
<tr>
<td>Intertidal</td>
<td>5 x 10⁴</td>
<td>12</td>
<td>36</td>
</tr>
<tr>
<td>Sea grass beds</td>
<td>1.1 x 10⁶</td>
<td>20</td>
<td>41</td>
</tr>
<tr>
<td>Estuaries and embayments</td>
<td>2 x 10⁶</td>
<td>7</td>
<td>26</td>
</tr>
<tr>
<td>Shelf-depositional</td>
<td>1.1 x 10⁷</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>Shelf-non-depositional</td>
<td>1.4 x 10⁷</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Upwelling</td>
<td>1.3 x 10⁵</td>
<td>7</td>
<td>19</td>
</tr>
<tr>
<td>Upper slope (200-1000 m)</td>
<td>1.6 x 10⁷</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Lower slope (1000-2000 m)</td>
<td>1.5 x 10⁷</td>
<td>0.4</td>
<td>1</td>
</tr>
<tr>
<td>Rise (2000-3000 m)</td>
<td>2.2 x 10⁷</td>
<td>5 x 10⁻²</td>
<td>2</td>
</tr>
<tr>
<td>Abyss (3000-4000 m)</td>
<td>7.1 x 10⁷</td>
<td>3 x 10⁻²</td>
<td>1</td>
</tr>
<tr>
<td>Abyss (4000-5000 m)</td>
<td>1.2 x 10⁸</td>
<td>4 x 10⁻³</td>
<td>0.4</td>
</tr>
<tr>
<td>Abyss (&gt; 5000 m)</td>
<td>8.8 x 10⁷</td>
<td>3 x 10⁻³</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Seeps can also be located in the deep sea, but seep organisms rely more on reduced compounds transported with the uprising deep subsurface fluids (e.g. methane, higher hydrocarbons) than on organic matter produced in the photic zone. The aerobic turnover or reduction of nitrate, manganese or iron are only of minor importance for the methane turnover at seeps due to the low penetration depth of oxidized compounds into the sediment, caused by high consumption rates and upward fluid flow (de Beer et al. 2006). This results in high methane-initiated sulfate turnover rates that often exceed SR rates (e.g. Treude et al. 2003; Niemann et al. 2006b) of shelf sediments with high loads of organic matter or of the common deep sea seafloor (Tab. 1).

In marine sediments or anoxic water columns, AOM is obligately associated with SR. However, sulfate reducing bacteria can also use higher hydrocarbons (e.g. propane, butane) as electron donors in anaerobic environments. As soon as higher hydrocarbons are available in sufficient amounts, the AOM syntrophy is out-competed by sulfate uptake (Orcutt et al. 2005; Niemann et al. 2006a; Omorogie et al. 2009), because of its lower energy yield compared to the anaerobic oxidation of higher hydrocarbons (Kniemeyer et al. 2007; Widdel et al. 2007).

1.4.3. Oxygen biogeochemistry

From the thermodynamical point of view, oxygen is the most favorable electron acceptor in the marine system. It is produced only in the photic zone of the water column, but even chemoautotrophic seep organisms in the deep sea often require oxygen for their metabolism. The oxygen concentration in seawater is low and oxygen is soon depleted in the sediment, thus SR becomes more important for
organic matter degradation. However, oxygen is important because most of the sulfide is re-oxidized via the redox cascade in the sediment, with oxygen as the terminal electron acceptor (Kasten and Jørgensen 2005).

Fig. 5: Relative distribution of ocean areas, oxygen uptake, and sulfate reduction rates in marine sediments. Most of the consumed oxygen in marine sediments is used to re-oxidize sulfide, an end product of sulfate reduction (Kasten and Jørgensen 2005).

At continental shelves, half of the entire benthic oxygen consumption is directly or indirectly used for the re-oxidation of sulfide (Fig. 5, Kasten and Jørgensen 2005). In the deep sea, the organic matter input is lower and mainly consumed aerobically (see chapter 1.4.2). Although cold seeps can also be located in the deep sea, seep sediments have similar or even higher sulfide production rates compared to shelf sediments. Therefore, it is likely that a similar fraction of the oxygen consumption at seeps is used for the re-oxidation of sulfide. Budget estimations of seeps discussing the ratio of SR to oxygen consumption rates are rare (Sommer et al., 2009, Niemann et al., 2006). One reason is that only a small data set of sulfate turnover rates at seeps exists, but even more important, the number of studies investigating oxygen consumption rates at seeps are only recently increasing (Linke et al. 2005; de Beer et al. 2006; Niemann et al. 2006b; Sommer et al. 2009a; Sommer et al. 2009b).

In general, benthic oxygen consumption has been commonly used to quantify in situ respiration and mineralization rates during the last decades (reviewed by Glud 2008). Such studies were mainly done in coastal areas with a few focusing on the abyssal deep sea plains (Glud et al. 2000; Wenzhöfer and Glud 2002; Glud et al. 2005). At seeps, oxygen consumption can be also useful to quantify benthic activity and indirectly quantify consumption rates of methane, which is often the sole electron donor. Especially in seep ecosystems, in situ measurements of biogeochemical processes have great advantages compared to ex situ measurements, because the recovery of gas-saturated sediment cores can cause disturbances of the geochemical gradients due to depressurizing and degassing of gases.

In situ benthic oxygen uptake rates can be measured one-dimensionally with a microprofiler, two-dimensionally with planar optode, or three-dimensionally with a benthic chamber (Fig. 6, Boetius and Wenzhöfer 2009). For the studies described in Chapter 2, microprofiler and benthic chamber were mainly used. Both techniques are discussed in detail in the following paragraphs.
The microprofiler module is usually equipped with numerous microsensors, which can measure various parameters, e.g. oxygen concentration, sulfide concentration, pH and temperature. Vertical high-resolution concentration profiles of the different parameters (Fig. 7A) are gained from measurements in the sediment. The depth resolution of those profiles is usually in the range of hundred micrometers and thus steep vertical chemical gradients can be investigated.

The benthic chamber encloses a defined area of sediment and the overlying bottom water. The concentration changes in the enclosed water body are recorded during the incubation time (Fig. 7B) and used to determine total areal exchange rates of oxygen and methane, but also of other dissolved compounds. For the oxygen measurements, minisensors are mounted to the chamber or water samples are retrieved at preset time intervals to determine concentration changes. The obtained fluxes of methane, dissolved inorganic carbon and nutrients can be used to quantify the methane release at seeps (e.g. Sommer et al. 2009b).
Fig. 7: (A) A typical microsensor profile gained from measurements of a sulfide oxidizing bacterial mat at the Amon mud volcano (a detailed description is found in subchapter 2.2. (B) During a benthic chamber incubation at a bacterial mat (Håkon Mosby mud volcano), the oxygen concentration decreased while the methane content increased over time.

Microprofilers are used to determine the diffusive oxygen uptake (DOU) in the sediment. It is calculated by assuming a steady state and applying Fick’s 1st law of diffusion to the linear oxygen concentration gradient in the diffusive benthic boundary layer (DBL). The DBL is a thin layer of 0.2 - 1 mm thickness above to the sediment, through which molecular diffusion is the dominate transport process (Jørgensen and Marais 1990). In sediments with high oxygen consumption rates, the transport across the DBL can be the limiting factor. The thickness of the DBL can vary according to hydrodynamic processes and small scale seafloor topography. Microsensor measurements along a horizontal transect in coastal sediments showed that DOU differs by less than 10% considering the sculptures surface as a flat plane (Røy et al. 2002).

In contrast to the DOU, the total oxygen uptake (TOU) is determined with the benthic chamber (Fig. 7). DOU does not consider faunal respiration or bioturbation. Previous investigations showed that DOU can underestimate the benthic consumption by a factor of 1.5 - 2.0 (Aller and Aller 1992; Rysgaard et al. 2000; Berg et al. 2001). Filter-feeding ciliates in microbial mats or carbon-rich sediments can have a large effect and enhance solute transport of small molecules by a factor of 1.1 - 10 (Glud and Fenchel 1999). Meiofauna activities influence the transport of solutes, e.g. oxygen, into the sediment mainly by affecting the porosity and subsequently the diffusion coefficient. Furthermore, TOU is also higher than DOU in macrofauna influenced sediments. This is not only caused by faunal respiration, but also by irrigation and bioturbation (Fig. 8), which stimulates aerobic microbial activity and chemical oxidation (Glud 2008). In conclusion, the microbial respiration of the sediment is better represented by DOU and the difference between DOU and TOU can be ascribed to faunal activity (respiration, bioirrigation).
Fig. 8: Processes responsible for the total oxygen uptake (TOU) in coastal sediments. The data shows that faunal initiated respiration and irrigation can be important processes for oxygen budgets of marine sediment (Glud 2008).
References:


Fujikura, K., S. Kojima, K. Tamaki, Y. Maki, J. Hunt, and T. Okutani. 1999. The deepest chemosynthesis-based community yet discovered from the hadal zone, 7326 m deep, in the Japan Trench. Marine Ecology-Progress Series 190: 17-26


Chapter 1

Introduction


Sommer, S. and others 2009b. Seabed methane emission and the habitat of frenulate tubeworms on the Captain Arutyunov mud volcano (Golf of Cadiz). Marine Ecology-Progress Series 382: 69-86


2. Results

This PhD-thesis includes five manuscripts. Two of them have been submitted to international journals and three of them will be submitted within the next three months. All manuscripts appear in the order described in the general introduction. In the following I specify my contribution to each article.

2.1. Contribution to publications

(I) Transport and consumption of oxygen and methane in different habitats of the Håkon Mosby Mud Volcano (HMMV)

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Sediment sampling and measurements of methane and sulfate turnover rates were accomplished by Antje Boetius and Janine Felden, or cited from earlier publications. The in situ work to measure total oxygen uptake and methane emission was performed by Janine Felden and Frank Wenzhöfer. Tomas Feseker carried out all temperature measurements. The manuscript was written by Janine Felden with contribution of Antje Boetius and Frank Wenzhöfer.

(II) Biogeochemical processes associated with mud volcanism on the Nile Deep Sea Fan - The Amon Mud Volcano

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Radiotracer incubations for sulfate reduction and methane oxidation rates were performed by Janine Felden. In situ measurements were carried out by Janine Felden, Anna Lichtschlag, Frank Wenzhöfer and Dirk deBeer. Pore water extraction and analyses were performed by Anna Lichtschlag and Gerd deLange. Janine Felden analysed total cell numbers. Antje Boetius initiated the study and planned the ROV operations. The manuscript was written by Janine Felden with support and help of Antje Boetius, Frank Wenzhöfer and Anna Lichtschlag.
(III) Evidence for the deepest known anaerobic methanotrophic microbial community at Calyptogena colonies (Japan Deep Sea Trench)

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The study was a Japanese-German collaboration that was initiated by Fumio Inagaki and Frank Wenzhöfer. Sediment sampling, all radiotracer incubations and 16S rDNA work were accomplished by Janine Felden. The in situ work was carried out by Frank Wenzhöfer and Janine Felden. Lipid analyses, solid phase analyses and methane concentration as well as carbon isotopic composition measurements were done by Tobias Mohr under the supervision of Kai-Uwe Hinrichs. The manuscript was written by Janine Felden with support and input from Frank Wenzhöfer and Tobias Mohr.

(IV) Geochemical processes and primary productivity in different thiotrophic mats of the Håkon Mosby Mud Volcano (Barents Sea)

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All in situ measurements were conducted by Anna Lichtschlag and Dirk deBeer. Pore water sampling and geochemical analyses of pore water and sediment as well as nitrate uptake experiments, and the S-isotope analyzes were done by Anna Lichtschlag. Sulfate reduction rates were determined by Antje Boetius and Janine Felden. The manuscript was written by Anna Lichtschlag with the contribution from Dirk deBeer, Antje Boetius and Volker Brüchert.

(V) Methane consumption and sulfide production in permanent anoxia: in situ study of a cold seep site in the Black Sea

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The field work and ROV dives were planned and carried out by Antje Boetius with all coauthors. The in situ measurements were done by Anna Lichtschlag, Dirk deBeer, Janine Felden and Frank Wenzhöfer. Pore water sampling and analyses were performed by Anna Lichtschlag. Samples for carbon isotopes and methane concentrations were obtained and analyzed by Florence Schubotz and Tobias Ertefai. The manuscript was written by Anna Lichtschlag with help and support by Dirk deBeer and Antje Boetius.
Chapter 2.2

Transport and consumption of oxygen and methane in different habitats of the Håkon Mosby Mud Volcano (HMMV)

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Abstract

The Håkon Mosby Mud Volcano (HMMV) is located on the SW Barents Sea slope at a water depth of approximately 1250 m. It is a highly active cold seep ecosystem comprising different chemosynthetic habitats surrounding its central area such as mats of thiotrophic bacteria and siboglinid tubeworm assemblages. This study focused on in situ measurements of methane efflux to the hydrosphere in the different mud volcano habitats, in comparison to microbial methane as well as total benthic oxygen consumption. By targeted in situ quantification of oxygen, methane and sulfide fluxes during several expeditions to the HMMV we achieved a spatial budget for the four main habitats on a scale of 10-1000 m.

At the HMMV, the range of dissolved methane efflux (770 - 2 mmol m^-2 d^-1) from the center to the outer rim was associated with a decrease in temperature gradients (46 - 1°C m^-1), indicating that spatial variations in fluid flow controls the distribution of benthic habitats and activities. Accordingly, total oxygen uptake (TOU) varied between the different habitats by one order of magnitude from 15-161 mmol m^-2 d^-1. However, the highest TOU was associated with the lowest fluid flow and methane efflux. Because of the high fluid flow rates controlling the benthic activities at the HMMV, the microbial sink for methane in the seabed was strongly limited, accounting for <22% of the dissolved methane emission. However, previously overlooked surface-near processes, including the aerobic oxidation of methane in the center and Beggiatoa mats, as well as meiofauna and tubeworm respiration contribute considerably to the benthic filter against methane as recorded by total oxygen consumption measurements with benthic chambers.
1. Introduction

Active submarine mud volcanoes (MVs) are found worldwide at convergent and passive continental margins (Dimitrov 2002). They are characterized by fluid and gas outflows fueling cold seep communities, and are often associated with the occurrence of gas hydrates. The total number of deep-sea MVs is estimated to be in the range of $10^3$-$10^5$ with a release of 5-20 Tg methane per year of which an unknown fraction may escapes to the atmosphere (Kopf 2002; Kvenvolden and Rogers 2005; Sauter et al. 2006).

In cold seeps ecosystems such as those associated with mud volcanism, the chemical energy in hydrocarbons emitted as fluids and gases is used as energy source for diverse chemosynthetic communities (Olu et al. 1997; Olu-Le Roy et al. 2004; Jørgensen and Boetius 2007). Benthic oxygen and sulfate consumption is often 1-2 orders of magnitude higher at cold seeps compared to non-seep seafloor. Hence, oxygen and sulfate become rapidly depleted in the upper millimeters or centimeters of cold seep seafloor, respectively (Treude et al. 2003; de Beer et al. 2006; Sommer et al. 2006). Most cold seeps release methane and other hydrocarbons into the water column either as free gas or dissolved, and microbial assemblages present at and in the sediment oxidize a significant fraction of the subsurface hydrocarbon flux. The flux of methane to the seafloor depends mostly on geological and physical processes in the subsurface seabed, but emission and consumption rates are considerably influenced by biological activities. Unfortunately, very few quantitative estimates of the balance between methane emission and consumption by aerobic and anaerobic oxidation of methane and other hydrocarbons are available today (Niemann et al. 2006; Sommer et al. 2006; Reeburgh 2007).

Two main pathways of methane oxidation in marine settings have been identified: The aerobic oxidation (MOx) with oxygen performed by methanotrophic bacteria, the anaerobic oxidation of methane (AOM) with sulfate mediated by methanotrophic archaea associated with sulfate reducing bacteria (Knittel and Boetius 2009). Sulfide and carbonate are the end products of AOM coupled to sulfate reduction (SR) (Boetius et al. 2000). Sulfide produced by AOM is the main electron donor for sulfide oxidizing bacteria, free living ones such as the giant mat-forming thiotrophs, and endosymbionts such as those in siboglinid tubeworms and several taxa of bivalves (Olu et al. 1997; Cordes et al. 2003; Teske and Nelson 2006). The presence of these chemosynthetic organisms at the seafloor is therefore used as an indicator of active AOM/SR microbial communities in the underlying seabed, fueled by high methane fluxes (Treude et al. 2003; Niemann et al. 2006).

Four main types of methane-dependent communities occur at the Håkon Mosby Mud Volcano (HMMV), which is located northwest of Norway in the Barents Sea (72° N, 14° 44’ E) at a water depth of 1250 m. (e.g. Milkov et al. 2004; de Beer et al. 2006; Kaul et al. 2006; Niemann et al. 2006; Van Gaever et al. 2006; Jerosch et al. 2007). These four habitats differ morphologically and biogeochemically, and include the central area populated by aerobic methanotrophs, two types of thiotrophic bacterial mats, and dense accumulations of siboglinid tubeworms at the outer rim of the mud volcano (de Beer et al. 2006; Niemann et al. 2006; Jerosch et al. 2007). All habitats are methane-
rich, but have been found to differ in fluid flow velocity and sulfide production rates (de Beer et al. 2006). Hence, the HMMV represents an ideal natural laboratory to investigate the relationship between fluid flow, methane flux and benthic activity. In our study, we combined data from six different cruises from 2001-2009 to investigate spatial variations of biogeochemical processes within and between the different HMMV habitats. We investigated for the first time diffusive methane efflux and total oxygen consumption for HMMV habitats characterized by different transport processes. Based on these data, the efficiency of the biological filter against methane and sulfide could be estimated. Furthermore, we inquired the relation between diffusive and total oxygen consumption, as well as between temperature gradients, oxygen, sulfide and methane fluxes.

2. Material and Methods

2.1. Site description

Based on previous studies (Niemann et al. 2006; Jerosch et al. 2007, Lichtschlag et al., in review) and on visual investigations, four habitats were identified and chosen for repeated sampling and in situ measurements: the mud volcano center, comprising a northern zone of ca. 100 m diameter with highly disturbed seabed, surrounded by an apparently older mud with a smooth surface (I), the adjacent *Beggiatoa* mats (II), a transition zone marked by patchy bacterial mats of gray color (III) and dense assemblages of siboglinid tubeworms occupying the hummocky outer zone of the HMMV (IV) (Fig.1; 2). The center of the HMMV is characterized by high temperature gradients, methane emission and mud extrusion, and the absence of sulfide production and chemosynthetic macrofauna (de Beer et al. 2006; Niemann et al. 2006). Adjacent to the active center, dense *Beggiatoa* mats cover large parts of the flat seafloor characterized by high fluid flow, gassy sediments and high sulfide flux. Another bacterial mat type with a grayish color was found above gas-saturated sediments in the transition zone towards the hummocky area surrounding the center. These mats are characterized by the absence of fluid flow but associated with high sulfide production in the sediments (de Beer et al. 2006, Lichtschlag et al., in review). The hummocky zone surrounding the HMMV is inhabited by two siboglinid tubeworm species that can reach high biomasses of 1-2 kg wet weight m$^{-2}$ (Smirnov 2000; Milkov et al. 2004; Niemann et al. 2006). These tubeworms irrigate the seafloor and push the anoxic zone into deeper sediment strata (Lösekann et al. 2008).

The patchy colonization of chemosynthetic organisms (e.g. tubeworms, thiotrophic bacteria) at the seafloor creates mosaic-like distribution patterns in each habitat. Therefore, within most habitats, measurements were carried out on those patches covered with characteristic benthic assemblages and also on adjacent patches lacking these, covering a spatial scale of decimeters to meters. For example, measurements in the *Beggiatoa* mat habitat were performed on sediment densely covered by bacteria and additionally “next to” the bacterial mat, where no mat was observed at the seafloor but the sediment could still be influenced by seepage.
Chapter 2.2.  Methane transport and consumption at the HMMV

2.2. Sampling

Sediment sampling and measurements were performed during six cruises: HMMV (R/V L’Atalante, 2001), ARK XIX-3b (R/V Polarstern, 2003), AWI-ROV (L’ Atalante 2005), Vicking...
cruise (R/V “Pourquoi pas”, 2006), ARK XXII-1b (R/V Polarstern, 2007) and ARK XXIV-2 (R/V Polarstern, 2009). Targeted sampling, precise positioning and operation of the in situ tools at the seafloor were performed with the remotely operated vehicles (ROVs) “Victor 6000” (IFREMER, France) and “Quest 4000” (Marum, University Bremen, Germany). Sampling locations, sample labels and all performed measurements are summarized in Tab.4 (appendix) and are available in the PANGAEA database (http://www.pangaea.de).

Fig 2.: Different habitats of the HMMV. (A) Push core sampling in the center where the highest temperature gradients occur; (B) The benthic chamber is deployed on a smooth mudflow of the center; (C) Beggiatoa mats; (D) Gray mat habitat; (E) Gray mat close up with temperature probe; (F) Siboglinid tubeworm habitat. All pictures are copyright of MARUM (University of Bremen, Germany), except (D) and (E) which are copyright IFREMER (France).

Sediment samples from the upper most sediment horizons (top 20-30 cm) were taken either with a video-guided multicoring device (MUC) or with pushcores (PCs) collected with the manipulator of the ROV. After recovery, the PC and MUC tubes were transferred to a cold room that was cooled to in situ temperature (0°C). The HMMV sediment is highly gas saturated, and outgassing during retrieval of the samples often caused disturbance of the bacterial mats. Therefore, respective cores were stored for 1-2 days at in situ temperature to reestablish bacterial mats and geochemical gradients as shown by microsensor profiles (de Beer et al. 2006). Afterwards, the cores were vertically sub-sampled with small sub-cores tubes (d=26 mm).

To access sediment depths below 30 cm, a gravity corer (GC) was equipped with a POSIDONIA (IXSEA SAS; FRANCE) positioning system for targeted sampling of the same habitats. Sediments from the GC were subsampled with small glass tubes (60 mm length, 10 mm diameter) immediately after the core recovery.
2.3. Methane oxidation and sulfate reduction rates

Sediment cores for measurements of methane oxidation (MOx/AOM) and sulfate reduction (SR) were subsampled on board with 2-4 replicates per sample station. The rates were measured according to Treude et al. (2003). 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 into the sub-cores (whole core injection method, Jørgensen 1978). The sediment was incubated in the dark at in situ temperature for 12-48 h (PC, MUC). After the incubation, all sediment cores were cut into 1-2 cm sections. Each section was fixed in 25 mL NaOH (2.5%, w/v) or in 20 mL zinc acetate solution (20%, w/v) for methane oxidation and sulfate reduction rate measurements, respectively. Subsamples from the gravity cores were treated the same way by either injecting 25 µL $^{14}$CH$_4$ (2.5 kBq) or 5-10 µL carrier-free $^{35}$SO$_4^{2-}$ (50 kBq) into the glass tubes. These sediments were incubated for 36 h and then preserved as described for the sub-core sections. In the home laboratory, sulfate and methane turnover were quantified according to Kallmeyer et al.(2004) and Treude et al.(2003), respectively. The radioactivity of the labeled constituents was determined by scintillation counting. The substrate concentrations (methane, sulfate) were measured by gas chromatography (5890A, Hewlett Packard) and anion exchange chromatography (Waters I.C.-Pak™ anion column 50x 4.6 mm, Waters 430 conductivity detector), respectively.

Methane turnover rates (MOx and AOM) were calculated according to the following equation:

$$\text{methane oxidation} = \frac{^{14}\text{CO}_2}{(^{14}\text{CH}_4 + ^{14}\text{CO}_2)} \times \frac{\text{CH}_4}{V \times t}$$

where CH$_4$ is the methane concentration, $^{14}$CO$_2$ is the activity of the produced carbon dioxide, $^{14}$CH$_4$ is the activity of the radioactive methane, t is the incubation time and V is the volume of the samples. The method itself does not distinguish between MOx and AOM but it can be assumed that MOx occurs only in the top oxygenated sediment horizon (0-2 cm), based on previous in situ microsensor measurements (de Beer et al. 2006).

Sulfate reduction (SR) rates were calculated with the following equation:

$$\text{SR} = \frac{\text{TRI}^{35}\text{S}}{(^{35}\text{SO}_4^{2-} + \text{TRI}^{35}\text{S})} \times \frac{\text{SO}_4^{2-}}{V \times t}$$

where SO$_4^{2-}$ is the sulfate concentration, TRI$^{35}$S is the activity of the reduced sulfur compounds and $^{35}$SO$_4^{2-}$ is the radioactive sulfate (Treude et al. 2003).

2.4. Total benthic oxygen uptake

The total benthic oxygen uptake (TOU) and the methane emission rates were determined with a cylindrical benthic chamber module (Fig. 2B). The measurements were conducted in 2007 (ARK XXII-1b) and 2009 (ARKXXXIV-2). The benthic chamber was operated by the ROV, and the water height inside the chamber was determined by visual observation with the ROV camera system. The stirred chamber (radius 9.5 cm) enclosed a seafloor area of 284 cm$^2$ together with 10-15 cm
(equivalent to 4-6 L) of overlying bottom water. A valve in the chamber lid ensured the release of overpressure while placing the chamber gently into the sediment avoiding any disturbance of the sediment surface. Two Clark-type oxygen mini-electrodes mounted in the chamber lid continuously monitored the oxygen concentration in the enclosed water. A two-point calibration of the reading of the mini-electrodes was performed. The reading at zero $O_2$ concentration was taken on board at in situ temperature. Values for the bottom water $O_2$ concentration were determined in situ at the seafloor and the respective determination of water samples by Winkler titration or oxygen optode readings (Recording Current Meter (RCM) 11, Aanderaa; Bergen, Norway). Additionally to the sensor readings, five water samples were taken with 50 mL syringes at preprogrammed time intervals to determine the dissolved methane concentration. After retrieval of the chamber, the water samples were immediately preserved by adding 40 mL to sealed glass vials with NaOH pellets. The samples were stored at 4°C until further analyses in the home laboratory. Methane concentrations were measured by gas chromatography (5890A, Hewlett Packard).

The total oxygen uptake (TOU) and methane emission rates were calculated from the linear regression of the concentration versus time (mmol m$^{-2}$ d$^{-1}$):

$$\text{TOU} / \text{methane emission} = \frac{dC}{dt} \times \frac{V_{\text{chamber}}}{A_{\text{chamber}}}$$

where $dC/dt$ (µmol L$^{-1}$ h$^{-1}$) are the changes of concentrations over the incubation time, $V_{\text{chamber}}$ (cm$^3$) is the volume of the overlying water in the enclosed chamber, and $A_{\text{chamber}}$ (cm$^2$) is the area of the sediment enclosed by the chamber. Calculating the potential replacement of oxygen rich bottom water with oxygen free subsurface water during the incubation of the chamber indicates, that < 1% of the enclosed water volume could have been replaced by subsurface fluids during the 4-6 h incubation time.

### 2.5. In-situ sediment temperature measurements

In-situ temperature measurements at shallow sediment depths using ROV-operated temperature probes were conducted during the cruises ARK-XIX-3b of R/V Polarstern in 2003, AWI-ROV of R/V L’Atalante in 2005, VICKING of R/V Pourquois Pas? in 2006 (Feseker et al. 2008), and ARK-XXII-1b of R/V Polarstern in 2007 (Pérez-Garcia et al. 2009). The mechanical design of the probes and the number of temperature sensors varied between the different cruises, but all temperature measurements covered the interval from around 0-50 cm below the seabed. The ROV-operated lance was equipped with eight sensors, which were calibrated to a precision of 0.002 °C (cf. Feseker et al. 2008; Pérez-Garcia et al. 2009).

### 2.6. Areal budget calculations

For a budget of total oxygen and methane fluxes at the entire HMMV geostructure and within the different habitats, measurements from five cruises were compiled (Fig.1). Previous estimates of
methane budgets for mud volcano systems (Linke et al. 2005; Niemann et al. 2006; Sauter et al. 2006; Wallmann et al. 2006; Sommer et al. 2009) have relied on a small number of measurements at selected locations. Here we have integrated rate measurements over 9 years at HMMV, assuming that the different assemblages of benthic organisms are associated with persistent biogeochemical and geophysical settings (Fig. 1), as described previously (de Beer et al. 2006; Niemann et al. 2006; Lösekann et al. 2007, Lichtschlag et al. in review). Videographic observations during ROV sampling indicated minor changes in overall habitat distribution within this time period, hence we have based the areal calculations on the detailed habitat map obtained in 2003 (Jerosch et al. 2007). The gray mat habitat (III) was only recently described (de Beer et al. 2006, Lichtschlag et al., in review) and not considered as separate habitat in the previous habitat analyses (Jerosch et al. 2007). Based on the published data and our video-observa-tions, we assumed that on average 75% of the hummocky area is inhabited by siboglinid tubeworms. The remaining 25% of this area is devoid of tubeworms but hosts patchily distributed gray mats (III).

To account for the heterogeneous distribution of organisms for areal budget calculations, we distinguished between those zones marked by a 100% coverage of the seafloor by chemosynthetic organisms and zones with a patchy coverage (Tab. 2). The benthic chamber measurements for oxygen uptake and methane emission rates were performed in the different zones of each habitat, i.e. on seafloor with 100% coverage with bacterial mats, and “next to” the mats in zones with more patchy distributions. Subsequently, we used both measurements in the respective proportion to calculate the total areal fluxes. Areal estimates of sulfate and methane turnover were gained by depth integration of sulfate reduction and methane oxidation rates in the respective habitats for the upper meter of seabed. This depth was chosen to account for the high subsurface AOM and SR rates below the roots of the siboglinid tubeworms (Lösekann et al. 2008).

3. Results

3.1. In situ temperature measurements

A total of 94 in-situ sediment temperature measurements were carried out in specific habitats (e.g. Fig. 2E) based on visual observations recorded as digital photographs during the ROV dives. The locations of all measurements are shown in Fig. 3, together with the temperature gradients associated with the different habitats. All temperature measurement within the HMMV showed elevated gradients compared to the normal geothermal gradient of the Norwegian margin (0.07°C m\(^{-1}\); Tab. 1). In the HMMV central area (I), 53 in-situ temperature measurements were carried out. The highest temperature gradients were reached in an area characterized by a disturbed seafloor surface (Tab. 1; Fig. 2A), less than 100 m in diameter, and north of the geometrical center of the mud volcano. In this “hot center” area, the temperature gradients ranged between 10°C and 46°C m\(^{-1}\) with a mean of 25.9°C m\(^{-1}\). Around the “hot center”, smoother mud deposits form a large flat area reaching up to 150 m south
and east of the hot center. These smooth mud deposits were associated with much lower temperature
gradients between 1.5 and 17 °C m⁻¹ (Tab. 1) and are subsequently called “warm center” area (Fig 2B).

Tab.1: Shallow sediment temperature gradients of the different HMMV habitats (n=number of replicates). Mean and
median (not shown) values of the temperature measurements were not significantly different.

<table>
<thead>
<tr>
<th>Habitat</th>
<th>minimal</th>
<th>maximal</th>
<th>mean</th>
<th>confidence interval (95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I) center</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hot (n= 16)</td>
<td>10.3</td>
<td>46.0</td>
<td>25.9</td>
<td>21.3 - 0.6</td>
</tr>
<tr>
<td>warm (n= 37)</td>
<td>1.5</td>
<td>17.2</td>
<td>6.9</td>
<td>5.7 - 8.0</td>
</tr>
<tr>
<td>(II) <em>Beggiatoa</em> mat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Beggiatoa</em> mat (n= 11)</td>
<td>1.2</td>
<td>20.8</td>
<td>4.7</td>
<td>1.2 - 8.3</td>
</tr>
<tr>
<td>“next to” (n= 9)</td>
<td>0.9</td>
<td>11.3</td>
<td>4.8</td>
<td>2.4 - 7.1</td>
</tr>
<tr>
<td>(III) gray mat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gray mat (n= 8)</td>
<td>0.6</td>
<td>6.7</td>
<td>4.0</td>
<td>3.0 - 4.9</td>
</tr>
<tr>
<td>“next to” (n= 7)</td>
<td>2.0</td>
<td>6.0</td>
<td>3.5</td>
<td>2.0 - 5.0</td>
</tr>
<tr>
<td>(IV) <em>siboglinid</em> tubeworms</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tubeworms (n= 4)</td>
<td>0.9</td>
<td>3.7</td>
<td>2.0</td>
<td>0.9 - 3.3</td>
</tr>
<tr>
<td>“next to” (n= 2)</td>
<td>3.4</td>
<td>8.3</td>
<td>5.9</td>
<td>1.1 - 10.7</td>
</tr>
</tbody>
</table>

Measurements in the white (II) and grey mat (III) habitats showed lower average temperature
gradients than in the hot and warm center, but were not significantly different from each other (Fig. 3,
Tab 1). In the *Beggiatoa* mat habitat (II) 20 in-situ temperature measurements were conducted. The
temperature gradients varied between 1.2 and 20.8 °C m⁻¹ inside the white mats and between 0.9 and
11.3 °C m⁻¹ next to the white mats. The difference between the average temperature gradients inside
and next to white mats was not significant. From the transition zone where gray mats (III) occur, 15
in-situ temperature measurements were obtained. The temperature gradients ranged from 0.6 to 6.7 °C
m⁻¹ next to gray mats and from 2 to 6 °C m⁻¹ inside grey mats. The difference between the average
temperature gradients inside and next to grey mats was not significant. In the siboglinid tubeworm
habitat north of the center (IV) six in-situ temperature measurements were conducted. Here, the
temperature gradients were clearly reduced compared to the other habitats. Four measurements in
tubeworm patches revealed low temperature gradients of between 0.9 and 3.7 °C m⁻¹ compared to 3.4
and 8.3 °C m⁻¹ measured next to tubeworm patches.
3.2. Methane and sulfate turnover in different HMMV habitats

**Center (I):**

In the center, methane oxidation and sulfate reduction rates were measured in 2001 and 2003 and were similar in both years (Fig. 4 A-B). Furthermore, methane and sulfate turnover were not significant different in the “warm” and “hot” center. At the sediment surface where oxygen penetrates in the most upper horizon (de Beer et al. 2006) methane oxidation of rates of up to 385 nmol cm$^{-3}$ d$^{-1}$ (hot center) were observed. Sulfate reduction (SR) was low (< 20 nmol cm$^{-3}$ d$^{-1}$) in all replicates over the entire vertical profile down to 19 cm below seafloor (bsf) and was less than 1 nmol cm$^{-3}$ d$^{-1}$ below 20 cm. On average, the integrated rates of methane oxidation over 1 m of seafloor in the hot and warm center were 8.3 (n = 4, 4.2 standard deviation, SD) and 10 mol m$^{-2}$ d$^{-1}$, respectively (Tab. 2). For SR the depth integrated rates were only 1 (n = 4; ± 0.2 SD) and 1.2 mol m$^{-2}$ d$^{-1}$ (n = 4; ± 0.3 SD) for the hot and warm center area.

**Beggiatoa mat (II):**

Below the *Beggiatoa* mats, the highest methane consumption rates of up to 418 nmol cm$^{-3}$ d$^{-1}$ were found in the anoxic 1-2 cm sediment layer, decreasing with increasing depth (Fig. 4 C-D). The sulfate reduction rates followed the same trend with a peak of up to 1353 nmol cm$^{-3}$ d$^{-1}$ in 1-3 cm depth, reaching < 20 nmol cm$^{-3}$ d$^{-1}$ below 15 cm. The depth integrated mean values for AOM and SR were 10.7 (± 6.5 SD; n=17) and 14.8 (± 6.6 SD; n = 32) mmol m$^{-2}$ d$^{-1}$, respectively (Tab. 2).
Chapter 2.2. Methane transport and consumption at the HMMV

Fig. 4: Rates of sulfate reduction (left) and methane oxidation (right) in different habitats. A-B center (I; hot and warm center show similar rates); C-D Beggiatoa mat (II); E-F gray mat (III); G-H siboglinid tubeworm (IV) habitat. The symbols of the different years are color-coded: yellow-2001; red-2003, green-2006 and blue-2007. Note the different depth scales of the panels G and H. For A-F only PC measurements are shown, the GC measurements had rates <10 nmol cm$^{-3}$ d$^{-1}$ down to one meter sediment depth.
Tab. 2: Average temperature gradients and fluxes in different habitats of the HMMV. All numbers are given in mmol m\(^{-2}\) d\(^{-1}\) if not indicated otherwise. The sulfate reduction (SR) and methane oxidation rates (MOx and AOM) were integrated over the top meter of sediment. Total oxygen uptake (TOU) and methane emission (CH\(_4\)-flux) rates were measured with the benthic chamber. The methane efflux refers only to diffusive transport of methane from the seafloor to the hydrosphere. Total methane flux is calculated as the sum of CH\(_4\) efflux and oxidation; the efficiency of the biological methane sink is calculated as the % consumption of methane from the total methane flux. n=number of replicates; n.d.= not determined.

<table>
<thead>
<tr>
<th>Temperature gradients [°C m(^{-1})]</th>
<th>hot spot</th>
<th>warm</th>
<th>Beggiaota mat</th>
<th>‘next to’</th>
<th>gray mat</th>
<th>‘next to’</th>
<th>siboglinid tubeworm</th>
<th>‘next to’</th>
</tr>
</thead>
<tbody>
<tr>
<td>temperature gradients 25.9 °C m(^{-1})</td>
<td>6.9 °C m(^{-1})</td>
<td>4.7 °C m(^{-1})</td>
<td>4.8 °C m(^{-1})</td>
<td>4.0 °C m(^{-1})</td>
<td>3.5 °C m(^{-1})</td>
<td>2.0 °C m(^{-1})</td>
<td>5.9 °C m(^{-1})</td>
<td></td>
</tr>
<tr>
<td>total CH(_4)-flux &gt; 500</td>
<td>67-84</td>
<td>89</td>
<td>n.d.</td>
<td>36</td>
<td>&gt;280 (rates n.d.)</td>
<td>22</td>
<td>n.d.</td>
<td></td>
</tr>
<tr>
<td>CH(_4)-efflux 220 / 777 (n=2)</td>
<td>58 / 75 (n=2)</td>
<td>78 (n=1)</td>
<td>n.d.</td>
<td>25 (n=1)</td>
<td>251 / 315 (n=2)</td>
<td>2 (n=1)</td>
<td>n.d.</td>
<td></td>
</tr>
<tr>
<td>sedimentary MOx/AOM 8.3 ± 4.2 (n=4)</td>
<td>10</td>
<td>10.7 ± 6.5 (n=17)</td>
<td>n.d.</td>
<td>10.7 ± 9.4 (n=7)</td>
<td>n.d.</td>
<td>19.8 ± 3.3 (n=14)</td>
<td>n.d.</td>
<td></td>
</tr>
<tr>
<td>sedimentary SR 1 ± 0.2 (n=4)</td>
<td>1.2 ± 0.3 (n=4)</td>
<td>14.8 ± 6.6 (n=32)</td>
<td>n.d.</td>
<td>43.3 ± 34.5 (n=11)</td>
<td>n.d.</td>
<td>53.9 ± 74.0 (n=12)</td>
<td>n.d.</td>
<td></td>
</tr>
<tr>
<td>TOU 15 / 66 (n=2)</td>
<td>57 / 72 (n=2)</td>
<td>101 / 114 (n=2)</td>
<td>28 (n=1)</td>
<td>60 (n=1)</td>
<td>33 / 78 (n=2)</td>
<td>161 (n=1)</td>
<td>n.d.</td>
<td></td>
</tr>
</tbody>
</table>
Gray mat (III):

In 2003 and 2007, methane oxidation and sulfate reduction rates were measured in the transition zone between the warm center and the tubeworm-covered hills of the HMMV, characterized by the occurrence of patchy gray mats (Fig. 2 D, E). The highest consumption rates of sulfate and methane were found distributed over the uppermost 10 cm of the sediment (Fig. 4 E-F). Minimal and maximal AOM and SR rates were covering a range of 10 to 402, and 8 to 1918 nmol cm\(^{-3}\) d\(^{-1}\), respectively, over the years. The average depth integrated AOM and SR rates were 10.7 (± 9.4 SD; n = 7) and 43.3 (± 34.5 SD; n = 11) mmol m\(^{-2}\) d\(^{-1}\) (Tab. 2).

Siboglinid tubeworms (IV):

Consumption of methane and sulfate in sediments inhabited by siboglinid tubeworms were determined in 2001 and 2003 (Fig. 4; G-H). Down to 45 cm bsf, methane oxidation (n = 14) and sulfate reduction (n = 12) rates were very low < 5 nmol cm\(^{-3}\) d\(^{-1}\). Below this depth, around the roots of the tubeworms, AOM and SR rates increased markedly with maximal SR rate of nearly 1700 nmol ml\(^{-1}\) d\(^{-1}\) at 45 cm depth. The highest AOM rate detected in the top meter of the sediment was only 178 nmol ml\(^{-1}\) d\(^{-1}\). On average the integrated AOM and SR rates were 19.8 (± 3.3 SD; n = 14) and 53.9 (± 74.0 SD; n = 12) mmol m\(^{-2}\) d\(^{-1}\) (Tab. 2).

3.3. Fluxes of oxygen and methane at the HMMV

Benthic chamber measurements were performed in all four HMMV habitats in 2007, and in 2009 additionally in the hot center (Tab. 2). The highest temperature gradients (Tab. 1) correlated with the highest methane efflux (498 mmol m\(^{-2}\)d\(^{-1}\)) at the hot center (Tab. 2), the lowest with the siboglinid tubeworm area. Average temperature gradients and methane efflux to the hydrosphere decreased with increasing distance from the hot center, in the order of hot center > warm center > gray mats > Beggiatoa mats > siboglinid tubeworms. The total CH\(_4\) flux was highly correlated with the average T gradient (20 mmol m\(^{-2}\)d\(^{-1}\) per 1°C/m; R\(^2\) = 0.99), except in the transition zone north of the hot center towards the siboglinid tubeworm zone, where the second highest methane efflux was found next to the gray mats (Including this point the correlation was 18 mmol m\(^{-2}\)d\(^{-1}\) per 1°C/m; R\(^2\) = 0.74). Both the microbiological oxidation of methane (R\(^2\) = 0.3) and reduction of sulfate (R\(^2\) = 0.4) were negatively correlated with the temperature gradient (Tab 2). In contrast, no correlation was detected between the temperature gradient and TOU, although the lowest TOU was found in the “hot center” sediment, and the highest was associated with the siboglinid tubeworms. Furthermore, the in situ oxygen uptake exceeded by far the ex situ measured AOM/SR rates indicating that aerobic methane and sulfide oxidation at the sediment surface could be underestimated by the whole core ex situ rate measurements.
Comparing the TOU of sediment areas populated by microbial mats to their direct surrounding on a scale of decimeter to meter, a substantial difference was detected. Accordingly, the TOU of sediment densely covered by *Beggiatoa* mats (II: 108 mmol m^{-2} d^{-1}) was more than 3-times higher than that of the bare sediment next to the mat. Between the two mat communities, *Beggiatoa* mats showed higher oxygen uptake and methane emission rates than gray mats, but methane consumption was similar in both habitats.

4. Discussion

Spatially across the HMMV fluid flow rates have been found to vary strongly, creating different niches for benthic assemblages, characterized by specific transport regimes and biogeochemical settings (de Beer et al. 2006; Jerosch et al. 2007; Feseker et al. 2008). Some temporal variability of mud volcanism was indicated by changes in absolute seafloor temperature between the different years (Feseker et al. 2008), and variations in the microbathymetry of mud flows in the central and southern HMMV (Foucher et al. 2009). However, the main biogeochemical habitats appear to be rather stable in their spatial distribution and function, supporting chemosynthetic communities and associated benthic fauna (Van Gaever et al. 2006; Van Gaever et al. 2009a).

By targeted in situ quantification of oxygen, methane and sulfide fluxes during several expeditions to the HMMV we achieved a spatial budget for the four main habitats on a scale of 10-1000 m, including a first estimate of internal patterns (< 10 m scale). It is known that the fragmented habitat structure of cold seep systems and the dynamics of methane fluxes cause a high spatial heterogeneity within the targeted habitats (Sibuet and Olu 1998). Using spatially replicated measurements in four different habitats of the HMMV, we investigated 1) the link between seafloor heat gradients, methane efflux and oxygen consumption, 2) the internal relation between oxygen, methane and sulfide fluxes; and 3) the efficiency of the biological filter against methane emission to the hydrosphere.

4.1. Relation between temperature gradients, methane flux and oxygen consumption at HMMV

In-situ temperature measurements from three cruises in 2003, 2005, and 2006 suggest that the sediment temperature distribution at HMMV is controlled in large part by fluid flow (Feseker et al. 2008). Warm pore water rising through a conduit from subsurface depths transports high amounts of methane to the seafloor. Rates of vertical specific discharge are estimated to increase from less than 1 m/year at the border of the central area to more than 5 m/year in the warm center associated with temperature gradients above 4°C m^{-1} (Feseker et al. 2008). For the disturbed seafloor area with the highest temperature gradient, fluid flow has not been estimated yet. The temperature measurements reported in this paper attest to the high activity of HMMV. Here we have obtained the first in situ measurement of methane efflux for the main habitats of HMMV, which show a strong relation with
the temperature gradient (Tab 2) and hence most likely also fluid flow. Previously, it was debated whether the spatial and temporal variation of temperature gradients at HMMV are caused by fluid flow dynamics or local patterns in mud eruptions (Feseker et al. 2008). Simple analytical models were applied in order to test these hypotheses, and suggest that temperature gradients are mainly related to upward fluid flow. The spatial correlation between dissolved methane efflux and temperature gradients support this hypothesis.

Overall, the dissolved methane efflux at HMMV of up to 777 mmol m\(^{-2}\) d\(^{-1}\) in the hot center (Tab. 2) is among the highest emissions recorded previously in cold seep ecosystems (<265 mmol m\(^{-2}\) d\(^{-1}\), polychaete assemblage, Hikurangi Margin (New Zealand), (Sommer et al. accepted); 1.9-100 mmol m\(^{-2}\) d\(^{-1}\); Beggiatoa mats, Hydrate Ridge; (Torres et al. 2002; Sommer et al. 2006); <1 mmol m\(^{-2}\) d\(^{-1}\); tubeworm habitat, Capt. Artyunov MV, Sommer et al. 2009). However, the methane efflux decreased together with the temperature gradient rapidly from the hot center to the border of the warm center, within a distance of ca 200 m. At the rim of the center, Beggiatoa mats and the gray mats of the transition zone showed similar temperature gradients and methane effluxes of around 60-100 mmol m\(^{-2}\) d\(^{-1}\), matching the high end of fluxes measured in Beggiatoa mats at Hydrate Ridge (Sommer et al. 2006). Methane efflux was much lower in the outer hummocky area populated with tubeworms, where methane efflux was 2 mmol m\(^{-2}\) d\(^{-1}\). Most interestingly, even when gray mat patches occurred in the hummocky zone, tubeworms were absent, indicating that they are limited in their distribution by high fluid flow indicated by temperature gradients > 4°C m\(^{-1}\) (Tab. 2).

The areal integration of dissolved methane efflux from the entire geostructure to the hydrosphere resulted in an estimate of 37 x 10\(^3\) mol d\(^{-1}\), or 14 x 10\(^6\) mol yr\(^{-1}\) for the HMMV. Previously only total gas bubble emission has been recorded in 2003 with a rate of 8-35 x 10\(^6\) mol CH\(_4\) yr\(^{-1}\), based on measurements at three active vents of the HMMV (Sauter et al. 2006). The release of gas bubbles can vary by orders of magnitude spatially, depending on fluid flow patterns. Hence, the finding that dissolved methane efflux from mud volcanoes may be as high as gaseous efflux, is very important for future exploration and biogeochemical studies of cold seep ecosystems.

In this study, we also investigated the relationship between methane efflux and total oxygen consumption. Where methane flux limits the activity and growth of cold seep communities, one could expect a strong relationship with total oxygen consumption. However, for the HMMV it was previously proposed that due to the very high fluid flow rates of > 1m yr\(^{-1}\), the availability of electron acceptors to benthic communities may be exerting the strongest control of their distribution and activity (Niemann et al. 2006). Accordingly, methane flux and oxygen consumption rather show a negative correlation across the HMMV ecosystem, with lowest consumption rates in the hot center characterized by the highest temperature gradients (and fluid flow rates), and the highest consumption rates in the Beggiatoa mats and tubeworm fields, associated with low fluid flow rates.
4.2. Dissolved and total oxygen uptake versus methane and sulfate consumption

Oxygen is the terminal electron acceptor of aerobic and anaerobic processes in marine sediments (cf. Glud 2008). At methane seeps, one can assume that oxygen consumption can be used to quantify the total biological sink for methane because oxygen is used either directly for the aerobic methane oxidation or indirectly for re-oxidation of reduced compounds like sulfide, a product of the AOM process. The recycling of chemosynthetic biomass production derived from sulfide or methane oxidation could also be a sink for oxygen (Lichtschlag et al., in review).

Previously, total oxygen consumption at cold seep ecosystems was found to be very high, exceeding TOU of non-seep benthic communities by 1-2 orders of magnitude. For example, at Hydrate Ridge, TOU rates of 38-53 mmol m$^{-2}$ d$^{-1}$; (Sommer et al. 2006) were found, and even higher TOU were reported from seeps of the Peru and Cascadia margin (Suess et al. 1999). At the HMMV, TOU varied between the different habitats by one order of magnitude from 15-161 mmol m$^{-2}$ d$^{-1}$, but even the lowest rates measured in the center were still 5-fold higher than in sediments from the pelagic Norwegian margin (3.1 mmol m$^{-2}$d$^{-1}$; Sauter et al. 2001). The TOU gained from benthic chamber incubations considers not only total microbial oxygen consumption in the sediments, but also faunal respiration, fauna-mediated consumption induced by bioturbation and bioirrigation and small scale sediment topography (Røy et al. 2002; Wenzhöfer and Glud 2002; Glud et al. 2003).

Studies of coastal and continental slope sediment demonstrated that TOU can be up to 4-fold higher than DOU. Differences in the oxygen consumption rates obtained by the two methods were found to correlate with abundances of in- and epifauna in these habitats (Wenzhöfer and Glud 2002; Glud 2008). Only little is known about the relationship between dissolved and total oxygen consumption with methane and sulfate consumption at cold seeps. Cold seeps support high benthic biomass and activities, which were found to be related to total methane efflux, and most likely sulfide production from AOM (Sibuet and Olu 1998). Here we tested if we could find relationships between dissolved and total oxygen consumption, and with methane and sulfate consumption as the main energy-yielding processes at the HMMV. In theory, the stoichiometric relationship between TOU and AOM/SR should be 1:2, under the assumption that the system is in balance, methane is the sole electron donor, all methane is oxidized anaerobically by sulfate reduction to sulfide, and all sulfide is respired with oxygen. Deviations could result from an efflux of methane into the aerobic zone, fueling aerobic methanotrophy with a stoichiometry of oxygen to methane consumption between 1:1 and 1:2 (depending on growth efficiency, Naguib 1976), and from variations in the trophic food web. The significant efflux of methane measured in all habitats, and a large variation in benthic community composition and biomass, suggested no stoichiometric link between TOU and anaerobic oxidation of methane.

However, the main problem for such a quantitative comparison is the difference between in situ assessment of TOU and the ex situ quantification of methane and sulfate consumption, the latter likely suffering artifacts from the difference in sulfate and methane availability after retrieval of the cores. In
the following, the difference in the relationship between TOU and sulfide production from methane oxidation is discussed in detail for each habitat.

**Center (I)**

In the warm center, methane is mainly oxidized aerobically in the top few cm with oxygen in a ratio of 1:2 (Fig. 2 A; B). However, the in situ TOU (65 mmol m\(^{-2}\) d\(^{-1}\)) measured was more than 20-times higher than the ex situ methane oxidation rates and 5-fold higher compared to previous DOU measurements (de Beer et al. 2006, Lichtschlag et al., in review). Sulfide production provided only a very small amount of reducing power for TOU, indicating that AOM is largely absent due to the high upward flow rates of sulfate-free subsurface fluids (de Beer et al. 2006). Interestingly, highest methane efflux in the hot center correlated with higher heat gradients and lower TOU compared to the warm center.

In the absence of sulfide production, TOU at the HMMV center was an order of magnitude higher than sediments from the pelagic Norwegian margin, indicating that methane is the main electron donor for oxygen consumption. Previously, Lösekann et al. (2007) as well as Elvert and Niemann (2008) found abundant aerobic methane oxidizing bacteria in surface sediments of the center. Also, the observed differences between TOU and DOU could not be explained by the presence of benthic fauna, which had only very low abundances (Van Gaever et al. 2006). Hence, we suggest that the assemblage of aerobic methane-oxidizing bacteria associated with surface sediments of the mud volcano center (Lösekann et al. 2008) may cause considerable oxygen consumption that was not detected with microsensors.

**Beggiatoa mat (II):**

Below the *Beggiatoa* mats, the consumption rates of sulfate and methane in the mud volcano sediments appear stable over time (Niemann et al. 2006), both temporally as well as spatially. The measurements indicate an active AOM-SR zone close to the sediment surface mainly in the first three centimeter of the sediment (Fig. 2; C,D) with a relatively close coupling of sulfate and methane turnover. However, also in the *Beggiatoa* mat habitat, considerable differences between DOU/TOU, and TOU/sulfide production were found.

Previously measured DOU (22 mmol m\(^{-2}\) d\(^{-1}\); (de Beer et al. 2006, Lichtschlag et al., in review) were only 20% of TOU (Tab. 2) suggesting that benthic fauna could play a major role in the oxygen consumption, and/or that considerable reoxidation of methane and sulfide occurs above the seafloor, not detected by microsensors. The investigation of the faunal community structure showed that the meiofauna consists nearly exclusively of the nematode species *Halomonhystera desjuncta* (Van Gaever et al. 2006; Van Gaever et al. 2009a). This small invertebrate (~1 mm) is not associated with any microbial ecto- or endosymbionts. Isotopic data suggest rather that these nematodes are heterotrophic, feeding on the biomass of the sulfide oxidizing bacteria (Van Gaever et al. 2006; Van Gaever et al. 2009a). This nematode is highly abundant (11 x 10\(^6\) individuals m\(^{-2}\)) in the HMMV
Beggiatoa mat habitats (Van Gaever et al. 2006). Considering an average deep sea nematode respiration rate of 2.7 nmol O$_2$ d$^{-1}$ individual$^{-1}$ (Shirayama 1992), the entire nematode community could consume approximately 30 mmol O$_2$ m$^{-1}$ d$^{-1}$. These nematodes were far less abundant in the gray mat habitat (III), which also showed a lower TOU despite the higher sulfide fluxes. Hence, we conclude that the TOU in both mat habitats correlates with the abundance of invertebrates, rather than with upward sulfide flux.

However, the TOU of the Beggiatoa mat was three times higher than the total sulfide production estimated from SR, indicating that aerobic oxidation of methane in the mat also played a significant role, as in the center habitat. Previously, aerobic methane oxidizing bacteria were found associated with the Beggiatoa mat (Elvert and Niemann 2008), supporting this hypothesis. The methane flux (78 mmol m$^{-2}$d$^{-1}$) across the sediment surface would be sufficient to fuel oxygen consumption in the top few µm of the bacterial mat (de Beer et al. 2006, Lichtschlag et al. submitted).

Gray mat (III)

Patches of gray mats consisting of diverse bacteria involved in sulfur cycling occur in the transition zone between the hot center (I) or the Beggiatoa mat (II) and the siboglinid tubeworm zone (IV), as well as within the siboglinid tubeworm area (IV) (Fig. 1B). Some of the gray mats appear overgrown by Beggiatoa indicating that they could be in succession to Beggiatoa mat communities. According, the meiobenthos of the HMMV gray mats (IV) consists mostly of the nematode species H. desjuncta, but in much lower numbers than in the Beggiatoa mats. Applying the mean deep sea nematode respiration rate of 2.7 nmol O$_2$ d$^{-1}$ individual$^{-1}$ (Shirayama 1992) to their abundance (1.1 x 10$^6$ individual m$^{-2}$) within the gray mats (Van Gaever et al. 2009b), the oxygen consumption of the meiobenthos could be in the range of 3 mmol m$^{-2}$ d$^{-1}$. The TOU rates of the gray mats and their surrounding sediment was quite variable, and on average lower than in the Beggiatoa mats (II), but similar to the center sites (I). The gray mat TOU (average in and next to gray mats: 55 mmol m$^{-2}$ d$^{-1}$) was not much higher than the DOU rates (de Beer et al. 2006, Lichtschlag et al., in review) and too low to account for the reoxidation of the produced sulfide (39 mmol m$^{-2}$ d$^{-1}$) in the sediments, which, however, also showed an enormous spatial heterogeneity of the gray mat habitat (Fig. 2 E, F).

Siboglinid tubeworms (IV)

The by far largest area of the HMMV is inhabited by siboglinid tubeworms (IV) (Fig. 1D), extending their roots deep into the sediments to take up hydrogen sulfide from the AOM zone above methane hydrates (de Beer et al. 2006; Niemann et al. 2006; Lösekann et al. 2008). The sulfide is oxidized by their thiotrophic endosymbionts (Naganuma et al. 1999; Naganuma et al. 2005; Niemann et al. 2006; Lösekann et al. 2008), and the worms finally release sulfate back into the seafloor (Dattagupta et al. 2006). Investigations in the Gulf of Mexico showed that the tubeworms have the
potential to excrete 70-90% of the recycled sulfate again to the surrounding sediment and therefore enhance further the sulfide production (Dattagupta et al. 2008). Hence, we found the highest areal AOM and SR rates as well as TOU in the tubeworm habitat (Tab 2).

The main energy source for the worm’s endosymbionts is sulfide and it can be assumed that the consumed oxygen (161 mmol m\(^{-2}\) d\(^{-1}\)) is mainly used for sulfide oxidation. This would result in SR and thus AOM rate of approximately 80 mmol m\(^{-2}\) d\(^{-1}\), which is higher than the range of the measured ex situ methane and sulfate turnover rate (Tab.2). Investigations in tubeworm inhabited sites at the Captain Arutyunov mud volcano (Gulf of Cadiz) indicate that aerobic methane oxidation by free-living bacteria is a relevant process due to bioirrigation of the sediments by tubeworms (Sommer et al. 2009).

4.3. The efficiency of the biological filter: Methane efflux versus methane consumption

Comparing methane fluxes across all habitats (Tab. 2 and 3), it can be concluded that the center (I) is the main emission site of dissolved methane at the HMMV. This is coherent with high bottom water methane concentrations of more than 10\(^4\) nM, which were reported before from the central HMMV area (Sauter et al. 2006). Previously, the efficiency of the microbial filter against methane at HMMV was estimated by comparing methane consumption rates with gaseous methane release, and a efficiency in the range of 1-24% was suggested for the different habitats (Niemann et al. 2006). Here we have calculated habitat specific efficiencies in the removal of methane based on the ratio between aerobic or anaerobic microbial methane consumption and the total dissolved methane fluxes (Tab 2).

In the center (I) of the HMMV, over 14 x 10\(^3\) mol methane per day are released (Tab. 3) and less than 11% of the total methane flux is consumed, mostly by aerobic oxidation of methane. This low ratio is caused by shallow penetration depth of oxygen and sulfate that restricts aerobic and anaerobic methane oxidation to a small horizon at the sediment surface (de Beer et al. 2006; Niemann et al. 2006; Lösekann et al. 2007). In the Beggiatoa mats (II), despite lower fluid flow velocities, the diffusion of electron acceptors into the sediment is still limited, resulting also in low turnover of 12% of the total dissolved flux, mostly by AOM (Tab. 3). In the gray mats (III), fluid flow is nearly absent, increasing the penetration of sulfate. As result, more of the methane flux (30%) is consumed in the seafloor compared to the center (I) or the Beggiatoa mats (II). In the siboglinid tubeworm (IV) habitat, the low methane emission rate (Tab. 2) suggests low upward fluid flow velocities. However, with 90% turnover, the tubeworm communities are also the most efficient filter against methane due to the bioirrigation of the sediments by the tubeworms (Sommer et al. 2009).

For the entire geostructure, 22 - 55% of the total dissolved methane flux was removed in the seafloor based on our measurements of methane oxidation or total sulfide production (sulfate reduction) rates (Tab. 3). Taking also into account the gaseous methane release, which was previously found to be in the same range (Niemann et al. 2006; Sauter et al. 2006), the microbial filter for methane is only 10-25%. However, assuming that oxygen is the ultimate sink for all methane derived reduced energy, including the previously overlooked contribution of aerobic oxidation of methane in
the mats and the recycling of chemosynthetic biomass in the food web (Van Gaever et al. 2009a, Lichtschlag et al., in review), the benthic filter would account for almost all of the total methane flux (dissolved + gaseous).

5. Conclusions

By targeted in situ quantification of oxygen, methane and sulfide fluxes during several expeditions to the HMMV we achieved a spatial budget for the four main habitats on a scale of 10-1000 m. The dissolved methane efflux at HMMV of up to 777 mmol m\(^{-2}\) d\(^{-1}\) (mud volcano center) is among the highest emissions recorded previously in cold seep habitats. For the entire mud volcano, the total dissolved methane efflux sums up to 14 x 10\(^6\) mol yr\(^{-1}\), compared to a gaseous emission of 8-35 x 10\(^6\) mol yr\(^{-1}\), as previously estimated (Sauter et al. 2006). The four main habitats of the HMMV were correlated with different temperature gradients ranging from 46 to 1°C m\(^{-1}\). The temperature gradients were related with the efflux of dissolved methane to the hydrosphere, declining from the northern center of the HMMV towards the outer rim populated by thiotrophic tubeworms. The decrease in methane emission was associated with an increase in oxygen consumption, reflecting the increasing efficiency of chemosynthetic organism to consume methane and sulfide at decreasing upward fluid flow conditions. Within and between the main habitats we recorded differences in methane, sulfide and oxygen fluxes of an order of magnitude, indicating the relevance of targeted spatial sampling in cold seep ecosystems.
At the high fluid flow rates dominating the HMMV, the microbial efficiency in consuming methane in the seabed was strongly limited, accounting for $< 22\%$ of the dissolved methane emission. However, previously overlooked surface-near processes, including the aerobic oxidation of methane in the center and Beggiatoa mats, as well as meiofauna and tubeworm respiration appear to contribute considerably to the methane filter as recorded by in situ total oxygen consumption measurements with benthic chambers.
References:


### Appendix:
Tab 4: Overview of all measurements and PC sampling stations (MOx = methane oxidation; SR = Sulfate Reduction) investigated in this study. The data are found in the PANGAEA database.

<table>
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<th>year</th>
<th>cruise</th>
<th>Habitat</th>
<th>measurements</th>
<th>PANGAEA database event labels</th>
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<td>&quot;next to Beggiatoa mat&quot;</td>
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</table>
Chapter 2.3

Biogeochemical processes associated with mud volcanism on the Nile Deep Sea Fan - The Amon Mud Volcano

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Abstract

The highly active Amon Mud Volcano (MV), located at 1118 m water depth between the Central and Eastern province of the Nile Deep Sea Fan, was investigated during the BIONIL expedition with RV Meteor (M70-2) in autumn 2006. The Amon MV can be subdivided into four habitats: a central dome (I); the surrounding hummocky area with patches of bacterial mats (II); a wide slope area covered by biogenic mounds (III); and a lateral mud flow at the flank of the Amon MV (IV). Here, we investigated differences in the biogeochemistry of these four habitats and their relation to fluid flow regimes. Total and diffusive oxygen uptake were quantified in situ with a ROV-operated benthic chamber and a microsensor-profiler, respectively. Microbial sulfate and methane consumption were measured by radiotracer incubations. Pore-water chemistry was investigated to gain an understanding about the flow patterns and composition of subsurface fluids. Our results show that the concentric structure, morphology and biogeochemistry of these habitats are mainly caused by spatial variations in gas and fluid flow on scales of meters to hundreds of meters. The central dome of the Amon MV was characterized by high temperature gradients and gas oversaturation, but low rates of microbial methane and sulfate consumption were detected despite a high availability of methane and sulfate. The biogeochemical hot spots of Amon MV were the patchy bacterial mats surrounding the central dome, with high rates of hydrocarbon oxidation, sulfide production and oxygen consumption (20-55 mmol m$^{-2}$ d$^{-1}$). Another benthic hot spot habitat was found at the southwestern flank of the Amon MV: a lateral outflow of sulfidic, briny muds partly covered by thiotrophic bacterial mats and siboglinid tubeworms. Here, the high oxygen uptake was fueled by sulfide transport rather than by microbial production. Compared to other mud volcanoes, the Amon MV presents a unique case as here despite a high availability of sulfate as electron acceptor, microbial methane consumption is only of low efficiency, causing a high methane emission to the hydrosphere.
1. **Introduction**

Submarine mud volcanoes form when gravitational or tectonical forces induce vigorous mud discharge accompanied by fluid and gas emissions from deep subsurface strata (Milkov 2000; Kopf 2002). When degassing continues after the eruption event, cold seep habitats form on the surface of the mud volcano, supporting diverse chemosynthetic communities (Sibuet and Olu 1998; Werne et al. 2004; Niemann et al. 2006b). In cold seep ecosystems, the anaerobic oxidation of methane (AOM) with sulfate is the key biogeochemical process (Boetius et al. 2000). The products of AOM, sulfide and carbonate, are relevant in shaping the cold seep habitats for different reasons. Sulfide is used as energy source by thiotrophic bacteria that are either free-living or symbiotically associated with invertebrates (Jørgensen and Boetius 2007). High sulfide fluxes attract nitrate-reducing giant sulfide oxidizers like *Beggiatoa* and *Thiomargarita*, which often form extensive bacterial mats above gassy sediments (de Beer et al. 2006, Lichtschlag et al., in review). When excess carbonate derived from AOM precipitates in the surface sediments of cold seeps, it attracts hard-bottom fauna such as some chemoautotrophic tubeworms, bivalves and diverse filter feeder communities (Jørgensen and Boetius 2007).

The active ridges and passive continental margins of the Eastern Mediterranean Sea are known to host numerous cold seep systems such as mud volcanoes, pockmarks, gas vents, and brine seeps (Loncke et al. 2004; Huguen et al. 2009). In recent years, several highly active cold seeps have been discovered on the Nile Deep Sea Fan (NDSF) (Loncke et al. 2004; Mascle et al. 2006; Dupré et al. 2007). The NDSF is one of the world’s largest deep-sea fans (90,000 km²). It was formed in the late Miocene and comprises large subsurface gas and oil reservoirs (Mascle et al. 2006 and references therein). The mud volcanoes of this region are often connected to salt tectonics and gravitational forces causing movements of the thick sedimentary package. One of them is the Amon mud volcano (MV) that is located on the border between the Central and Eastern province of the NDSF. This 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. Previous studies of the Amon MV were based on bathymetric and visual investigations (Mascle et al. 2001; Dupré et al. 2007; Dupré et al. 2008), and first analyses of hydrocarbon emission and turnover at its center (Mastalerz et al. 2007; Mastalerz et al. 2009; Omorogie et al. 2009). The aim of this study was to quantify the key biogeochemical processes in all main habitats of the Amon MV, and to investigate the relation of their structure and activity to mud volcanism.
2. **Materials & Methods**

2.1. **Sampling**

The Amon MV (E 31°42.6’; N 32°22.2’) is located at a water depth of about 1118 m and has a diameter of 2 km (Fig. 1). Its summit has a height of 90 m above the surrounding seafloor. The slope decreases gently from the central mound to the outer edges (Dupré et al. 2007). Sampling and in situ measurements were performed at locations indicated in Fig. 1 during the M70-2 BIONIL cruise with the RV Meteor from 21.10. - 23.11.2006.

The precise positioning and operation of the in situ tools as well as the targeted sampling were achieved with the remotely operated vehicle (ROV) “Quest 4000” (Marum, Bremen, Germany). All performed measurements, sample locations and labels are summarized in Table 1 and are available in the PANGAEA database (http://www.pangaea.de).

At all habitats, the upper 30 cm of the sediment were sampled with push cores (PCs) by the ROV. On board of the ship, the PCs were immediately transferred to a tempered room that was cooled to in situ bottom water temperature of 14°C. Samples from the center of Amon MV contained a lot of gas and their retrieval caused disturbance of the sediment layers by gas ebullition. To reestablish the geochemical gradients the cores were stored at in situ temperature until the mud settled and the bacterial mats recovered.

In addition to ROV-operated push core sampling, sediments were also sampled by gravity-coring down to 380 cm below seafloor (bsf). The device was positioned with the help of the position system POSIDONIA (IXSEA SAS; Marly-le-Roi, France). After recovery the gravity cores (GCs) were immediately processed.

![Fig. 1: Positions of all in situ instrument deployments as well as push core and gravity core sampling at the Amon MV. 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).](image-url)
Table 1: Overview of all sampling stations and measurements (PW = pore-water analyses including ex situ methane concentration measurements; AOM = anaerobic oxidation of methane; SR = sulfate reduction; AODC = Acridine Orange Direct Counts) of this study and their respective PANGAEA event label. All data of this study were submitted to the PANGAEA database.

<table>
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<tr>
<th>habitat</th>
<th>measurements</th>
<th>PANGAEA database event labels</th>
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<td>AODC</td>
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<td>SR</td>
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<td>AODC</td>
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<td>M70/2b_825_CALMAR-1</td>
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2.2. Turnover rates of methane and sulfate

Ex situ turnover rates of methane and sulfate were determined according to Treude et al. (2003) and references therein. PC sediments were vertically subsampled with small subcore liners (diameter = 2.8 cm). For each method and sampling station 3-4 replicate subcores were used. On board, either 25 µl 14CH4 (dissolved in water, 2.5 kBq) or 5-10 µL carrier-free 35SO42- (dissolved in water, 50 kBq) were injected in 1 cm intervals through silicon-sealed holes into the subcores (whole core injection method, Jørgensen 1978). The sediments were incubated in the dark for 12 hours at in situ temperature. Afterwards the subcores were 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 sulfate reduction rate measurements were fixed in 20 mL zinc acetate solution (20%, w/v).

The turnover rates of methane and sulfate were determined in the home laboratory by scintillation counting as previously described (Treude et al. 2003; Kallmeyer et al. 2004). The substrate concentrations were measured by gas chromatography (5890A, Hewlett Packard) and anion exchange chromatography (see pore-water section) for methane and sulfate, respectively.

Methane oxidation rates were calculated according to the following equation:

\[
\text{methane oxidation} = \frac{{14} \text{CO}_2}{{14} \text{CH}_4 + {14} \text{CO}_2} \times \frac{\text{CH}_4}{V \times t}
\]

where CH4 is the methane concentration, 14CO2 is the radioactivity of the produced carbon dioxide, 14CH4 is the radioactivity of the labeled methane, t is the incubation time and V is the volume of the sample. Sulfate reduction (SR) rates were calculated with the following equation:

\[
\text{SR} = \frac{\text{TRI}^{35}S}{({35} \text{SO}_4^{2-} + \text{TRI}^{35}S)} \times \frac{\text{SO}_4^{2-}}{V \times t}
\]

where SO42- is the sulfate concentration, TRI35S is the activity of the reduced sulfur compounds and 35SO42- is the radioactive sulfate.

2.3. Geochemistry

2.3.1 Methane concentration

Methane concentrations were determined by slicing sediment cores in 2-3 cm intervals and transferring 3 mL of each sediment horizon quickly 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 the sediment was extracted with Rhizons (Rhizon CSS: length 5 cm, pore diameter 0.1 µm; Rhizosphere Research Products, Wageningen, Netherlands) with a resolution of 1 cm. For each sampling station, pore water was extracted from two replicate PCs. After extraction the pore-water was immediately fixed in 5% zinc acetate for sulfate, chloride and sulfide analyses. Furthermore, samples for measuring dissolved inorganic carbon (DIC) were preserved by adding HgCl₂ and stored without headspace in glass vials. All samples were stored at 4°C until analyses in the home laboratory.

The extraction of pore water from GC sediments was either performed by centrifugation according to de Lange (1992) or also with Rhizons. The extracted pore water was then preserved and measured like the PC pore-water samples. Only the pore-water salinity was determined with a conventional refractometer on board.

### 2.4. Pore-water analyses

After filtration and dilution, the sulfate and chloride concentrations were measured by non-suppressed anion exchange chromatography (Waters IC-Pak anion exchange column, waters 430 conductivity detector).

The total sulfide concentrations (H₂S + HS⁻ + S²⁻) 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).

### 2.5. Acridine orange direct counts (AODC)

To determine the total number of single cells with Acridine Orange (AO), sediment sections were preserved in artificial seawater with 2% formaldehyde. The AO-staining was performed in the home laboratory based on previously described methods (Meyer-Reil 1983; Boetius and Lochte 1996). For each sample, replicate filters and at least 30 grids per filter were randomly counted. The AO direct counts included only single cells.

### 2.6. In situ measurements

#### 2.6.1 Benthic chamber

The total benthic oxygen uptake (TOU) was determined with a benthic chamber module operated by the ROV “Quest 4000” to ensure targeted measurements at selected spots. A valve in the chamber lid ensured the release of enclosed water during its placement. The centrally stirred chamber had a radius of 9.5 cm and hence enclosed an seafloor area of 284 cm² together with 10-20 cm (equivalent to 4-6 L) of overlying bottom water. The exact water height inside the chamber was determined by visual inspection with the ROV camera system. The oxygen concentration in the enclosed water volume was continuously recorded with two Clark-type oxygen mini-electrodes that were mounted to the chamber lid (Glud et al. 2009). The sensors were calibrated against bottom water samples and a zero reading recorded at in situ temperature on board. During the chamber deployment at the sulfur band, water samples with 50 mL syringes were taken in preprogrammed time intervals.
The samples were preserved at the seafloor in 5 mL 5% ZnAc solution per syringe, which was added to the syringes before the deployment.

TOU (mmol m\(^{-2}\) d\(^{-1}\)) was calculated from the linear regression of the oxygen concentration versus time (Wenzhöfer and Glud 2002):

\[
3. \quad \text{TOU} = \frac{dC}{dt} \times \frac{V_{\text{chamber}}}{A_{\text{chamber}}}
\]

where \(dC/dt\) (µM h\(^{-1}\)) is the change of the concentration over the incubation time, \(V_{\text{chamber}}\) (cm\(^3\)) is the volume of the enclosed water, and \(A_{\text{chamber}}\) (cm\(^2\)) is the area of the sediment enclosed by the chamber.

### 2.6.2 Microprofiler

The deep-sea microprofiler module was operated by the ROV to obtain high-resolution pore-water profiles at visually targeted sites. During the autonomous measurement program, the microsensors mounted to the microprofiler were driven vertically in 250 µm steps to 10 cm sediment depth (de Beer et al. 2006). At each deployment, two pH, two sulfide and two oxygen microsensors (Revsbech et al. 1983; Revsbech and Ward 1983; Jeroschewski et al. 1996) and one macro-temperature sensor (Pt100, UST Umweltsensorentechnik GmbH) were used. All sensors were calibrated on board according to previously published methods (Wenzhöfer et al. 2000; de Beer et al. 2006).

Fluxes of the different constituents were calculated by using Fick’s first law of diffusion. The linear oxygen gradient in the diffusive boundary layer was used to calculate oxygen fluxes with the specific diffusion coefficient for oxygen (\(D = 1.6 \times 10^{-9}\) m\(^2\) s\(^{-1}\)) and the following equation (Jørgensen and Revsbech 1985):

\[
4. \quad J = D \frac{dC}{dz}
\]

where \(J\) is the diffusive flux (mmol m\(^{-2}\) d\(^{-1}\)), \(D\) is the diffusion coefficient in water (m\(^2\) d\(^{-1}\)) corrected for temperature and salinity (Li and Gregory 1974) and \(dC/dz\) is the concentration gradient (dC (mmol m\(^{-3}\)); dz (m)). To calculate fluxes in the sediment, the diffusion coefficient (\(D\)) had to be corrected for porosity (\(\phi\)) of the sediment (Iversen and Jørgensen 1993). The following equation was used:

\[
5. \quad D_{\text{Sed}} = \frac{D}{1 + 3(1 - \phi)}
\]

Interfacial fluxes were calculated from the highest concentration gradients in the profile with the specific diffusion coefficient for sulfide (\(D_{\text{Sediment}} = 6.63 \times 10^{-10}\) m\(^2\) s\(^{-1}\)) and the following equation (Iversen and Jørgensen 1993):

\[
6. \quad J = \phi D_{\text{Sed}} \frac{dC}{dz}
\]
2.6.3 Planar optode

The two-dimensional oxygen distribution in the sediment was measured with an autonomous in situ planar optode module (Glud et al. 2001; Glud et al. 2005) in a bacterial mat and biogenic mound habitat. The planar optode was produced according to the method described by Precht and coworkers (2004). The oxygen-sensitive layer was based on a platinum(II)mesotetra-(pentafluorophenyl)porphyrin (Frontier Scientific, Inc.) embedded in polystyrene and immobilized on a transparent polyester support foil by a knife-coating procedure. The sensing layer had a thickness of 15-20 µm.

The planar optode foil was placed in front of the inverse periscope of the planar optode module (detailed description see Glud et al. 2001; Wenzhöfer and Glud 2004; Glud et al. 2005). The oxygen-quenched fluorescence emitted by the fluorochrome was recorded using a modulated digital charge coupled device (CCD) camera (Sensi Cam, PCO Computer Optics). The planar optode was calibrated on board with oxygen saturated and anoxic seawater at in situ temperature. The calibrated oxygen image covered an area of 6 x 8 cm with a spatial pixel resolution of 128 µm (CCD camera chip size, 1280 x 1024 pixel, two pixels horizontal and vertical binning). Oxygen images were calculated based on a modified version of the Stern-Vollmer equation (Holst et al. 1998). The image analysis and the calculations of lifetime and oxygen images were done with homemade software using the program MATLAB 6.5 (MatchWorks Inc.)
3. Results

Based on visual observations with ROV “QUEST4000”, the total surface area of the dome-shaped Amon MV (ca 3.2 km², Fig. 1) can be subdivided into the following concentric habitats (Fig. 2): (I) The central dome area (12,000 m²) is characterized by gas expulsion and mud breccia extrusions. We observed the release of gas bubbles upon ROV touch down and sampling. Blocks of grayish mud with sharp edges were separated by cracks and troughs of several meters in width and height indicating recent processes of seafloor displacement. One of the stations sampled (Ia) was at the northern boundary of the central dome. (II) The central dome is surrounded by a hummocky area of ripples and troughs of 2-3 m height with a smooth sediment surface, covered by patches of bacterial mats and black sediment spots of decimeter to a few meter diameters. These sediments were also highly gassy. (III) The largest part of Amon’s surface is littered with large biogenic mounds (20-50 cm in height and width). Here, a thin layer of pelagic, oxidized sediment covers grayish mud. (IV) The fourth habitat, a lateral outflow of briny mud, called “sulfur band”, was detected at the flank of the Amon MV (southwestern area). Further, the outer area of the Amon MV showed patchy distributions of carbonate crusts, which were not investigated in this study.

Fig. 2: Four different habitats were visually distinguished at the Amon MV. (A) At the central dome (I) degassing of the seafloor creates bathymetric anomalies of several meters in height. (B) Around the central dome, the seafloor becomes smoother and bacterial mats (II) are found covering the hummocks. (C) Biogenic mounds of up to half a meter in height (III) comprise the largest habitat of the Amon MV. (D) A unique feature of the AMV is the lateral outflow of sulfidic muds at the outer rim (“sulfur band”). The two green ROV laser points (C) mark a distance of 20 cm.
3.1. Methane and sulfate turnover

The consumption of methane and sulfate was investigated in all four habitats (Fig. 3). In the gas-oversaturated sediments of the central dome of Amon (I), the SR values varied considerably over the investigated sediment horizon with subsurface maxima between 3 and 15 cm reaching up to 156 nmol mL\(^{-1}\) d\(^{-1}\) (Fig. 3 A). AOM was lower than SR with a maximum of 28 nmol mL\(^{-1}\) d\(^{-1}\) (Fig. 3 B). AOM took mainly place in the top 4 cm of the sediment, except for one replicate, where methane oxidation was found over a wide depth range between 4 and 10 cm below seafloor (bsf). Overall, the central dome of Amon showed integrated sulfate and methane turnover rates of 5.1 mmol m\(^{-2}\) d\(^{-1}\) (n = 4; 1.6 standard deviation, SD) and 0.6 mmol m\(^{-2}\) d\(^{-1}\) (n = 4, SD = 0.6), respectively, in the top 15 cm of sediment.

At the northern edge of the central dome (Ia) we could not observe any indications of recent gas ebullition or bacterial mats at the seafloor. The sulfate turnover was two orders of magnitude lower (maximum 6 nmol mL\(^{-1}\) d\(^{-1}\)) than in gassy sediments of the central dome (data not shown). The 0-15 cm depth integrated SR rate was only 0.2 mmol m\(^{-2}\) d\(^{-1}\) (n = 3; SD = 0.01).

In the small zone surrounding the central dome where patchy bacterial mats were found (II), very high SR rates of up to 3590 nmol mL\(^{-1}\) d\(^{-1}\) were measured below the mats (Fig. 3 D). One of the investigated sampling stations (ROV 125-825; 106-3590 nmol mL\(^{-1}\) d\(^{-1}\)) had up to one order of magnitude higher sulfate turnover rates than the other (ROV 113-760; 32-654 nmol mL\(^{-1}\) d\(^{-1}\)), indicating a high heterogeneity on small spatial scales. The averaged depth integrated (0-15 cm) SR rates were 96.8 (n = 2; SD = 58.2) and 25.4 (n = 3; SD = 7.0) mmol m\(^{-2}\) d\(^{-1}\) for the two sites sampled (ROV 125-825 and ROV 113-760), respectively. All measurements indicated that the highest sulfate turnover occurred in the upper 5 cm and that SR decreased with increasing depth. The AOM rates (Fig. 3 E reached 478 nmol mL\(^{-1}\) d\(^{-1}\), and the maxima were also mostly found in the top 5 cm. The averaged depth integrated AOM rate of the 0-15 cm sediment horizon was 17.2 mmol m\(^{-2}\) d\(^{-1}\) below the bacterial mats (n = 6; SD = 14.1), i.e. about 20-70% of SR.

At the Amon biogenic mounds (III), due to the rather deep oxygen penetration in the surface sediments, SR and AOM rates (Fig. 3 G, H) were mostly at the detection limit in the top 15 cm. Hence, the averaged depth (0-15 cm) integrated rates of SR and AOM were low with 0.2 (n = 4; SD = 0.2) and 0.1 mmol m\(^{-2}\) d\(^{-1}\) (n = 4; SD = 0.1), respectively.

At the sulfur band (IV), briny muds covered by a whitish microbial mat were sampled for turnover rate measurements (Fig. 3 J, K). At this site, sulfate and methane turnover were restricted to the upper 3 cm of blackish sediment. The highest SR rates were found directly below the sediment surface (maximum 183 nmol mL\(^{-1}\) d\(^{-1}\)), but decreased sharply with depth. The averaged depth integrated SR rate of the 0-15 cm depth interval was 0.5 mmol m\(^{-2}\) d\(^{-1}\) (n = 3; SD = 0.2). The integrated AOM rates were only 0.1 mmol m\(^{-2}\) d\(^{-1}\) (n = 3; SD = 0.01).
Fig. 3: Biogeochemistry of the different habitats at Amon MV. From left to right: sulfate reduction, anaerobic oxidation of methane, and cell counts at (I) the central dome (A, B, C); (II) bacterial mats (D, E, F); (III) biogenic mounds (G, H, I) and (IV) sulfur band (J, K, L). Replicates of one station are represented with the same symbol. Note: The insets in graph A, B, G, H, J, K show the SR and AOM rates on a different (x-axis) scale.
3.2. Geochemistry (methane concentration and pore-water data)

The sediments from the central dome (I) were gas saturated at a water depth of 1120 m and at 14°C in situ temperature, equivalent to a concentration of about 80 mM at the seafloor. After retrieval, methane concentrations of only about 2 mM were measured throughout the entire cores (data not shown), due to depressurization and subsequent degassing. The sulfide concentration profiles (Fig. 4) indicated sulfide production in the top 15 cm of sediment. In agreement with the rate measurements, highest sulfide concentrations were reached beneath the bacterial mats (II), but up to 1.3 mM sulfide was also present in the core from the central dome. The sulfate concentration showed an increase from 30 mM of up to 40 mM in the top 14 cm (Fig. 4), indicating an upflow of sulfate-rich fluids. The DIC content of the pore water (0-14 cm bsf) increased also with increasing depth up to 18 mM (Fig. 4). Additionally, pore-water concentrations below 20 cm sediment depth were obtained from gravity cores at the Amon center (Fig. 5). These analyses confirmed a transport of sulfate- and DIC-rich subsurface fluids to the surface of the mud volcano, with a sulfate concentration of up to 96 mM (3-times seawater concentration) at about 300 cm bsf. In contrast, chloride concentrations decreased from close to seawater values at the sediment surface to less than 130 mM at 250 cm bsf (Fig. 5).
Fig. 4: Pore-water data of sulfate, chloride, sulfide and DIC from the four main Amon MV habitats: 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.

At the northern edge of the central dome (Ia), ex situ methane concentrations in the sediment were low (maximum 0.007 mM) and increased only slightly with depth (data not shown). The sulfate (averaged 34 mM) and chloride (averaged 547 mM) concentrations were constant over the analyzed depth interval (Fig. 4 A, B). No sulfide was detected down to 14 cm bsf and the DIC concentration of only 3 mM did also not change with depth.

Below the bacterial mats (II) sulfate was consumed down to about 13 mM in the top 15 cm (Fig. 4). The chloride concentration in the pore water did not change (~ 600 mM) over the analyzed depth interval.
sediment depth interval (0-20 cm). The sulfide concentrations increased with depth and reached a maximum of 5 mM at around 10 cm bsf indicating sulfate turnover at these sediment horizons. Similar to sulfide, the DIC concentration increased almost linearly with depth to more than 20 mM without reaching a maximum.

In the sediment from the biogenic mounds (III) no methane was detected. Furthermore, the concentrations of the analyzed pore-water constituents did not change with depth. Sulfate (32 mM), chloride (620 mM) and DIC (3 mM) had values close to seawater concentrations. No sulfide was detected over the entire investigated sediment horizon (Fig. 4).

At the sulfur band (IV), in an area covered by a microbial mat, the methane concentration was slightly elevated at the surface but methane was not detected deeper than 3 cm bsf. Furthermore, no decrease in sulfate or increase in DIC (Fig. 4) in the pore water was observed in the sediment layers underlying the top briny mud.

Fig. 5: Sulfate and chloride concentrations as well as salinity from the central dome of the Amon MV. Closed symbols are GC 804, open symbols are GC 791.

3.3. Cell counts

At the Amon central dome (I), total cell numbers in the top 15 cm were relatively low with a values between 0.05 - 0.3 x 10^9 cells mL^-1 sediment and showed a decrease with increasing depth (Fig. 3 C). In the sediment below the bacterial mats (II), the abundance of microbial cells (Fig. 3 F) was elevated compared to the central dome sediments. The two examined bacterial mat stations showed the same trend of higher cells numbers at the sediment surface (2 x 10^9 cells mL^-1 sediment) and a sharp decrease in the top 5 cm. Below 5 cm sediment depth, the numbers of cells remained nearly constant (approximately 0.7 x 10^9 cells mL^-1). The total number of cells in the sediments of the biogenic mounds (III) were about 0.5 x 10^9 cells mL^-1 sediment and decreased only slightly with depth in the investigated sediment horizons (Fig. 3 I). The cell numbers at the sediment surface were even below the cell abundance of a reference core taken outside of the Amon MV at a similar water depth (~ 1 x 10^9 cells mL^-1 sediment). At the sulfur band (IV), covered by a bacterial mat, the number of single
cells at the surface \((3 \times 10^9 \text{ cells mL}^{-1} \text{ sediment})\) were as high as at the bacterial mat of the Amon center and decreased with depth (Fig. 3 L).

### 3.4. In situ measurements

#### 3.4.1 Benthic chamber

At the Amon MV the benthic chamber was deployed at the bacterial mat (II), the biogenic mounds (III) and the sulfur band (IV) (Tab. 2). The total oxygen uptake (TOU) was one order of magnitude higher at the bacterial mat (II) site \((50 \text{ mmol m}^{-2} \text{ d}^{-1})\) than at the biogenic mounds (III). At the sulfur band (IV), the TOU ranged from \(35 - 71 \text{ mmol m}^{-2} \text{ d}^{-1}\) indicating similar high benthic oxygen consumption rates as at the bacterial mats (II). With the chamber, we recorded sulfide fluxes of \(2.5 - 13 \text{ mmol m}^{-2} \text{ d}^{-1}\) escaping from the sulfur band sediment, which were in the same range as the fluxes determined with the microprofiler (Tab. 2).

#### 3.4.2 Microprofiler

The microprofiler was placed at the Amon central dome (I) and at a bacterial mat on the hummocks surrounding the center (II) (Fig. 6). Furthermore, in situ microsensor measurements were performed at the sulfur band (IV).

At the central dome (I), oxygen penetrated \(3 \text{ mm}\) into the sediment and the diffusive oxygen uptake (DOU) was \(10 \text{ mmol m}^{-2} \text{ d}^{-1}\) (Tab. 2). No sulfide was detected in the top \(2 \text{ cm}\) and pH showed a continuous decrease from \(8.3\) to \(7.6\) in the investigated sediment depth interval. The temperature increased from \(13.50^\circ\text{C}\) to \(13.75^\circ\text{C}\) in the upper \(2 \text{ cm}\) with a temperature gradient of \(10^\circ\text{C m}^{-1}\) (Fig. 6A).

![Fig. 6: Microsensor measurements](image)

Fig. 6: Microsensor measurements from the (A) central dome (I), (B) the bacterial mat (II) site and (C) averaged microsensor profiles from three deployments at the sulfur band (IV). The profiles show oxygen \((\text{O}_2)\) (spheres), total sulfide \((\text{H}_2\text{S})\) (gray line) concentrations, pH (triangles) and temperature (T, dashed line).
At the bacterial mat (II), oxygen did not diffuse into the sediment and was consumed within the mat at the sediment surface (Fig. 6B). In comparison to the central dome (I), the DOU was enhanced (42 mmol m$^{-2}$ d$^{-1}$) and the sulfide profiles indicated a substantial production of sulfide below the bacterial mats. The sulfide concentration profile showed a small peak at 0.5 cm. Below this depth sulfide concentration further increased with increasing depth without reaching a maximum. Sulfide reaching the sediment surface was entirely consumed within the bacterial mat layer where oxygen and sulfide overlapped. The pH decreased between the zone of sulfide oxidation and production but remained at a higher level than in the central dome sediments. The temperature gradient (2.1°C m$^{-1}$) indicates a lower transport of fluid and heat at the bacterial mat (II) site than in the central dome (I).

At the sulfur band (IV), the microprofiler was deployed three times on the bacterial mat covering the reduced mud flow (Fig. 6C). The DOU was in the range of 23 to 46 mmol m$^{-2}$ d$^{-1}$. The sulfide concentrations in the sulfur band sediments were lower (maximum 1.7 mM) compared to the bacterial mat (II) site in the center of the Amon MV.

Table 2: All numbers are given in mmol m$^{-2}$ d$^{-1}$. Total oxygen uptake (TOU) and the total sulfide flux were measured with the benthic chamber. The dissolve oxygen uptake (DOU), and upward diffusive sulfide flux were calculated based on in situ microsensor measurements. The averaged sulfate reduction (SR) and anaerobic methane oxidation rates (AOM) were integrated over the first 15 cm. (n.d.= not determined; n = numbers of replicates if available); * unpublished data Wenzhöfer et al.

<table>
<thead>
<tr>
<th></th>
<th>central dome (I)</th>
<th>bacterial mat (II)</th>
<th>biogenic mounds (III)</th>
<th>sulfur band (IV)</th>
<th>reference</th>
</tr>
</thead>
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<tr>
<td>TOU</td>
<td>n.d.</td>
<td>50 (n = 1)</td>
<td>5 (n = 1)</td>
<td>35-71 (n = 2)</td>
<td>n.d</td>
</tr>
<tr>
<td>DOU</td>
<td>10 (n = 1)</td>
<td>40 / 44 (n = 2)</td>
<td>n.d.</td>
<td>23 - 46 (n = 4)</td>
<td>1.3* (n =1)</td>
</tr>
<tr>
<td>J (upward total sulfide-flux)</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>3-13 (n = 2)</td>
<td>n.d</td>
</tr>
<tr>
<td>J (upward diffusive sulfide-flux)</td>
<td>0</td>
<td>40 (n = 1)</td>
<td>n.d.</td>
<td>1-23 (n=4) / 3-13 (n=2)</td>
<td>0* (n = 1)</td>
</tr>
<tr>
<td>SR</td>
<td>5.1± 1.6 (n = 4)</td>
<td>54.1± 49 (n = 5)</td>
<td>0.2 ± 0.2 (n = 4)</td>
<td>0.5 ± 0.2 (n = 3)</td>
<td>0 (n = 3)</td>
</tr>
<tr>
<td>AOM</td>
<td>0.6 ± 6.6 (n = 4)</td>
<td>17.2 ± 14.1 (n = 6)</td>
<td>0.1± 0.1 (n = 4)</td>
<td>0.1± 0.01 (n = 3)</td>
<td>0 (n = 3)</td>
</tr>
</tbody>
</table>

3.4.3 Planar optode

The two dimensional oxygen concentration measurement at the bacterial mat (II) site (Fig. 7) showed a homogenous distribution of oxygen in the bacterial mat and the complete consumption of oxygen within the mat. At the biogenic mounds (III) oxygen penetration into the sediments was also limited to a few millimeters. However, associated with the mud shrimp borrows, oxygen penetration reached several centimeters into the seafloor (Fig. 8).
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Fig. 7: Two dimensional oxygen distribution of a bacterial mat (II) sediment (scale-oxygen saturation %). The picture results from averaging nine oxygen pictures taken during one deployment (scale bar represents 1 cm).

Fig. 8: Oxygen distribution at a biogenic mound (II) (scale-oxygen saturation %). During the deployment of nearly 6 hours the oxygen content in the mud shrimp burrow was decreasing. Active pumping of the mud shrimp during this time was not recorded. The scale bar represents 1 cm.

4. Discussion

The Amon MV can be bathymetrically, visually and biogeochemically subdivided in four habitats: the central dome characterized by hills and troughs of very disturbed highly gassy sediments (I); the surrounding hummocky area with patches of bacterial mat (II); the relatively flat slope covered by biogenic mounds (III); and a lateral mud flow at the flank of the MV (IV). The concentric structure, morphology and associated communities of these habitats appeared to be mainly caused by spatial variation in gas and fluid flow on scales of meters to hundreds of meters.
4.1. The central dome of Amon

The central dome has a diameter of ca. 125 m (area 0.012 km²) (Dupré et al. 2008). It is the source of gas flares above the Amon (Mastalerz et al. 2007), and shows the strongest temperature gradient (Dupré et al. 2007; Dupré et al. 2008). The elevated mud breccia blocks making up most of the seafloor indicate massive gas outbursts in the past and illustrate the importance of gas flow at the Amon MV. At the edges of the central dome, the surface of the seafloor changes from a highly disturbed landscape (Fig. 2A) to smoother hummocks partially covered by bacterial mats (Dupré et al. 2007). At the northern edge of the central dome, there is an abrupt transition from highly gassy sediments to gas-free surface sediments.

Most interestingly, despite the high availability of methane and sulfate in the top meters of the gas saturated central dome (Fig. 4 and 5), SR and AOM rates were comparatively low. Sulfide concentrations (Fig. 4) in the subsurface sediments increased to max. 1 mM, and turnover rate measurements showed that SR and AOM were mainly restricted to the top 4-15 cm of sediment (Fig. 3 A). With in situ microsensor measurements, we did not detect sulfide production in the top 2 cm (Fig. 6). This indicates that AOM and other processes of anaerobic hydrocarbon oxidation are inhibited in the central dome area, presumably by other factors than energy availability.

In the pore water of the Amon central dome sediments (1), the salinity decreased with increasing depth, whereas the sulfate concentration increased to nearly 100 mM in the top 2 m (Fig. 5). Like large parts of the Nile Deep Sea Fan, it is likely that the Amon MV is located above deep subsurface Messinian evaporates (Loncke et al. 2004). The dissolution of Messinian gypsum by deep subsurface fluids of low salinity rising upwards could lead to this increase in sulfate content. Low salinity fluids rising from the deep subsurface were also described from other mud volcanoes of the Mediterranean Sea such as Milano and Amsterdam mud volcano (Dahlmann and de Lange 2003; Haese et al. 2006). It was concluded that this freshening is caused by clay mineral dehydration in the deep subsurface during their geothermal transformation at high temperature and pressure (Dahlmann and de Lange 2003 and references therein). It was previously found that in MV centers with high seepage rates > 1m, the upward flow of sulfate-depleted fluids disables the diffusion of sulfate from the water column into deeper sediment strata, thus limiting SR and AOM to the top few centimeter (de Beer et al. 2006, Lichtschlag et al. in review). This explanation does not work for the sulfate-enriched subsurface fluids of the Amon MV. Maybe, the uprising Amon MV fluids contain a not yet identified compound that is toxic for sulfate-reducing bacteria, or the central dome area is covered by a recent mud flow which has not been populated by AOM-mediating microbial communities. This hypothesis is supported by the very low total cell numbers found in the disturbed surface sediments, which are about an order of magnitude lower than cell numbers usually found at active cold seeps (Knittel and Boetius 2009).

The isotopic signature and composition of hydrocarbons transported by the rising fluids with a specific wetness ratio of 4-8% (Mastalerz et al. 2009) indicates a thermogenic source. The presence of high amounts of higher hydrocarbons also explains the higher SR rates compared to AOM with a ratio of almost 5:1. Such ratios are common at oily hydrocarbon seeps where SR is primarily fueled by non-
methane hydrocarbons (cf. Orcutt et al. 2005; Niemann et al. 2006a). Sulfate-reducing bacteria prefer the oxidation of higher hydrocarbons because of the higher energy yield (Kniemeyer et al. 2007). At the Amon MV, the gas chemistry suggests the preferential degradation of C_3 and C_4 compounds compared to C_1 (Mastalerz et al. 2009).

In the central dome area, oxygen fluxes were rather high, especially when considering the absence of mega- and macrofauna, and the relatively low biogeochemical activity. The DOU (10 mmol m^{-2} d^{-1}) can only be partly explained by sulfide oxidation (according to integrated SR rates of 2 mmol m^{-2} d^{-1}). Therefore, aerobic hydrocarbon oxidation in the top few millimeters of the seafloor may be a relevant biogeochemical process in this area. The DOU of the Amon MV center is in the same range as those from the Håkon Mosby MV (HMMV) (de Beer et al. 2006), where it was previously shown that aerobic methanotrophs are an important component of the biogeochemistry of this mud volcano (Niemann et al. 2006b, Felden et al. in prep.).

4.2. **Bacterial mats of the central dome**

The visual observations in 2006 indicated that the hummocks with the bacterial mat patches occur within a ring of about 50 m diameter around the inner dome structure (area 0.027 km^2). The bacterial mats themselves occupied about 10 - 20% of this seafloor area. The variable distribution and size of the mat patches in the central area suggest a spatially heterogeneous outflow of seep fluids in this habitat. The gas and fluid flow below the bacterial mat-covered patches appeared already significantly reduced compared to the inner dome structure, as indicated by the pore-water fluid composition (Fig. 4) and the less steep temperature profile (Fig. 6 B). However, the underlying sediments still contained relatively high concentrations of methane and other hydrocarbons (Mastalerz et al. 2009), which fueled high AOM and SR rates (Fig. 3 D, E).

The SR rates below the bacterial mats (II) were as high as 3500 nmol mL^{-1} d^{-1}, i.e. about one order of magnitude higher than previously detected here (Omoregie et al. 2009). Sulfate was present over the entire investigated sediment horizon (0-30 cm bsf), showing some consumption in the top 10 cm to concentrations of around 10 mM. The microsensor profiles and rate measurements indicate a hotspot area for anaerobic oxidation of hydrocarbon below 2 cm. Microsensor measurements also show that oxygen is entirely consumed by sulfide oxidation in the mat and sulfide does not reach the water column (Fig. 6 B). Depth integrated SR rates matched the calculated sulfide flux into the mat (40 mmol m^{-2} d^{-1}) and were in the same range as the diffusive oxygen and total oxygen uptake (Tab. 2). Our microscopic analyses indicate that the mats are formed by giant vacuolate sulfide oxidizers, which are able to utilize nitrate for sulfide oxidation (Teske and Nelson 2006; Preisler et al. 2007, Lichtschlag et al., in review). This process may explain the depletion of sulfide in the mat, contributing to about 30-50% of the sulfide consumption based on the TOU and DOU measurements.

The benthic oxygen uptake of the bacterial mat (II) (Tab. 2) is one of the highest found at the Amon MV. Interestingly, the TOU was only slightly elevated compared to the DOU (Tab. 2). It is
commonly assumed that differences of TOU and DOU can be explained by faunal respiration (cf. Glud 2008) and thus similar uptake rates indicate low abundances of benthic fauna in the mat patches.

4.3. Biogenic mounds formed by deep-sea mud shrimp

Outside the steeper center, the relatively flat slope of the Amon MV is densely covered by biogenic mounds (III). Interestingly, a similar structure and sequence of habitats were observed at the Isis mud volcano (Dupre et al. 2008). The biogenic mound habitat was found to comprise the largest area of Amon MV (1.9 km²), but also of Isis MV on the NDSF (Dupré et al. 2008). The mounds were 20-50 cm high and wide, and often show signs of recent burrowing activities, with greyish reduced sediments thrown on top of the beige hemipelagic sediments. By chance we collected a thalassinid mud shrimp from the biogenic mound area and conclude that these organisms are the engineers of this landscape.

The animal as well as the morphology of the mounds resembled coastal thalassinid mud shrimps like *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). Suspension feeding is also discussed for some species (Li et al., 2008). A fossil cold seep community with an association between chemosynthetic bivalves and mud shrimps was described recently (Amano and Kiel 2007). However, the nature of the association of deep-sea mud shrimps with mud volcanoes of the Nile Deep Sea Fan remains unknown.

The burrowing activity of the shrimp leads to a partial oxygenation of the first centimeters (Fig. 8), and flushing of the burrow system may lead to a strong mixing of pore-water constituents. Accordingly, the top 15 cm seafloor of the biogenic mound area contained only very low levels of methane or sulfide and showed very low SR and AOM rates (Fig. 3 G, H), and no consumption of sulfate or production of DIC, respectively (Fig. 4). We observed rather low cell numbers (Fig. 3 I) in comparison to other Amon MV habitats or other seeps (Treude et al. 2003; Niemann et al. 2006b; Omoregie et al. 2008). These biogeochemical features seem to be the result of the mud shrimp bioturbation and feeding activity. It is possible that in deeper sediment layers the flux of hydrocarbons is high enough to sustain higher biomasses of microorganisms, which may be the main food source of the shrimp. Accordingly, the total benthic oxygen consumption rate (5 mmol m⁻² d⁻¹) in the biogenic mound area was higher than background values of pelagic non-seep influenced sediment in the Eastern Mediterranean at similar water depths outside of the Amon MV (DOU 1.3 mmol m⁻² d⁻¹).

A previous study investigated the effect of mud shrimps on benthic oxygen consumption rate by replicate chamber deployments. On average the TOU of sediments with mud shrimps was higher than without, but the difference was not statistically significant. The authors argued that only a minor fraction of the incubated water was exchanged during the measurement based on the borrow irrigation rate (Hughes et al. 2000). By considering a similar borrow irrigation rate (36 cm³ h⁻¹) and our incubation time of 5.6 h, the animal could circulate 200 cm³ of water, which is only 5% of the total enclosed water (~4,300 cm³). Hence, we conclude that the biogeochemical activities in the biogenic
mound areas associated with the NDSF mud volcanoes are directly or indirectly elevated by fluid flow processes in subsurface sediments.

4.4. Sulfuric mud flow at the base of Amon

Another unique habitat influenced by mud volcanism was found at the southwestern flank of the Amon MV. Here, a lateral outflow of blackish, highly reduced muds and brines from the Amon MV was found (Dupré et al. 2008). The mud flow investigated here was three to six meters wide and was visible for 50-60 meters before it disappeared below a layer of pelagic sediments. Most of the black mud was covered by a whitish bacterial mat (Fig. 2 D). The detailed morphological and biogeochemical features of this bacterial mat (IV) will be discussed in detail elsewhere (Girnth et al., in prep). In the surrounding of the mud flow, we found active chemosynthetic communities characterized by Lamellibrachia tubeworms (Duperron et al. 2009) and various bivalves associated with carbonate crusts. The source area of the mud flow was littered with shells of Thyacosirid bivalves, indicating that the flank of the Amon MV was once a highly active chemosynthetic ecosystem.

The low rates of SR (0.5 mmol m⁻² d⁻¹) and AOM detected in the sulfur band (IV) were restricted to the top 4 cm of the sediment, but the microsensor profiles and benthic chamber measurements indicated a substantial release of sulfide from the mud into the hydrosphere. Sulfide flux was highest at the source of the mud flow and decreased with increasing distance along the sulfur band (Girnth et al. in prep). This indicates that the sulfide efflux is mostly due to its transport with the subsurface muds and subsequent release when these are exposed to bottom waters. Hence, biogeochemical processes of the sulfur band are not fueled by vertical transport of seep fluids like in the central dome area, but rather by horizontal outflow of brine and sulfidic mud which gets chemically and biologically oxidized when reaching the pelagic seafloor. The observation of bushes of large thiotrophic tubeworms associated with the mud flow suggests that the transport phenomenon must persist for decades (Duperron et al. 2009). Moreover, the finding of such lateral mud flows supporting chemosynthetic communities indicate that cold seep habitats are not restricted to the central areas of MVs, and illustrates the need for systematic surveys of seafloor structures like mud volcanoes.

4.5. Comparison to other mud volcanoes

The morphology, seepage intensity and composition of seep fluids varies considerably between the different submarine mud volcanoes known, but many of them have patterns in the distribution of chemosynthetic habitat in common (Olu et al. 1997; Kopf 2002; Niemann et al. 2006b; Dupré et al. 2007; Dupré et al. 2008; Huguen et al. 2009; Sommer et al. 2009). The habitat distribution of MVs is mainly caused by fluid flow intensity and biogeochemical transport processes. Generally, three more or less concentric habitats can be distinguished at most active MVs (Fig. 9): 1) a gas and fluid-emitting central zone overlying the main channel for the upward transport of mud, fluids, and gas from a deep subsurface reservoir. Here, fluid flow rates above 1 m per year can limit microbial hydrocarbon
degradation by constraining the downward diffusion of electron acceptors into the anoxic sediments; II) a surrounding bacterial mat zone establishing where more stable environmental conditions and lower fluid flow rates allow the growth of anaerobic sulfide-producing hydrocarbon degrader communities which fuel diverse assemblages of thiotrophic bacteria at the seafloor; and III) an outer zone often characterized by abundant carbonate cements, where animals such as tubeworms, bivalves, polychaetes or mud shrimps are adapted to access the reduced energy stored in deeper sediment strata. A key factor is the transport of sulfate and sulfide in the sediments. At low fluid flow regimes, the seep fauna access subsurface sulfide sources by burrowing, enhancing sulfate penetration depth and hence sulfide production rates (Cordes et al. 2005; Dattagupta et al. 2006; Dattagupta et al. 2008). In this way an efficient biofilter is formed that prevents the release of greenhouse gases like methane, or toxic sulfide, into the water column (Felden et al., in prep).

However, at the Amon MV we found at least two habitats, which represent leaks for methane and sulfide. Lateral mud flows such as the one sampled at the southwestern flank of Amon release sulfide into the water column when they get exposed to the seafloor. In the central dome, despite the overlapping occurrence of hydrocarbons and sulfate, large quantities of methane escape to the water column, because of the apparent inhibition of anaerobic hydrocarbon oxidizer communities, which needs further investigation.

Fig. 9: Schematic drawing of habitats at the Amon mud volcano. The habitats are not scaled consistently.
Reference:


Chapter 2.3. Biogeochemical processes at the Amon Mud Volcano


Chapter 2.4

Evidence for the deepest known anaerobic methanotrophic microbial community at *Calyptogena* colonies in the Japan Deep Sea Trench

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Abstract

*Calyptogena* species are typical members of cold seep communities associated with tectonic faults where active venting of fluids and gases takes place, which are used by their endosymbiotic sulfide oxidizing bacteria as energy source. We investigated a *Calyptogena* colony in the Japan Trench at 5360 m water depth in order to understand the main biogeochemical processes that define the locally restricted clam colonies. An integrated approach of biogeochemical and molecular techniques was used combining in situ and ex situ measurements. In the center of the clam field, low sulfate reduction (SR) rates (max. 128 nmol ml$^{-2}$ d$^{-1}$) were restricted to the upper sediment horizon and were closely coupled to the anaerobic oxidation of methane (AOM). Bioturbation of *Calyptogena* and the active transport of sulfate by the clams influence SR and AOM over a depth range of 15 cm, resulting in a depth integrated rate of 6 mmol m$^{-2}$ d$^{-1}$. The oxygen consumption of the chemosynthetic community was in the order of 19 mmol m$^{-2}$ d$^{-1}$. A distinct separation of the seep site to the surrounding seafloor is indicated by steep horizontal geochemical gradients at the colony rim. The sediment below the *Calyptogena* colony is dominated by ANME-2 and their sulfate reducing partner. Fluid flow and activity of microbes is rather low but sufficient to support the chemosynthetic community and to build up high biomasses.
1. **Introduction**

Methanotrophic communities establish where tectonic or gravitational forces thrust free gas, methane-rich pore water and/or mud upward into sulfate-penetrated surface sediments (Judd and Hovland 2007). The high energy availability in such ecosystems supports enormous biomasses of chemosynthetic organisms such as siboglinid tubeworms, mytilid and vesicomyid bivalves or giant sulfide-oxidizing bacteria (Sibuet and Olu 1998; Sahling et al. 2002). In methane-rich seep sediments, bacterial sulfate turnover is closely coupled to the anaerobic oxidation of methane (AOM) performed by *Euryarchaeota*. Although no pure cultures of these organisms are available yet, presence and activity of these microbes have been intensively studied with different molecular tools. Thus, it is known that AOM-performing Archaea and their bacterial partners often form aggregates (Knittel et al. 2005; Knittel and Boetius 2009b). Based on their 16S rRNA genes and different membrane lipids, three main clades of Archaea are distinguished: ANME-1, ANME-2 and AMNE-3 (Hinrichs et al. 1999; Elvert et al. 2002; Elvert et al. 2003). Each of them is associated with a specific group of sulfate-reducing bacteria (SRB). For instance, ANME-2 is found together with bacteria that are closely affiliated to *Desulfosarcina* spp. and *Desulfococcus* spp. (Knittel et al. 2003; Knittel et al. 2005).

The Japan Trench has a maximum depth of 9000 m, created by subduction of the Pacific plate beneath the Eurasian plate (Suyehiro and Nishizawa 1994). In this tectonically active area, numerous *Calyptogena* spp. and other chemosynthetic bivalves were discovered, forming round and elongate shaped colonies (Sibuet et al. 1988; Ogawa et al. 1996). It is assumed that these colonies mark fracture zones through which gasses and fluids from the deep subsurface rise up, nourishing chemosynthetic communities at the sediment surface (Juniper and Sibuet 1987; Sibuet et al. 1988; Ogawa et al. 1996; Fujikura et al. 1999; Kobayashi 2002). In the Japan Trench, chemosynthetic communities are described for water depths of 3850 to 6500 m (Juniper and Sibuet 1987; Sibuet et al. 1988; Ogawa et al. 1996). Most of these sites are dominated by one group of chemosynthetic bivalves, building colonies with up to 700 individuals per m² (24 - 42 kg m⁻² wet weight) (Juniper and Sibuet 1987; Ohta and Laubier 1987). The deepest known clam colonies are located at 6437 m water depth and mainly consist of *Calyptogena phaseoliformis* (Ogawa et al. 1996). Mats of sulfide-oxidizing bacteria at the seafloor, which usually indicate sediments with high microbial sulfate reduction associated to AOM (e.g. Treude et al. 2003; Niemann et al. 2006b), however, were absent in these areas of the Japan Trench (Ogawa et al. 1996; Fujikura et al. 1999), suggesting a rather low sulfide flux to the seafloor.

Despite the existence of chemosynthetic clam colonies at the Japan Trench, not much is known about the underlying biogeochemical processes and related microbial community fueling these seep communities. In this study, we investigated sediment sections underlying a *Calyptogena* colony from the Japan Trench to (1) understand key biogeochemical processes that support the spatial restricted clam occurrence, and (2) to characterize the composition and ecological importance of the associated microbial community. An integrated approach combining analyses of sediment pore water chemistry, total organic carbon, methane and sulfate turnover rate measurements as well as PCR- and lipid-based
community analyses was used. Results are discussed in terms of fluid flow and turnover rates and are compared to other clam seep sites.

2. Material & Methods

2.1. Seafloor observations and sampling

During the cruise YK06-05 on RV Yokosuka, a clam colony composed of *Calyptogena phaseoliformis* and *Calyptogena fossajaponica*, found at 5346 m water depth in the Japan Trench, was investigated. Beside the well-defined *Calyptogena* colonies present in this area, no other chemosynthetic communities, such as mat-forming, sulfide-oxidizing bacteria, were observed. The *Calyptogena* colonies were, however, associated with different groups of benthic organisms, including sea anemones and tubeworms. Typically, the clam patches were round and had diameters ranging from a few decimeters to two meters (Fig. 1). The distance between the widespread colonies was about a few tens in meters.

During the cruise, targeted sampling and precise positioning of the in situ instruments were achieved with the manned research submersible SHINKAI 6500 (JAMSTEC; Yokusuka, Japan).

One large *Calyptogena* colony (Fig. 1; 39º 6.3560’ N, 143º 53.5619’ E) was studied in detail in situ as well as ex situ with an interdisciplinary biogeochemical approach. In order to collect
undisturbed sediment, sampling was first performed close to the rim and then at the center of the clam patch (Fig. 1). Immediately after pushcore recovery, the sediment was sub-sampled for ex situ rate measurements or was preserved for later analyses.

2.2. Geochemistry

Ex situ pore water concentrations of sulfate and dissolved inorganic carbon (DIC) were measured along with the concentration and isotopic composition of dissolved methane, total organic carbon (TOC) content and turnover rates of sulfate and methane. In addition, in situ benthic oxygen uptake rates were measured with a microprofiler and a benthic chamber module.

2.2.1 Ex situ measurements

To measure pore water constituent concentrations, push cores (PCs) were subsampled in one centimeter intervals. To extract pore water, the sediment was quickly transferred to squeezers (Reeburgh 1967) equipped with 0.45 µm hydrophilic Durapore Membrane filters (Millipore, Bedford, USA). For each depth we achieved a pore water volume of 1 - 5 ml that was immediately preserved and stored at 4°C until the measurements in the home laboratory. To determine sulfate concentrations, 0.5 - 1 ml pore water were fixed in 1 ml 2% ZnAc. Samples were diluted and filtered before concentrations were determined with non-suppressed anion exchange chromatography (Waters IC-Pak anion exchange column, waters 430 conductivity detector). For measuring DIC concentrations, the pore water was preserved with HgCl2 solution and was stored headspace-free. DIC content of the samples was measured with the flow injection method (detector VWR scientific model 1054) according to Hall and Aller (1992). Dissolved methane concentrations and isotopic compositions were determined with the headspace method according to Kvenvolden (1986) and Ertefai et al. (submitted) using gas chromatography and isotope ratio mass spectrometry, respectively. Carbon isotopic ratios are reported in the δ-notation as per mill (‰) deviation from Vienna Pee Dee Belemnite standard (VPDB). Standard deviations of δ13C values were obtained from repeated measurements and were usually less than ±1.0‰. Pyrite and carbonate content of the sediment were measured by x-ray diffractometry described in Ertefai et al. (submitted). Total organic carbon (TOC) contents were measured from dry and homogenized sediment samples using a Leco CS 200. Prior to the analysis, samples were treated with 12.5% HCl to remove inorganic carbon.

Sulfate reduction (SR) and anaerobic oxidation of methane (AOM) were measured ex situ by radiotracer incubations. PCs were first vertically subsampled with small acryl subcore liners (diameter of 2.8 cm; three subcores for each method) and then incubated at in situ temperature (1.5°C). Sulfate and methane turnover rates were determined according to Treude et al. (2003). Briefly, either 25 µl 14CH4 (dissolved in water, 2.5 kBq) or 5 - 10 µl carrier-free 35SO4 (dissolved in water, 50 kBq) were injected in one centimeter intervals through silicon-sealed holes into the subcores (whole core injection method, Jørgensen 1978). After incubation for 48 hours, the sediment of the subcores was sliced in one centimeter intervals and fixed in 25 ml NaOH (2.5%, w/v) or 20 ml ZnAc (20%, w/v) for
AOM or SR rate measurements, respectively. In the home laboratory, radioactivity of all constituents was determined by scintillation counting (Treude et al. 2003; Kallmeyer et al. 2004). The substrate concentrations (sulfate, methane) were measured with anion exchange chromatography and gas chromatography (5890A, Hewlett Packard). SR and AOM rates were calculated according to the following equations:

\[
SR = \frac{\text{TRI}^{35}\text{S}}{(\text{SO}_4^{2-} + \text{TRI}^{35}\text{S})} \times \frac{\text{SO}_4^{2-}}{V \times t}
\]

and

\[
\text{AOM} = \frac{14\text{CO}_2}{(14\text{CH}_4 + 14\text{CO}_2)} \times \frac{\text{CH}_4}{V \times t}
\]

where \(\text{SO}_4^{2-}\) and \(\text{CH}_4\) are substrate concentrations, \(\text{TRI}^{35}\text{S}\) is the activity of the reduced sulfur compounds, \(14\text{SO}_4^{2-}\) is the radioactive sulfate, \(14\text{CO}_2\) is the radioactivity of the produced carbon dioxide, \(14\text{CH}_4\) is the radioactivity of the labeled methane, \(t\) is the incubation time and \(V\) is the volume of the sample.

2.2.3 In situ measurements

Total oxygen uptake (TOU) and dissolved oxygen uptake (DOU) were measured at the center and the rim of the *Calyptogena* colony, respectively. The difference between these two oxygen uptake measurements is commonly dedicated to faunal-mediated consumption, including bioirrigation and bioturbation as well as the animal respiration itself (Glud 2008 and references therein). TOU of the clam patch center was determined with a small, centrally stirred, cylindrical benthic chamber (Boetius and Wenzhöfer 2009; Treude et al. 2009), which enclosed a sediment area of 284 cm\(^2\) (radius = 9.5 cm) together with 15 cm of the overlying bottom water (equivalent to approx. 5 L). Two Clark-type minielectrodes continuously recorded the oxygen concentration of the enclosed water body during incubation. The sensors were calibrated against bottom water oxygen concentration (determined by Winkler titration) and a zero reading recorded at in situ temperature on board. TOU (mmol m\(^{-2}\) d\(^{-1}\)) was calculated from the linear change in oxygen concentration versus time:

\[
\text{TOU} = \frac{\delta C}{\delta t} \times \frac{V_{\text{chamber}}}{A_{\text{chamber}}}
\]

where \(\delta C/\delta t\) (µM h\(^{-1}\)) is the change of the concentration over the incubation time, \(V_{\text{chamber}}\) (cm\(^3\)) is the volume of the enclosed overlying water, and \(A_{\text{chamber}}\) (cm\(^2\)) is the area of the sediment enclosed by the chamber (Wenzhöfer et al. 2002).

Oxygen penetration depth and DOU outside of the *Calyptogena* colony were measured with a small deep-sea microprofiler module (Boetius and Wenzhöfer 2009; Treude et al. 2009), carrying 3 oxygen Clark-type microsensors (Revsbech et al. 1983) and one macro-temperature sensor (Pt100, UST Umweltsensorentechnik GmbH). The oxygen sensors were calibrated according to previous publications (Wenzhöfer et al. 2000; de Beer et al. 2006). Oxygen fluxes were calculated using Fick’s first law of diffusion (Jørgensen and Revsbech 1985):

\[
J = D \frac{\partial C}{\partial z}
\]
where $J$ is the diffusive flux (mmol m$^{-2}$ d$^{-1}$), $D$ is the diffusion coefficient in water (m$^2$ d$^{-1}$) corrected for temperature and salinity ($1.26 \times 10^{-9}$ m$^2$ s$^{-1}$, Li and Gregory 1974) and $\delta C/\delta z$ is the linear gradient within the diffusive boundary layer ($\delta C$ (mmol m$^{-3}$); $\delta z$ (m)).

### 2.3. Microbial community

The microbial community was explored by 16S rRNA gene and cell membrane lipid analyses. On board, sediment cores were sectioned into 1 - 5 cm intervals and frozen at -20°C.

For phylogenetic analyses, sediment from 0 - 10 cm depth was pooled and total DNA was directly extracted from 5 g of wet sediment according to the method described in Zhou et al. (1996). Crude DNA was then purified with the Wizard DNA clean-up kit (Promega, Madison, WI). Domain-specific primers were used to amplify almost full-length 16S rRNA genes by PCR. Bacterial primers used included GM3F (Muyzer) and EUB1492 (Kane et al. 1993), and for Archaea primers 20f (Massana et al. 1997) and Uni1392 (Lane et al. 1985) were applied. All subsequent steps, including cloning and plasmid sequencing, were performed according to Niemann et al. (2006a). Sequencing was performed by Taq cycle sequencing with a model ABI377 sequencer (Applied Biosystems). Sequence data were phylogenetically analyzed with the ARB software package (Ludwig et al., 2004; Pruesse et al., 2007).

Before intact and free cell membrane constituents were analyzed by liquid and gas chromatography, freeze-dried sediment was spiked with internal standards and lipids extracted using a modified Bligh and Dyer method (Sturt et al. 2004). The total lipid extract (TLE) was separated chromatographically on a glass column using 3 g of silica gel (60 mesh) into three fractions: a non-polar fraction (dichloromethane), a glycolipid fraction (acetone), and a phospholipid fraction (methanol). The phospholipid fractions were analyzed for intact polar lipids (IPLs), which were measured by high performance liquid chromatography/electrospray ionization-multiple stage-mass spectrometry (HPLC/ESI-MS$^n$) as previously described in Ertefai et al. (2008). The non-polar fractions were further separated for GC analyses following standard protocols for separation, derivatization and transesterification (e.g. Elvert et al. 2000; Elvert et al. 2003) with methods described in Ertefai et al. (2008).
3. Results

3.1. Solid phase

The recovered sediment cores were visually differentiated into an upper (0-10 cm bsf), middle (10-25 cm bsf) and lower (>25 cm bsf) section. The upper 10 cm showed a light brown color and were characterized by living *Calyptogena* spp. being partly buried into the slightly sandy sediment (Fig. 2). The middle section of the core was black with broken shells and a sulfidic smell was noticed during subsampling. Below 25 cm depth, the sediment displayed a uniform grey color. The differentiation of the sediment into different horizons was also reflected in the pyrite, carbonate, and TOC contents of the sediment. In the upper sediment horizon (0 - 10 cm), pyrite was absent and carbonate was low (5 - 7 wt-%). In the middle section, the amount of pyrite and carbonate increased to up to 8 and 32 wt-%, respectively. The carbonate content declined again in the lower section in contrast to pyrite, which reached values of up to 12 wt-%. TOC content in the sediment was constant in the upper 15 cm (~1.7 wt-%), decreased in the middle section of the core (14 - 18 cm bsf) and stayed constant again in the lower section (Fig. 2B).

Fig. 2: Stratigraphy and solid phase of the sediment sampled in the center of the *Calyptogena* colony. (A) Core image and sketch. (B) Total organic carbon (TOC).
3.2. Geochemistry

3.2.1 Pore water geochemistry

In the center and at the rim of the *Calyptogena* colony, pore water sulfate, DIC and dissolved methane concentrations were determined as well as the methane isotopic composition. In the center of the *Calyptogena* patch, sulfate concentration decreased to less than 1 mM at 12 cm bsf (Fig. 3A). The DIC concentration profile mirrored the sulfate concentration by a first increase with depth and then nearly constant DIC concentrations of more than 100 mM below 10 cm sediment depth. In contrast, at the clam colony rim, sulfate penetrated much deeper into the sediment (18 cm) as compared to the center (Fig. 3B) and the maximum DIC concentration (~100 mM) was found at 22 cm bsf. Dissolved methane was analyzed in all three lithostratigraphic horizons (Fig. 3). Concentrations and isotopic compositions varied with sediment depth and showed differences between sampling spots. The center revealed higher dissolved methane contents than the rim with a maximum between 25 and 33 cm bsf (Fig. 3A). At the center, δ¹³C values of dissolved methane ranged from -84 to -79‰. The most ¹³C-enriched values were found in the middle section of the core at 15-20 cm bsf (Fig. 3). At the rim, the dissolved methane was less depleted in ¹³C (-72 to -66 ‰, Fig. 3B).

3.2.1 Methane oxidation and sulfate reduction rates

Turnover rates of sulfate and methane were measured at the rim and the center of the *Calyptogena* colony (Fig. 3). At the center, averaged SR values were scattered over the investigated depth and ranged from 19 to 62 nmol ml⁻¹ d⁻¹. The averaged depth integrated SR rate (0-16 cm) was 6.3 mmol m⁻² d⁻¹. At the colony rim, sulfate turnover was lower (1- 42 nmol ml⁻¹ d⁻¹) with a maximum at about 5 cm bsf, but rates extended deeper into the sediment as compared to the colony center. Methane turnover at the colony rim decreased with increasing depth with values ranging from 2 to 25 nmol ml⁻¹ d⁻¹. The averaged depth (0-16 cm bsf) integrated turnover rates of methane and sulfate at the rim were in the same range with values of 2.4 (n=3) and 2.4 (n = 3) mmol m⁻² d⁻¹, respectively.
Fig. 3: **left panel:** Sulfate (black triangles) and DIC (white triangles) concentrations were measured in the pore water from the center (A) and the rim (B) of the *Calyptogena* colony. Potential SR and AOM horizons according to pore water concentrations are highlighted. Furthermore, methane concentration (black dots) and isotopic composition (white dots) were determined at both locations. **right panel:** SR (black dots) and AOM (white triangles) rates from the center and the rim of the clam colony. All presented values are averages of three replicates, except for the SR plot of the rim with seven replicate measurements.

**A** clam patch center

**B** clam patch rim
3.2.1 In situ oxygen uptake measurements

The microprofiler module was placed on the sediment next to the *Calyptogena* colony. A direct placement of the instrument on the clams was not possible due to the fragile glass sensors. Next to the clam field (about 20 cm away from the rim), the averaged oxygen penetration depth was 1.64 cm (n=3), and the averaged diffusive oxygen uptake (DOU) was 1.9 mmol m\(^{-2}\) d\(^{-1}\) (Fig. 4). No temperature gradient was recorded and the temperature remained constant at about 1.3°C for the entire length of the profile. In contrast to the microprofiler, the benthic chamber was placed directly on the clam patch enclosing about 20 clams (Fig. 4). A total oxygen uptake (TOU) of 21 mmol m\(^{-2}\) d\(^{-1}\) was measured, which is one order of magnitude higher than the DOU outside of the clam patch. Assuming that the DOU represents the benthic oxygen consumption of pelagic sediment in the Japan Trench at 5360 m we can calculate the oxygen consumption related to the chemosynthetic community by subtracting DOU from TOU (e.g. Wenzhöfer and Glud, 2002, Glud 2008) (chemosynthetic community oxygen consumption = TOU – DOU). For our investigated clam community this would result in an oxygen consumption of 19 mmol m\(^{-2}\) d\(^{-1}\) or \(\sim 0.3\) mmol d\(^{-1}\) per clam.

Fig. 4:  *left:* During the benthic chamber incubations, about 20 clams were enclosed at the colony center and the oxygen concentration decreased in the enclosed water column. *right:* The high resolution microsensor measurements outside of the *Calyptogena* colony showed an oxygen penetration (channel 1-3) depth of >1 cm and constant temperature throughout the entire profile.
3.3. Microbial community

3.3.1 Phylogenetic diversity

Bacterial and archaeal 16S rRNA gene libraries were constructed from surface sediments (0-10 cm depth) in order to gain a first insight into microbial community composition associated with the investigated Calyptogena colony at the Japan Trench. In total 85 and 89 bacterial clones were analyzed from the clam colony center and rim, respectively. Most of the obtained sequences were closely affiliated with seep-endemic and uncultured microorganisms (Tab. 1a, 1b). In the center sediment more phylogenetic groups (17) were found than at the rim of the clam field (6), suggesting a higher bacterial diversity in the sediment underlying the central clams (Tab. 1a). Here, most bacterial clones (32%) were associated with different sulfate-reducing bacteria (SRB), subgroup of the Deltaproteobacteria. The majority of these clones was affiliated with SEEP-SRB3 and SEEP-SRB4, which are related to the genera Desulfobulbus and Desulfotalealalus, respectively (Knittel et al. 2003).

Only 1% of the clam center sequences belonged to the SEEP-SRB1 group, which include the sulfate-reducing bacterial partner of ANME-1 and ANME-2. Further, many clam center sequences were affiliated to uncultured Bacteroidetes (19%) or with the Epsilonproteobacterium Arcobacter cibarius (14%). In contrast, most of the clam rim clones were affiliated with Gammaproteobacteria (53%). In addition, a high number of rim clones was associated with the Firmicutes (26%) and Bacteroidetes (15%). Only a few sequences (4%) clustered with the Deltaproteobacteria (SEEP-SRB3 and SEEP-SRB4).

The diversity of Archaea in the center and at the rim of the Calyptogena colony was investigated with 70 and 80 clones, respectively (Tab. 1b). In contrast to the bacterial diversity, the archaeal diversity was higher at the rim than at the center. The majority of the sequences from the center were related to ANME-2 archaea (1% ANME-2a; 88% ANME-2c), which are known to mediate AOM (Boetius et al. 2000; Orphan et al. 2002; Knittel et al. 2005). The remaining archaeal sequences (10%) in the center were all closely affiliated with known methanogens of the genus Methanococcoides. Most of the rim sequences (63%) were related to the methanogenic archaea and only a minor fraction to anaerobic methane oxidizers of ANME-2. Furthermore, rim sequences were affiliated to crenarchaeotal groups such as the marine benthic group B (MBGB), which is often found in methane-rich surface and subsurface sediments (Knittel et al. 2005). Other retrieved sequences mainly clustered in the marine Group 1 that includes Candidatus Nitrosopumilus maritimus, a cultivated aerobic ammonium oxidizer.
Tab. 1a: Phylogenetic affiliation and frequencies of bacterial 16S rRNA genes in clone libraries of the *Calypogena* clam patch center and rim sediments (0-10 cm bsf; marker 43): (n= total numbers of clones).

<table>
<thead>
<tr>
<th>Phylogenetic affiliation</th>
<th>close cultivated relatives or uncultivated group</th>
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3.3.2 Biomarker analyses

Intact membrane and free extractable microbial lipid analyses were performed from sediment below the *Calyptogena* in the center of the clam field (Fig. 5). The HPLC-MSN analysis revealed phosphate-based intact polar lipids (IPLs) in the form of hydroxyarchaeol (OH-Ar) with phosphatidylglycerol (PG) and phosphatidylserine (PS) as polar headgroups. Both IPL species increased with sediment depth from 5 to 62 µg kg⁻¹ dry sediment and were most abundant in the sediment horizon between 12 and 18 cm (141 µg kg⁻¹) before their concentration declined to 35 µg kg⁻¹ with sediment depth (Fig. 5). Dialkylglycerolethers (di-ether lipids), occurring as phosphatidylethanolamine (PE) and PS, were absent in the upper 6 cm, but increased with sediment depth and peaked in the sediment horizon at 12 to 18 cm (27 µg kg⁻¹) before declining to 4 µg kg⁻¹ dry sediment. The free extractable lipids revealed (OH)-Archaeol and monoalkyl glycerol ethers (MAGEs) supporting the LC-MS analysis. δ¹³C values of free extractable lipids varied with sediment depth and showed most depleted values at 12-18 cm bsf (Fig. 5).

Fig. 5: Microbial lipid profiles in the center of the *Calyptogena* colony. Concentrations of archaeal and bacterial lipids are shown on the left (A) and the isotope composition on the right (B) panel.
4. Discussion

_Calyptogena_ clams belonging to the family of Vesicomyidae and members of this family are commonly found at cold seeps (e.g. Monterey Bay, Barry et al. 1997; Hydrate Ridge, Sahling et al. 2002) and hydrothermal vents (Dubilier et al. 2008). There are more than 50 _Calyptogena_ species known that are symbiotic associated with carbon fixing and sulfide oxidizing endosymbiotic bacteria. Dual symbioses with thiotrophic and methanotrophic symbionts, as know for Bathymodiolus species, has not been described (Dubilier et al. 2008). Like most Vesicomyidae members _Calyptogena_ species own only a reduced gut system and thus rely nearly entirely on their sulfide oxidizing symbionts for nutrient and energy supply (Childress et al. 1993; Goffredi and Barry 2002 and references therein). These clams use their food to dig for sulfide in the sediment, while their siphon lies in the oxygenated water (Dubilier et al. 2008). Sulfide, bound to specific serum proteins, and oxygen are transported by their blood system to their endosymbionts (Childress et al. 1993). The sulfide binding ability of some species is very efficient and they are able to concentrate the sulfide in their body of up to 60-time above ambient concentrations (Childress et al. 1993; Barry and Kochevar 1998). As result, _Calyptogena_ spp. are found in habitats with sulfide concentrations in the range of 0.6 to nearly 20 mM (Barry et al. 1997). The sulfide is mainly produced by SR coupled to AOM in these seep sediments (Knittel and Boetius 2009a). The digging for sulfide enhances also the sulfate transport from the water column into the sediment resulting in SR at sediment depths, which would be otherwise sulfate limited if only diffusion would occur (Wallmann et al. 1997; Levin et al. 2003).

4.1. Confined sulfate and methane turnover in seep sediment underneath _Calyptogena_

Even detailed physiological studies are missing for _Calyptogena phaseoliformis_ and _Calyptogena fossajaponica_, it is likely that those animals show a similar physiological behavior as other _Calyptogena_ sp.. Hence, these clams are indicator for sulfide-enriched sediments caused by SR most likely coupled to AOM at the here investigated Japan Trench site. Indeed, geochemical gradients and turnover rates indicate an active SR and AOM community in the upper 15 cm of the sediment. This sediment horizon correlates with the depth range that can be affected by various clam activities, like bioirrigation and digging, based on their averaged body length (15-17 cm) and the fact that they were generally buried up to four fifth in the sediment.

In the sediment from the colony center, decreasing sulfate and increasing DIC concentrations (Fig. 3A) indicate sulfate and methane turnover in the top 15 cm. Side products of SR coupled to AOM are sulfide and carbonate that can chemically react with iron and calcium to form pyrite and calcium carbonate. In the center sediment, highest concentrations of pyrite and calcium carbonate were found between 10 and 20 cm bsf, confirming the presence of AOM and SR. Elevated SR rates were found over the entire investigated sediment depths without indications of a main production zone. The measurements might be partly biased by subsampling of the sediment containing broken shells with small sub cores. However, the scattered values could also suggest that the sulfate reducing microbes are dispersed over the entire analyzed depth range. Such a homogenous depth distribution of
SR and AOM mediating microbes over several centimeter was also recently described for *Calyptogena* colonies in the REGAB pockmark of the Gabon continental margin (Cambon-Bonavita et al. in press). According to the pore water analyses, sulfate concentration would be sufficient to perform SR over the entire depth range in the center of the colony. Hence, bioturbation of the clams seems to result in increasing sulfate availability over a wide depth range. A mixed upper sediment layer would also explain the relative homogenous TOC content in the first 15 cm of the sediment. In contrast, the decreasing sulfate pore water profile of the colony center indicates sulfate consumption mainly above 10 cm bsf. Pore water analyses and radiotracer incubations are both ex situ measurements. Thus, the sulfate pore water profile might be already affected by the inactivation of the clams during the sampling procedure, which under in situ conditions actively supply deeper sediment horizons with sulfate. In contrast, during the ex situ radiotracer incubations, the activity of the sulfate reducing bacteria had not changed, showing sulfate reduction down to 20 cm bsf. Apart from methodical biases, the concaved shaped sulfate concentrations profile could also be caused by upward fluid flow, which reduces sulfate diffusion into the center sediment (de Beer et al. 2006). The active sulfate transport by clams would not be influenced by this upward fluid flow and therefore responsible for the constant supply of deeper sediment horizons with sulfate. The fast sulfate turnover associated with methane oxidation in these deeper layers together with the upward fluid flow might thus be responsible for the low pore water sulfate concentration but high SR rates.

At the clam field rim, sulfate penetrates much deeper into the sediment as compared to the center (Fig. 3). Neither upward fluid flow nor clam activities seems to influence the pore water gradient suggesting that sulfate is mainly transported by diffusion into the sediment. Both, the pore water sulfate concentration profile and the SR rates show a main sulfate turnover horizon between 5 and 15 cm bsf. However, turnover rates were also measured close to the surface and down to 20 cm depth, which might be partly biased by the sediment sampling from more than 5000 m water depth due to warming and depressurizing. Although methane and sulfate turnover rates were low, the ratio of the depth integrated rates were close to one indicating a tight coupling of these two processes in the sediment of *Calyptogena* colonies in the Japan Trench (Treude et al. 2003; Niemann et al. 2006b).

### 4.2. Microbial diversity in the seep sediment of the *Calyptogena* colony

The coupling of SR to AOM at our investigated *Calyptogena* colony was also confirmed by the microbial community structure. Furthermore, we found direct evidence by lipid and 16S rDNA genes analyzes for the, to our knowledge, deepest AOM community. In the center of the *Calyptogena* sp. colony, the analysis of intact and free extractable microbial cell membrane lipids revealed diagnostic biomarkers for specific bacterial and archaeal groups. The exclusive appearance of Archaeol-species proves the presence of Archaea, and more specific, the high ratios of OH-Archaeol vs. Archaeol indicate the ANME-2 clade to be active (Niemann 2008). The presence of the syntrophic SR bacteria involved in AOM, is evidenced by the $^{13}$C depleted di-ether IPLs and MAGE species and more
specific the high amounts of MAGEs are characteristic for the sulfate reducing partners of ANME-2, forming the SEEP-SRB1 cluster (Hinrichs et al. 2000; Ertefai et al. 2008; Niemann 2008). The increasing IPL concentrations indicate an increase of prokaryotic cell abundances at the depth of the geochemical reaction zone between sulfate and methane (Fig. 4).

The interpretation of the microbial biomarker analyses is in accordance with the DNA data (Table 1). Most of the center sequences (0-10 cm bsf) were affiliated with sulfate reducing bacteria. However, only 1% of all bacterial sequences clustered in the ANME-2 associated SEEP-SRB1 group, even ANME-2 sequences were highly abundant in the clone library. This might be a methodological bias of the PCR based DNA approach, or at our study site the ANME-2 archaea might be associated with SEEP-SRB3 and 4 members because many of their sequences were found in the center sediment. Their functions in the sediment are not known but it was assumed that these bacteria might be involved in AOM (Knittel et al. 2003). A microbial community study at a Calyptogena sp. colony in the Nankai Trough (south of the Japan Trench) found also ANME-2a and ANME-2c to be the dominant archaeal groups in the sediment (Arakawa et al. 2005). In contrast, in sediments below Calyptogena sp. clams at Hydrate Ridge and at the REGAB pockmark, ANME-2 and their sulfate reducing partners were accompanied by ANME-1 and even ANME-3 (Knittel et al. 2003; Knittel et al. 2005; Cambon-Bonavita et al. in press). It was assumed that ANME-1 is less oxygen tolerant than ANME-2 and thus ANME-1 dominates methanotrophic communities under permanent anoxic conditions like in the Black Sea (Knittel et al. 2005). At Hydrate Ridge, the abundance of ANME-1 was increasing with increasing depth, and thus decreasing sediment irrigation by clams (Knittel et al. 2005). At our Japan clam colony, the top 10 cm of the sediment were light brown (Fig. 2) and pyrite was absent, which might indicate temporal presence of oxygen due to faunal activity and explains the exclusively presence of ANME-2 in this sediment horizon. Below the faunal irrigated horizon, ANME-1 could find optimal growth conditions but the lipid analysis found no evidence for ANME-1 in these sediments.

Overall, at the rim the diversity of archaeal groups, especially of non-ANME-2 phyla, was larger compared to the center of the clam patch. In contrast, SRB were more divers in the center of the colony (Tab. 1). This might indicate that the microbial community is shifting towards the rim from a methane oxidizing seep-community to an organic matter degrading community.

4.3. Seepage intensity at Japan Trench clam colony

At the investigated Japan Trench site, seepage seems to be restricted to small seafloor areas indicated by the clam patch sizes and the microprofiler measurements next to the colony. The distribution of the Calyptogena clams is strongly influenced by the biogeochemical process present in the sediment, which themselves are controlled by the seepage of methane enriched fluids from below. Oxygen and temperature profiles however, point to a focused release of seep fluids. The relatively deep penetration of oxygen together with the straight temperature profile 20 cm next to the clams indicate that fluid flow from the deep subsurface is absent (Fig. 4). Similar measurements at the
Nankai Trough (Kobayashi 2002) and at the Peruvian margin (Olu et al. 1996) found increasing temperatures with increasing depths in the center of clam patches suggesting an upward flow of deep subsurface fluids. In addition, the oxygen penetration depth (1.6 cm), is rather in the range of non-seep than seep influenced sediments (Wenzhöfer and Glud 2002; de Beer et al. 2006). In seep sediments oxygen diffusion is usually limited and even at Calyptogena sites restricted to the top few millimeters (Levin et al. 2003).

At the here investigated Calyptogena colony in the Japan Trench, the focused outflow must have been taken place for a long time in order to establish such a sulfide enriched sediment horizon and macrofauna community. Using averaged growth rates of other Calyptogena species (Barry and Kochevar 1998) and the measured shell sizes, we estimate an average age of 10-15 year for the living Calyptogena sp. clams. The mixture of alive animals and empty shells indicated that the clam accumulation exits since more than 15 years and subsequently seepage has to occur since decades at this Japan Trench site. Biodiversity of cold seep communities might reflect the ecosystem variability in space and time (Sibuet and Olu-Le Roy 2002). The observed macrofauna diversity of only two Calyptogena species (C. phaseoliformis, C. fossajaponica) indicates that constant environmental conditions (fluid flow intensity) occur over longer time periods. Fluctuation in seepage and thus heterogeneous ecosystems, would have enabled different organism adapted to various abiotic conditions to settle and thus would have increased the diversity of the chemosynthetic fauna (Sibuet and Olu-Le Roy 2002).

The here observed abundance of living clams suggests that fluid flow is sufficient to maintain this seep community. However, our Japan Trench SR rates are up to two orders of magnitude lower compared to other Calyptogena habitats such as Hydrate Ridge (maximal SR 3000 nmol ml⁻¹ d⁻¹) (Treude et al. 2003; Boetius and Suess 2004), where also higher methane concentrations (1 to 10 mM) were reported (Torres et al. 2002; Boetius and Suess 2004). In the Japan Trench, the dissolved methane concentrations are lower with a maximum of 303 µM in the colony center but this concentrations are not extremely low for Calyptogena sites (Barry et al. 1997; Wallmann et al. 1997; Cambon-Bonavita et al. in press). Low methane concentration often result in low sulfide concentrations, but which still can be sufficient for clams due to their ability to enrich sulfide in their body fluids above ambient concentrations (Childress et al. 1993; Barry et al. 1997; Barry and Kochevar 1998). A constant supply of sulfide seems to be more important than the absolute concentration (Dubilier et al., 2009). If the sulfide concentrations would be too low, Calyptogena clam could still move to sediments with higher sulfide content (Sibuet et al. 1988; Olu et al. 1996; Levin 2005). During our exploration, such moving clams were sometimes observed at the seafloor but the majority was associated with well-defined patches.

Our low methane concentration in the sediment could also result from efficient methane consumption that prevents methane enrichment in the sediment. Indeed, AOM and SR rates could be slightly higher in situ than our ex situ rates (6.3 mmol m⁻² d⁻¹ in the center) taking the in situ TOU as a
measure for the overall activity. Assuming that oxygen is used for sulfide oxidation, which is mainly produced by SR coupled to AOM, TOU can be used as an approximation for the methane consumption within the sediment. For our investigated Calyptogena colony this would result into a methane consumption of 9.5 mmol m⁻²d⁻¹. Considering this TOU based methane oxidation rate for the entire patch size (1.8 m²) of the here investigated clam colony and assuming that the uprising methane is completely oxidized, the methane flux from the deep subsurface would be 17 mmol d⁻¹. Overall, during our sampling of the Calyptogena colony the seepage rate was relatively low as indicated by methane concentrations and turnover rates. This could be a temporal effect because fluid flow can slightly varied over time (Olu et al. 1996) or the seep community is well adapted to use efficiently this low but constant methane supply to build up high biomasses.

4.4. Origin of the used methane at the clam patches

Calyptogena patches in the Japan Trench are commonly assigned to seepage through fracture zones (Juniper and Sibuet 1987; Sibuet et al. 1988; Ogawa et al. 1996). The sharp boundary of seep communities indicates the occurrence of focused upflow of nutrient-rich fluids through cracks and faults (Kobayashi, 2002). Studies from the Monterey Canyon however, found that chemosynthetic benthic communities are most common on steep slopes where seafloor erosion have occurred rather than associated with tectonically driven flow through faults (Paull et al. 2005). Removal of the upper sediment layer by slope-failure events would result in an exposure of the methane-rich and sulfate-free deeper sediment to the oxygenated bottom water. The subsequent exposure to oxygen but also now sulfate-containing seawater in oxygen-depleted sediment layers would lead to the production of sulfide near sediment surfaces, necessary to support chemoautotrophic communities. This scenario might be also possible for the Japan Trench, where earthquakes occur regularly. Such down-slope movements of sediment surfaces triggered by earthquake shocks have been observed for this region (Kobayashi 2002). The here investigated area of Calyptogena colonies is also located at a slope and thus seafloor erosion processes could have triggered the development of these chemosynthetic communities. The isotopic composition, in the center of the clam colony shows methane δ¹³C values less than -80‰, indicating biogenic methane formation from CO₂ and H₂ rather than a thermogenic processes (Whiticar 1999). However, such isotopic ratios and methane concentrations below 1 mM were also found at other Calyptogena faults sites (Olu et al. in press). At our the Japan Trench no bacterial mats were observed in contrast to the Monterey Canyon (Paull et al. 2005). This could either indicate that the area is in a later succession stage after a land slide event, when methane enriched sediment horizons are found several decimeter below the surface or that the seep community indeed mark faults through which deep subsurface fluids advect slowly. The outflow via fracture zones would also support the observed clam patch sizes and confined expansion indicated by the steep horizontal gradients at the patch rim.
Chapter 2.4. AOM at Calyptogena colonies in the Japan Trench

References:


Chapter 2.4. AOM at Calyptogena colonies in the Japan Trench


Chapter 2.4. AOM at *Calyptogena* colonies in the Japan Trench


Chapter 2.5

Geochemical processes and chemosynthetic primary production in different thiotrophic mats of the Håkon Mosby Mud Volcano (Barents Sea)

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Abstract

In this study we have investigated if in a cold seep methane or sulfide is used for chemosynthetic primary production and if significant amounts of the sulfide produced by anaerobic oxidation of methane (AOM) are oxidized geochemically, hence are not available for chemosynthetic production. Geochemically controlled redox reactions and biological turnover were compared in different habitats of the Håkon Mosby Mud Volcano. The center of the mud volcano is characterized by the highest fluid flow, and most primary production by the microbial community depends on oxidation of methane. The small amount of sulfide produced is oxidized geochemically with oxygen or is precipitated with dissolved iron. In the medium flow peripheral Beggiatoa habitat sulfide is largely oxidized biologically. The oxygen and nitrate supply is high enough that Beggiatoa can oxidize the sulfide completely, and chemical sulfide oxidation or precipitation is not important. An internally stored nitrate reservoir with average concentrations of 110 mmol L\(^{-1}\) enables the Beggiatoa to oxidize sulfide anaerobically. The pH profile indicates sequential sulfide oxidation with elemental sulfur as intermediate. The third habitat investigated were gray thiotrophic mats associated with perturbed sediments. These showed a high heterogeneity in sulfate turnover and high sulfide fluxes, which are balanced by the opposing oxygen and nitrate fluxes so that biological oxidation dominates over geochemical sulfide removal processes. The three habitats indicate substantial small-scale variability in carbon fixation pathways either through direct biological use of methane or through indirect carbon fixation of methane-derived carbon dioxide by chemolithotrophic sulfide oxidation.
1. Introduction

Geochemical and microbiological processes at cold seeps like mud volcanoes, surface gas hydrate deposits and methane-laden pockmarks differ significantly from processes in organic detritus-fueled deep-sea sediments. At cold seeps, methane is abundant near the sediment-water interface and methane-consuming microbial communities control its discharge to the water column (e.g. Boetius and Suess 2004; Niemann et al. 2006; Wallmann et al. 2006). The key process in methane-rich seafloor sediment is the microbially mediated anaerobic oxidation of methane (AOM) coupled to sulfate reduction (Boetius et al. 2000). One product of this process is dissolved sulfide. However, not much is known about the fate of AOM-produced sulfide in cold seep sediments, but previous work indicated that it is almost completely oxidized within the seabed (Sahling et al. 2002; de Beer et al. 2006).

Both biological and geochemical processes can remove sulfide. In many cold seep ecosystems different thiotrophic communities gain energy from the chemolithoautotrophic oxidation of sulfide, such as the symbiotic siboglinid tubeworms (Cordes et al. 2005; Lösekann et al. 2008) and diverse bivalves including mytilids, lucinids and vesicomyids (Sibuet and Olu 1998; Duperron et al. 2005). Free-living thiotrophic bacteria form extensive mats and representatives of these mat-forming organisms are giant sulfur-oxidizing $\gamma$-proteobacteria like *Beggiatoa*, *Thiomargarita* or *Thioploca*. Many members of these groups are able to store both nitrate and elemental sulfur intracellularly (McHatton et al. 1996; Teske and Nelson 2006). *Thiomargarita* are sessile, spherical bacteria, whereas *Beggiatoa* and *Thioploca* are motile filamentous bacteria able to glide. They can move downward to a sediment zone where dissolved sulfide is abundant and use the stored nitrate as electron acceptor, either oxidizing sulfide to sulfate (Eq. 1) or to elemental sulfur (Eq. 2):

$$\text{HS}^- + \text{NO}_3^- + \text{H}^+ + \text{H}_2\text{O} \rightarrow \text{NH}_4^+ + \text{SO}_4^{2-} \quad \text{(Eq. 1)}$$

$$4\text{HS}^- + 3\text{NO}_3^- + 6\text{H}^+ \rightarrow 4\text{S}^0 + \text{NH}_4^+ + 3\text{H}_2\text{O} \quad \text{(Eq. 2)}$$

In the upper zone of the sediment where oxygen and nitrate are available but sulfide is depleted, they refill their vacuoles with nitrate and oxidize the internally stored sulfur aerobically:

$$2\text{S}^0 + 3\text{O}_2 + 2\text{H}_2\text{O} \rightarrow 2\text{SO}_4^{2-} + 4\text{H}^+ \quad \text{(Eq. 3)}$$

The most important abiotic process is the precipitation of sulfide with iron-oxides. Iron-oxides react with sulfide, forming mainly elemental sulfur and metastable, amorphous iron-sulfides. These metastable iron-sulfides can be converted to the stable species pyrite ($\text{FeS}_2$) e.g. by reaction with sulfide (Drobner et al. 1990) or by reaction with polysulfides (Luther 1991). Iron-sulfides are either buried in the sediment or remain part of the sulfur and iron cycle by transport to the oxic zone, e.g. by bioturbation, where they are re-oxidized. The oxidized iron is again available as an electron acceptor for sulfide. In some marine settings such as coastal zones geochemical sulfide precipitation can totally
dominate over microbial oxidation by *Beggiatoa* (Preisler et al. 2007). In the sediments of Limfjorden (Denmark) *Beggiatoa* is responsible for up to 50% of the sulfide oxidation (Mussmann et al. 2003). *Thioploca* off the coast of Chile mediate maximally one third of the total sulfide oxidation (Ferdeman et al. 1997). The causes for this variation in the partitioning of biotic and abiotic sulfide oxidation in marine sediments are not well constrained, and at cold seeps in deep-waters this has not been investigated.

The Håkon Mosby Mud Volcano is characterized by large gradients in fluid flow velocities and sulfide fluxes (de Beer et al. 2006), and hence represents an ideal natural laboratory to study the processes involved in sulfide consumption at cold seeps. The different fluid flow zones include: I) the central mud flows (fluid flow velocity > 3-6 m yr\(^{-1}\)) dominated by aerobic methane oxidation, II) *Beggiatoa* mats (fluid flow velocity: 0.3-1 m yr\(^{-1}\)) and III) areas covered by gray mats (de Beer et al. 2006; Niemann et al. 2006; Lösekann et al. 2007). We have not studied the large outer rim of the Håkon Mosby Mud Volcano populated by thiotrophic siboglinid tubeworms (Lösekann et al. 2008) as we could not obtain in situ microsensor measurements from the dense subseafloor system of tubeworm roots. Part of the primary production at the Håkon Mosby Mud Volcano is based on the energy generation from microbial sulfide oxidation, and geochemical sulfide oxidation with iron-oxides or other electron acceptors could potentially reduce the sulfide supply to bacteria, and hence the energy and carbon supply to this chemosynthetic ecosystem.

Thus our aims were 1) to determine if geochemical or biological processes dominate sulfide removal, 2) whether methane or sulfide is used for chemosynthetic biomass production, and 3) to estimate the areal primary production of the different microbial habitats. To answer these questions, the key pathways in sulfide oxidation were investigated, including the turnover of the geochemically and biologically relevant components ($\text{SO}_4^{2-}$, $\text{O}_2$, $\text{NO}_3^-$, $\text{CH}_4$), the efflux of the emanating products (mainly $\text{HS}^-$, DIC) and the distribution of sulfide oxidizers.
2. Material and Methods

2.1. Sampling site

The Håkon Mosby Mud Volcano is situated on the Norwegian-Barents-Svalbard continental margin (72°00.3’ N, 14°44.0’ E). It is located at a water depth of 1250 m, has a concentric shape with a diameter of 1 km and an elevation above the seafloor of 8-10 m (Vogt et al. 1997). The sediment layer thickness above the oceanic crust is more than 6 km and the deposits consist of Eocene-Pliocene preglacial hemipelagic sediments and Late Pliocene-Pleistocene glacial-marine sediments (Hjelstuen et al. 1999). The pore water rising in the Håkon Mosby Mud Volcano is highly enriched in methane and gas hydrate form around the central conduit for fluid flow (Milkov et al. 2004; Niemann et al. 2006).

Fig. 1: Map of the Håkon Mosby Mud Volcano showing the sampling and measurement positions in the different habitats. The topographic map was generated during the ARK XIX/3b by the Ifremer, Brest (Foucher et al. 2009).

2.2. Sampling

Sampling and measurements were performed in 2003 during the ARK XIX/3b cruise with the R/V Polarstern (PS64), in 2005 with the R/V L’Atalante (ATL05/02-3), and in 2006 during the HERMES cruise Viking (IFREMER) with the R/V Pourquoi pas?. On all expeditions the remotely operated vehicle (ROV) Victor 6000 (IFREMER) was used for sampling and positioning of instruments. Sampling and measurement details are summarized in Table 1 and sites are displayed in Fig. 1.
Push core samples with a sediment core length of 15-20 cm were obtained with the hydraulic ROV manipulator arm. Cores with 25-30 cm sediment length were retrieved with a multiple corer device (MUC), equipped with a POSIDONIA positioning system. As high gas load and decrease in pressure caused outgassing and sediment surface disturbances during retrieval, the sediment-filled liners were stored at in situ temperature (0°C) for 1 day so that the mats appeared again and the geochemical gradients re-established. Subsequently the cores were transferred to a 4°C room for pore water extraction with Rhizons (type: CSS, Rhizosphere Research Products) connected to a peristaltic pump and glass syringes. The Rhizons with a filter pore diameter of 0.1 µm were horizontally inserted into the cores per 1 cm depth interval through predrilled holes that were sealed with diffusion-tight tape before coring. The extracted pore water was immediately fixed in 5% ZnAc for sulfate, chloride and sulfide analyses or in 2 mol L⁻¹ HCl for analyses of dissolved iron. Samples for dissolved inorganic carbon (DIC) were fixed in HgCl₂ and stored at 4°C without gas bubbles in glass vials with an additional butyl layer.

After pore water extraction, the remaining sediment was immediately cut in sections of 1 cm (depth interval 1-10 cm) or 2 cm (depth below 10 cm) slices and frozen at -20°C in nitrogen flushed sampling bags. Before freezing, subsamples for porosity and elemental sulfur analyses were taken. For analysis of elemental sulfur, about 0.2 g sediment from each depth interval was put in 0.5 mL 5% ZnAc, 9.5 mL methanol were added and the mixture was vortex mixed. The samples were placed on a shaker for circa 12 h, decanted and transferred to glass vials.
Table 1: List of samples from all targeted habitats. Samples from measurements and experiments are labeled according to the PANGAEA database, where the geochemical data is deposited (http://www.pangaea.de, doi:10.1594/PANGAEA.715022). Included are investigations during the Viking cruise 2006 (VKG), the L’Atalante cruise 2005 (ATL05/02-3) and the Polarstern cruise 2003 (PS64). Sediment was sampled either by a multiple corer (MTB-no.) or with pushcores (PC-no./PUC-no.). Also in situ deployments of the microprofiler (MIC) are included.

<table>
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<td>S-isotopes</td>
<td>VKGD276/PC-8</td>
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</tr>
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<td>sulfate reduction</td>
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<td>nitrate uptake</td>
<td>VKGD277/PC-7</td>
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<td>S-Isotopes</td>
<td>VKGD277/PC-7</td>
</tr>
<tr>
<td></td>
<td>microscopy</td>
<td>VKGD277/PC-8</td>
</tr>
<tr>
<td>‘next to gray mat’</td>
<td>MIC</td>
<td>VKGD278/MIC-10</td>
</tr>
</tbody>
</table>

2.3. Microscopy and determination of internally stored nitrate and sulfur

To be able to evaluate the role of Beggiatoa in the environment, the morphology of the filaments and the internal storage capacity of substrates were assessed. The morphological diversity of the microorganisms from the gray and Beggiatoa mats was studied by transmitted light microscopy. Between 139 and 161 filaments were picked from four cores of the Beggiatoa habitat. The average biovolume and biomass of the filaments was calculated from their length and radius, assuming a cylindrical shape of the organisms. The filaments were transferred to vials with 250 µL of demineralized water and immediately frozen at -20°C. This procedure breaks the cells and releases the vacuolar nitrate, which was measured after reduction to N₂ with a gas chromatograph-mass spectrometer (Hinck et al. 2007). Elemental sulfur was extracted from subsamples with methanol and
analyzed as described below. In addition, sediment densely covered with *Beggiatoa* filaments was vertically sampled in high resolution, frozen and the nitrate content was measured as described below.

### 2.4. Geochemistry

**Solid phase.** Different iron and sulfur solid phase species were identified and quantified. Elemental sulfur was determined on the methanol extracts of biological and sediment samples by high-performance liquid chromatography (Zopfi et al. 2004). The concentrations of AVS (acid volatile sulfides: FeS, some greigite: Fe₃S₄) and CRS (chromium reducible sulfur: FeS₂ and S⁰, remaining greigite) were assessed with the modified two step acid distillation method (Fossing and Jørgensen 1989). In the following, the AVS and CRS will be referred to as their main components FeS and pyrite (FeS₂), respectively. Iron was extracted with the dithionite method (Canfield 1989) and the ascorbic acid method (Ferdelman et al. 1991). The dithionite method extracts amorphous iron-oxides, crystalline iron-oxides, some iron-bearing silicates and some AVS (Canfield 1989; Kostka and Luther 1994). Triplicate samples of frozen sediment were extracted in 10 mL dithionite solution (0.5 g L⁻¹ sodium dithionite in 0.35 mol L⁻¹ acetate/0.2 mol L⁻¹ sodium citrate) on a shaker for 48 h at room temperature and the extracts were filtered afterwards. The extracts were left for at least 72 h to oxidize the remaining dithionite and were then analyzed with the Ferrozine method (see below) with 1% (w/v) hydroxylamine hydrochloride as reducing agent. To determine the amount of dithionite-soluble manganese, subsamples of the same extracts were measured with a Perkin Elmer 3110 flame atomic absorption spectrophotometer (AAS). The ascorbic acid method extracts the most reactive, amorphous iron-oxides, some AVS and some iron bound in clay minerals (Kostka and Luther 1994). Triplicates of frozen sediment samples were extracted in 10 mL solution (10 g sodium citrate, 10 g sodium bicarbonate, 4 g ascorbic acid in 200 mL anoxic, demineralized water, adjusted to pH 8). Samples were shaken at 60°C for 24 h, filtered and iron concentrations were measured with the Ferrozine method.

**Pore water.** Sulfate and chloride concentrations were measured by non-suppressed anion exchange chromatography (Waters IC-Pak anion exchange column, Waters 430 Conductivity detector). As eluent isophtalic acid (1 mmol L⁻¹, pH 4.6) containing 10% v/v methanol with a constant flow rate of 1 mL min⁻¹ was used. Total dissolved sulfide concentrations (H₂S + HS⁻ + S²⁻) were determined with the diamine complexation method (Cline 1969). For the determination of dissolved iron Ferrozine was used (1 g L⁻¹ Ferrozine in 50 mmol L⁻¹ HEPES buffer, adjusted to pH 7) and the concentration was measured spectrophotometrically (Stookey 1970). DIC concentrations were assessed by flow injection (Hall and Aller 1992) with 30 mmol L⁻¹ HCl and 10 mmol L⁻¹ NaOH as eluent and a conductivity detector (VWR scientific, model 1054).
2.5. Microsensor Measurements and Fluxes

High resolution in situ microsensor measurements were carried out with a deep-sea microprofiler as described previously (Wenzhöfer et al. 2000, see Fig. 2a). The ROV Victor was used to precisely position the microprofiler, to start the autonomous profiling routine and for the instrument retrieval. The microsensors were stepwise driven from the water phase into the sediment to a depth of up to 8 cm. On the profiler electronic unit three pH, three oxygen, two sulfide microsensors (Revsbech and Ward 1983; Jeroschewski et al. 1996; de Beer et al. 1997) and one temperature sensor (3 mm diameter, Pt100, UST Umweltsensorentechnik GmbH) were mounted and calibrated on board of the ship as described previously (Wenzhöfer et al. 2000; de Beer et al. 2006).

In this study altogether seven deployments of the profiler were carried out at the center habitat, the *Beggiatoa* habitat and at the gray mat site of the Håkon Mosby Mud Volcano (Fig. 1, Table 1). Measurements ‘next to *Beggiatoa*’ were conducted about 1 m next to a large *Beggiatoa* mat; ‘next to gray mat’ was 20 cm away from a gray mat patch. As the surface was sometimes slightly undulating, the tilt of the profiler was estimated by image analyses and used to determine the position of the sediment surface.

Solute fluxes were calculated according to Fick’s first law of diffusion, assuming steady state conditions. Oxygen fluxes were calculated from the oxygen gradients through the diffusive boundary layer according to Jørgensen and Revsbech (1985):

\[
J = D \frac{dc}{dz} \quad \text{(Eq. 4)}
\]

where \(J\) = flux [mmol m\(^{-2}\) d\(^{-1}\)], \(D\) = diffusion coefficient in water [m\(^2\) d\(^{-1}\)] corrected for temperature and salinity (Li and Gregory 1974) and \(dc/dz\) = concentration gradient (dc [mmol m\(^{-3}\); dz [m]). Sulfide fluxes were calculated from gradients in the sediment according to following equation:
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\[ J = \phi D_{\text{Sed}} \frac{dc}{dz} \]  
(Eq. 5)

where \( \phi \) = porosity and \( D_{\text{Sed}} \) = diffusion coefficient in the sediment \([\text{m}^2 \text{d}^{-1}]\). The diffusion coefficient \( D_{\text{Sed}} \) in the sediment was calculated according to (Iversen and Jørgensen 1993):

\[ D_{\text{Sed}} = \frac{D}{1 + 3(1 - \phi)} \]  
(Eq. 6)

The diffusion coefficient \( D \) at a salinity of 35‰ and a temperature of 0°C was calculated to be 9.1\times10^{-5} \text{m}^2 \text{d}^{-1} (Unisense Table, Seawater and Gases) for oxygen and of 6.9\times10^{-5} \text{m}^2 \text{d}^{-1} for sulfide (Li and Gregory 1974). Calculated diffusive in situ fluxes were corrected for advective upflow according to de Beer et al. (2006).

Fluxes of advected solutes were calculated as

\[ J = v c \]  
(Eq. 7)

where \( v \) is the fluid upflow velocity \([\text{m d}^{-1}]\) and \( c \) is the concentration of the solute in the advected fluid. Fluid upflow velocities \( v \) of the different habitats can be found in de Beer et al. (2006) and in this manuscript.

2.6. Sulfate Reduction

Sulfate reduction was measured by the ex situ whole core injection method according to Jørgensen (1978) adapted as in Treude et al. (2003). On board 5-10 \( \mu \text{L} \) \( ^{35}\text{SO}_4^{2-} \) (dissolved in water, 50 kBq) radioactive tracer was injected into replicate subcores in depth intervals of 1 cm. Subcores were incubated in the dark at in situ temperature for 24 h. To stop the incubation, the sediment was sliced in 1 cm sections and fixed in 20 mL 20% ZnAc. Rates were determined with the single step cold distillation method (Kallmeyer et al. 2004).

2.7. Stable Sulfur Isotope Analyses of AVS and CRS

To determine potential sinks of reduced sulfide, subsamples of precipitated ZnS from the AVS and CRS distillations were filtered through 0.2 \( \mu \text{m} \) cellulose nitrate filters and converted to Ag2S in a 0.1 mol L\(^{-1}\) AgNO3 solution. The Ag2S precipitates were dried, weighed and mixed with approximately 3.5 mg vanadium pentoxide in tin cups. The stable sulfur isotope composition of the samples was determined by isotope ratio mass spectrometry (irmMS) using a Eurovector elemental analyzer connected to a Finnigan Delta Plus gas isotope mass spectrometer via a Finnigan Conflco II split interface. Sulfur isotope compositions were determined for the center area, the Beggiatoa habitat, and the gray mat site. Isotopic ratios are displayed in the standard \( \delta \)-notation (\( \delta^{34}\text{S} \)) with respect to the Vienna Canyon Diablo Troilite (V-CDT). Accuracy and precision of the isotope mass spectrometer
were tested after every tenth sample using IAEA Standard S2 (δ34S 20.3‰ vs. V-CDT). Standard deviation based on all replicates was 0.4 ‰.

2.8. Nitrate Uptake

Defined amounts of nitrate were added to the overlying water of retrieved sediment cores. The water column was gently stirred with a jet stream. Water samples were taken after different time intervals, acidified and stored at 4°C. Nitrate concentrations were measured with a chemiluminescence NOx analyzer (Thermo Environmental Instruments) based on reduction of NO2⁻ and NO3⁻ and reoxidation of the produced NO by ozone (O3) (Braman and Hendrix 1989). Random samples were checked for nitrite. No nitrite was detected and all NOx was assumed to be NO3⁻.

3. Results

3.1. Characteristics of the Different Habitats

To study the effect of methane fluxes and upflow velocities on biological versus geochemical methane and sulfide conversions, we concentrated our study on three main habitats: the center site, the Beggiatoa habitat and the gray mat fields.

Center. Sediments in the center area were devoid of macrofauna and microbial mats. Based on visual observations we differentiated between an ‘active’ center in the northwest (Fig. 1) with a few sites of methane bubble release, disturbed sediments and small cracks at the sediment surface. Southeast of this ‘active’ center, the seafloor showed a slightly undulating surface littered with small holes (diameter 2 cm). In this ‘less active’ center no gas bubble escape was observed.

Beggiatoa habitat. Sediments covered with Beggiatoa mats were sampled southeast of the center (Fig. 1) in an area with a dense mat of 1-2 mm thickness. The mats extended for hundreds of meters, with patches of several centimeter to decimeter large uncovered sediment (Fig. 2a). Some Beggiatoa filaments reached a few millimeter into the sediment, but most filaments were present in the mat covering the sediment surface. Beggiatoa filaments had an average length of 1.3 mm and an average filament radius of 4.8 µm, resulting in a biovolume of 1.02x10⁻¹⁰ L per filament. Filaments contained on average 120 mmol L⁻¹ elemental sulfur and 110 mmol L⁻¹ nitrate (Table 2).

Table 2: General morphology and characteristics of Beggiatoa filaments picked from the surface mat of four different cores of the Beggiatoa habitat (ATL05/02-3/PUC-14, PUC-15, PUC-24, PUC-23); samples were taken in two different Beggiatoa patches close to each other.

<table>
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<tr>
<th>length (µm)</th>
<th>radius (µm)</th>
<th>biovolume (µL)</th>
<th>biomass (µg)</th>
<th>internal S⁰ (mmol L⁻¹)</th>
<th>internal nitrate (mmol L⁻¹)</th>
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<td>ncores=4</td>
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<td>1.02 x10⁻¹⁰</td>
<td>0.09</td>
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<td>nfilaments=</td>
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<td>(± 0.5 x10⁻⁵)</td>
<td>(± 0.3 x10⁻⁵)</td>
<td>(± 0.05)</td>
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<tr>
<td></td>
<td>with</td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>average: 120</td>
<td>(±113)</td>
<td>average: 110</td>
<td>(± 36)</td>
<td></td>
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</table>
In sediments densely covered with *Beggiatoa*, a high total (internal plus pore water) nitrate content of on average 0.3 mmol L\(^{-1}\) was measured in the uppermost 1.6 mm. This mainly originated from internally stored nitrate released after freezing and thawing and subsequent centrifugation. Below 1.6 mm the total nitrate content decreased rapidly (Fig. 3a). *Beggiatoa*-covered sediments, incubated at in situ temperature, lost all nitrate in the upper 2 cm of the sediment within 6 days (Fig. 3b).

![Graph](image)

**Fig. 3**: Distribution of the total nitrate content in (a) a fresh sediment core densely covered with *Beggiatoa* filaments and (b) a sediment core covered with *Beggiatoa* filament that stood for 6 days at in situ temperature; (total nitrate content = pore water nitrate + nitrate released from disrupted *Beggiatoa* filaments). Note the different scales. Error bars display the standard error (n=3).

**Gray mat area.** Patches of gray mats of 0.5-5 m diameter (Fig. 2b) occurred northwest of the center. Within a single patch the color changed from white to dark gray; often a gray mat was surrounded by a white mat. In this habitat about 50% of the sediment was covered by mats and the sediment surface was flat. At the seafloor some of the mats appeared quite thick and gelatinous, but in retrieved cores the average mat thickness was only approximately 1 mm. Microscopy showed a morphologically highly diverse community as described previously (de Beer et al. 2006).

None of the sediment cores from the different habitats showed a visible color transition associated with redox gradients, the sediment color was typically dark grayish over the entire core length.
3.2. Geochemistry

Solid phase. The results of all measured solid phase parameters are summarized in Figure 4. Concentrations of dithionite and ascorbic acid extractable iron, AVS and CRS were similar in all habitats over the entire core lengths. Only in the sediment under the gray mat, the pyrite (CRS) concentration was slightly lower in the top 4 cm, below which it increased to somewhat higher concentrations. In the center site and the gray mat habitat concentrations of FeS (AVS) slightly decreased with depth. The amount of dithionite-extractable manganese in the sediment was below 2 µmol cm$^{-3}$ in all habitats (data not shown). No subsurface peaks of manganese were detected in the surface sediments, indicating the absence of manganese-oxides. Clear differences between the habitats were observed in elemental sulfur concentrations and distributions. In the center site and in the ‘next to Beggiatoa’ sediments the elemental sulfur levels were less than 0.2 µmol cm$^{-3}$ throughout the core (Fig. 4c, k), whereas they were as high as 0.6 µmol cm$^{-3}$ in the near-surface sediments of the gray mat and the Beggiatoa site (Fig. 4g, n).

Pore water chemistry. Results from pore water analyses are summarized in Figure 5. The sulfate gradients were steeper in the Beggiatoa site (Fig. 5f) than in the ‘less active’ center and the ‘next to Beggiatoa’ site (Fig. 5a, k). Sulfate concentrations decreased only slightly with depth in gray mat sediments (Fig. 5p). As chloride is not reactive, the steepness of its gradient can be used as a measure for upflow of mud and pore water with different chloride content (as e.g. in Aloisi et al. 2004; Wallmann et al. 2006). The chloride gradients differed only slightly between the ‘active center’, the Beggiatoa, and the ‘next to Beggiatoa’ sediments (Fig. 5c, h, m). In the top 15 cm of sediment below the gray mat chloride did not decrease (Fig. 5r). Sulfide concentrations were up to a magnitude lower in the extracted pore water than as measured in situ by microsensor measurements, due to stripping by degassing methane during retrieval. Below the sulfidic zones (Fig. 5b, g, i) dissolved iron was present (Fig. 5d, i, n). In zones with high sulfide content dissolved iron concentrations sometimes varied, likely due to sampling artifacts. DIC pore water profiles were scattered in the gray mats (Fig. 5t), probably due to disturbances by outgassing. In all other habitats DIC increased with depth (Fig. 5e, j, o).
Fig. 4: Iron and sulfur geochemistry, and S-isotope distribution in the solid phase at different habitats ('less active' center: a-d, *Beggiatoa* site; e-h, 'next to *Beggiatoa*’ site; i-k, gray mat area: l-o); CRS: chromium reducible sulfur (FeS₂, S⁰, some greigite), AVS: acid reducible sulfur (FeS, some greigite), FeAsc: ascorbic acid extractable iron, FeDith: dithionite extractable iron, S⁰: elemental sulfur.
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3.3. S-Isotope Composition

The stable isotope composition of the pyrite (CRS) was similar at the three sites and varied only between -5 to 0‰ vs. V-CDT with sediment depth (Fig. 4d, h, o). In contrast, the isotope composition of AVS varied strongly. In the top 2 cm of the gray mat sediments, AVS was enriched in $^{34}\text{S}$ (+5‰ vs. V-CDT) relative to CRS, but relatively depleted at depth (-5‰ vs. V-CDT). An inverse relationship was observed in the sediments from the ‘less active’ center, where AVS was more depleted relative to
pyrite at the top, but became gradually more enriched with increasing sediment depth (Fig. 4d, h, o). Lastly, in the *Beggiatoa*-covered sediments, $\delta^{34}S_{FeS}$ was $^{34}$S-enriched relative to pyrite throughout the sediment core and varied little with depth.

3.4. In Situ Microsensor Measurements

The results from the in situ measurements are shown in Figures 6-8. In the ‘active’ center, where only pH and temperature measurements were successful, temperature increased sharply with depth (Table 3). The steep sediment temperature gradients indicate an upflow of warm fluids from the deeper subsurface. The pH decreased pronouncedly in the upper 5 cm (Fig. 6a).

![Fig. 6: In situ measured microprofiles in sediments from (a) the ‘active’ center and (b) the ‘less active’ center.](image)

The pH profile in the ‘less active’ center showed a similar shape, but the decrease with depth was not as strong as in the ‘active’ center (Fig. 6b). Oxygen was present in the upper 1.3 mm of the sediment. Sulfide concentrations were low overall, peaked at 2 cm depth, and were below detection below 3 cm. Sulfide and oxygen profiles overlapped. The temperature gradient was less than 25% of the ‘active’ center temperature gradient (Table 3).

At the *Beggiatoa* site, oxygen penetration was only 0.5 mm (Table 4), and all oxygen is consumed within the mat. The sulfide profile showed highest concentrations at 2-4 cm (Fig. 7a), as
reported previously (de Beer et al. 2006). All three replicate pH profiles showed a pH minimum near the mat surface and a pH maximum at 4 mm depth. The pH maximum coincided with the zone where the upward diffusing sulfide disappeared. This pH undulation was not observed before at this site.

In the ‘next to Beggiatoa’ site (Fig. 7b) oxygen penetrated 3.0 mm. This was the deepest oxygen penetration depth measured during our in situ deployments at the Håkon Mosby Mud Volcano, associated with the lowest temperature gradient. Sulfide and oxygen profiles overlapped. Sulfide concentrations were much lower than in the directly adjacent Beggiatoa covered sediments. The pH showed a gradual decrease from the overlying seawater.

![Graphs showing oxygen, sulfide, pH, and temperature profiles](image)

**Fig. 7**: Oxygen, sulfide, pH and temperature profiles measured in situ in (a) sediments densely covered with Beggiatoa filaments and (b) in sediments without a visible Beggiatoa mat: ‘next to Beggiatoa’.

Sulfide concentrations were always very high in gray mat sediments with the steepest gradients near the sediment surface (in Fig. 8a: average sulfide profile of two deployments). Oxygen penetration was comparable to the Beggiatoa-covered sediment, but sulfide and oxygen profiles overlapped. The pH profile showed a small peak just below the sediment surface; below this the pH decreased gradually.

In the ‘next to gray mat’ site (Fig. 8b) sulfide reached peak values that were 10 times lower than in the gray mat-covered area. The pH decreased only slightly from seawater values in the upper 6 cm. Oxygen penetrated only 0.3 mm into the sediment. Temperature gradients in both the gray mat and in the ‘next to gray mat’ sediments were higher than in the Beggiatoa habitat.
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Fig. 8: In situ microprofiles from sediments covered by (a) a gray mat and (b) 20 cm aside a gray mat: ‘next to gray mat’. The sulfide profile in (a) the gray mat is an average value gained during two microprofiler deployments.

Table 3: Temperature gradients measured in situ with the microprofiler.

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<th>habitat</th>
<th>temperature gradient (°C m⁻¹)</th>
</tr>
</thead>
<tbody>
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<td>13.9</td>
</tr>
<tr>
<td>‘less active’ center</td>
<td>3.2</td>
</tr>
<tr>
<td>Beggiatoa</td>
<td>2.8</td>
</tr>
<tr>
<td>‘next to Beggiatoa’</td>
<td>1.6</td>
</tr>
<tr>
<td>gray mat</td>
<td>2.8, 4.6</td>
</tr>
<tr>
<td>‘next to gray mat’</td>
<td>3.2</td>
</tr>
</tbody>
</table>

3.5. Oxygen and Sulfide Fluxes

The fluxes calculated from in situ microsensor measurements (oxygen flux into the sediment and upward directed sulfide flux), corrected for upward advection, and the averaged oxygen penetration depths of all assessed habitats are shown in Table 4. Oxygen fluxes were highest in the gray mat habitat, slightly smaller in the ‘next to gray mat’ habitat and the Beggiatoa fields and strongly reduced
in the ‘next to Beggiatoa’ site and the center sediments. Sulfide fluxes followed a similar trend as those of oxygen, but the lowest were found ‘next to gray mats’.

### 3.6. Sulfate Reduction Rates

Sulfate reduction was measured in the Beggiatoa habitat, the gray mat site and the ‘less active’ center area. In Beggiatoa-covered sediments the highest sulfate reduction rate, 1200 nmol cm\(^{-3}\) d\(^{-1}\), occurred in the 1-3 cm depth interval and strongly decreased below 4 cm depth. In the gray mats, sulfate reduction occurred irregularly in the top 15 cm with values ranging from 100-1400 nmol cm\(^{-3}\) d\(^{-1}\). At the center site sulfate reduction was low with maximally 20 nmol cm\(^{-3}\) d\(^{-1}\) and decreased strongly with depth. Integrated sulfate reduction rates for the upper 10 cm varied by a factor of 200 between habitats and ranged from an average of only 0.5 mmol m\(^{-2}\) d\(^{-1}\) in the ‘less active’ center to 59 mmol m\(^{-2}\) d\(^{-1}\) in the gray mat (Table 4). Despite the considerable small scale variability associated with the mat habitats, the average sulfate reduction rates measured explain very well the average sulfide fluxes measured in situ with the microsensors.

Table 4: Summary of oxygen fluxes \(J_{O_2}\) (with standard deviations), oxygen penetration depths, upward directed sulfide fluxes \(J_{\text{sulfide upward}}\), and sulfate reduction rates (SRR) from different habitats of the Håkon Mosby Mud Volcano. Calculations and measurements are based on the in situ deployments of the microprofiler and on the ex situ sulfate reduction rates integrated over the upper 10 cm. Displayed fluxes were calculated as diffusional fluxes and afterwards corrected for advection with a fluid upflow velocity as determined in (de Beer et al. 2006). For the ‘less active’ center an upflow rate of \(1\) m yr\(^{-1}\) was assumed; only at an upflow rate of \(\leq 1\) m yr\(^{-1}\), sulfate can penetrate to a depth of 2 cm and a sulfide peak can be formed (Fig. 6b). An upflow velocity of \(0.5\) m yr\(^{-1}\) was used for the Beggiatoa and ‘next to Beggiatoa’ site; no correction was applied to the gray mat and ‘next to gray mat’ site (upflow velocity: 0 m yr\(^{-1}\), see Discussion). The negative notation of the fluxes stands for fluxes from the water column into the sediment, the positive notation stands for fluxes from the sediment in direction of the overlying water column. n.d.= not determined. Standard deviations and averages are derived from replicate measurements \(n\); for \(J_{O_2}\) and \(O_2\) penetration depth \(n=6\) for gray mat, \(n=3\) for ‘less active’ center, Beggiatoa and ‘next to gray mat’ and \(n=2\) for ‘next to Beggiatoa’. For SRR \(n=5\) for ‘less active’ center, \(n=8\) for Beggiatoa and \(n=2\) for gray mat.

<table>
<thead>
<tr>
<th>habitat</th>
<th>(J_{O_2}) (mmol m(^{-2})d(^{-1}))</th>
<th>(O_2) penetration (mm)</th>
<th>(J_{\text{sulfide upward}}) (mmol m(^{-2})d(^{-1}))</th>
<th>SRR (mmol m(^{-2})d(^{-1}))</th>
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</thead>
<tbody>
<tr>
<td>‘less active’ center</td>
<td>-11.4 (± 3.7)(^*)</td>
<td>1.3</td>
<td>0.7(^1)</td>
<td>0.2-1.1 average: 0.5(^1)</td>
</tr>
<tr>
<td>Beggiatoa</td>
<td>-33.7 (± 8.8)(^{**})</td>
<td>0.5</td>
<td>11.6(^{**})</td>
<td>2.8-23.1 average: 11.2(^{**})</td>
</tr>
<tr>
<td>‘next to Beggiatoa’</td>
<td>-9.2 (± 0.9)(^{**})</td>
<td>3.0</td>
<td>7(^{**})</td>
<td>n.d.(^{**})</td>
</tr>
<tr>
<td>gray mat</td>
<td>-45.2 (± 8.4)(^{**})</td>
<td>0.5</td>
<td>17/131</td>
<td>9.5-108 average: 59(^{**})</td>
</tr>
<tr>
<td>‘next to gray mat’</td>
<td>-35.2 (± 13)</td>
<td>0.5</td>
<td>4.8</td>
<td>n.d.(^{**})</td>
</tr>
</tbody>
</table>
3.7. Experimental Determination of Nitrate Uptake

Nitrate uptake was higher in Beggiatoa mats than in gray mats and the center sediments (Table 5). The initial uptake rate of starved Beggiatoa was up to eight times higher than after one hour of exposure. For the calculation of the nitrate flux the regression of the gradients (Fig. 9) after this initial hour was used. In the Beggiatoa sediments the nitrate uptake rate increased with higher nitrate concentrations in the water overlying the core. Increasing the seawater nitrate concentration 20-30 fold still resulted in constant decline after 66 h exposure (Fig. 9). As the nitrate decrease in the water is caused by both a) nitrate uptake by bacteria and b) molecular diffusion of nitrate from the water column into the sediment, the purely diffusional flux at given concentrations and time intervals was modeled (COMSOL multiphysics model) and subtracted from the value calculated from the regression of the gradient. This value then corresponds to the nitrate uptake by bacteria.

Table 5: Summary of nitrate uptake experiments with the calculated nitrate fluxes. Uptake was calculated from linear nitrate regression after a time period > 60 min; values for nitrate loss due to diffusive uptake into the sediment were achieved with the COMSOL metaphysics model and have been subtracted.

<table>
<thead>
<tr>
<th>habitat</th>
<th>nitrate concentration before addition (µmol L⁻¹)</th>
<th>amount of nitrate added (µmol L⁻¹)</th>
<th>Jₙₒₙ⁻ (mmol m⁻² d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘less active’ center</td>
<td>9</td>
<td>29 (2 x environmental concentration)</td>
<td>-7</td>
</tr>
<tr>
<td>Beggiatoa</td>
<td>4</td>
<td>14 (environmental concentration)</td>
<td>-10.8</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>29 (2 x environmental concentration)</td>
<td>-26</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>52 (4 x environmental concentration)</td>
<td>-102</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>270 (18 x environmental concentration)</td>
<td>-40</td>
</tr>
<tr>
<td></td>
<td>184</td>
<td>310 (30 x environmental concentration)</td>
<td>-74</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>no nitrate addition</td>
<td>-1.5</td>
</tr>
<tr>
<td>gray mat</td>
<td>9</td>
<td>28 (2 x environmental concentration)</td>
<td>-4.5</td>
</tr>
<tr>
<td>control</td>
<td>13</td>
<td>29 (2 x environmental concentration)</td>
<td>-1.4×10⁻⁴</td>
</tr>
</tbody>
</table>

(seawater without sediment)
Fig. 9: Uptake of nitrate by *Beggiatoa* was measured in bottom water overlying a densely *Beggiatoa* covered sediment. Different amounts of nitrate were added: (a) 14 µmol L⁻¹, (b) 270 µmol L⁻¹, (c) 310 µmol L⁻¹ (see also Table 5).
4. Discussion

At the Håkon Mosby Mud Volcano the presence and absence of thiotrophic mats can be explained by the sulfide fluxes from AOM. High fluxes of sulfide >10 mmol m$^{-2}$ d$^{-1}$ are exploited by thiotrophic mats. At a sulfide flux of 7 mmol m$^{-2}$ d$^{-1}$ and less, these mats do not form (Table 4). The patchiness of the mats can vary on scales of centimeter to 100 m. This indicates a strong spatial heterogeneity in fluid upflow and methane supply to the seafloor, and hence spatially variable AOM activity. A third possible limitation of thiotrophic primary production could be the competition between geochemical and microbial sulfide oxidation. However, we found bacterial processes to be responsible for both the sulfide production as well as the removal, showing that a substantial fraction of the AOM associated DIC production is efficiently channeled into chemosynthetic carbon fixation.

4.1. Beggiatoa Habitat

Sulfide production. Sulfate reduction rate measurements showed that in the Beggiatoa habitat most sulfide formed in the upper 3 cm. Also sulfide microprofiles indicate a hot spot of AOM at 2-4 cm bsf. (Fig. 7a). The averaged depth integrated sulfate reduction rate was close to the sulfide flux obtained from the microprofiles (Table 4). Thus very little sulfide is consumed between the source of the sulfide and the zone of maximal thiotrophic consumption at 4 mm depth. Precipitation of iron-sulfides is minor and most of the sulfide is available for consumption by thiotrophic bacteria. To confirm that in the Beggiatoa habitat sulfide oxidation is mainly performed by thiotrophic bacteria, we addressed the questions I) whether Beggiatoa are actually capable of oxidizing the total sulfide supply and II) if indeed no geochemical sulfide oxidation is detected from the distribution of iron-sulfides and -oxides in the sediment.

Biological sulfide oxidation. As in situ microsensor measurements in the Beggiatoa habitat showed a pronounced gap between the depths of oxygen depletion and sulfide appearance, direct aerobic sulfide oxidation can be excluded. This characteristic gap was not observed in the other habitats and was likely caused by migrating Beggiatoa via anaerobic oxidation of sulfide with nitrate. At ambient nitrate concentrations the nitrate flux into the Beggiatoa mats was as high as the upward sulfide flux (Table 4, 5), thus the nitrate supply was sufficient to consume the total sulfide flux (Eq. 1, 2). Beggiatoa stored nitrate up to a concentration of 110 mmol L$^{-1}$ (Table 2), which is about 7,500-fold higher than the environmental background concentration of 0.015 mmol L$^{-1}$. From the storage capacity of individual filaments (Table 2) and the total nitrate content in the sediment we calculated that 2.7x10$^4$ Beggiatoa filaments are present per cm$^3$ sediment in the zone with the high nitrate content (Fig. 3a, 0.3 mmol L$^{-1}$ nitrate / 1.12x10$^{-8}$ mmol nitrate per 1 filament). The mat itself (thickness: 1.6 mm) hosts 4.2x10$^7$ Beggiatoa filaments per m$^2$. Beggiatoa has a wet biomass of 3.8 g m$^{-2}$ (4.2x10$^7$ filaments m$^{-2}$ x 9x10$^{-8}$ g wet weight per filament), corresponding to 0.8 g m$^{-2}$ dry weight assuming a water content of 80%. All nitrate disappeared from the sediment within 6 days (Fig. 3b), thus the
vacuolar nitrate decrease was at least 18,000 mmol m$^{-3}$ d$^{-1}$ (110 mmol L$^{-1}$ / 6 days), similar to a value reported previously (Preisler et al. 2007).

The unique shape of the pH profile can be caused by sequential sulfide oxidation. Anaerobic sulfide oxidation with nitrate is a proton-consuming process (Eq. 1, 2) leading to the observed local pH increase at around 4 mm sediment depth where the upward diffusing sulfide disappears (Fig. 7a). The pH minimum located at the lower boundary of the oxic zone can be a consequence of oxidation of $S^{0}$ to sulfate:

\[
2S^{0} + 3O_2 + 2H_2O \rightarrow 2SO_4^{2-} + 4H^+ \quad (\text{Eq. 8})
\]

Indeed a high $S^{0}$ content of 120 mmol L$^{-1}$ was found in the mat and thus $S^{0}$ will be the principal electron donor for *Beggiatoa* in the sulfide-depleted sediment and mat layer. The oxygen flux (Table 4) is high enough to oxidize large amounts of $S^{0}$ (Eq. 8). When gliding between the oxic and the sulfidic zone internally stored energy sources (nitrate and $S^{0}$) will be used. Accordingly, the circa 0.4 $\mu$mol cm$^{-3}$ surplus sulfur in the surface sediments will be due to the intracellular sulfur pool of the filaments.

Spatially separated oxidation steps were used to explain pH microprofiles measured in laboratory incubated *Beggiatoa* mats (Sayama et al. 2005; Kamp et al. 2006). However, also a series of geochemical reactions can explain similar pH profiles. Preisler et al. (2007) reported an intense manganese and iron cycle in methane-containing sediments (Eckernförde Bay, Baltic Sea), where the chemical oxidation of sulfide with oxidized iron causes an increase in pH; produced Fe$^{2+}$ diffuses upwards and reduces manganese-oxides, and the concurrent iron-oxide precipitation causes a pH minimum.

**Potential role of iron for sulfide oxidation.** In the mud volcano fluids Fe$^{2+}$ is transported upwards with a flux of 0.05 mmol m$^{-2}$ d$^{-1}$. Dissolved iron will precipitate near the AOM zone as FeS and/or pyrite. However, this process does not balance the dissolved sulfide flux ($J_{\text{sulfide}} = 11.6$ mmol m$^{-2}$ d$^{-1}$, Table 4), which is 230 times higher. The manganese concentration is too low to play a geochemical role in iron or sulfide oxidation. Bioturbating and bioirrigating organisms were absent in all sampled habitats, thus do not drive an iron oxidation and reduction cycle in the suboxic zone.

Potentially reactive dithionite and ascorbic acid extractable iron remain the only detected species available for sulfide oxidation. Deposition or transport of rising mud from a deep reservoir most probably supplies each habitat with similar iron mineral species. In highly sulfidic environments, more iron-sulfide minerals than oxidized iron minerals are expected. Conversely, in habitats with little sulfide or suboxic habitats, more oxidized iron and a minor amount of iron-sulfides would be expected. However, different extraction methods showed similar concentrations of dithionite and of ascorbic acid extractable iron in all habitats investigated here (Fig. 4), as could be the result from a relatively recent mud flow forming the inner flat crater of the Håkon Mosby Mud Volcano. Even the sediments from the ‘less-active’ center that had virtually no free sulfide showed the same extractable iron

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concentrations as the highly sulfidic gray mat sediments. Thus available iron-minerals are not or only slowly reacting or the applied extractions overestimate the actual amount of reactive iron available for sulfide oxidation in the sediments of the Håkon Mosby Mud Volcano. As ascorbic acid and dithionite extract some iron-containing silicates (Kostka and Luther 1994) and the extruded material has iron-containing minerals like chlorite (Lein et al. 1998), in this setting the methods might extract a larger amount of other iron minerals than previously thought. Most iron-containing silicates have a low reactivity with sulfide ranging from hundreds to thousands of years (Canfield et al. 1992).

Since the precipitation of pyrite produces sulfur isotope fractionations smaller than 1‰, the isotope composition of pyrite is a good approximation of the isotope composition of the source sulfide derived from sulfate reduction. In sediments with sulfate reduction rates of a similar magnitude as those reported here, pyrite that forms directly at the sediment surface is generally more depleted in $^{34}$S than in the Håkon Mosby Mud Volcano sediments (e.g. (Lyons 1997; Schenau et al. 2002)) due to biological isotope fractionation (Habicht and Canfield 1996). Strong $^{34}$S-enrichment of sedimentary sulfides is usually indicative of precipitation from $^{34}$S-enriched dissolved sulfide (Brüchert et al. 2003; Dale et al. 2009). However, the homogeneity of the observed values with depth and between the three different sites supports a common allochthonous origin of the pyrite. We suggest that the pyrite in the mud volcano sediments derives largely from deeply-buried subseafloor deposits and is transported upwards with the mud/gas/fluid mixture. The slightly heavier $\delta^{34}$SAVS in the sediments under the Beggiatoa mat indicates the reservoir effect of an evolving dissolved sulfide pool due to ongoing bacterial sulfate reduction. The much stronger $^{34}$S-enrichment of near-surface AVS, e.g. compared to the sediments from the ‘less active’ center (Fig. 4d, h), indicates a smaller isotope effect from combined sulfate reduction and sulfide oxidation, in agreement with recent modeling studies on sediment inhabited by the phylogenetically related large sulfur bacteria Thiomargarita (Brüchert et al., 2003; Dale et al., 2009). AVS is generally considered as an intermediate in the formation of pyrite and as an active intermediate during sulfide oxidation (Berner 1970; Schippers and Jørgensen 2002). The overall low concentrations of AVS throughout the cored sediment indicate that AVS, like pyrite, is not an important sulfide sink. Although we cannot provide quantitative estimates of the turnover time of the AVS fraction, it is likely that the iron-sulfides make up only a small fraction of the total sulfide turnover in these sediments because the rate of supply of detrital reactive iron and the lack of bioturbating organisms limit the availability of reactive iron for iron-sulfide formation.

**Chemosynthetic biomass production.** We observed that most of the sulfide oxidation at the Håkon Mosby Mud Volcano is carried out by sulfide-oxidizing bacteria. Marine chemolithotrophic Beggiatoa gain a biomass of 8.4-15.9 g dry weight per mole sulfide oxidized (Nelson et al. 1986; Hagen and Nelson 1997). Thus a sulfide flux of 11.6 mmol m$^{-2}$ d$^{-1}$ (equal to 4.2 mol m$^{-2}$ yr$^{-1}$) corresponds to 1.5-2.8 mol C production per m$^2$ in one year, assuming that 50% of the dry weight is carbon. On the whole densely Beggiatoa covered area of 38,244 m$^2$ (> 50% dense bacterial coverage,
Jerosch et al. 2007) biomass production due to sulfide oxidation by *Beggiatoa* will then be $5.7 \times 10^4 - 1.1 \times 10^5$ mol C yr$^{-1}$. A complete turnover of thiotrophic biomass occurs within 4 days (0.8 g dry weight m$^{-2}$ respectively $1.2 \times 10^3$ mol C in the total habitat / $1.1 \times 10^5$ mol C yr$^{-1}$ biomass gain). This is realistic as the experimentally determined doubling time for *Beggiatoa* is approximately 1 day (Kamp et al. 2008) and high grazing pressure might control the standing stock efficiently (Van Gaever et al. 2006).

With the oxidation of one mole sulfide *Beggiatoa* fixes 0.35 mol CO$_2$ (Nelson et al. 1986). Thus 1.5 mol CO$_2$ is fixed per m$^2$ in one year ($4.2 \text{ mol m}^{-2}\text{ yr}^{-1} \times 0.35$) respectively $5.6 \times 10^4$ mol CO$_2$ is fixed in the total *Beggiatoa* habitat per year.

Compared to biomass production from sulfide oxidation, the biomass yield from AOM is low with a gain of 0.03 mol C fixed per mole methane oxidized (Nauhaus et al. 2007). Assuming methane consumption equals sulfide production ($11.6 \text{ mmol m}^{-2}\text{ d}^{-1}$ or $4.2 \text{ mol m}^{-2}\text{ yr}^{-1}$), then $0.11 \text{ mol C m}^{-2}\text{ yr}^{-1}$ is produced by AOM. This is in total $4 \times 10^3$ mol C yr$^{-1}$ for the *Beggiatoa* habitat. In the surface sediment of this habitat also aerobic methanotrophs are abundant (Lösekann et al. 2007). Biomass yield by aerobic methane oxidation is more difficult to calculate, as the amount of aerobically oxidized methane and the yield from methane oxidation at in situ conditions is not properly determined. Leak and Dalton (1986) came up with approximately 8 g biomass fixed per mole methane oxidized through the aerobic pathway. Assuming 10% of the oxygen consumption is due to aerobic methane oxidation as 10% of the microbes in the upper sediment layer of the *Beggiatoa* habitat are methanotrophs (Lösekann et al. 2007), 0.2 mol C m$^{-2}$ yr$^{-1}$ or $7.7 \times 10^3$ mol C yr$^{-1}$ in the whole *Beggiatoa* area is fixed (note: stoichiometry CH$_4$:O$_2$ = 1:2). In summary, in this habitat the largest amount of biomass is produced by *Beggiatoa* via the oxidation of sulfide, whereas AOM and aerobic methane oxidation contribute maximally 20%.

### 4.2. Gray Mat Habitat

*Sulfide production.* Large differences in sulfide fluxes and sulfate reduction rates and variable but relatively high temperature gradients (Table 3, 4) indicate large heterogeneity of the gray mat habitat which is most likely associated with dissociating gas hydrate. Sulfide concentrations were high and sulfide penetrated deep. This can be explained by absence of upward fluid flow, and diffusion as dominant mass transport process. Assuming 1) steady state, 2) absence of advective flow, and 3) complete conversion of sulfate to sulfide as only S-converting process (e.g. no sulfide is oxidized), then at any depth below the oxic zone:

$$C_x_{\text{sulfide}} = C_{0\text{sulfide}} - (C_{x\text{sulfide}} - C_{0\text{sulfate}}) \times \frac{D_{\text{sulfate}}}{D_{\text{sulfide}}} \quad \text{(Eq. 9)}$$

where $C_{0\text{sulfide}}$ and $C_{0\text{sulfate}}$ are sulfide and sulfate concentrations at the sediment-water interface, $C_{x\text{sulfide}}$ and $C_{x\text{sulfate}}$ are sulfide and sulfate concentrations at sediment depth $x$ and $D_{\text{sulfide}}$ and $D_{\text{sulfate}}$ are molecular diffusion coefficient of the solutes in seawater ($D_{\text{sulfate}} = 4.3 \times 10^{-5}$ m$^2$ d$^{-1}$, Li and Gregory 1974). With a seawater sulfate concentration of 28 mmol L$^{-1}$ and with sulfide in the water
column and sulfate at large depth are zero, the maximal concentration of sulfide can be 17 mmol L⁻¹. Regarding the high heterogeneity in this habitat, this can be seen as close to what we measured. Thus the three assumptions for the above equation are valid: in gray mat sediments only little or no advective pore water flow occurs, the system is close to steady state and sulfide is not consumed in the anoxic zone. From the shape of the sulfide profile and the above equation it can be concluded that sulfate does not penetrate more than 2 cm. Diffusion as dominant mass transport process in an otherwise advective geosystem can occur e.g. by spatial restricted blockage of fluid upflow by gas hydrates.

However, pore water profiles showed deeper sulfate penetration (Fig. 5p). This is in conflict with the high sulfate reduction and sulfide production rates and the estimated sulfate penetration depth. In diffusive systems maximum sulfate penetration depth ($Z_{\text{sulfate}}$) is controlled by sulfate reduction and can be approximated according to Jørgensen et al. (2004):

$$Z_{\text{sulfate}} = \frac{D_{\text{sed sulfate}} \phi C_{0\text{sulfate}}}{J_{\text{sulfate}}} \quad (\text{Eq. 10})$$

with variables as defined previously and $J_{\text{sulfate}}$ is equal to the areal sulfate reduction rate. Maximum sulfate penetration depth at the averaged depth integrated sulfate reduction rate (59 mmol m⁻² d⁻¹) is approximately 1 cm, which is close to the penetration depth estimated before from the sulfide profile. The discrepancy to the measured pore water concentrations can be explained by mixing of water column sulfate with the gassy sediments during core recovery, which caused the sediments to bubble forcefully due to the pressure loss.

**Biological sulfide oxidation.** Whereas the sulfide flux measurements were variable, oxygen uptake rates were constant. As oxygen and sulfide profiles overlapped, part of the sulfide is oxidized aerobically and is fuelling the mixed thiotrophic mat. Oxidation of sulfide to sulfate with oxygen as terminal electron acceptor occurs in a ratio of 2:1:

$$\text{HS}^- + 2\text{O}_2 \rightarrow \text{SO}_4^{2-} + \text{H}^+ \quad (\text{Eq. 11})$$

Since the measured flux ratio was less than 1:1, the oxygen flux is too small to oxidize sulfide completely to sulfate. Elevated elemental sulfur concentrations in the upper part of the sediment indicate that sulfide is instead oxidized to elemental sulfur (Eq. 12), which can in turn be used either as electron acceptor by sulfate-reducing bacteria or can be further oxidized to sulfate by sulfide-oxidizing bacteria.

$$2\text{HS}^- + \text{O}_2 + 2\text{H}^+ \rightarrow 2\text{S}^0 + 2\text{H}_2\text{O} \quad (\text{Eq. 12})$$
Active nitrate uptake by the gray mat was indicated by the nitrate uptake experiments (Table 5). Since no sulfide leaked from the sediment, the sum of oxygen and nitrate fluxes should approximate the opposing sulfide flux if precipitation or chemical oxidation with iron is insignificant. Assuming an oxidation of sulfide to elemental sulfur, the nitrate flux into the gray mat habitat therefore will be maximally $\frac{1}{4}$ of the sulfide flux (Eq. 2). The morphological diversity and patchy white (sulfur storing filaments) and gray (non sulfur storing filaments) mats hint to a patchwork of sulfide-oxidizing processes in this habitat. The density of motile bacteria that convert nitrate in the anoxic zone is too low to produce a gap between the oxic and the sulfidic zone.

**Potential role of iron in sulfide oxidation.** Sulfide oxidation takes place in the upper centimeter of the sediment (Fig. 8a). Only a very active iron oxidation cycle could supply the system with sufficient iron-oxides to consume the high sulfide fluxes. No elevated concentrations of dithionite or ascorbic acid extractable iron were found in the top centimeter of the sediment. Oxygen penetration depth (0.5 mm) was smaller than the mat thickness (1 mm) and thus all oxygen was consumed within the mat leaving no oxygen for the chemical oxidation of sedimentary iron-sulfides.

If reactive iron minerals had reacted with sulfide, elemental sulfur would have formed over the full depth and not only in the upper centimeter were the thiotrophic community is present. $S^0$ concentrations in the deeper sediment layers were in the same range as in the center site. Although pyrite concentrations were slightly elevated in the gray mat habitat, the isotopic composition does not show an AOM-derived signal for pyrite sulfur. As in the *Beggiatoa* habitat, the $\delta^{34}S$ values imply that pyrite originated from a deep source, and was not recently formed.

For the role of iron-oxides in sulfide oxidation in the gray mats the same arguments as for the *Beggiatoa* habitat hold: geochemical oxidation of sulfide does not limit bacterial primary production. The most important characteristic of this habitat seems to be its heterogeneity concerning sulfate reduction and thus sulfide flux and diffusion as dominant mass transport processes. However, the gray mat habitat covers only a very small fraction of the Håkon Mosby Mud Volcano and may represent pioneer communities in transient AOM hotspots above dissociating gas hydrate.

### 4.3. ‘Less Active’ Center Site

**Sulfide production.** The decreased temperature gradient suggests substantially less fluid upflow in the outer rim NE and S of the ‘active’ center, here referred to as ‘less active’ center, and marked by small elevations in the seafloor bathymetry (Fig. 1; Table 3). Low sulfate reduction rates were measured at the northern site and sulfide was detected in pore water and in situ microsensor measurements in the southern ‘less active’ site. Obviously, decreased fluid upflow in contrast to the ‘active center’ allows some sulfate to penetrate the sediment and enables AOM-driven sulfate reduction to some extent (de Beer et al. 2006).
Chapter 2.5. Primary production at a cold seep

**Geochemical and biological turnover and chemosynthetic biomass production.** In the ‘less active’ center site Fe²⁺ is migrating upwards with the mud volcano fluids with a flux of 0.1 mmol m⁻² d⁻¹ and is vanishing at the interface concurrent with sulfide. As the sulfide flux in this area is substantially lower than in all other habitats (Table 4), chemical immobilization with dissolved iron is significant and removes around 10% of the sulfide (Fig. 10). Precipitation of dissolved iron with isotopically light sulfide derived from AOM is visible in the sulfur isotope distribution (Fig. 4d). The isotope composition of AVS indicates the active involvement of this iron-sulfide pool in the sulfur cycling near the sediment surface. The strong ³⁴S-depletion of AVS in the sediments from the ‘less active’ center reflects the strong fractionation effect of sulfate-reducing bacteria under conditions where sulfate is not limiting (Canfield 2001). Iron-sulfides extracted in the AVS fraction are known to exchange sulfide rapidly with ambient pore water (Fossing and Jørgensen 1989). The continuous enrichment in AVS with depth in these sediments therefore indicates the exchange of iron-bound sulfide with coexisting hydrogen sulfide that becomes more ³⁴S-enriched with depth. Below 5 cm depth FeS showed an isotopic composition as in other habitats and this displays background values from the deep sediment source. No thiotrophic mat was visible on top of the center sediments. Sulfide and oxygen profiles did overlap (Fig. 6b) and 20% of the oxygen flux will be used for oxidation of the remaining sulfide flux. This process might be purely chemical as Lösekann et al. (2007) reported that the oxic part of the center sediment is clearly dominated by methylotrophic γ-proteobacteria (affiliated with *Methylobacter, Methylophaga*). Indeed major oxygen uptake will be by aerobic methanotrophs. Assuming the same growth yields for aerobic methane oxidation as stated above, maximally 0.6 mol C m⁻² yr⁻¹ will be produced. In the mud volcano center without the active parts (115,000 m² (Jerosch et al. 2007) − 10,000 m² active area as estimated from visual observations) maximally 6.1x10⁴ mol C yr⁻¹ will be produced by aerobic methane oxidation. Biomass production due to AOM (calculated from sulfide fluxes) will be as small as 6.7x10² mol C yr⁻¹.

In the ‘active’ center only aerobic methane oxidation occurs. Here in total 1.9 mol m⁻² yr⁻¹ methane are consumed (Niemann et al. 2006). This produces a biomass of 0.6 mol C m⁻² yr⁻¹ or 6x10³ mol C yr⁻¹. In total approximately 6.8x10⁴ mol C yr⁻¹ will be formed in the center habitat of the Håkon Mosby Mud Volcano, mainly by aerobic oxidation of methane. These are rough estimates as the subdivision in ‘active’ and ‘less active’ center is only by sediment surface structure and the in situ energy yield for aerobic methane oxidation has yet not been determined properly.
Fig. 10: Schema of the most important geochemical and microbiological processes in the three investigated areas of the Håkon Mosby Mud Volcano.
5. Conclusions

At the Håkon Mosby Mud Volcano habitats associated with methane-fueled sulfide production > 10 mmol m$^{-2}$ d$^{-1}$ were populated by *Beggiatoa* or thiotrophic bacteria forming gray mats. Geochemical sulfide oxidation with e.g. iron-oxides or manganese-oxides which could reduce the primary production by the thiotrophic bacteria is of minor importance. Fluxes of oxygen and nitrate are sufficient for a complete consumption of sulfide by the thiotrophs, which assimilate a substantial amount of the AOM-derived CO$_2$ for growth. Sulfide availability depends on the upflow regime, which varies on spatial scales of cm to hundreds of meters. Advectorive upflow shapes the *Beggiatoa* habitat; in the gray mat area mass transport is mainly by diffusion resulting in high sulfide concentrations close to the sediment surface. Although methane oxidation coupled to sulfate reduction is mandatory for the sulfide supply, primary productivity in the *Beggiatoa* and gray mat habitat of the Håkon Mosby Mud Volcano is in general dominated by thiotrophic bacteria, and methanotrophs contribute < 20%. Because sulfate is limiting sulfide production in both hotspot habitats, AOM leaves a relatively $^{34}$S-enriched isotope signature in the iron monosulfide pool. Pyrite, however, does not represent a significant sink for sulfide formed at the sediment surface. In the zones of the center area with reduced fluid flow, some sulfate can penetrate into the sediment, which results in some sulfide production by AOM-coupled sulfate reduction. The rates, however, appear too low to support thiotrophic mats and chemical oxidation of the upward directed sulfide flux with oxygen and dissolved iron predominates. Thus in the center of the Håkon Mosby Mud Volcano geochemical sulfide oxidation processes predominate and biomass production is largely limited to direct energy conservation from aerobic and anaerobic methane oxidation.
References:


Chapter 2.6

Methane and sulfide fluxes in permanent anoxia: in situ studies at the Dvurechenskii mud volcano (Sorokin Trough, Black Sea)

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Abstract

Benthic fluxes of methane and sulfide, and the factors controlling transport, consumption and production of both compounds within the sediment were investigated at a cold seep in permanently anoxic waters (Dvurechenskii mud volcano (DMV), 2060 m water depth, Black Sea). The otherwise pie shaped mud volcano showed temperature anomalies as well as solute and gas fluxes indicating high fluid flow at a small elevation north of the geographical center. This is probably the missing source of the previously reported methane flares above the DMV. The anaerobic oxidation of methane (AOM) coupled to sulfate reduction (SR) was excluded from this zone due to fluid-flow induced sulfate limitation. Consequently the biological methane consumption by AOM did not function at this site, hence methane escaped into the water column with a rate of 0.44 mol m\(^{-2}\) d\(^{-1}\). Fluid flow and total methane flux was decreased in the outer center of the mud volcano, correlating with an increase in sulfate penetration into the sediment, and with higher SR and AOM rates. Additional signs of seepage were seen at the edges of the mud volcano. Outside the summit area between 50-70% of the methane flux (0.07-0.1 mol m\(^{2}\) d\(^{-1}\)) was consumed within the upper 10 cm of the sediment. The total amount of dissolved methane released from the DMV into the water column was still significant with a discharge of 1.4x10\(^7\) mol yr\(^{-1}\). The DMV maintains also high areal rates of methane-fueled sulfide production of on average 0.05 mol m\(^{-2}\) d\(^{-1}\). However, we concluded that sulfide and methane emission into the hydrosphere from deep water mud volcanoes does not significantly contribute to the sulfide and methane inventory of the Black Sea.
1. Introduction

Below the chemocline in the permanently anoxic waters of the Black Sea, oxygen, nitrate and most of the reactive iron- and manganese-oxides are depleted. Sulfate is the principle electron acceptor in organic matter mineralization in the water column and the upper seabed (Eq. 1). This leads to an accumulation of sulfide in the anoxic water column to concentrations of up to 370 µmol L⁻¹ (Neretin et al., 2001).

\[
2\text{CH}_2\text{O} + \text{SO}_4^{2-} \rightarrow \text{HS}^- + 2\text{HCO}_3^- + \text{H}^+ \quad \text{(Eq. 1)}
\]

At methane seeps like the Dvurechenskii mud volcano (Bohrmann et al., 2003) or the many gas vented areas of the slope off Crimea (Kruglyakova et al., 2002; Michaelis et al., 2002), another metabolic pathway with significant sulfide production is the anaerobic oxidation of methane (AOM) coupled to sulfate reduction (SR). This process is performed by methane oxidizing archaea and associated sulfate reducing bacteria (Hinrichs et al., 1999; Boetius et al., 2000), which gain energy by the following reaction:

\[
\text{CH}_4 + \text{SO}_4^{2-} \rightarrow \text{HS}^- + \text{HCO}_3^- + \text{H}_2\text{O} \quad \text{(Eq. 2)}
\]

At many active gas and fluid seeps, methane oxidation is limited by the availability of sulfate and oxygen, which must diffuse against an upward pore water flow (de Beer et al., 2006; Niemann et al., 2006). Hence, fluid flow velocities control the amount of methane and sulfide that is consumed or released into the water column, and their fluxes often differ locally depending on the geological structure of the cold seep system.

Here we investigated how spatial variations in fluid flow and the absence of oxygen as an electron acceptor influence fluxes of methane and sulfide from an active mud volcano to the Black Sea hydrosphere. Our target site, the Dvurechenskii mud volcano (DMV), is a large cold seep structure located in the central part of the Sorokin Trough at a water depth of 2060 m. It has a wide flat center with an elliptic shape of 1200 by 800 m diameter, and a steep outer edge with an elevation 80 m above the seafloor. Mud is diapirically rising from a deep source originating in the Maikopian formation (Oligocene-Lower Miocene) (Woodside et al., 1997). Fluids ascend from up to 3 km depth and are formed by thermogenic organic matter and silicate alteration processes, resulting in enrichment in Ba²⁺, I⁻, Cl⁻, Sr²⁺, Li⁺, and ammonium. The fluids are depleted in sulfide and sulfate and have a higher salinity than seawater. Particulate organic matter content in surface sediments is with 2-6 wt.% relatively high at the seaﬂoor, decreasing with sediment depth (Aloisi et al., 2004; Wallmann et al., 2006). Temperature anomalies in the water column and in the sediments of the DMV indicated areas with increased fluid and/or mud upflow (Bohrmann et al., 2003). The distribution of pore water
components in retrieved cores could be fitted to a transport model assuming upward flow velocities from 0.08 to 0.25 m yr⁻¹, with highest upflow in the central part of the mud volcano, decreasing towards the edge (Aloisi et al., 2004; Wallmann et al., 2006). Methane oxidation rates were also highest in the central part, and the model predicted a consumption of up to 80% of the average methane flux (Wallmann et al., 2006). Gas flares extended up to 1300 m into the water column above the DMV (Greinert et al., 2006), but their source was not located. The expelled gas was mainly composed of methane with low amounts of ethane and propane. Previous investigations from other seep structures have shown that modeling of ex situ pore water gradients tends to underestimate fluid flow rates, and that the relationship between fluid flow and methane consumption is not linear (de Beer et al., 2006; Niemann et al., 2006). In this study we revisited the DMV to investigate the spatial variation of fluid flow, methane and sulfide fluxes as well as associated biogeochemical processes using in situ methods. We aimed to find the hot spot of the DMV with regard to methane emission. We further tested the hypotheses that 1) fluid flow, methane efflux and consumption decrease radially from the source to the outer edge, 2) that methane consumption and sulfide production are controlled by fluid flow and 3) that mud volcanoes are a significant source for methane and sulfide to the Black Sea hydrosphere.

2. Material and methods

2.1. Sampling

The DMV was visited during the RV Meteor M72/2 cruise in February/March 2007. High quality videographic observation at this site was accomplished using the cameras of the remotely operated vehicle (ROV) Quest 4000 (MARUM, Bremen), and recording of high resolution topographic maps was carried out with the sonar of the research vessel (Fig. 1). In a small area (N 44° 17.03'; E 34° 58.88') north of the geographical center of the mud volcano the multibeam maps showed an elevation of the seafloor of around 2-3 m above the surrounding area. Here temperature loggers mounted on the ROV frame recorded a temperature anomaly of +0.014 °C in the bottom water (Feseker et al., in press). Elevated water temperature is a reliable indicator for discharge of warm fluids, hence we planned sampling transects from the DMV summit as an area of increased fluid seepage to the outer edge of the mud volcano. Sediments were sampled from the summit (St. 1), the geographical center (St. 2), the edge (St. 3, St. 8), and sites north and south of the DMV (St. 9, St. 10), with short sediment cores (push cores: 10-20 cm sediment length) operated by the ROV manipulator, with a TV-guided multiple corer (TV-MUC, 20-40 cm sediment core length) equipped with a POSIDONIA positioning system or with a gravity corer (up to 4 m sediment length). Sampling locations are displayed in Fig. 1 and samples were labeled according to the international geoscience database PANGAEA (Table 1).
In situ microsensor measurements and flux calculations

High resolution geochemical gradients were measured in situ with a deep sea microprofiler (Fig. 2a), a similar unit as mounted on free-falling lander (Gundersen and Jørgensen, 1990; Wenzhöfer et al., 2000). However, instead of recording one set of profiles, the program could be restarted by a button (activated by the ROV arm), allowing multiple recordings per deployment (Treude et al., 2009). For measurements the profiler was precisely positioned at the seafloor and profiling was started with the ROV manipulator. The microprofiler was equipped with three H₂S sensors, two pH sensors, one N₂O sensor and one 3 mm thick temperature sensor (PT 100, Umweltsensorentechnik GmbH, Germany) (Jeroschewski et al., 1996; de Beer et al. 1997, Andersen et al., 2001). The sensors were calibrated at in situ temperature as described previously (Wenzhöfer et al., 2000; de Beer et al., 2006). The results from the H₂S sensors were converted to total sulfide concentrations with a $pK_1$ for sulfide in seawater after Goldhaber and Kaplan (1975) and will further be referred to as sulfide ($= H_2S+HS^-+S^{2-}$).

Eight microprofile measurements were done along a transect from the geographical center (St. 2, in the ‘outer center’) as the most southwards measuring point towards the northern edge (St. 3) (Fig. 1). Here the profiler was deployed as close as possible (10 cm) to a crack in the sediment that was filled with white matter (further referred to as ‘white patches’). The transect crossed the summit twice (St.
1a, b). Between the summit and the station at the northern edge, four other stations (St. 4-7, ‘outer center’) were sampled along the transect.

Diffusive sediment-water exchange fluxes of sulfide were calculated from the gradients in the diffusive boundary layer (DBL) using Fick’s first law of diffusion:

\[
J_{\text{diff}} = D \frac{dc}{dz} \quad \text{(Eq. 3)},
\]

where \( J_{\text{diff}} \) = diffusive flux \([\text{mmol m}^{-2} \text{d}^{-1}]\), \( D \) = diffusion coefficient in water \([\text{m}^2 \text{d}^{-1}]\) corrected for temperature and salinity (Li and Gregory, 1974) and \( \frac{dc}{dz} \) = concentration gradient \([\text{mmol m}^{-3} \text{m}^{-1}]\). A diffusion coefficient \( D \) for HS\(^{-}\) of 1.05x10\(^{-4}\) \text{m}^2 \text{d}^{-1} \) was used. Fluxes in the sediment were calculated from the gradients:

\[
J_{\text{sed}\, \text{diff}} = \phi D_{\text{sed}} \frac{dc}{dz} \quad \text{(Eq. 4)},
\]

where \( \phi \) = porosity and \( D_{\text{sed}} \) = diffusion coefficient in the sediment \([\text{m}^2 \text{d}^{-1}]\). The diffusion coefficient \( D_{\text{sed}} \) in the sediment was calculated according to Iversen and Jørgensen (1993):

\[
D_{\text{sed}} = \frac{D}{1 + 3(1 - \phi)} \quad \text{(Eq. 5)}
\]

2.3. **Benthic chamber measurements**

A benthic chamber module constructed for the deployment by an ROV as described in Glud et al. (2009) was positioned at St. 1 on the DMV summit (Fig. 2b). Subsamples of the water enclosed in the chamber were taken with syringes after certain time intervals. The total deployment time of the benthic chamber was 8 hours. After retrieval of the chamber module, subsamples of each water sample were immediately fixed in ZnAc (2%) for sulfate and chloride analyses (1 mL sample in 0.5 mL ZnAc), stored in sealed glass vials for methane concentration measurements, or frozen for nutrient analyses. Nutrients were measured with a Skalar Continuous-Flow Analyzer according to the method of Grasshoff et al. (1983). Concentrations of chloride and sulfate were measured with non-suppressed ion-chromatography (Waters 510 HPLC Pump; Waters IC-Pak 50 x 4.6 mm anion exchange column; Waters 430 Conductivity detector). Methane was measured in the headspace of the glass vial after heating to 60 °C for 30 minutes with a gas chromatograph (5890A, Hewlett Packard) coupled to a flame ionization detector (HP 5890 GC-FID). A GFT-Poropak-Q column (6 ft., 80/100) was installed for the chromatographic separation using the following temperature program: initial oven temperature:
40 °C, hold for 1 minute, heating rate of 20 °C min⁻¹ to 200 °C (1 minute). The precision of the gas chromatography measurements was ±5%.

2.4. **Bottom water and sediment pore water analyses**

Seawater was sampled at defined spots 10 cm above the seafloor with the ROV fluid sampling system KIPS (Schmidt et al., 2007). The samples were enclosed in PFA (perfluoralkoxy) flasks, where they stayed during the dive. The pH was measured immediately after retrieval of the water samples. Subsamples for nutrient concentrations analyses were treated and measured as described above.

For analyses of sediment solutes either push cores or TV-MUC cores were taken north and south of the DMV (St. 9, St. 10) and close to the microprofiler stations at the summit (St. 1), the geographical center (St. 2) and the northern and the western edge with the conspicuous white patches on the seafloor (St. 3, St. 8). For methane analyses sediments were sampled with a gravity corer at the same stations. Pore water was extracted from the sediment with Rhizons (type: CSS, Rhizosphere Research Products, filter capacity of 0.1 µm). To allow Rhizon insertion, holes were drilled in the cores at depth intervals of 1 cm and sealed with diffusion-tight PVC tape before sampling. The Rhizons were horizontally inserted into the sediment through the tape covering the predrilled holes and pore water was extracted gently with a syringe. Pore water subsamples for dissolved inorganic carbon (DIC) were quickly transferred into glass vials and poisoned with HgCl₂ for concentration measurements or sterile filtered for the determination of the DIC isotopic composition. Further subsamples (1 mL) were transferred into plastic vials with 0.5 mL ZnAc (2%) for sulfate, chloride and total sulfide determination. Sulfate and chloride were measured as described above. The photometric methylene blue method (Cline, 1969) was used to measure the sulfide concentration (= H₂S+HS⁻+S²⁻). DIC concentrations were measured with a conductivity detector (VWR scientific, model 1054) with 30 mmol L⁻¹ HCl and 10 mmol L⁻¹ NaOH as eluent according to the method of Hall and Aller (1992).

For methane concentration measurements 3 mL wet sediment samples were taken in cut off syringes, extruded into 20 mL vials and closed gas tight. 100 µL subsamples were taken from the gas phase with gas tight syringes and measured as described above. For onshore isotopic analysis headspace gas (5 mL) was transferred into vials filled with saturated NaCl-solution using gas tight syringes and volume-volume exchange with hypodermic needles, and stored up-side-down.

The DIC isotopic composition was measured with a GasBench automated sampler, interfaced to a Finnigan MAT 251 mass spectrometer. Prior to analysis a pore water aliquot (~0.5 to 1 mL) was flushed with helium and subsequently acidified with phosphoric acid to allow CO₂ extraction. Analyses were calibrated with a known standard of defined isotopic value, primary standardization occurred by tank CO₂. Standard deviation was estimated to be less than 0.1‰. The stable carbon isotopic compositions of methane was analyzed by gas chromatography-isotope ratio mass spectrometry using a Thermo Finnigan Trace GC ultra coupled to a Thermo Finnigan Deltaplus XP mass spectrometer via a Thermo Finnigan GC Combustion III interface. The GC was equipped with a
Supelco Carboxen 1006 Plot fused-silica capillary column (30 m x 0.32 mm in diameter). The initial oven temperature was set to 40 °C, held for 4 minute, raised by 20 to 50 °C min\(^{-1}\) to 240 °C, held for 1 minute. Standard deviations were usually less than 1‰. Isotope ratios are given in δ-notation relative to the Vienna Peedee Belemnite Standard (V-PDB).

2.5. **Sulfate reduction (SR) and anaerobic methane oxidation (AOM)**

Rate measurements of anaerobic methane oxidation and sulfate reduction were done on retrieved cores with the whole core injection method. Samples were digested and analyzed as described before (Jørgensen, 1978; Treude et al., 2003; Kallmeyer et al., 2004). For sulfate reduction rate measurements 10 µL \(^{35}\text{SO}_4^{2-}\) (75 kBq activity in water) and for anaerobic methane oxidation rate measurements 20 µL \(^{14}\text{CH}_4\) (1.4 kBq activity in water) were injected. Measurements were carried out on sediment samples from the summit (St. 1), the geographical center (St. 2), the western edge in an area with white patches on top of the sediment (St. 8), as well as north (St. 9) and south (St. 10) of the DMV.

2.6. **Acridine orange direct counts (AODC)**

Sediments were sliced in 1 cm intervals, fixed in formalin/seawater and the total number of microbial cells present in 1 cm\(^3\) sediment was determined with acridine orange direct counts (AODC) via epifluorescence microscopy (Meyer-Reil, 1983; Boetius and Lochte, 1996). Cell counts include only single cell and no aggregates.
Table 1: Sampling and analysis details from the different areas of the Dvurechenskii mud volcano (DMV). Samples and in situ instruments are labeled as in the PANGAEA database (http://www.pangaea.de). SR: sulfate reduction, AOM: anaerobic methane oxidation, AODC: acridine orange direct counts, DIC isotopes: \( \delta^{13}C \) of dissolved inorganic carbon.

<table>
<thead>
<tr>
<th>Station</th>
<th>Mud volcano area</th>
<th>Coordinates</th>
<th>Measurement and PANGAEA event label</th>
</tr>
</thead>
<tbody>
<tr>
<td>St. 1a, St. 1b</td>
<td>Summit</td>
<td>44° 17.03' N, 34° 58.88' E</td>
<td>Microsensor measurements (M72/2_309_MICP-1, MICP-8), benthic chamber deployment (M72/2_319_CHAM-1), SR, AOM (M72/2_309_PUC-68, -36), Geochemical analyses (M72/2_309_PUC-51, -36), M72/2_319_PUC-2, M72/2_310), AODC (M72/2_309_PUC-36),</td>
</tr>
<tr>
<td>St. 2</td>
<td>Outer center: geographical center of the DMV</td>
<td>44° 16.95' N, 34° 58.94' E</td>
<td>Microsensor measurements (M72/2_309_MICP-2), SR, AOM (M72/2_270), Geochemical analyses (M72/2_269, M72/2_300), AODC (M72/2_270),</td>
</tr>
<tr>
<td>St. 3</td>
<td>Northern edge</td>
<td>44° 17.14' N, 34° 58.84' E</td>
<td>Microsensor measurements (M72/2_309_MICP-3), Geochemical analyses (M72/2_319_PUC-43)</td>
</tr>
<tr>
<td>St. 4</td>
<td>Outer center</td>
<td>44° 17.12' N, 34° 58.85' E</td>
<td>Microsensor measurements (M72/2_309_MICP-4)</td>
</tr>
<tr>
<td>St. 5</td>
<td>Outer center</td>
<td>44° 17.10' N, 34° 58.86' E</td>
<td>Microsensor measurements (M72/2_309_MICP-5)</td>
</tr>
<tr>
<td>St. 6</td>
<td>Outer center</td>
<td>44° 17.06' N, 34° 58.87' E</td>
<td>Microsensor measurements (M72/2_309_MICP-6)</td>
</tr>
<tr>
<td>St. 7</td>
<td>Outer center</td>
<td>44° 17.04' N, 34° 58.88' E</td>
<td>Microsensor measurements (M72/2_309_MICP-7)</td>
</tr>
<tr>
<td>St. 8</td>
<td>Western edge</td>
<td>44° 16.97' N, 34° 58.59' E</td>
<td>SR, AOM (M72/2_282_PUC-26, -67), Geochemical analyses (M72/2_282_PUC-27)</td>
</tr>
<tr>
<td>St. 9</td>
<td>North of DMV</td>
<td>44° 17.26' N, 34° 58.98' E</td>
<td>SR, AOM (M72/2_280), Geochemical analyses (M72/2_279, M72/2_314), AODC (M72/2_280)</td>
</tr>
<tr>
<td>St. 10</td>
<td>South of DMV</td>
<td>44° 16.58' N, 34° 58.64' E</td>
<td>SR, AOM (M72/2_291), Geochemical analyses (M72/2_291, M82/2_306) AODC (M72/2_291)</td>
</tr>
</tbody>
</table>
3. Results

3.1. Site and sediment description

The videotransects showed spatial differences in the surface morphology of the DMV (Fig. 2). At the geographical center (St. 2) the sediment surface was uneven with small hills (maximal heights of 20 cm). A beige-brown fluffy layer of sedimented detritus of a thickness of about 1 cm was visible on top of the sediment. At the summit (St. 1) the sediment surface was even more hummocky, and ripple-like structures with a wavelength of approximately 50 cm were present. Here, no fluff layer was observed on top of the seafloor. At all other sites around the inner center (St. 4 - St. 7) the seafloor was flat and the fluffy layer was covering the seafloor completely. At the northern edge (St. 3) and the western edge (St. 8) the thickness of the fluffy layer increased to 2 cm. Here, the sediment surface was again very hummocky and many cracks in the seafloor were observed. Sediments retrieved from the site north of the DMV (St. 9) were covered with up to 7 cm of the fluff. Here, no ROV dives were carried out.

The material forming the fluffy layer comprises deposits of marine snow often found on top of Black Sea sediments. It was stratified with interspersed brown-green layers separating about 1 mm thick whitish zones representing the sedimentation of the coccolithophorid blooms. Sediment cores recovered from the DMV were homogeneous over the whole core length, with a dark, olive green sediment color, a fine grained sediment texture and the presence of gas bubbles from methane oversaturation after retrieval. In the inner center of the DMV (between St. 1, 2, 6 and 7) gas bubbles were released from the sediment upon touching the seafloor with our instruments and samplers (Fig. 2e), indicating that methane was oversaturated in situ, at a depth of 2060 m. This is related to the high in situ methane concentrations of 118 mol L$^{-1}$ in the warm summit and close-by sediments (Feseker et al., in press). A lower methane concentration of 85 mmol L$^{-1}$ was estimated for the DMV for the presence of methane hydrates in equilibrium with pore water in the outer center area with lower in situ temperatures (Wallmann et al., 2006). At the northern, western and southern edge of the DMV video observation showed white patches on the seafloor (Fig. 2f). Microscopy of recovered samples from these white patches showed needle shaped minerals (possibly barite). The white material did not contain sulfide-oxidizing bacteria, which are usually forming conspicuous whitish mats at cold seeps that reside in oxic and suboxic bottom waters.
Fig. 2: a) Microprofiler measurements, b) benthic chamber module deployment and c) sediment sampling at the summit lacking the fluffy layer on top of the sediment; d) sediment sampling at the outer center with a thick fluffy layer on top of the sediment, e) gas bubble escape during coring at the geographical center and f) white patch at the western edge (diameter of about 2 m).
3.2. In situ microsensor measurements and flux calculations

3.2.1 Temperature

With our microprofiler the highest sediment temperature gradients of up to 10.2 °C m⁻¹ were found at the summit of the DMV (St. 1; Fig. 3b, c). Here the temperature in the water column was also slightly elevated (+0.1 °C, 5 cm above the sediment). At St. 7 the temperature gradient was already reduced to 15% of the value at the summit (Fig. 3d) and declined further towards the northern edge. The temperature gradient at the geographical center (St. 2) was 1.2 °C m⁻¹, in between those of St. 6 and St. 5. At the outer edge (Fig. 3h) the gradient was only 2% of that of the summit. Sediment temperature gradients are summarized in Table 2.

3.2.2 Sulfide

Sulfide concentrations were the lowest in sediments of the summit of the DMV (St. 1, Fig. 3). The stations at the edge (St. 3) and outer center (St. 2, 4-7) showed elevated concentrations of sulfide in the sediment indicating local production. Neither the sulfide depth distributions, nor the concentration maxima of sulfide showed a gradual succession with distance from the summit, in contrast to the temperature gradients. Interestingly, at most sites two sulfide peaks were found in the sediment: one at the sediment-water interface and a second one at 3 - 15 cm bsf (below surface). Below the second concentration maximum, the sulfide steadily decreased with sediment depth. The peaks indicate zones of net production separated by a sulfide sink. From the sum of the diffusive fluxes below and above these zones, an average volumetric consumption rate of 1,300 mmol m⁻³ d⁻¹ was calculated from profiles with dips. The time needed for leveling of the two concentration maxima into a straight line by diffusion, was modeled using a 1-dimensional Comsol-Multiphysical modeling suite.

Sulfide fluxes are summarized in Table 2 and are displayed as (I) fluxes calculated from the DBL and thus representing the sulfide that is released from the sediment into the water column and (II) upward directed sulfide fluxes of the second, deeper located sulfide peak. The DBL sulfide fluxes (I) showed an increase along the transect from the summit to the northern edge. The sulfide fluxes calculated from the gradients in the subsurface sediment (II) were substantially lower with lowest fluxes at the summit, intermediate fluxes in the outer center area and highest fluxes at the edge.

Below the lower sulfide peak downward diffusion equals upward advection, in steady state. Fluid upflow velocities can then be calculated from the shape of the sulfide profiles below the peak (de Beer et al., 2006):

\[
    c_x = c_0 e^{\frac{-v x}{D_{sed}}} \quad (Eq. 6),
\]

where \(c_x\) is the solute concentration at sediment depth \(x\), \(c_0\) the concentration at the source (in this case the maximal concentration of the lower sulfide peak), \(v\) is the upflow velocity (m yr⁻¹) and all
other variables as described before. Modeling of the fluid upflow velocities from sulfide profiles was possible at St. 1 a, b, St. 2, and St. 3. The upflow velocities obtained by this modeling approach are displayed in Table 2. To estimate the effect of fluid flow on the sulfide fluxes, which in Table 2 are displayed as diffusive fluxes \( J_{\text{diff}} \), the total flux \( J_{\text{tot}} \) was calculated as described previously (de Beer et al., 2006):

\[
J_{\text{tot}} = J_{\text{diff}} + J_{\text{adv}} = D \frac{dc}{dx} + vc \tag{Eq. 7},
\]

where \( J_{\text{adv}} \) is the advective flux and \( c \) is the local concentration of the solute. The interfacial fluxes appeared to be hardly influenced by the upflow velocities obtained with Equation 6. Less than 5% of the total sulfide flux across the DBL was due to the advective term. Depending on the upflow velocity used in Equation 7, the efflux from the second peak was 40-90% higher at the summit, and 25% higher at the geographical center. As this is in the range of the standard deviations of the measurements, in the further discussion the diffusive fluxes are considered accurate.

3.2.3. pH

At one deployment at the DMV summit (St. 1a) both pH sensors recorded a very distinct profile with a dip right below the sediment surface followed by a peak at 3.5 cm bsf, and a gradual decline below this depth (Fig. 3b). The replicate measurement at the summit (St. 1b, about 15 m away from the first one), showed a gradual decline of pH (Fig. 3c). At the outer center area north of the summit the pH was more or less constant with depth (Fig. 3d-g). The pH profiles at the edge (Fig. 3h) showed a steady decrease with depth with a similar profile as in St. 1b. At the geographical center (St. 2, outer center) there was a clear and strong peak in pH at a depth of 2 cm (Fig. 3a).

3.2.4. \( N_2O \)

The signal of the \( N_2O \) sensor (data not displayed) showed a strong increase with depth at the summit and at the edge, but not in the stations in between. As no \( N_2O \) can be present in the sediments of the DMV, the sensor must have responded to another gaseous species. In laboratory experiments, the sensor showed a response to sulfide, methanethiol but not to dimethyl sulfide. At the summit, where the \( N_2O \) sensor showed a response, sulfide was absent, so sulfide cannot have caused the strong signal of the \( N_2O \) sensor. Although it is unknown what substance the sensor has measured, the locations suggest release of a very labile, possibly oxidizing, compound in seepage areas of the DMV.
Fig. 3: a-h) High resolution in situ microsensor profiles of sulfide ($H_2S + HS^- + S^{2-}$), pH and temperature. Graphs are arranged in geographical order from most southwards station (outer center, geographical center, St. 2), crossing the summit (St. 1a, 1b), the outer center (St. 7-4) and ending at the northern edge (St. 3). Sulfide profiles from St. 1 to St. 6 are averages of results from three different sensors. At St. 7 only two sensors worked. Except for St. 1 only one of the two pH sensors was functional.
3.3. **Benthic chamber measurements**

The summit of the DMV emits large amounts of methane, ammonium and chloride, as indicated by their concentration increase with time in the benthic chamber (Fig. 4; Table 3). Using the effluxes and pore water concentrations of these solutes, an upflow velocity of the mud volcano fluid of 1-3 m yr\(^{-1}\) was estimated, assuming no consumption of the solutes in the sediment (Table 3).

Table 3: Results from the 8 hour deployment of the benthic chamber at the summit of the DMV (St. 1). The upflow was calculated from the flux of solutes into the chamber and the solute fluid concentrations. *Methane concentration at the summit at equilibrium at ambient salinity, temperature and pressure as in Feseker et al. (in press); it was assumed that no methane was consumed in the summit sediments (see discussion); ** ammonium concentration of the fluid as in Aloisi et al. (2004).

<table>
<thead>
<tr>
<th></th>
<th>methane</th>
<th>ammonium</th>
<th>chloride</th>
</tr>
</thead>
<tbody>
<tr>
<td>total emission (mmol m(^{-2}) d(^{-1}))</td>
<td>440</td>
<td>40</td>
<td>6.6 x10(^3)</td>
</tr>
<tr>
<td>concentration in mud volcano fluid (mmol L(^{-1}))</td>
<td>118*</td>
<td>20**</td>
<td>810</td>
</tr>
<tr>
<td>upflow (m yr(^{-1}))</td>
<td>1.2</td>
<td>0.7</td>
<td>2.8</td>
</tr>
</tbody>
</table>

Fig. 4: Results from analyses of a) methane, b) ammonium and c) chloride in water collected inside the benthic chamber during the 8 hour deployment at the summit. Fluxes are calculated from the linear increase of the compounds inside the chamber.
3.4. **Geochemistry of bottom water and pore water**

The pH of the Black Sea water at 10 cm above the seafloor of the DMV was 8.0. No nitrate or nitrite was found. Ammonium was equally high in the water above the summit and the geographical center. At the edge ammonium values were reduced to half of the amount of the other sites.

Solute distributions in recovered sediment cores differed substantially between the sampling stations. In the core from the summit (St. 1) a strong outgassing was observed after retrieval. In this core sulfate was detected to a depth of up to 10 cm after retrieval (Fig. 5a). In the less gaseous geographical center (St. 2) sulfate was found only to a depth of 5 cm (Fig. 5g). From the rather variable data it is clear that the pore water in the mud volcano is depleted in sulfate, and is elevated in chloride and DIC (Fig. 5). Outside the volcano (St. 9) these concentrations were almost constant, and close to seawater values. The sulfide concentrations (Fig. 5c, i, o, s) were always lower than as measured in situ with the microprofiler, probably an effect of degassing during retrieval of the sediment cores. Methane concentrations after retrieval exceeded atmospheric saturation levels in all cores from the DMV (data not shown). The δ13C signature of methane retrieved from the summit of the DMV was around -60‰ V-PDB. The DIC carbon isotope signature was similar over the whole sediment core (+8‰ V-PDB, Fig. 5d), and the same as found in several meters depth (data from gravity core 310, not shown), thus conversion of methane to DIC is slow at the summit compared to the transport of DIC from the subsurface. At the geographical center the DIC concentration increased to maximally 17 mmol L-1 in 10 cm depth. Here the lowest DIC δ13C value (-30‰ V-PDB) was found 2 cm below the sediment surface, and the signature became less depleted with depth (Fig. 5j), thus part of the DIC originated from AOM. Also at the edge stations the DIC was increased (Fig. 5p, t). At the reference site (St. 9) outside the DMV the δ13C signature was constant over the upper 10 sediment depth (Fig. 5z), and methane concentrations were < 0.1 mmol L-1 in the top 100 cm (data not shown).

3.5. **Rates of sulfate reduction (SR) and anaerobic oxidation of methane (AOM)**

Depth integrated rates (0-10 cm) of SR and AOM are summarized in Table 2. At the summit (St. 1, Fig. 5e) SR and AOM were very low in the upper 10 cm, but above detection limit. In the geographical center of the DMV (St. 2, Fig. 5k) SR reached 20 mmol m⁻² d⁻¹. The highest SR was detected directly at the seafloor-water interface, where it exceeded AOM significantly. Below the first cm, AOM and SR were similar. AOM rates were similar also at the western edge (St. 8), but SR exceeded AOM considerably (Fig. 5u). All sites except the summit showed considerably higher SR and AOM rates than the sites outside the DMV (Table 2, St. 9, St. 10, Fig. 5aa; St. 10, not included in Fig. 5). Outside the mud volcano, integrated SR rates were only 0.2-1% of those in the outer center, but slightly higher than those of the summit.
# Table 2: Results from in situ microsensor measurements and ex situ AOM and SR rate measurements. For the sulfide fluxes averages are given with standard deviation (SD).

<table>
<thead>
<tr>
<th>Soil Depth</th>
<th>Sulfide Flux</th>
<th>AOM Integrated</th>
<th>SR Integrated</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2 ± 0.1</td>
<td>10.0 ± 0.2</td>
<td>12.0 ± 0.3</td>
<td>14.0 ± 0.4</td>
</tr>
<tr>
<td>0.2 ± 0.1</td>
<td>8.0 ± 0.2</td>
<td>9.0 ± 0.3</td>
<td>10.0 ± 0.4</td>
</tr>
<tr>
<td>0.2 ± 0.1</td>
<td>3.0 ± 0.2</td>
<td>4.0 ± 0.3</td>
<td>5.0 ± 0.4</td>
</tr>
</tbody>
</table>

(Units: mol m⁻² d⁻¹)

**Notes:**
- nd = not determined.
3.6. **AODC counts**

Cell counts from the summit showed an unusual depletion in cells in the first 10 cm of the sediment with values of on average $10^7$ cells per cm$^3$ sediment (Fig. 5f). This is one to two orders of magnitude lower than in the same sediment depth at the sites north and south of the DMV (Fig. 5 ab). Below the depth of 10 cm the amount of cells in sediments from the summit increased to $10^9$ cells per cm$^3$ sediment. At the geographical center the number decreased with depth from $10^9$ cells per cm$^3$.
sediment at the sediment surface to $10^7$ cells per cm$^3$ sediment in 18 cm depth (Fig. 5l). Numbers of cells were constantly high over the upper 10 cm at the western edge (St. 8). These cell counts always comprise only single cells and not aggregated microbial cells.

4. **Discussion**

4.1. **Patterns of fluid flow and methane flux**

The Dvurechenskii mud volcano has the shape of a flat mud pie with a steep outer edge, and consists of a warm inner mud and fluid flow channel with a decreasing temperature gradient to the outside (Feseker et al., in press). Previous ex situ biogeochemical measurements at the DMV indicated a radial zonation of the DMV with highest fluid upflow and most intense anaerobic oxidation of methane in the central part, gradually decreasing towards the outer zone (Wallmann et al., 2006). During our investigations we studied the geochemical structure of the DMV with in situ tools to circumvent artifacts during recovery such as disturbances by degassing of cores. The in situ analyses and higher sampling resolution of our study revealed a more complex distribution and relationship of fluid flow and microbial methane and sulfur turnover than previously observed. This may reflect temporal and spatial changes in volcanic activity or a more precise picture of consistent local patterns.

Combining detailed bathymetry and temperature probing, we found a hotspot of fluid flow and mud transport, associated with a small elevation (summit) north of the geographical center of the DMV, characterized by a high methane emission and low microbial methane consumption. This is most likely the source of the flare previously reported (Greinert et al., 2006), as we observed intensive gas ebullition after local disturbances of the seafloor during sampling. This hot spot area was not found before and thus was not investigated geochemically. Here, a fluid upflow velocity of 0.6-0.9 m yr$^{-1}$ was calculated from in situ microsensor measurements. From benthic chamber measurements the fluid flow was found to be 0.7-2.8 m yr$^{-1}$, so of the same order of magnitude. At the geographical center (St. 2) and outer edge (St. 3) we calculated from the lower peak of the in situ sulfide profile a fluid flow of 0.3 m yr$^{-1}$, similar to velocities previously observed in this area (Aloisi et al., 2004; Wallmann et al., 2006). In the summit area very low SR and AOM rates were found while outside the summit area, we measured considerable SR and AOM rates. However, the AOM and SR rates did not follow the radially decreasing temperature gradients (Fig. 6). Although the fluid upflow velocity was similar as in the outer center, the temperature gradient was substantially lower at the edge than in all other zones of the DMV (Table 2). This may indicate different sources for warm and cold mud volcano fluids. Differences in sources could also explain the white patches on top of the sediment of the outer edge, which consist of mineral precipitations (presumably barite) and not of bacterial mats as previously thought (Bohrmann et al., 2003). Alternatively, the water seeping out at the edge has lost more heat than the water seeping out of the center, because of longer pathways or because it was cooled by methane hydrates (Feseker et al., in press).
4.2. Factors controlling methane consumption and sulfide production

Most mud volcanoes release methane in dissolved and gaseous phases. At the DMV we did not find active gas vents, but observed the release of gaseous methane upon disturbances of the seafloor such as the touch down of the ROV or coring (Fig. 2e). Measuring emission of dissolved methane in situ with the benthic chamber at the summit of the DMV resulted in estimates of a methane seepage rate of 440 mmol m$^{-2}$ d$^{-1}$. This is in the upper range of methane emission rates reported from other cold seeps, e.g. 15 mmol m$^{-2}$ d$^{-1}$ off the coast of Costa Rica (Mau et al., 2006), or maximally 100 mmol m$^{-2}$ d$^{-1}$ at Hydrate Ridge (Torres et al., 2002). Consumption of methane was virtually absent in the upper 15 cm of the summit sediment (Fig. 5e). The constant $\delta^{13}$C DIC values with depth and low DIC pore water concentrations (Fig. 5d) confirm that no methane oxidation occurred in the sediment. In the more peripheral areas of the DMV, sulfate reduction and AOM reached around 10-100 mmol m$^{-2}$ d$^{-1}$, similar to the range previously reported from the DMV (Wallmann et al. 2006), or from other mud volcanoes like the Håkon Mosby Mud Volcano (Niemann et al., 2006) and the Kazan Mud Volcano (Haese et al., 2003). The discrepancy between sulfate reduction and methane oxidation rates (Table 2,
Fig. 5) can be caused by the high patchiness of the sedimentary processes as observed from the in situ sulfide profiles.

Two factors may be responsible for the low AOM rates despite high methane concentrations at the summit. A striking difference with other methane seeps is the low number of cells in the upper 10 cm of the summit sediment, of on average $10^7$ cells per cm$^3$ (Fig. 5f). As a comparison, the sediments of the active Håkon Mosby Mud Volcano area and sediments of Hydrate Ridge contain up to $10^9$-$10^{10}$ cells per cm$^3$ (Knittel et al., 2003; Lösekann et al., 2007). At the reference site north of the DMV 50 times more cells were present than in the upper 10 cm of the summit sediment (Fig. 5 ab). Such a cell number anomaly could have been caused by the extrusion of a ‘microbe-depleted’ subsurface mud package that flew over the former seafloor, diluting out the slowly growing anaerobic methane oxidizers. Fresh mud flow at certain areas of the DMV was proposed earlier (Bohrmann et al., 2003; Aloisi et al., 2004; Wallmann et al., 2006) and also explains the absence of the fluffy layer on top of the seafloor at the summit. Consequently, sulfate reduction was even lower than at the reference station outside the DMV, where the contribution of organoclastic sulfate reduction was around 0.2 mmol m$^{-2}$ d$^{-1}$.

An additional factor explaining the low AOM rates at high methane flux could be sulfate limitation by high upward flow of sulfate-depleted fluids, preventing diffusion of seawater sulfate into the seafloor (de Beer et al., 2006; Niemann et al., 2006). At the high fluid upflow velocity as calculated for the summit (Table 2, 3) in situ sulfate penetration would be restricted to the upper few centimeter of the sediment, hence limiting AOM. High rates of AOM and a clear geochemical signature of DIC production from AOM was observed in the sediments of the DMV around the summit, which are subjected to lower upflow velocities of around 0.3 m yr$^{-1}$. Accordingly, DIC concentrations increased considerably with depth and, associated with the peak in methane oxidation, DIC carbon showed the lowest $\delta^{13}$C values at 2 cm bsf ($\delta^{13}$C DIC: -30‰ V-PDB). This value comprises the isotopic signature of the DIC of the advected mud volcano fluids ($+8$‰ V-PDB), the DIC diffusing into the sediment from the overlying seawater (Black Sea seawater $\delta^{13}$C DIC: +0.8‰ V-PDB, (Fry et al., 1991)), and the $^{13}$C depleted DIC produced in the sediment from methane oxidation ($\delta^{13}$C-methane: -60‰ V-PDB).

As the diffusion coefficient of sulfate is around 1.7 times lower than that of sulfide, the maximal sulfide concentration at steady state conditions, under diffusional transport and complete sulfate conversion without re-oxidation, can reach 10 mmol L$^{-1}$ in Black Sea sediments (maximal sulfate concentration is 17 mmol L$^{-1}$). This value was approximated in some profiles of the DMV outer center, and the fluid upflow or the sulfide concentration might be an overestimation here. Interestingly, the sediment depths of the sulfide peaks are different in all center sites and this implies irregularities in the fluid flow across the mud volcano surface, and disturbances by mud flow.

The concentration minima in the sulfide profiles are highly unusual and difficult to explain. Minima between peaks in the sulfide profiles indicate a sink for sulfide, by oxidation or precipitation.
Oxygen and nitrate are absent below the chemocline of the Black Sea. However, atypical for Black Sea sediments, about 20-80 $\mu$mol cm$^{-3}$ amorphous and crystalline Fe(III)oxides or iron silicates and up to 150 $\mu$mol L$^{-1}$ dissolved iron were detected in the summit (data not shown) and might be responsible for the sulfide concentration minimum. Outside the summit no dissolved iron was found, concentrations of Fe(III)oxides were much lower and sulfide fluxes significantly higher. However, also here sulfide concentration minima were observed (St. 4-7). There is no dissolved iron and not enough Fe(III) to sustain the volumetric sulfide consumption of on average 1,300 mmol m$^{-3}$ d$^{-1}$ for a prolonged period. With a concentration of reactive iron of maximally 20,000 mmol m$^{-3}$ as found for the geographical center (data not shown), consumption can be maintained for about two weeks. Thus, we may have sampled a fresh mud flow not yet in steady state, or other processes must be responsible for consuming sulfide. A recent deposition and decomposition of fresh organic matter during a winter or spring bloom of Emiliania huxleyi as common in the Black Sea (Sorokin, 1983; Hay et al., 1990) could cause a non-steady state situation with two sulfide peaks. However, the sulfate reduction rates outside the mud volcano were not elevated compared to other observations in the abyssal part of the Black Sea (Jørgensen et al., 2001; Weber et al., 2001). Mud volcanoes are dynamic environments and transient state can be caused e.g. by changes in upward fluid flow velocity, hydrate dissociation, and mud flow events. However, over short distances of several centimeter, leveling of the two concentration maxima into a straight line by diffusion is in the order of days and the dip must be a transient phenomenon.

4.3. Mud volcanoes as source for methane and sulfide to the Black Sea

We identified an elevated zone with a radius of approximately 50 m (7800 m$^{2}$), which showed evidence of high fluid flow velocities and methane seepage. As no methane was consumed in the upper seafloor, the methane emission at the summit equaled a methane flux of 1.3x10$^{6}$ mol yr$^{-1}$ (0.44 mol m$^{-2}$ d$^{-1}$ x 7800 m$^{2}$ x 365, Table 3). Methane emission in the surrounding center was not measured in situ, but can be calculated from the upflow velocity and the ambient concentration of dissolved methane. At the in situ methane concentration as estimated for methane hydrates in equilibrium with methane (0.085 mol L$^{-1}$, Wallmann et al., 2006), we determined an average methane transport of 0.07 mol m$^{-2}$ d$^{-1}$ or 1.9x10$^{7}$ mol yr$^{-1}$ for the entire DMV center area and edge (7.5x10$^{5}$ m$^{2}$). However, as gas bubbles were released from the sediment, the in situ concentration will be higher and at the methane concentration as calculated for the ambient conditions (0.118 mol L$^{-1}$, Feseker et al., in press) the methane flux could be up to 0.1 mol m$^{-2}$ d$^{-1}$ or 2.7x10$^{7}$ mol yr$^{-1}$ in the DMV center area. In the sediments of the DMV, methane utilization as inferred from the averaged sulfide flux (0.05 mol m$^{-2}$ d$^{-1}$, average of fluxes except from the summit) was 1.4x10$^{7}$ mol CH$_{4}$ yr$^{-1}$, i.e. between 50-70% of the total methane flux was consumed. Accordingly, from the summit plus the outer center up to 1.4x10$^{7}$ mol CH$_{4}$ yr$^{-1}$ was discharged in form of dissolved methane, which is about an order of magnitude higher than previous estimates of 1.9x10$^{6}$ mol CH$_{4}$ yr$^{-1}$ (Wallmann et al., 2006). The Black Sea is the world's
largest surface water reservoir of dissolved methane, with concentrations of around 11 μmol L⁻¹, and a methane inventory of 6x10¹² mol with 5-20 yr turnover time (Reeburgh et al., 1991). Less than 15% of the methane comes from methanogenesis in the sediment, and seeps and gas hydrates are thought to provide the remainder (Kessler et al., 2006). Even with our higher estimate for methane discharge at least 15,000 mud volcanoes with similar emission rates as the DMV would be needed to account for the Black Sea water column methane flux of 2.3 - 3.5x10¹¹ mol yr⁻¹ as estimated from Kessler et al. (2006). To date the presence of more than 65 submarine mud volcanoes is known for the total Black Sea (Kruglyakova et al., 2002). Thus mud volcanoes are not relevant for the methane budgets of the Black Sea. Instead, gas seeps with strong and focused methane gas venting such as frequently found on shelves and slopes of the Black Sea (Polikarpov et al., 1992; Luth et al., 1999; Dimitrov, 2002; Michaelis et al., 2002) play the dominant role in the methane inventory of the Black Sea.

Also the amount of sulfide produced in the DMV is low compared to the total sulfide inventory of 1.4x10¹⁴ mol of the Black Sea (Neretin et al., 2001). It was previously estimated that the largest contribution to the sulfide inventory (8.8x10¹¹ - 1.5x10¹² mol yr⁻¹) comes from the water column and only 9.4x10¹⁰ - 1.5x10¹¹ mol yr⁻¹ sulfide is derived from the sediment (Neretin et al., 2001). The 1.4x10⁷ mol yr⁻¹ sulfide released from the DMV contributes maximally 0.01% to the total sediment derived sulfide content. Thus, although sulfate reduction and sulfide production are significant processes in the sediments of the DMV, the sulfide flux from mud volcanoes does not significantly add to the sulfide inventory of the Black Sea.

5. Conclusions

Our results show that the Dvurechenskii mud volcano represents an active methane seep and a highly dynamic geobio-system. Fluid and mud flow have a significant impact on the efflux of methane and sulfide, as well as on the anaerobic oxidation of methane. Medium to low fluid upflow allowed high sulfate and methane consumption and reduced the methane emission to the water column by circa 50-70%. High fluid upflow > 1 m yr⁻¹, and the extrusion of subsurface muds depleted in microbial assemblages prevented methane oxidation and led to very high methane emission rates of > 400 mmol m⁻² d⁻¹ at the summit of the DMV. This rather small elevation north of the geographical center of the DMV showed the highest temperature gradients, but almost no microbial sulfide production was detected. Hence, while temperature gradients, fluid flow, methane emission and consumption affected each other, they were not directly correlated in space. Our results suggest that deep-water mud volcanoes have only a small contribution on the methane and sulfide inventory of the Black Sea, and that most methane is derived from the abundant gas vents in shallower areas of the Black Sea margin.
Reference:


3. Discussion

Cold seeps like mud volcanoes and pockmarks are highly interesting ecosystems, from both biological and geological perspectives. The rising fluids, muds and gases represent a window between the deep geosphere and the biosphere. A complex interplay of biological, geochemical, and geological processes shapes these environments, creating highly dynamic cold seep ecosystems at the seafloor. Associated with fluid outflow at seeps are chemosynthetic communities that utilize the chemical energy of reduced components such as sulfide, methane, and other hydrocarbons. The production of biomass by these communities can be several orders of magnitude higher than at non-seep sites on the nearby ocean floor. This PhD thesis aims to provide an overview on and a comparison of differences and similarities of various cold seep geostructures in terms of biodiversity, biogeochemical turnover rates, and fluxes on a geostructural level as well as on a regional and global scale. It combines results from different cold seep systems and geographical regions. Data from various geostructures clearly show that cold seeps are heterogeneous ecosystems in which abiotic as well as biotic processes are strongly influenced by fluid and gas fluxes, often varying in space and time. In situ measurements reveal that at a geostructural scale specific habitats develop, in which distinct oxygen consumption and methane emission rates can be identified. The obtained results improve our understanding of the development and distribution of different habitats (e.g. bacterial mats, chemosynthetic fauna), the oxidation of methane, and the release of methane into the hydrosphere. The data also improve our global budget estimates of consumption rates (e.g. oxygen) and methane release from the deep-sea floor.

3.1 Methane consumption and emission in cold seep ecosystems

Cold seeps are recognized as important sources of methane emission to the ocean (Hinrichs and Boetius 2002; Reeburgh 2007), and thus the investigation of transport and consumption processes is crucial for the overall oceanic methane budget. In this PhD thesis, three mud volcanoes and one fault-related seep community with various fluid flow intensities and habitat structures have been investigated, focusing on the methane cycle and related processes in the sediment. The aim was to study biogeochemical key processes (SR, AOM) within the seep sediment, as well as fluxes (oxygen, methane) across the sediment-water interface at different habitats in order to gain insights into the ecosystem functioning. The obtained turnover rates and fluxes were compared to each other and related to other seep locations to get a better understanding of the relevance of cold seeps for the global methane cycle.
Tab. 1: Averaged depth integrated sulfate (SR) and methane (AOM/MOx) turnover rates, diffusive oxygen uptake (DOU), total oxygen uptake (TOU) and methane efflux measured at various cold seep ecosystems in the framework of this PhD thesis. All values are given in mmol m\(^{-2}\) d\(^{-1}\). For comparison some values were obtained from literature or personal communication (\(^{a}\)de Beer et al. 2006; \(^{b}\)Lichtschlag et al. in review; \(^{c}\)Lichtschlag et al., submitted; \(^{d}\)Grünke et al. in prep.; \(^{e}\)Sommer et al. 2009; \(^{f}\)Sommer et al. 2008; \(^{g}\)Sommer et al. 2006; \(^{h}\)Torres et al. 2002; \(^{i}\)Treude et al. 2002; \(^{j}\)Wenzhöfer et al. unpublished; \(^{k}\)Linke et al. 2005).

<table>
<thead>
<tr>
<th>Location</th>
<th>Habitat</th>
<th>water depth [m]</th>
<th>AOM / MOx</th>
<th>SR</th>
<th>methane efflux</th>
<th>TOU</th>
<th>DOU</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HMMV</strong> (Nordic Margin)</td>
<td>bacterial mat (I)</td>
<td>1250</td>
<td>9</td>
<td>1</td>
<td>58-770</td>
<td>15-72</td>
<td>11(^{a,b})</td>
</tr>
<tr>
<td></td>
<td>“next to” bacterial mat (I)</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>28</td>
<td>91(^{b})</td>
<td></td>
</tr>
<tr>
<td></td>
<td>bacterial mat (II)</td>
<td>11</td>
<td>15</td>
<td>78</td>
<td>101 / 114</td>
<td>22(^{a,b})</td>
<td></td>
</tr>
<tr>
<td></td>
<td>“next to” bacterial mat (II)</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>60</td>
<td>45(^{b})</td>
<td></td>
</tr>
<tr>
<td></td>
<td>siboglinid tubeworms</td>
<td>20</td>
<td>54</td>
<td>2</td>
<td>161</td>
<td>n.d.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>“next to” tubeworms</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>21</td>
<td>n.d.</td>
<td></td>
</tr>
<tr>
<td><strong>Amon MV</strong> (Eastern Mediterranean Sea)</td>
<td>center</td>
<td>1220</td>
<td>0.6</td>
<td>5.1</td>
<td>n.d.</td>
<td>n.d.</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>bacterial mat</td>
<td>17</td>
<td>54</td>
<td>n.d.</td>
<td>50</td>
<td>40 / 44</td>
<td></td>
</tr>
<tr>
<td></td>
<td>biogenic mounds</td>
<td>0.1</td>
<td>0.2</td>
<td>n.d.</td>
<td>5</td>
<td>n.d.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>sulfur band</td>
<td>0.1</td>
<td>0.5</td>
<td>n.d.</td>
<td>35-71</td>
<td>23 - 46</td>
<td></td>
</tr>
<tr>
<td><strong>Japan Deep Sea</strong></td>
<td>Calyptogena colony</td>
<td>5380</td>
<td>n.d.</td>
<td>6</td>
<td>n.d.</td>
<td>21</td>
<td>n.d.</td>
</tr>
<tr>
<td><strong>Trench</strong></td>
<td>summit</td>
<td>2030</td>
<td>0.05(^{e})</td>
<td>0.07(^{e})</td>
<td>440(^{e})</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>outer center</td>
<td>9(^{c})</td>
<td>20(^{c})</td>
<td>n.d.</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Pockmarks (Eastern Mediterranean Sea)</strong></td>
<td>bacterial mat</td>
<td>1700</td>
<td>4(^{d})</td>
<td>30(^{d})</td>
<td>81(^{h})</td>
<td>71(^{d}) - 174(^{d})</td>
<td>27(^{d})</td>
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<tr>
<td></td>
<td>“next to” bacterial mat</td>
<td>&lt; 0.1(^{d})</td>
<td>0.1(^{d})</td>
<td>24(^{d})</td>
<td>1.4(^{d}) - 6(^{d})</td>
<td>0.6(^{d})</td>
<td></td>
</tr>
<tr>
<td><strong>Captain Arutyunov MV</strong> (Gulf of Cadiz)</td>
<td>clast (center)</td>
<td>1320</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.27</td>
<td>4</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td>siboglinid tubeworms</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.001(^{e})</td>
<td>13(^{d})</td>
<td>n.d.</td>
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<tr>
<td><strong>Hydrate Ridge</strong></td>
<td>bacterial mat</td>
<td>600-800</td>
<td>99</td>
<td>32</td>
<td>1.9-100(^{h})</td>
<td>48(^{g})</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td>Calyptogena clam field</td>
<td>56(^{e})</td>
<td>65(^{e})</td>
<td>0.6(^{g})</td>
<td>4(^{g})</td>
<td>n.d.</td>
<td></td>
</tr>
</tbody>
</table>
Until recently, only a few in situ studies on off-shore cold seeps dealing with methane consumption and efflux have been published (Linke et al. 2005; de Beer et al. 2006; Niemann et al. 2006; Sommer et al. 2008; Sommer et al. 2009; Sommer et al. accepted). This limits our possibilities to estimate the contribution of seep systems to the ocean methane budget. However, detailed investigations of cold seeps showed that they are indeed rather heterogeneous ecosystems, concerning the spatial distribution of fluid flow intensity and benthic biogeochemical activity (Olu et al. 1996; Treude et al. 2002; Olu-Le Roy et al. 2004; Niemann et al. 2006; Foucher et al. 2009). Thus, it is necessary for a well constrained methane budget to investigate various seep systems and to perform high numbers of measurement iterations.

In the framework of this PhD work, such detailed investigations of methane-associated processes were performed on three MVs (Håkon Mosby Mud Volcano (HMMV), Amon Mud Volcano (MV), Dvurechenskii mud volcano (DMV)). All geostructures were highly active cold seeps with respect to methane and sulfate turnover, as well as oxygen consumption (except DMV which is situated in permanently anoxic waters), in comparison to other cold seep ecosystems (Tab. 1) or even highly productive coastal settings such as salt marches or mangroves (Canfield et al. 2005). A large fraction (up to 70% at the DMV) of the total methane flux is consumed in the sediment by the so-called biological methane filter. However, methane consumption rates vary not only between the different habitats of a geostructure, but also between different MVs (Tab.1), ranging from 4 to 90 %. Methane turnover and benthic activity at seeps are strongly affected by the fluid flow, with flow intensity controlling the diffusion of suitable electron acceptors (oxygen, sulfate) into the sediment for methane oxidation. Furthermore, the chemical composition of uprising gases and fluids can also influence methane turnover rates. For instance, at the Amon MV sulfate-reducing bacteria oxidizing higher hydrocarbons compete successfully for sulfate with microbes mediating anaerobic methane oxidation.

By comparing fluid flow intensity, methane flux and methane consumption in cold seep sediments, roughly four different scenarios can be distinguished:

(I) High fluid flow rates resulting in high methane fluxes and low benthic methane consumption rates, as found at the central outflows (“hot spots”) of the HMMV, the Amon MV, and the DMV.

(II) Medium fluid flow rates enabling high methane consumption rates in the sediment and thus reducing methane discharge into the water column. This methane turnover was strongly coupled to sulfate reduction and was found mainly below the bacterial mats at the HMMV and the Amon MV.

(III) Nearly absent fluid flow in combination with high methane consumption rates, resulting in a very low release of methane. Those sediments were inhabited by chemosynthetic macrofauna such as siboglinid tubeworms (HMMV). However, abundant macrofauna at the seafloor not always correlates to high methane turnover rates, for example methane consumption was low in the sediment of fault-related Calyptogena colonies in the Japan Deep Sea Trench or of the Amon MV biogenic mounds.

(IV) Medium fluid flow rates combined with medium consumption rates in the sediment, but missing chemosynthetic organisms at the sediment surface, resulted in medium methane emission as
observed at the DMV. Chemosynthetic biota usually use oxygen to oxidize reduced compounds such as sulfide, and are present as soon as the sulfide concentration in the sediment is sufficient. However, the DMV is located in anoxic waters of the Black Sea, and thus the common chemosynthetic seep biota are absent.

3.2 Methane budget of cold seeps

To improve future methane budget estimations of single geostructures, and also of the oceans, the different scenarios of methane release and consumption should be considered. Therefore, detailed ecosystem investigations, such as those performed in the framework of this PhD thesis, are necessary to further improve the knowledge about the contribution of marine cold seeps to methane emission.

It is assumed that methane emission from MVs is more significant on a global scale compared to other cold seep ecosystems, because of their individual sizes and geological activity (Reeburgh 2007). Therefore, the following discussion focuses on the methane release from deep-sea MVs.

In recent years, the contribution of seeps and especially offshore MVs to the oceanic and global atmosphere methane budget was intensively discussed (Judd 2003; Milkov et al. 2003; Judd 2004; Kvenvolden and Rogers 2005; Etiope et al. 2008). However, the methane discharge of submarine MVs was mainly indirectly quantified, using model approaches or methane emission rates from terrestrial MVs due to missing data sets from deep-sea MVs (Milkov et al. 2003). One direct measurement of the advective methane transport was the quantification of the gas bubble discharge from three sites at the HMMV (Sauter et al. 2006). The estimated efflux from bubble streams (14 x 10^6 mol yr^-1) is in the same range as the diffusive methane discharge of the HMMV (Chapter 2.2).

In situ methane discharge rates obtained from different cold seep sites during this PhD study increased the global data significantly, because only a few similar seep investigations have been performed before (for an overview see Tab. 1). For instance, in situ measurements from the Captain Arutyunov MV (Sommer et al. 2009) showed only low diffusive methane discharge rates (0.27 mmol m^-2 d^-1). In the course of this PhD work, a wide range of diffusive methane emission rates (2 to 700 mmol m^-2 d^-1) were measured (Tab.1). It has to be mentioned however, that the different studies represent differently active MVs. While the DMV and HMMV are active MVs, less active MVs such as the Captain Arutyunov MV exhibit low methane consumption and emission rates. Additionally, it was shown that methane emission and consumption are controlled by the flow velocity of uprising fluids and gases (Chapter 2.2). The highest fluid flow rates, in conjunction with high sediment temperatures, are found in the central areas of MVs (HMMV, Amon MV, and DMV) characterized by low methane consumption but high methane emission rates. The fluid flow intensity is then decreasing towards the rim of the geostructure. In consequence, habitats associated with specific chemoautotrophic organisms, such as bacterial mats or tubeworms, are concentrically distributed around the central area according to their physiological requirements. This biogeochemical difference of the various habitats
also affects the efficiency of the biological filter to consume methane (Tab.1). At the siboglinid tubeworms habitat (HMMV) up to 90% of the methane is removed before it is released to the water column. The anaerobic methane oxidation seems to be the most important pathway, even if the amount of consumed methane varies between <12 (bacterial mat at the HMMV, pockmark Eastern Mediterranean Sea) and up to 70% (at the DMV) (Tab. 1). Sediments covered by mats of sulfide-oxidizing bacteria can have high methane consumption rates, but the efficiency in removing the methane can be low, depending on the fluid flow velocity.

Overall, the average in situ diffusive methane discharge of the here investigated MVs (DMV, HMMV) is 14 x 10^6 mol per year, which is significantly higher compared to the less active Captain Arutyunov MV (0.006 x 10^6 mol yr^-1) (Sommer et al. 2009). Estimated numbers of deep-sea mud volcanoes vary between 10^3 - 10^5 (Milkov 2000). Taking into account an average methane emission of 9.3 x 10^6 mol yr^-1 (HMMV, DMV, Captain Arutyunov MV), the estimated global annual methane release from deep-sea MVs would be in the range of 0.9 - 93 x 10^10 mol (0.14 - 14.9 x 10^12 g CH4 yr^-1).

The amount of methane released from MVs to the ocean water in previous global estimations was similar (3.2 - 27 x 10^12 g CH4 yr^-1) (Judd 2003; Milkov et al. 2003). However, it has to be noticed that the here presented estimation is based solely on diffusive methane discharge measurements. As shown for the HMMV, advective methane discharge by gas bubble streams can significantly enhance the methane release of geostructures. Hence, the estimation represents rather a minimum of the total MV methane emission rate, indicating that deep-sea mud volcano contribution to the global budget might be underestimated. It is, however, argued that deep-sea MVs are not relevant for the global atmospheric methane budget, because most of the released methane is either consumed in the water column before it is released from the oceans or is stored as gas hydrates in the seep sediment (Judd 2004). Large amounts of methane might indeed be removed by aerobic methane oxidation in the water column, but investigations of dissolved methane in the water column above the HMMV showed that the decrease in methane concentration in seawater is rather caused by dilution than by consumption (Damm and Budeus 2003). Gas hydrates are formed under specific pressure and temperature conditions and constitute a dynamic methane reservoir in marine sediments. However, they are not necessarily associated with deep-sea MVs, as for example they are found at the HMMV and DMV (Milkov et al. 2004; Feseker et al. in press), but are not reported from the Amon MV.
3.3 General outlook

One aim of this PhD study was the detailed investigation of methane fluxes and consumption processes in different habitats of cold seeps. These studies (e.g. HMMV and the Amon MV) showed that cold seeps are heterogeneous ecosystems controlled by the spatial variability of fluid flow intensities. These variations are visualized by the distribution of different chemosynthetic organisms at the seafloor. However, temporal fluctuations in fluid flow intensities could not be investigated during this PhD thesis. The continuous revisiting of the HMMV over years showed that the overall distribution of chemosynthetic habitats seems to be stable, but there might be changes in seep activities, which could not be resolved by our sampling intervals. An indication for temporal fluid, mud and/or gas flow variability was observed in the HMMV center. Gas bubble streams were identified in 2003 (Sauter et al. 2006), but these emission sites could not be observed in 2007. Long-term temperature measurements at the HMMV also showed that temperature gradients, and thus upward fluid flow, changed significantly within months (Feseker et al. 2008). However, temporal changes in diffusive methane release and consumption rates are not yet quantified.

Therefore, one major goal of future seep studies should be the investigation of temporal changes of methane fluxes and methane-related biogeochemical processes. For such an approach, the available technology needs to be improved and new technology has to be developed. For instance, in situ measurements are currently time-limited due to sensor and battery lifetime. One first approach is the establishment of long-term observatories at seep sites. Recently, the first deep-sea observatory was installed at the HMMV within the framework of the EU project ESONET (LOOME; http://www.esonet-noe.org/about_esonet).

Additional information about temporal variability at cold seep ecosystems, such as mud volcanoes, will further improve our knowledge about the contribution of seeps to the global methane budget. Already the detailed spatial analyses of MV habitats within the framework of this PhD thesis showed that previous approaches might have underestimated the contribution of cold seeps to the global methane budget.
References:


4. Other scientific activities during my PhD study

4.1. Further publications during my PhD study


Anne-Christin Girnth, Stefanie Grünke, Anna Lichtschlag, Janine Felden, Katrin Knittel, Frank Wenzhöfer, Dirk de Beer and Antje Boetius., in prep.. A novel, mat-forming Thiomargarita population associated with a sulfidic fluid flow from a deep-sea mud volcano

4.2. Poster and oral presentations during my PhD study

Oral presentations:

J. Felden, A. Lichtschlag, F. Wenzhöfer, D. deBeer, J.P. Foucher, A. Boetius
In situ and ex situ measurements in methane enriched sediments of Amon Mud Volcano (Nile Deep Sea Fan),
EGU General Assembly, Vienna, Austria, 15.04.-20.04.2007

J. Felden, G. Wegener, M. Bowles, F. Wenzhöfer, F. Schubotz, K.U. Hinrichs, M. Zabel, G. Bohrmann and A. Boetius
In situ rates of hydrocarbon and sulfide oxidation at the Chapopote asphalt volcano (Campeche Knolls, Gulf of Mexico),
International Conference and 97th Annual Meeting of the Geologische Vereinigung e.V., Bremen, Germany 01.10. - 05.10. 2007

J. Felden, A. Lichtschlag, H. Niemann, D. deBeer, F. Wenzhöfer, A. Boetius
AOM in cold seep ecosystems - implications on flux rates and methane emission;
Anaerobic Methane Oxidation - Exchange Meeting; 21st - 22nd February 2008; Aselage-Herzlake

J. Felden, A. Lichtschlag, H. Niemann, D. de Beer, F. Wenzhöfer, A. Boetius
Methane, sulfide and oxygen fluxes at methane and brine seeps of the Nile Deep Sea Fan (Eastern Mediterranean); EGU General Assembly; Vienna, Austria, 13.04.-18.04.2008
J. Felden, A. Lichtschlag, H. Niemann, D. de Beer, F. Wenzhöfer, A. Boetius
Budgets of oxygen, sulfate and methane fluxes at an active mud volcano - The Håkon Mosby
9th International Conference on Gas in Marine Sediments; Bremen, Germany, 15.09.-19.09.2008

J. Felden and A. Boetius
From research cruises to PANGAEA: an example for interdisciplinary data processing
HERMIONE kickoff meeting; Sorrento, Italy, 06.04.-8.04.2009

Poster:

J. Felden, F. Zielinski, G. Schroll, R. Seifert, A. Boetius, N. Dubilier
Oxidation of methane in symbiotic bacteria of the vent mussel Bathymodiolus puteoserpentis
2nd Workshop SPP 1144; 28.06.-30.06.2005, Etelsen

J. Felden, A. Lichtschlag, F. Wenzhöfer, A. Boetius
Anaerobic oxidation of methane (AOM) in marine sediments
Hermes Graduate Training Workshop; 16.01-20.01.2006, Bremen

J. Felden, A. Lichtschlag, F. Wenzhöfer, D. De Beer, A. Boetius
In situ microbial habitat studies
1st Status Seminar-Seminar "Methane in the Geo/Bio-System" in the frame of the
GEOTECHNOLOGIEN program; Kiel, Germany 07.03.-08.03.2006

J. Felden, Frank Wenzhöfer, Frank Zielinski, Nicole Dubilier, Antje Boetius
Fluid Dynamic and Microbial Processes at Hydrothermal Vents
3rd Workshop SPP 1144; Etelsen, Germany 4.07.-06.07.2006

J. Felden, H. Niemann, F. Wenzhöfer, A. Boetius
Tracing changes in the community structure in sediments of the Håkon Mosby Mud Volcano by
molecular and biogeochemical methods
11th International Symposium on Microbial Ecology-ISME 11; Vienna, Austria 20.08.-25.08.2006

J. Felden, A. Lichtschlag, S. Grünke, F. Wenzhöfer, D. deBeer, A. Boetius
Chapter 4

Other scientific activities during my PhD study

Methane, sulfide and oxygen fluxes at methane and brine seeps of the Nile Deep Sea Fan (Eastern Mediterranean)
Goldschmidt 2007 - "atoms to planets"; Cologne, Germany 19.08. – 24.08. 2007

J. Felden, A. Lichtschlag, F. Wenzhöfer, D. deBeer, J.P. Foucher, A. Boetius
In situ and ex situ measurements in methane enriched sediments of Amon Mud Volcano (Nile Deep Sea Fan)
International Conference and 97th Annual Meeting of the Geologische Vereinigung e.V.; Bremen, Germany 01.10. - 05.10. 2007

J. Felden, A. Lichtschlag, H. Niemann, D. de Beer, F. Wenzhöfer, A. Boetius
Oxygen and methane budgets of the Håkon Mosby Mud Volcano (HMMV)
EGU General Assembly; Vienna, Austria, 13.04.-18.04.2008

4.3. Participation on research cruises during my PhD study

(1) M64-2 South Atlantic, Meteor , SPP 1144 Vom Mantel zum Ozean, 06.05.-06.06.2005
(2) Alkor 267, Alkor, MUMM2 & MarTech, 24.09.-03.10.2005
(3) M67-2b Fluid seepage in the Gulf of Mexico, Meteor, MUMM2, 03.04.-25.04.2006,
(4) YK06-06 Japanese Trench, RV Yokosuka, 24.05.-14.06.2006
(6) M72-2 MICROHAB- Black Sea, Meteor, MUMM2, HERMES, 23.02.-13.03.2007
(7) ARK XXII-1b - HMMV, Polarstern, MUMM2, HERMES, 21.06.-11.07.2007
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