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Biogeochemistry of a low-activity cold seep in the Larsen B area, western Weddell Sea, Antarctica

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Abstract

First videographic indication of an Antarctic cold seep ecosystem was recently obtained from the collapsed Larsen B ice shelf, western Weddell Sea (Domack et al., 2005). Within the framework of the R/V Polarstern expedition ANTXXIII-8, we revisited this area for geochemical, microbiological and further videographical examinations. During two dives with ROV Cherokee (MARUM, Bremen), several bivalve shell agglomerations of the seep-associated, chemo syntheticclam *Calyptogena* sp. were found in the trough of the Crane and Evans glacier. The absence of living clam specimens indicates that the flux of sulphide and hence the seepage activity is diminished at present. This impression was further substantiated by our geochemical observations. Concentrations of thermogenic methane were moderately elevated with 2 μM in surface sediments of a clam patch, increasing up to 9 μM at a sediment depth of about 1 m in the bottom sections of the sediment cores. This correlated with a moderate decrease in sulphate from 28 mM at the surface down to 23.4 mM, an increase in sulphide to up to 1.43 mM and elevated rates of the anaerobic oxidation of methane (AOM) of up to 600 pmol cm^{-3} d^{-1} at about 1 m below the seafloor. Molecular analyses indicate that methanotrophic archaea related to ANME-3 are the most likely candidates mediating AOM in sediments of the Larsen B seep (Domack et al., 2005; EOS 86, 269–276).

1 Introduction

Ocean research of the last decade has provided evidence for a variety of fascinating ecosystems associated with fluid, gas and mud escape structures. These so-called cold seeps are often colonized by thiotrophic bacterial mats, chemosynthetic fauna and associated animals(Jørgensen and Boetius, 2007). Cold seep sediments also harbour diverse microbial populations that degrade hydrocarbons anaerobically along fluid and gas escape pathways. Key agents of the anaerobic oxidation of methane (AOM) with sulphate are consortia of methanotrophic archaea (ANME) and sulphate-reducing bacteria (SRB) (Knittel and Boetius, 2009). These consortia effectively reduce the efflux of methane to the hydrosphere (Hinrichs and Boetius, 2002; Reeburgh, 2007), and are the source for sulphide which fuels the chemosynthetic seep fauna (Jørgensen and Boetius, 2007). So far, three archaeal groups of marine, anaerobic methanotrophs (ANME-1, -2 and -3) related to the methanogenic *Methanosarcinales* and *Methanomicrobiales* were discovered (Boetius et al., 2000; Orphan et al., 2001, 2002; Knittel et al., 2005; Niemann et al., 2006b; Lösekann et al., 2007). In addition to the sulphate-dependent ANME-1, -2 and -3 / SRB consortia, denitrifying bacteria of the candidate division 'NC10' were found to mediated AOM with nitrate or nitrite as the terminal electron acceptor in a bioreactor with sediments from a fresh water canal systems (Raghoebarsing et al., 2006; Ettwig et al., 2008).

Cold seep systems were discovered at almost all continental margins, from Arctic to tropical latitudes. No marine cold seep ecosystems were known in Antarctica until the recent discovery of a potential methane seep in the trough of the Evans and Crane glacier in the Larsen B area by Domack and co-workers (2005). After the collapseof the Larsen B ice shelf at the South-Eastern margin of the Antarctic Peninsula in 2002 (Rott et al., 1996; Shepherd et al., 2003; Rack and Rott, 2004), Domack et al. (2005) conducted a videographic survey of the seafloor in the now open water embayment of Larsen B in March 2005. At the time of observation, 50 to 70% of the explored seafloor (5540 m^2) were covered by some whitish material, which the authors interpreted as sulphide-oxidising microbial mats. Such mats are typical for highly active seeps where AOM leads to high sulphide concentration in near-surface sediment horizons (Boetius et al., 2000; Treude et al., 2003; Niemann et al., 2006b). Furthermore, the video-footage showed features that were interpreted as bubbles emanating from the seafloor and traces of mudflows. The authors also observed aggregations of large bivalve clam shells, which were most likely extinct populations of vesicomyid clams (see Fig. 2d, Domack et al., 2005). Just as the sulphide-oxidising bacterial mats, vesicomyid clams are indicative for highly reduced, sulphidic environments, because the known species harbour sulphide-oxidising symbionts in their gills tissue and depend on their chemosynthetic production (Fisher, 1990; Childress and Fisher, 1992; Goffredi and Barry, 2002).

In January 2007, we revisited the Larsen B area for videographic and geochemical examinations within the framework of the R/V Polarstern cruise ANTXXIII-8, an expedition contributing to the "Census for Antarctic Marine Life" (Gutt, 2008). To improve the biogeography of deep-water chemosynthetic ecosystems at a global scale, and to understand the processes driving these ecosystems, we aimed at estimating the extent of seep activity in the Larsen B area, and to identify the micro- and megafauna associated with methane seepage and chemosynthetic production.

2 Materials and methods

2.1 Bathymetric mapping and seafloor observations

Bathymetric mapping of the Crane and Evans glacier trough was performed with R/V Polarstern's multibeam echosounder system Hydrosweep DS2 (Atlas Hydrographic) operated in a 90° aperture angle and 59 hard beam mode. Recorded raw data were edited with the CARIS HIPS and SIPS and subsequently mapped using the Generic Mapping Tool (GMT) software packages. Visual observations of the seafloor were carried out with the ROV Cherokee (MARUM, University of Bremen) and a video-guided multi grab sampler (Gerdes et al., 1992). The video footage was used to select positions for sediment sampling (photographs as well as metadata to videos recorded can be found in the PANGEA data base – http://doi.pangaea.de/10.1594/PANGAEA.702077).

2.2 Sample collection and storage

Sediment samples were collected with a video-guided multiple corer (MUC) and a gravity corer (GC) at positions of clam beds, the surrounding area within the glacier trough

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and from a reference station outside the trough (Table 1, Fig. 1). Immediately upon corer retrieval, sediment samples were transferred into a cold room (2°C) and subsampled for concentration measurements of hydrocarbon gases, pore water constituents (sulphate, sulphide), AOM and sulphate reduction rate (SRR) measurements and the analysis of microbial diversity. Sediment subsampling for gases and DNA was performed with cut-off syringes (GC and MUC cores) and for AOM and SR rates with glass tubes (GC cores) and acrylic core liners (MUC cores) according to previously described methods (Treude et al., 2003; Niemann et al., 2006a). Pore water was extracted with rhizons connected to syringes through holes drilled into the core liners (Seeberg-Elverfeldt et al., 2005). A vertical sampling resolution of 2 cm was chosen for MUC-cores, and of 10 to 20 cm for GCs.

2.3 Methane and ethane concentrations

3 ml of sediment was fixed in 7 ml of saturated NaCl solution in a 20 ml gas-tight sealed glass vial. Shortly after the cruise (<3 month), methane and ethane concentrations were determined from the headspace by gas chromatography using an OPTIMA-5 capillary column (5 μM film thickness; 0.32 mm ID, 50 m length) in an Agilent 6890N gas chromatograph equipped with a split/splitless injector (operated in splitless mode) and a flame ionisation detector (FID). The carrier gas was heliumat a flow rate of 3 ml min^{-1}. Initial oven temperature was set to 45°C held for 4 min, subsequently increased at 15°C min^{-1} to 155°C, held for 2 min, then raised at 25°C min^{-1} to 240°C and finally held for 7 min. The chromatography system was calibrated for a concentration range of 1 to 100 μM methane and 0.1 to 10 μM ethane (final concentration in the sediment). Concentrations of hydrocarbons with more tan 2 carbon atoms (propane, butane etc.) were below detection/quantification limit.

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2.4 Sulphate and sulphide concentrations

Sulphate and sulphide concentrations were analysed according to modified methods from Small et al. (1979) and Fogo and Popowsky (1949), respectively, as described in detail elsewhere (Grasshoff et al., 1983). Briefly, for sulphate concentration measurements, 0.4 ml of filtered pore water was diluted with MilliQ water (1:50, v/v) and the sample was stored at −20°C till further analysis. Shortly after the cruise (<2 month), sulphate concentrations were determined by ion chromatography (IC) using a Metrosep Anion Dual2 column (4.6×70 mm) in a Metrohm 761 Compact IC equipped with a 732 IC Detector. $Na_2CO_3/NaHCO_3$ (3.2 mM/1 mM, respectively) was used as a solvent at a flow rate of 0.7 ml min^{-1}. For sulphide concentration measurements, 1.5 ml of pore water was fixed in 0.6 ml zinc acetate (ZnAC) solution (20%, w/v) and stored at 4°C till further analysis. According to the Methylene-Blue method, sulphide was determined photometrically at 660 nm using a Kontron Uvikon 922 photometer.

2.5 Ex situ AOM and SR rate measurements

AOM and sulphate reduction rates were determined by radiotracer incubations using ^{14}C-methane and ^{35}S-sulphate, respectively, as described previously (Jørgensen, 1978; Treude et al., 2003; Niemann et al., 2005). In short, aqueous tracer solutions (40 μl $^{14}CH_4$, 5 kBq or 5 μl $^{35}SO_4^{2-}$, 40 kBq) were injected into butyl rubber sealed glass tubes (GC core subsamples) or in 1 cm intervals into small push cores (MUC core subsamples). The incubations were carried out for 24 h at 1°C. Subsequently, AOM and SR rate samples were fixed in 25 ml NaOH (2.5%, w/v) and 20 ml ZnAC solution (20%, w/v), respectively. The analyses of radioactive substrates and products were performed as described previously (Fossing and Jorgensen, 1989; Treude et al., 2003). AOM and SR rates were calculated according to the following formulas:

$$\mathrm{AOM} = \frac{^{14}CO_2}{^{14}CH_4 + {}^{14}CO_2} \times \frac{[CH_4]}{t} \quad (1)$$

$$SRR = \frac{TRI^{35}S}{^{35}SO_4^{2-} + TRI^{35}S} \times \frac{[SO_4^{2-}]}{t} \quad (2)$$

Here, $^{14}CH_4$, $^{14}CO_2$, $^{35}SO_4^{2-}$ and $TRI^{35}S$ are the activities (Bq) of methane, carbon dioxide, sulphate and total reduced inorganic sulphur species, respectively. $[CH_4]$ is the average methane concentration between the beginning and end of the incubation and $[SO_4^{2-}]$ is the sulphate concentration at the beginning of the incubation. Aerobic methane oxidation (MOx) was determined in the same way as AOM. In both processes, the oxidized ^{14}C-methane is trapped as $^{14}CO_2$ but the availability of either oxygen or sulphate determines which process occurs (Niemann et al., 2006b).

2.6 DNA extraction and clone library construction

Total DNA was extracted from ca. 30 g of wet sediment recovered from the upper margin of the sulphate-methane transition zone (SMTZ) of core 706-4 (−110 cm below seafloor – bsf) according to the method described by Zhou et al. (1996). PCR amplification of 16S rRNA genes, sequencing and phylogenetic analysis of archaeal and bacterial 16S rRNA genes was performed according to previous works (Knittel et al., 2003, 2005). Sequences were checked for chimeras using the RDP CHIMerA CHECK version 2.7 (http://rdp8.cme.msu.edu/cgis/chimera.cgi?su=SSU). The sequence data reported here will be published in the EMBL, GenBank and DDBJ nucleotide sequence databases under the accession numbers XXX to XXX.

3 Results and discussion

3.1 Seafloor observations

Two years after the discovery of a potential cold seep habitat in the trough of the Evans and Crane glacier (Domack et al., 2005), we revisited the Larsen B area (Fig. 1a) for further videographic and the first biogeochemical examinations. The bathymetry in the Larsen B embayment was caused by glacier erosion forming a trough in an East-West direction with several depressions deeper than 800 m water depth. In the most eastern depression (Fig. 1b), we examined the seafloor during two ROV and multi grab transects (Fig. 1c), of which the North-South ROV transect was previously surveyed with a video sledge by Domack et al. (2005) in March 2005. The total area monitored in 2007 was ca. 5500 m^2 (assuming a 2 m wide field of vision) of which about 3000 m^2 were overlapping with the previous survey by Domack et al. (2005). However, we could not detect any whitish material (putative bacterial mats) in the wider surrounding of the clam shell accumulations. The seafloor was mainly covered by pelagic sediments and phytodetritus, giving the seabed a green-greyish appearance (Fig. 2a). A survey with R/V Polarstern's fisheries echosounder (Simrad™ EK60, frequencies: 38, 70, 120, 200 kHz, beam width: 7°, 1–2 kts survey speed, Sauter et al., 2006) could not detect gas bubbles in the water column (data not shown). However, our observations could confirm the presence of bivalve agglomerations of which we found about 10 dense beds and 3 more scattered aggregations of approximately 0.5 m in diameter (Figs. 1c, 2b, c). The bivalve shells belonged to the vesicomyid clam genus *Calyptogena* and were on average about 8 cm long (Fig. 2d). Living specimens of this chemosynthetic genus provide evidence for sulphidic conditions in near surface sediments (Fisher, 1990; Goffredi and Barry, 2002; Sahling et al., 2002), which are typically found at active cold seeps. Nevertheless, videographic observations (Fig. 2b, c) and samples recovered with a TV-guided MUC and multigrab only revealed empty shells. *Calyptogena* sp. occurs at seep habitats with low to medium methane fluxes, since it can reach into subseafloor sulphide zones down to 20 cm bsf (Sahling et al., 2005). Living clams tend to accumulate in patches of 0.5–5 m, and are oriented vertically in the sediments (Olu-Le Roy et al., 2007). However, the images from the video survey obtained by Domack et al. (2005) show accumulations of horizontally oriented and therefore most likely dead specimen, so that it remains unknown when sediments were sulphidic enough to support growth of these chemosynthetic bivalves. Furthermore, in 2007, we did not observe any whitish precipitates nor record signs of gas ebullition as described by Domack et al. (2005). However, due to the limited observation and sampling time in 2005 and 2007, we refrain from further speculations on the distribution of these potential seep indicators.

3.2 Geochemical observations andseep activity

During the expedition ANTXXIII-8, one TV-guided MUC was launched directly above a clam patch, one GC was recovered from a position where most clam beds had been observed and two GCs were recovered about 1.4 km to the west and 2.7 km to the south of the ROV transect intersection, respectively (Table 1, Fig. 1b, c). To compare the geochemical data from these cores potentially associated with methane seepage to the background biogeochemistry, one additional GC (core 702-6) was recovered from a reference station 73 km to the south-east of the glacier trough (Fig. 1a). Surface sediments within the clam patches (core 709-3, Fig. 3a) showed slightly elevated methane concentrations (<2 μM, Fig. 3a) in comparison to background levels (~0.1 μM throughout core 702-6, data not shown). Furthermore, moderately elevated methane concentrations in sediments deeper than −30 cm bsf were found in the GCs recovered in the glacial trough, with highest concentrations at the clam shell patches(up to 9.1 μM,core 706-4, Fig. 4a) and lower concentrations with distance to the shell aggregations (up to 7.5 and 4 μM in cores 711-4 and 711-5, respectively; Fig. 5a, b). In the core with most methane (core 706-4), a moderate decrease in sulphate down to 23.4 mM and an increase of sulphide up to 1.43 mM below −100 cm bsf was measured. Also MUC core 709-3 showed a decrease in sulphate down to 22.4 mM at −22 cm bsf (Fig. 3b). However, sulphide concentrations were not elevated in this core (Fig. 3c). Cold seeps and hydrothermal vents are often characterised by an advective upward flow of chloride-depleted geofluids, which can be traced by a conspicuous decrease in chloride concentrations with sediment depth (von Damm et al., 1995; Schulz and Zabel, 2006; Hensen et al., 2007). Chloride concentrations in core 706-4 were uniform with about 520 mM and only the bottom section was slightly depleted with a concentration of 492 mM (data not shown). This indicates that the advection of geofluids is very low and that diffusion is the dominant mass transport component of pore water solutes at Larsen B; at least in the upper metre of sediments. According to Fick's first law of diffusion, the methane, sulphate and sulphide gradients at station 706-4 (0.0003, 0.1, 0.04 μmol cm^{-4}, respectively) together with the diffusion coefficients (193, 117, 239 cm^2 yr^{-1}, respectively; porosity=80%, T=2°C; Boudreau, 1997) translate to diffusive fluxes of about 0.4, 97 and 70 mmol m^{-2} yr^{-1}. This already indicates that methane accounts only for a small fraction of the sulphate flux. Unfortunately, a layer of glacial gravel at about −100 cm bsf prevented the GCs to penetrate deeper into the sea floor. Hence, the core sample included only the upper boundary of the sulphate-methane transition zone (SMTZ) and fluxes of methane, sulphate and sulphide could be higher in deeper sediment layers.

In contrast to seep-influenced sediments, no apparent geochemical gradients could be detected at the reference station (core 702-6). Here, methane (~0.1 μM) and sulphide concentrations were below quantification/detection limit and sulphate and chloride concentrations were roughly 28 and 520 mM, respectively, throughout the core (down to −160 cm bsf, data not shown).

Methane consumption in surface sediments of the clam patch (600 pmol cm^{-3} d^{-1}, core 709-3, Fig. 3d) was not coupled to SR. Although we did not measure oxygen concentrations, the beige colour of the upper 8 cm of sediments (core 709-3) indicates the oxygen penetration depth. Similar resultshave been reported previously from Weddell Sea sediments between 500 and 2000 m water depth (Rutgers Van der Loeff et al., 1990). Hence, the detected methane consumption in surface sediments was most likely due to aerobic oxidation of methane (MOx). Furthermore, elevated rates of SR just beneath the MOx peak in core 709-3 (Fig. 3d) and at −33.5 cm bsf in core 706-4 (Fig. 4d) did not correlate with methane oxidation and were thus probably fuelled by and buried detritus. However, in the bottom section of core 706-4, we could also detect elevated, correlating rates of AOM and SR of about 600 pmol cm^{-3} d^{-1} (Fig. 4d).

The geochemical measurements indicate very low methane fluxes and oxidation rates at the Larsen B cold seep system, similar to some of the cold seep systems in the

Gulf of Cadiz which do not host thiotrophic mats or chemosynthetic bivalves, but sporadically populations of siboglinid tubeworms (Niemann et al., 2006a). In comparison, methane concentrations in surface sediments at active seeps supporting thiotrophic microbial mats or dense clam populations (e.g., Haakon Mosby Mud Volcano, the Gulf of Mexico, Hydrate Ridge) are in the millimolar range, and sulphate is consumed in the first few centimetres of the sediment, leading to high sulphide concentrations of >4 mM (Boetius et al., 2000; Torres et al., 2002; Joye et al., 2004; de Beer et al., 2006; Niemann et al., 2006b). In these systems, the rates of AOM and SR are orders of magnitude higher and may exceed 1 μmol cm^{-3} d^{-1} in surface sediment horizons often amounting to methane, sulphate and sulphide fluxes of some moles m^{-2} yr^{-1} (Boetius et al., 2000; Treude et al., 2003; Joye et al., 2004; Niemann et al., 2006b). Yet, the presence of *Calyptogena* sp. shells provides evidence for a higher seep activity in the past. In sediments below clam shell aggregations, micro to millimolar sulphide concentrations were detected only in sediment horizons below −50 cm bsf (Fig. 4c) and the sulphide flux was about 0.07 mol m^{-2} yr^{-1}. However, the maximum depth that *Calyptogena* sp. can reach is 20 cm bsf (Sahling et al., 2005 and references therein), and the lowest sulphide flux supporting *Calyptogena* sp. found so far is 0.3 mol m^{-2} yr^{-1} (Juniper et al., 1992; Grehan and Juniper, 1996; Sahling et al., 2005). Thus, from the time the Calyptogena populations at the Larsen B shelf were alive, the sulphide front has moved about 30 cm deeper into the sediment and the sulphide flux must have been about 4-fold higher.

3.3 Origin and composition of light hydrocarbons

In addition to methane, elevated ethane concentrations of up to 0.6 μM (core 711-4) were found in deeper sediment horizons (Figs. 4a, 5a). Interestingly, ethane and methane concentrations correlated. Comparable to previous findings (Niemann et al., 2006a), this indicates concurrent transport from a source and subsequent anaerobic consumption of higher hydrocarbons in the sediment possibly mediated by SRB (Kniemeyer et al., 2007). Ethane contributed 3.6, 7.2 and 2.5% to the analysed hydrocarbon gases in the bottom sections of cores 706-4, 711-4 and 711-5, respectively. Other gases were not analyzed and/or below detection limit. A distinction of thermogenic and microbial methane is usually based on the source gas compositionin combination with their stable isotope ratios (δ^{13}C, δD) because abiotic and biotic processes influence the molecular and isotopic composition of light hydrocarbons during production, migration and consumption in the seabed (James and Burns, 1984; Larter and di Primio, 2005; Stadnitskaia et al., 2006). AOM for instance leads to ethane enrichment relative to methane and a ^{13}C-enrichment of the residual methane (Whiticar, 1999; Hinrichs and Boetius, 2002). Although, we did not sample the gas source, the composition of light hydrocarbons at the Larsen B cold seep shows exceptionally low C_1/C_2 ratios (15 to 29) with δ^{13}C-CH_4 values (data not shown) of −32.0‰ V-PDB (−110 cm bsf) and −37.6‰ V-PDB (−120 cm bsf) in core 706-4. This indicates a thermogenic origin of methane at the study site, but probably also ^{13}C enrichment caused by its anaerobic consumption. A thermogenic origin of light hydrocarbons agrees well with the geological setting of the Larsen B area. The bedrock beneath the trough consists of Mesozoic marine shale, which is a potential source rock for hydrocarbons (Domack et al., 2005). The geological setting of the Larsen B area may even support abiotic hydrothermal production of methane (Lollar et al., 1993; Foustoukos and Seyfried, 2004). As part of a back arc basin, it is associated with the volcanic arc on the Antarctic Peninsula and a subduction zone in the Drake Passage (Anderson, 1999 and references therein; Hathway, 2000).

3.4 Identity of AOM communities

The composition of the microbial assemblagein the AOM horizon below a clam shell aggregation (core 706-4; −110 cm bsf) was studied by 16S rRNA gene phylogeny. A total of 118 archaeal and 71 bacterial 16S rRNA gene sequences were analysed (Table 2). Among the archaeal sequences, 5 major groups were detected. The largest archaeal clone group was affiliated with uncultivated Methanomicrobiales. Cultivated members of this group are methanogens (Garcia et al., 2006). The second largest group of OTUs was related to the Methanosarcinales and included sequences of uncultivated archaea related to ANME-3 and other AOM-associated archaea (ANME-AAA). Since neither ANME-1 nor ANME-2 sequences could be retrieved, ANME-3 archaea are most likely the dominant anaerobic methanotrophs at Larsen B. The seep site investigated here is the only AOM habitat described so far where no ANME-1 and ANME-2 sequences were found. Usually, ANME-1 and/or ANME-2 sequences are present in cold seep sediments and comprise the dominant group of anaerobic methanotrophs, while ANME-3 has been detected only rarely in these systems (Knittel et al., 2005; Omoregie et al., 2008; Roussel et al., 2009; Knittel and Boetius, 2009). So far, only one other cold seep has been found where ANME-3 is the dominant ANME type, the Arctic Haakon Mosby Mud Volcano (Niemann et al., 2006b; Lösekann et al., 2007). This raises the question whether ANME-3 is best adapted to the ice-cold temperature conditions of Arctic or Antarctic waters or if additional factors select for this particular group in these environments.

Other potential anaerobic methanotrophs might also be archaea belonging to the AOM associated archaeal cluster (AAA) (Knittel and Boetius, 2009). This cluster comprises many sequences from terrestrial and limnic sources, but also from a deep-sea sulphide chimney (Schrenk et al., 2003). However, the biogeochemical functioning of these archaea is not well constrained at present (Ettwig et al., 2008). Sequences of the Thermoplasmatales were also found. Their functioning is also unclear, but they have previously been found to co-occur with ANMEs (Hinrichs et al., 1999; Orphan et al., 2001; Inagaki et al., 2004; Girguis et al., 2005; Knittel et al., 2005; Wegener et al., 2008). Interestingly, no Crenarchaeota were found although they are common members of seep communities (Knittel et al., 2005; Lösekann et al., 2007).

The bacterial diversity was higher than the archaeal diversity. 71 sequences could be assigned to 29 OTUs of which 17 belong to the Deltaproteobacteria. Interestingly, we could not find the known partner sulphate-reducer *Desulfobulbus* which forms a consortium with ANME-3 at the Haakon Mosby Mud Volcano (Niemann et al., 2006b; Lösekann et al., 2007). This may indicate that ANME-3 mediates AOM with another partner, but we cannot exclude undersampling of the bacterial sequences with the applied set of methods. We detected 1 sequence of the *Desulfosarcina/Desulfococcus* Seep Cluster I, the partner bacteria of ANME-1 and -2, as well as 3 other sequences of *Desulfosarcina/Desulfococcusgroup*. Also, clones belonging to *Desulfobacterium indolicum*, *Desulfatirhabdium butyrativorans*, and *Desulfobacterium anilini*, which are related to the *Desulfosarcina/Desulfococcus* group were found. The function of these SRB at Larsen B remains speculative but some could be involved in the degradation of higher hydrocarbons. *Desulfobacterium anilini* relatives are known to degrade aromatic hydrocarbons (Widdel, 2009) and one of the clones without a close cultivated relative (clone Bac3) was affiliated with a butane degrading strain (strain BuS5, clone Butane 12-GMe, Kniemeyer et al., 2007). The most abundant bacterial sequences were distantly or closely affiliated with *Desulfocapsa* spp. The cultivated species *Desulfocapsa thiozyomogenes* and *Desulfocapsa sulfoexigens* grow well by disproportionation of sulphur (in the presence of a sulphide scavenger), thiosulphate and sulphite (Janssen et al., 1996; for a review see Rabus et al., 2006). In addition, *Desulfocapsa thiozymogenes* can grow by reduction of sulphate to sulphide. However, as with the *Desulfosarcina/Desulfococcus* relatives, the functioning of *Desulfocapsa* spp. at Larsen B cannot be determined at present.

Interestingly, a sequence of *Acrobacter* was found in the sediments as well. Typically, these sulphur oxidisers occur at oxic-anoxic interfaces (Wirsen et al., 2002), but were here found >1 m bsf in the sulphidic zone (core 706-4). In addition to *Proteobacteria* several strains of bacteria with unknown biogeochemical functioning but which are commonly encountered at cold seeps and/or hydrothermal vents could be found. These include members of the Firmicutes and clostridia as well as candidate divisions OP3, OP5 and JS1 (Teske et al., 2002; Knittel et al., 2003; Webster et al., 2007).

4 Conclusions

Based on videographic evidence, it was recently proposed that the former ice shelf area at Larsen B hosts the first cold seep system discovered in Antarctic waters (Domack et al., 2005). During R/V Polarstern cruise ANTXXIII-8, bivalve shells could be sampled and were identified as *Calyptogena* sp., a genus of chemosynthetic bivalves, which typically populatessulphidic sediments associated with intermediate to low methane seepage. However, neither living clam populations, nor thiotrophic bacterial mats or gas ebullition were observed, which indicates a reduction of seepage activity. Biogeochemical analyses of the seabed showed an elevated AOM activity at >1 m depth in the seafloor, and that methane, sulphide and sulphate transport is dominantly diffusive. The methane source for the Larsen B seep is thermogenic (and possibly hydrothermal), based on the hydrocarbon composition containing considerable amounts of ethane, and a stable isotope signature of −32 to −37‰ (V-PDB). The AOM zone hosted members of the ANME-3 clade of anaerobic methanotrophs as well as of a new group of archaea associated mostly with terrestrial AOM, and showed a high diversity of sulphate reducing bacteria.

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Table 1. Sediment cores recovered during expedition ANTXXIII-8 in the Larsen B area.

Location Device	Station	Lat. S	Long. W	Water Depth (m)	Date sampled (2007)
Glacier trough					
MUC (in clam patch)	709-3	65°26.18′	61°26.51′	852	Jan 15
GC	706-4	65°26.10′	61°26.49′	850	Jan 15
GC	711-4	65°26.14′	61°28.28′	841	Jan 17
GC	711-5	65°27.51′	61°26.02′	794	Jan 17
Reference station					
GC	702-6	65°54.52′	60°21.16′	429	Jan 12

Table 2. Phylogenetic affiliation and frequencies of 16S rRNA gene sequences obtained from sediments of core 706-4 (−110 cm bsf, upper margin of SMTZ). Representative clones of each OTU (operational taxonomic unit; 98% sequence similarity) are presented.

Domain	Phylogenetic affiliation	Closest relatives or uncultivated group	No. of clones within OTU	Clone representative	Acc. No.
Archaea	Methanosarcinales	*Methanimicrococcus* spp.	1	ANTXXIII_706-4 clone Arch200	XXX
	ANME-3	*Methanococcoides* spp.	5	ANTXXIII_706-4 clone Arch42	XXX
	AOM associated Archaea	no close cultivated relative	31	ANTXXIII_706-4 clone Arch67	XXX
		no close cultivated relative	1	ANTXXIII_706-4 clone Arch213	XXX
	Methanomicrobiales	*Methanospirillum* spp.	64	ANTXXIII_706-4 clone Arch216	XXX
	Thermoplasmatales	Marine Benthic Group D	2	ANTXXIII_706-4 clone Arch92	XXX
		no close cultivated relative	10	ANTXXIII_706-4 clone Arch81	XXX
			2	ANTXXIII_706-4 clone Arch265	XXX
			1	ANTXXIII_706-4 clone Arch282	XXX
			1	ANTXXIII_706-4 clone Arch35	XXX
	Subtotal Archaea		118		
Bacteria	Deltaproteobacteria	*Desulfocapsa* spp.	17	ANTXXIII_706-4 clone Bac49	XXX
		Desulfobacterium catecholicum	3	ANTXXIII_706-4 clone Bac76	XXX
		Desulfosarcina/Desulfococcus	1	ANTXXIII_706-4 clone Bac94	XXX
		Seep-SRB1 cluster			
		other *Desulfosarcina/Desulfococcus*	2	ANTXXIII_706-4 clone Bac79	XXX
			1	ANTXXIII_706-4 clone Bac9	XXX
		Strain BuS5-affiliated	2	ANTXXIII_706-4 clone Bac3	XXX
		Desulfobacterium indolicum,	2	ANTXXIII_706-4 clone Bac4	XXX
		Desulfatirhabdium butyrativorans			
		Desulfobacterium anilini	1	ANTXXIII_706-4 clone Bac59	XXX
			1	ANTXXIII_706-4 clone Bac14	XXX
			1	ANTXXIII_706-4 clone Bac41	XXX
		Desulfuromonas/Pelobacter	1	ANTXXIII_706-4 clone Bac26	XXX
		Syntrophus/Smithella	1	ANTXXIII_706-4 clone Bac7	XXX
			2	ANTXXIII_706-4 clone Bac54	XXX
			1	ANTXXIII_706-4 clone Bac6	XXX
			1	ANTXXIII_706-4 clone Bac19	XXX
			3	ANTXXIII_706-4 clone Bac45	XXX
		no close cultivated relative	1	ANTXXIII_706-4 clone Bac88	XXX
	Epsilonproteobacteria	*Arcobacter* spp.	1	ANTXXIII_706-4 clone Bac53	XXX
	Nitrospina	*Nitrospina gracilis*	1	ANTXXIII_706-4 clone Bac74	XXX
	Bacteriodetes – Chlorobium	*Chlorobium* spp.	1	ANTXXIII_706-4 clone Bac44	XXX
	Firmicutes	*Thermoanaerobacterium* spp.	1	ANTXXIII_706-4 clone Bac71	XXX
		Erysipelothrix	1	ANTXXIII_706-4 clone Bac93	XXX
		Desulfotomaculum spp.	1	ANTXXIII_706-4 clone Bac61	XXX
		Desulfosporosinus spp.	1	ANTXXIII_706-4 clone Bac5	XXX
	Actinobacteria	no close cultivated relative	1	ANTXXIII_706-4 clone Bac69	XXX
	OP3	no close cultivated relative	1	ANTXXIII_706-4 clone Bac64	XXX
	JS1	no close cultivated relative	15	ANTXXIII_706-4 clone Bac16	XXX
	OP5	no close cultivated relative	4	ANTXXIII_706-4 clone Bac50	XXX
	unaffiliated	no close cultivated relative	1	ANTXXIII_706-4 clone Bac1	XXX
	Subtotal Bacteria		71		

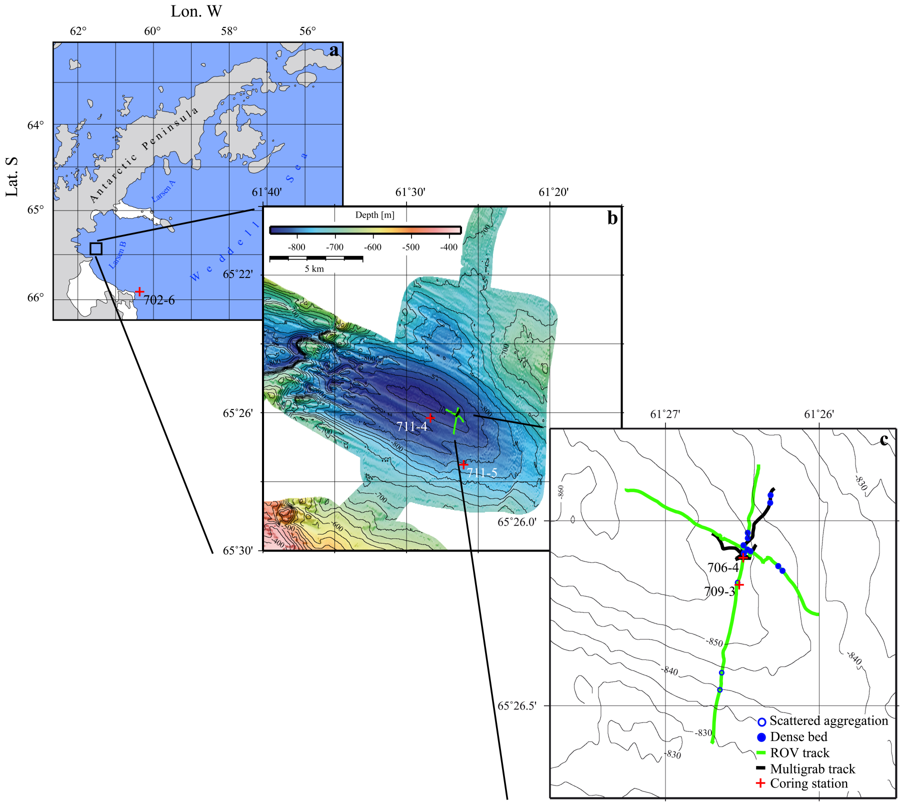

Fig. 1

Fig. 1. (a) Geographical map of the Larsen B embayment at the eastern site of the Antarctic Peninsula (permanently ice covered areas are highlighted in white). **(b)** Bathymetry of the eastern most basin of the Crane and Evans glacier trough. **(c)** Detailed chart of the videographic survey area. Survey tracks, clam agglomerations and coring stations are indicated.

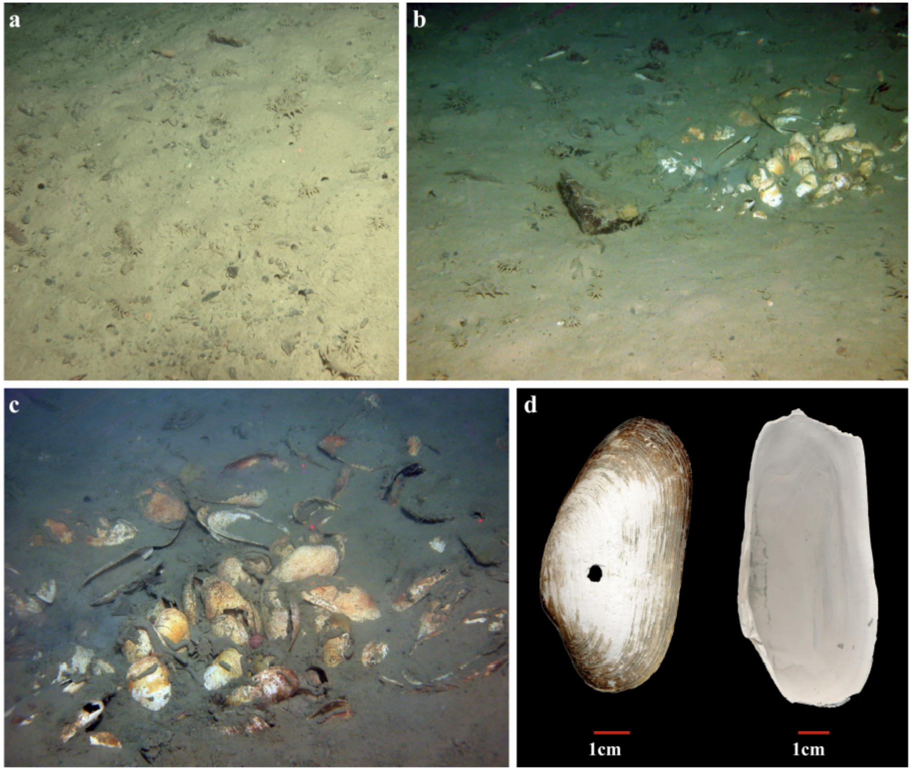

Fig. 2. Images showing **(a)** the sea floor typically encountered during video surveys in the trough of the Crane and Evans glaciers. **(b)** An agglomeration of *Calyptogena* sp. shells at the sea floor. Elasipod holothurians and drop stones are visible on image a and b. **(c)** A close up of the agglomeration showing only empty shells. Shell agglomerations were photographed on the north-south transect (Photographs: J. Gutt and W. Dimmler, © AWI/Marum, University of Bremen). **(d)** *Calyptogena* sp. shells recovered during R/V Polarstern expedition ANTXXIII-8.

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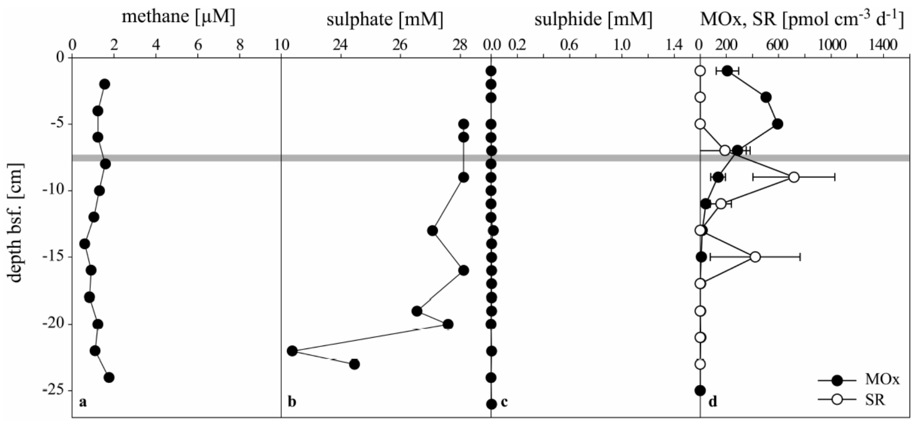

Fig. 3. Concentration profiles of **(a)** methane, **(b)** sulphate, **(c)** sulphide as well as rates of aerobic methane oxidation (MOx) and sulphate reduction (SR) in sediments of MUC-core 709-3, which was launched directly above a clam patch (see Fig. 1c for position). Concentrations of ethane were below detection limit. The oxygen penetration depth (grey line) was assumed with 8 cm bsf according to the beige sediment colour of this layer.

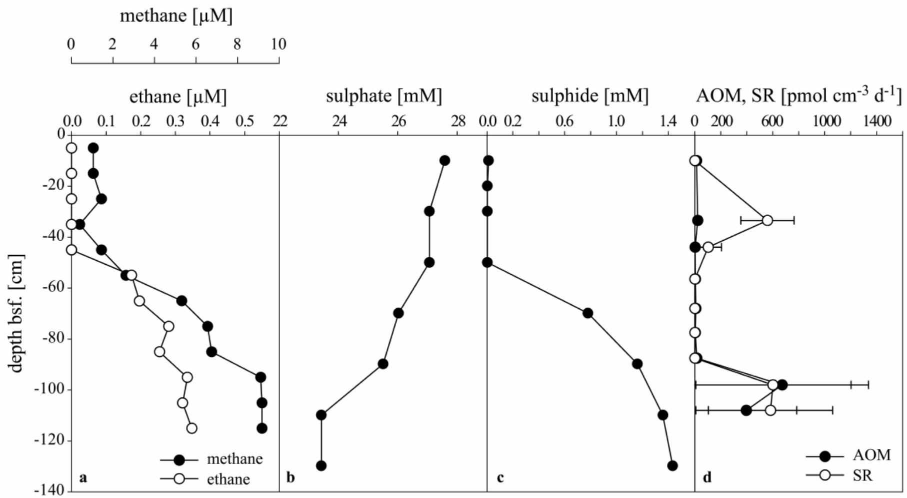

Fig. 4. Concentration profiles of **(a)** methane and ethane, **(b)** sulphate, **(c)** sulphide as well as rates of anaerobic methane oxidation (AOM) and sulphate reduction (SR) in sediments of gravity core 706-4 which was recovered from a position where many clam patches were observed during videographic examinations (see Fig. 1c for position). Chloride concentrations were uniform with about 520 mM throughout the core indicating that diffusion is the dominant mass transport phenomenon at the Larsen B seep.

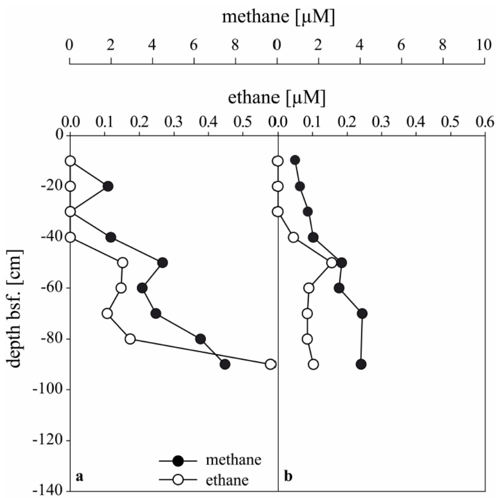

Fig. 5. Concentration profiles of methane and ethane in sediments of the gravity cores 711-4 **(a)** and 711-5 **(b)** (see Fig. 1c for positions).