FEATURE ARTICLE

Diversity and distribution of cold-seep fauna associated with different geological and environmental settings at mud volcanoes and pockmarks of the Nile Deep-Sea Fan

Bénédicte Ritt · Catherine Pierre · Olivier Gauthier · Frank Wenzhöfer · Antje Boetius · Jozée Sarrazin

Abstract The Nile Deep-Sea Fan (NDSF) is located on the passive continental margin off Egypt and is characterized by the occurrence of active fluid seepage such as brine lakes, pockmarks and mud volcanoes. This study characterizes the structure of faunal assemblages of such active seepage systems of the NDSF. Benthic communities associated with reduced, sulphidic microhabitats such as sediments and carbonate crusts were sampled by remotely operated vehicles during two cruises in 2006 (BIONIL) and 2007 (MEDECO). Environmental conditions and biological factors including family-level faunal composition, density and diversity were measured at local and regional scales. Significant differences were detected at different spatial scales: (1) the fauna of reduced habitats differed substantially in activity, diversity and biomass from the non-seep environment at similar water depth, (2) cold seep microhabitats showed differences in community structure and composition related to substratum type as well as to the intensity and location of fluid emissions.

Communicated by M. Kühl.

B. Ritt (✉) · O. Gauthier · J. Sarrazin (✉)
Ifremer, Centre de Brest, Département Etude des Ecosystèmes Profonds/Laboratoire Environnement Profond,
BP 70, 29280 Plouzané, France
e-mail: benedicte.ritt@ifremer.fr; benedicte.ritt@temple.edu

J. Sarrazin
e-mail: jozee.sarrazin@ifremer.fr

C. Pierre
LOCEAN, UMR 7159, Université Pierre et Marie Curie,
75005 Paris, France

O. Gauthier
LEMAR, UMR 6539, Université de Bretagne Occidentale,
Place N. Copernic, 29200 Plouzané, France

O. Gauthier
Ecole Pratique des Hautes Etudes CBAE, UMR 5059,
163 rue Auguste Broussonet, 34000 Montpellier, France

F. Wenzhöfer · A. Boetius
MPI, Habitat Group, Celsiusstrasse 1, 28359 Bremen, Germany

F. Wenzhöfer · A. Boetius
AWI, HGF MPG Research Group on Deep Sea Ecology
and Technology, 27515 Bremerhaven, Germany

Introduction

Since their discovery on the Florida Escarpment in the Gulf of Mexico (Paull et al. 1984), cold seeps have been reported along convergent plate boundaries on active as well as passive continental margins, where over-pressure in the sediments controls the emission of fluids enriched in methane or other hydrocarbons to the seafloor and hydrosphere (Milkov 2000; Dimitrov 2002). Depending on their geophysical settings, these cold seeps are associated with distinct geological features such as gas hydrates, pockmarks, brine lakes and mud volcanoes (MVs) (Milkov 2000; Hovland et al. 2002; Judd and Hovland 2007; Foucher et al. 2009). To date, hundreds of seep sites supporting chemosynthesis-based communities have been encountered throughout the world's oceans (Sibuet and Olu 1998; Levin 2005; Campbell 2006; Baker et al. 2010).

The Eastern Mediterranean Sea is one of the world's MV and pockmark hotspots (Kopf 2002; Foucher et al. 2009). Most of the MVs are associated with the active Mediterranean Ridge that stretches over more than 1,500 km (Cita et al. 1981) and the thickly sedimented Nile Deep-Sea Fan (NDSF) along the Mesozoic-rifted continental margin of northern Egypt (Bellaiche et al. 2001;

Mascle et al. 2001, 2006; Loncke et al. 2004). The present study focuses on sites of the NDSF that have been explored since 2003 in the frame of the European Science Foundation (ESF) MEDIFLUX program (2003–2007) and the European 6th Framework Program project HERMES (2005–2009).

The NDSF is divided into four morpho-structural provinces (Fig. 1a): (1) the Levantine province, a domain of salt-related folding; (2) the Eastern province, a domain of intense salt-related tectonics; (3) the Central province with pockmark fields and gas chimneys, distinguished by active sedimentary instability and fluid-related processes; and (4) the Western province with mud cones, characterized by active turbiditic processes, salt tectonics and fluid venting (Mascle et al. 2006; Huguen et al. 2009). The accumulation of organic-rich sediments, probably since the early Cenozoic, led to the formation of hydrocarbons in deep reservoirs that were partially sealed by the deposition of thick evaporites during the Messinian (Hsu et al. 1977). A mixture of hydrocarbons, water and mud is expelled through faults that may extrude salt deposits, which reach the seafloor (Loncke et al. 2004). Fluid seepage is recorded in geological features such as mud cones, caldera-like depressions, gas chimneys, brine pools and pockmarks (Gontharet et al. 2007; Bayon et al. 2009a). Chemosynthesis-based communities have been observed in association with these different features (Huguen et al. 2005; Zitter et al. 2005; Dupré et al. 2007).

Faunal assemblages associated with Mediterranean cold seeps from the NDSF are still relatively unknown. Only those from a few sites along the Mediterranean Ridge have been described, and symbiont-bearing species such as the siboglinid polychaete *Lamellibrachia anaximandri*, the lucinid *Lucinoma kazani* and the mytilid *Idas modiolaefomis* were identified (Salas and Woodside 2002; Olu-Le Roy et al. 2004; Werne et al. 2004; Duperron et al. 2008; Southward et al., in press). The distribution of fauna on the Amsterdam MV has been hypothesized to be linked to the amount of methane escaping from the mud flows, which decreases from the summit to the periphery (Olu-Le Roy et al. 2004). Likewise, biological zonation has also been observed on the Barbados prism (Olu et al. 1997) and the Håkon Mosby MV (Niemann et al. 2006; Jerosch et al. 2007). In both cases, the summit of the MV was covered by fresh gassy mud flows and devoid of visible epifauna. Communities dominated by sulphide-oxidizing bacterial
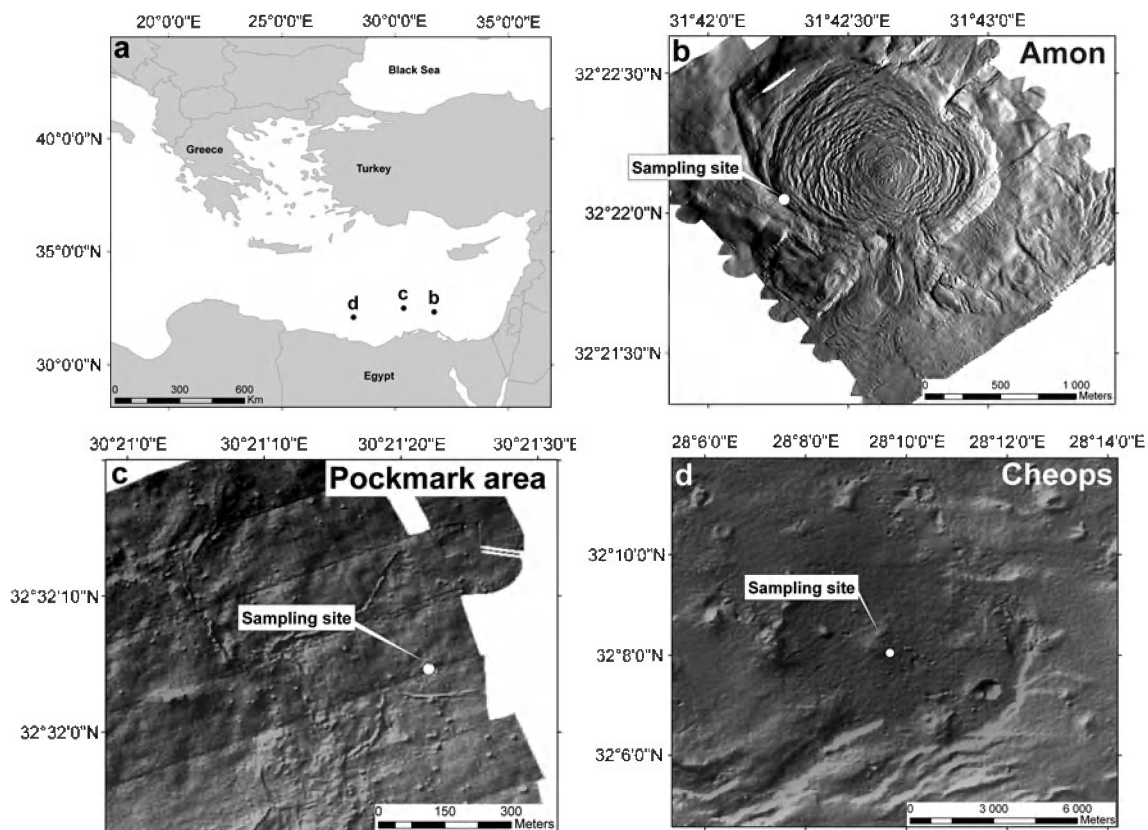

Fig. 1 **a** General map of the Eastern Mediterranean Sea with the localisation of the sampling sites in the three morpho-structural provinces: **b** the Amon mud volcano (*MV*) located in the Eastern province, **c** the Pockmark area located in the Central province and, **d** the Cheops MV in the Western province. Sampling sites are indicated on each MVs

mats were observed close to the summit, followed by symbiont-bearing fauna and heterotrophic fauna towards the periphery (Olu et al. 1997; Zitter et al. 2003; Niemann et al. 2006; Jerosch et al. 2007). Fresh mud flows are often characterized by very high rates of upward flux of reduced sulphidic fluids, excluding animals (de Beer et al. 2006). Older mud flows, located at the periphery, transport lower concentrations of methane and sustain sulphide production, fuelling chemosynthetic populations of siboglinid tube-worms or bivalves like *Acharax*. The latter migrate to deeper sediment layers to reach for reduced chemical compounds. This environment also constitutes a favourable habitat for heterotrophic fauna, which develop in response to a local increase in microbial production (Levin and Mendoza 2007).

The present study was the first opportunity to characterize the structure of the faunal assemblages in the NDSF seeps on two types of geological features: MVs and pockmarks. Prior to 2006, no ecological study had been performed on NDSF seep sites, with the exception of a few observations and samples taken during the NAUTINIL cruise in 2003 (Dupré et al. 2007; Bayon et al. 2009b; Huguen et al. 2009). The macro- and meiofaunal benthic communities associated with different microhabitats found in three provinces of the NDSF were sampled by remotely operated vehicles (ROVs) during two cruises: BIONIL in 2006 and MEDECO in 2007. The microhabitats were characterized with regard to their environmental conditions and faunal communities. We then compared faunal composition, density and diversity—mostly to family level—at local and regional scales to test the hypotheses that (1) reduced habitats support higher biomasses but lower diversity of meio- and macrofauna compared to surrounding oxygenated habitats, (2) carbonate crusts and reduced sediments bear different faunal assemblages, (3) faunal composition is related to microhabitat type rather than to the larger geological setting, (4) beta-diversity between the different microhabitats is related to differences in fluid flow and substratum type.

Materials and methods

Faunal sampling and habitat characterizations were done during BIONIL M70/2b aboard the German R/V *Meteor* with the ROV *Quest4000* (MARUM, University Bremen) in November 2006 and during leg 2 of MEDECO aboard the French R/V *Pourquoi Pas?* with the ROV *Victor6000* (Ifremer) in November 2007.

Study sites

The cold seep sites from three of the four provinces of the NDSF were investigated: (1) the Amon MV in the Eastern province, (2) a carbonate cemented area close to a pockmark field associated with large debris-flows in the Central province and (3) the Cheops MV in the Western province hosting large brine pools (Fig. 1). A single reference sample located 15 km away from the centre of the Amon MV and outside the influence of fluid emissions was sampled as a reference (hereafter marked 'Ref') for oxygenated deep-sea sediments not associated with fluid flow.

The Western province: the Amon MV

The Amon MV (32°22′05″N–31°42′27″E, Fig. 1b) is circular, approximately 3 km in diameter and 90 m high. It lies close to the limit of the Messinian platform, at 1,150 m water depth. The summit is covered with mud blocks and clasts and shows a disturbed, chaotically structured surface suggesting recent impacts of mud extrusion and gas expansion. Temperatures at the centre reach 45°C at 10 m below the seafloor, confirming high upward fluid flow at this MV (Dupré et al. 2007, 2008). The periphery of the Amon MV is characterized by highly bioturbated hemipelagic sediments. At its south-western rise, a lateral flow of reduced muds was identified ('sulphur-band') surrounded by carbonate crusts. Both types of substrata were sampled in 2006 during BIONIL. The carbonate crusts were rather thick, and we could not observe blackish muds or siboglinid colonies associated with them.

The Central province: the Pockmark area

The Central province hosts large carbonate-cemented areas associated with reduced debris-flows, and numerous small pockmarks located between 1,700 and 2,100 m depth (Fig. 1c). The pockmarks form circular depressions of a few metres in diameter and about 1 m deep, and are associated with the presence of authigenic carbonate crusts and reduced sediments (Loncke et al. 2004). During BIONIL, benthic communities were sampled from one reduced blackish sediment site at mid-slope (site 2A, 32°32′00″N–30°21′10″E 1,700 m) as well as from their surrounding carbonate cements. Blackish muds and living siboglinid colonies were observed between the cracks of the flat, thin carbonates.

The Eastern province: the Cheops MV

The Cheops MV is located within the Menes caldera at 3,000 m depth (32°08′05″N–28°09′67″E, Fig. 1d), above the Messinian platform. This caldera is a circular 50 m-deep depression 8 km in diameter. As previously observed in 2003 during the NAUTINIL cruise, a mixture of brine and mud was flowing from Cheops during the sampling

operations in 2007 (MEDECO). The mud is expelled from deep layers as indicated by high-temperature anomalies (Huguen et al. 2009). This MV is also characterized by numerous brine pools, covered by bright white matter, identified as microbial sulphur deposits (Dupré et al. 2007; Omoregie et al. 2008). Brine pools constitute direct evidence of fluid emissions, and the migration of fluids enriched in salt induces high sediment instability that may influence faunal composition and distribution on this MV. Faunal sampling and environmental characterizations were carried out during MEDECO. Sparse carbonate crusts were observed but not sampled.

Description of the microhabitats

At all cold seep sites visited, ROV surveys indicated a mosaic of visually distinguishable microhabitats, characterized by the presence of visible fauna or microbial mats.

Two types of reduced habitats were sampled (1) reduced blackish sediments (Red) covered with whitish bacterial mats or small tubeworms at the surface (Figs. 2a, c, e) and (2) carbonate crusts (CC) that were dark-coloured at Amon, or 'crumbly' and of whitish to light grey colour at the Pockmark area Figs. 2b, d).

Chemical characterization of the microhabitats was performed above the reduced sediments of Cheops MV during MEDECO. Water samples were taken for chemical analyses using the PEPITO water sampler above the organisms as close as possible to the seafloor using the Victor6000 manipulator arm. PEPITO collects water in 200-ml titanium bottles (Sarradin et al. 2009). Chemical measurements and sediment samples were taken during BIONIL as close as possible above the seafloor or in soft sediments with bottles (750 ml) and push cores (inner diameter 7.5 cm) at 2.5–50 m from the sampled microhabitats. Further sampling details are given in Table 1.

Fig. 2 Representative photographs of the microhabitats sampled in the three study sites. On the Amon MV: **a** reduced sediments and **b** carbonate crusts; in the Pockmark area: **c** reduced sediments and **d** carbonate crusts; on the Cheops MV: **e** reduced sediments. *Red* reduced sediments, *CC* carbonate crusts. All microhabitats were sampled during the BIONIL (2006) and MEDECO (2007) cruises. Photos **a–d**: MARUM, University Bremen, *QUEST 4000* and photo **e**: Ifremer, *Victor6000*
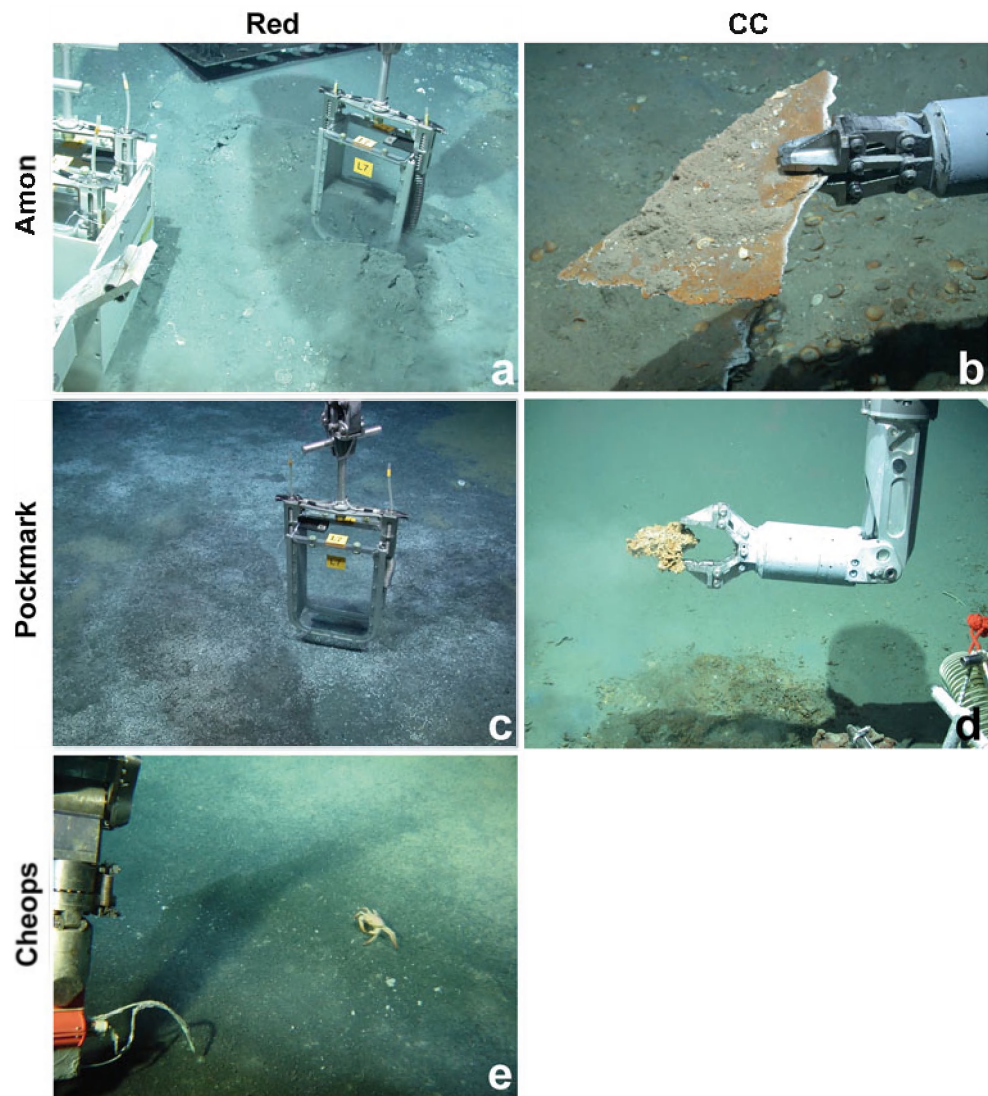
Table 1 Location, depth and tools used to perform physico-chemical and faunal sampling at each microhabitat from the different sampling sites of the Nile Deep-Sea Fan explored during the BIONIL (2006) and MEDECO (2007) cruises

Microhabitat types	Latitude (°N)	Longitude (°E)	Depth (m)	Physico-chemical measurements	Faunal sampling
Reference site [ca. 15 km away from Amon MV (BIONIL 2006)]					
Reference site (Ref)	32°21.42′	31°32.50′	1,000	3 tubes (multicorer) M70/2b_785	3 tubes (74 cm² each) Ref1 (20 cm) Ref2 (20 cm) Ref3 (20 cm)
Eastern province—Amon MV (BIONIL 2006)					
Reduced sediments (Red)	32°22.05′	31°42.27′	1,154	Microsensor, chamber and porewater samples PC15, 46, 47 M70/2b_765 (D115) M70/2b_790 (D121)	3 blade cores (200 cm² each) Red1 (BCROV-2, 10 cm) Red2 (BCROV-3, 10 cm) Red3 (BCROV-8, 10 cm)
Carbonate crusts (CC)	32°22.05′	31°42.27′	1,153	None	3 pieces of crust CC1 (SFS-11, 84 cm²) CC2 (SFS-111, 103 cm²) CC3 (SFS-2, 139 cm²)
Central province—Pockmark area, site 2A (BIONIL 2006)					
Reduced sediments (Red)	32°32.01′	30°21.13′	1,697	Microsensor, chamber and porewater samples PC9, 52 M70/2b_784 (D120) M70/2b_841 (D127)	3 blade cores (200 cm² each) Red1 (BCROV-3, 20 cm) Red2 (BCROV-7, 17 cm) Red3 (BCROV-8, 20 cm)
Carbonate crusts (CC)	32°32.00′	30°21.18′	1,696	None	3 pieces of crust CC1 (SFS-5, 48 cm²) CC2 (SFS-7, 81 cm²) CC3 (SFS-8, 34 cm²)
Western province—Cheops MV (MEDECO 2007)					
Reduced sediments (Red)	32°08.05′	28°09.67′	3,007	3×2 water sample: C^1H_4 MEDECO2_D343-PEPITO A-1 and PEPITO A-2, A-2, B-1, B-2, C-1 and C-2	3 blade cores (200 cm² each) Red1 (BL-2, 20 cm) Red2 (BL-4, 19 cm) Red3 (BL-6, 17 cm)

Sampling effort, length of each sediment cores and estimated surface of each piece of carbonate crusts are also reported. Further details of samples are archived in http://www.pangaea.de/PHP/CruiseReports.php?b=HERMES

Epi- and endofauna were sampled with blade corers on a surface of 200 cm² (Menot et al. 2010) on all soft sediment sites (Figs. 2a, c, d, g), and pieces of carbonate crusts were sampled using the claw of the ROV *Victor6000* (Figs. 2b, e). A reference site, located 15 km away from the active centre of Amon, has been sampled with a single multicorer. Three tubes, covering a surface of 74 cm² each, were analysed (pseudo-replicates). The number of samples for each microhabitat is reported in Table 1. Quantitative sampling of the crusts was difficult because they had to be broken off in pieces with the submersible manipulator. No crusts were obtained from Cheops.

Physico-chemical analyses

The 200-ml water samples were used to determine methane concentrations using the technique of headspace sampling gas chromatography with a thermal-conductivity detector (TCD) and a flame-ionisation detector (error of 4%; see method in Sarradin and Caprais 1996).

Sediments from push corers were split horizontally at 1-cm intervals on board, and porewater was extracted by centrifugation of the different sediment layers. After filtration and dilution, sulphate and chloride concentrations were measured by non-suppressed anion exchange chromatography (Water IC-Pak anion exchange column, waters 430 conductivity detector). Total dissolved sulphide concentrations were determined with the diamine complexation method by colorimetric method (Cline 1969). Intact cores, along with supernatant water, were used for pH, oxygen and total dissolved sulphide concentration measurements. Small-scale porewater concentration profiles for O_2, H_2S and pH were performed on push core samples from soft sediments using microelectrodes as described by de Beer et al. (2006). Microelectrodes with a tip diameter of ca. 20 μm were lowered into the sediment with a step resolution of 200 μm to monitor the concentration profiles within the upper sediment layer. Total oxygen consumption was measured in situ using a ROV-operated benthic chamber module (Treude et al. 2009). The chamber encloses an area of ca. 285 cm² together with approximately 10–15 cm of overlying bottom water. The change in O_2 concentration over time in the enclosed water volume was continuously monitored by two Clark-type mini-electrodes mounted in the chamber lid. This measurement integrates all relevant transport and consumption processes (diffusion, advection and fauna mediated transport as well as fauna respiration).

Faunal sorting and identification

The faunal samples were processed as described by Ritt et al. (2010). Sediments from blade corers were photographed and split horizontally (0–1, 1–3, 3–5, 5–10,

>10 cm) immediately after recovery. The range of the last slice depended on core lengths that varied from 10 to 20 cm (Table 1). Core slices were passed through a sieve column (2, 1 mm, 500, 250 μm), and the retained sediment was preserved in 10% buffered formalin. In the laboratory, all sediments up to 10 cm were rinsed and invertebrates were sorted under a dissecting microscope and identified to the lowest taxonomic level possible (here mostly family level). In this study, we considered macrofauna sensu stricto (>250 μm, Hessler and Jumars 1974), and any meiofaunal taxa such as Nematoda, Copepoda, Ostracoda and Acarina were analysed separately. As a consequence, meiofaunal samples contain only the largest fraction retained by a 250-μm mesh and do not include the 32 or 62 μm size limit usual for meiofauna (Hessler and Jumars 1974; Thistle 2003; Van Gaever et al. 2006).

The CC samples were preserved individually in 10% buffered formalin on board after recovery. In the laboratory, the carbonates crusts were treated by retrieving the organisms embedded or attached on them without breaking the crusts. Then they were washed over a 250-μm mesh, and the organisms retained were processed as those on the soft sediments. The surface of the sampled carbonate crusts were estimated using the IPLab Spectrum® image analysis software. Quantitative 2-D surface analyses were performed on video images of the upper face of the crusts, and we did it thrice to reduce error resulting from on-screen tracing (Sarrazin and Juniper 1999). Total surface area of the crust was used to calculate area-related indicators such as density and biomass. To do this, organisms were counted at both sides of the crust and in the holes, and total counts of individuals were integrated over the seafloor surface area covered by the crust, as for the integration of sediment horizons.

However, because it does not take topography into account, this method may underestimate total surface area and in turn overestimate density and biomass. The crusts from the Pockmark area had a more complex topography with numerous holes in comparison with the flat carbonates from Amon; hence, the two-side method may have added uncertainty in areal estimates in this case.

Vertical distributions

Vertical distributions within each reduced sediment microhabitat and the reference site were studied in the depth layers 0–1, 1–3, 3–5 and 5–10. Total densities were calculated by summing up the sediment layers up to 10 cm. This was repeated for relative abundance and biomass. Biomass was assessed by measuring the mean preserved wet weight (pww) for each microhabitat. To do so, individuals of all major macrofaunal taxa (bivalves, polychaetes, gastropods and crustaceans) were pooled, pat-dried on absorbent paper and weighed on a microbalance with an error of 0.1 mg.

Diversity measurements

Within-microhabitat (α-diversity) and between-microhabitat (β-diversity) diversity (Whittaker 1960; Gray 2000) were estimated for all microhabitats.

Alpha-diversity

The α-diversity analyses were performed at the family level, which was reached for most of the taxa except Zoantharia, Scyphozoa, Terebellida, Isopoda, Leptostraca, Nematoda and Acarina. Undetermined families, individuals and larvae were removed from the analysis because of the probability that they belong to a family already listed. This may have resulted in an underestimation of the taxonomic richness. Sample-based rarefaction curves (sensu Gotelli and Colwell 2001) were calculated on macrofaunal datasets for each of the three study sites (Sanders 1968). Individual-based rarefaction curves were also computed on macro-faunal datasets for each microhabitat type, regardless of the province of origin. These curves plot expected taxonomic richness against sampling effort and allow comparisons impossible with observed richness (S). All rarefaction curves use the expected number of individuals as the X-axis (Sanders 1968, corrected and modified by Hurlbert 1971; Gotelli and Colwell 2001; Gauthier et al. 2010). Observed within microhabitat, taxonomic diversity was evaluated using common diversity indices as well as more robust intrinsic-diversity-based ordering methods (Liu et al. 2007; Gauthier et al. 2010). Commonly used to define the α-diversity, the taxonomic richness (S), the number of taxonomic groups observed in each microhabitat, the Shannon's entropy (H'_e; Shannon 1948) and the Gini-Simpson diversity index (D_{GS}; Gini 1912; Simpson 1949) were calculated. They are presented along with their *numbers equivalents*, allowing straightforward comparisons between communities (Hill 1973; Patil and Taillie 1982; Jost 2006, 2007). Community evenness was also determined using Pielou's index of evenness (J'; Pielou 1969).

The right tail-sum method (RTS) is a diversity ordering method, which is more robust and stringent than other methods and allows graphical comparisons of communities (Patil and Taillie 1982; Tothmérész 1998; Liu et al. 2007). Communities are ordered in decreasing diversity from the top most curves to the lowest ones. No clear conclusions can be drawn when curves intersect (Liu et al. 2007).

Beta-diversity

Despite the modest number of samples, multivariate analyses were conducted to better illustrate the similarities and differences among faunal samples. The lowest available taxonomic level was used. Principal component analysis (PCA) and Ward's hierarchical clustering were used to indirectly evaluate the influence of habitat conditions on community structure variation within and between the different microhabitats and sites, but excluding the undetermined taxa as previously explained for the alpha-diversity. A Procrustean randomization test (Jackson 1995) was performed to compare the PCA results of the macrofaunal and meiofaunal datasets. Abundance data were first Hellinger-transformed to conserve Hellinger, rather than Euclidian, distances in PCA (Legendre and Gallagher 2001). Hellinger distances were also used for Ward's hierarchical clustering. This distance gives low weight to rare taxa in the analyses. In the deep-sea, rare species are often not well sampled, and their sporadic appearance in samples is mostly attributable to sampling error (Gauthier et al. 2010).

In the PCA, the equilibrium contribution circle was computed to identify the taxa having the most impact on the position of the samples in the ordination (Legendre and Legendre 1998). The Jaccard's similarity coefficient (S_{jacc}) was used to quantify similarity in terms of shared taxa among replicates within each microhabitat (Jaccard 1901), giving equal weight to all taxa. Mean Jaccard similarity was computed to evaluate within-microhabitat variation.

All analyses were performed in the R environment (R Development Core Team 2009). Rarefaction curves, diversity indices and diversity profiles were computed both with the Biodiversity R package (Kindt and Coe 2005) and functions in Gauthier et al. (2010). Multivariate analysis was carried out using the vegan package (Oksanen et al. 2008).

Results

Physico-chemical characterization of microhabitats

The length of the cores varied from 10 to 20 cm depending on the nature of the substratum (Table 1). All samples came from surface cold seep habitats at in situ bottom water temperature of 13.5°C, as at the reference site. The Red cores from the Pockmark area contained black sediments with a strong hydrogen sulphide odour. At Amon, a horizon of 8–10 cm of black sediments overlaid greyish to beige hemipelagic sediments. On Cheops, a black layer of only 1–2 cm was found on top of beige hemipelagic sediments. The cores of the reference site contained only beige sediments.

Chemical characterization at a larger scale around microhabitats

Bottom water measurements

All chemical measurements were taken directly on the bottom, at a few centimetres above the seafloor. Overall, the pH was slightly lower in reduced sediments than at the reference site (Table 2). According to microprofiler measurements, pH value reached 7.9 at the interface of reduced sediments on Amon while in the Pockmark area, it was around 8.1 (Table 2).

Oxygen bottom water concentrations were similar at all sites, including the reference site (200–230 μmol l^{-1}) with the exception of the reduced sediments at Amon, where a temporary depletion was observed, reaching values below 200 μmol l^{-1}. No free sulphide was detected in the bottom waters at any of the sites, but sulphide was found within the porewaters at two reduced sediment sites (Table 2). Low concentrations of methane were measured in the bottom waters of Amon and the Pockmark area (0 and 0.2–0.3 μmol l^{-1}, respectively), while they ranged between 3.6 and 9.3 μmol l^{-1} in the overlying water of Cheops samples (Table 2).

Sulphate values of 31 mmol l^{-1} and chloride values of 529 mmol l^{-1} were measured above and throughout the core from the reference site. Sulphate and chloride measurements above the reduced sediments on Amon showed values varying from 30 to 40 mmol l^{-1} and 404–580 mmol l^{-1} respectively (Table 2). Here, spatial heterogeneity between samples was high, despite the proximity of the cores (few dm, e.g. PC 46 and PC 47). In microbial mats from the Pockmark area, sulphate concentrations varied from 29 to 30 mmol l^{-1}, while chloride values were high, ranging from 606 to 630 mmol l^{-1} (Table 2).

Porewaters, oxygen consumption and sulphate reduction rates in sediments

Oxygen penetration depth measured by microsensors was >4 cm at the reference site (Table 2). The porewater samples from the reference site did not contain sulphide or methane. Accordingly, sulphate reduction rate at the reference site was not measurable, and oxygen consumption was <1 mmol m^{-2} day^{-1} (Table 2).

In contrast, oxygen microsensor profiles in reduced sediments of Amon, Cheops and the Pockmark area showed complete oxygen consumption within the first 1–2 mm of seafloor (Figs. 3a, b). Total dissolved sulphide profiles in reduced sediments of Amon showed a maximum concentration of 2.5 mmol l^{-1} at 12 cm depth and were <0.8 mmol l^{-1} in the top 5 cm (Table 2; Fig. 3c). The

Table 2 Physico-chemical characterization of the bottom water environment at the three study areas

	pH	[O$_2$] Bottom water (μmol l^{-1})	[O$_2$] Penetration depth (cm)	[O$_2$] Total benthic consumption (mmol m^{-2} day^{-1})	[SO$_4^{2-}$] Porewater (mmol l^{-1})	[Cl$^-$] Porewater (mmol l^{-1})	[CH$_4$] Bottom water (μmol l^{-1})	[ΣS] Porewater (mmol l^{-1})	[ΣS] Porewater top 5 cm/peak conc. (mmol l^{-1})	SR Sediments (mmol m^{-2} day^{-1})
Amon MV										
Reference site (Ref)	8.20[a]	230[a]	>4[a]	<1[a]	31.4[a]	529[a]	0.0[a]	0[a]	–	<0.2[a]
Reduced sediments (Red)	7.88[b]	150–200[b]	0.0–0.25[b]	10–46[c]	30.7–40.3[d]	404–580[d]	0.0[d]	<0.7[b]	0.8/2.5[d]	0.5 ± 0.2 (n = 3)[d]
Pockmark area										
Reduced sediments (Red)	8.11[b]	230[e]	0.1–0.2[b]	156–174[c]	29.1–29.8[d]	606–630[d]	0.2–0.3[e]	0.2[b] 0.7[d]	0.8/25[d]	22–41[d]
Cheops MV										
Reduced sediments (Red)	–	220[e]	–	111–130[e]	–	–	3.60–9.34[f]	–	–	5–14[d]

Only the methane data from the Cheops MV were obtained directly above the organisms in this study. Amon data were provided by Girnth et al. (2011), Pockmark data by Grünke et al. (in press). ΣS total dissolved sulphides (H$_2$S + HS$^-$ + S^{2-}), SR sulphate reduction rate. The reference site was ca. 15 km away from the active centre of the Amon MV

Devices used to acquire the data: [a] multicorer, [b] microprofiler, [c] benthic chamber, [d] PCs, [e] KIPS bottle, [f] PEPITO, (–) no available data

Fig. 3 a, b Dissolved oxygen and **c, d** sulphide profiles measured by microsensors and in porewater extracted from push corers (*PC*) taken in reduced sediments on the **a, c** Amon MV and in **b, d** bacterial mats covering reduced sediments in the Pockmark area. The measurements were performed during the BIONIL cruise (2006)
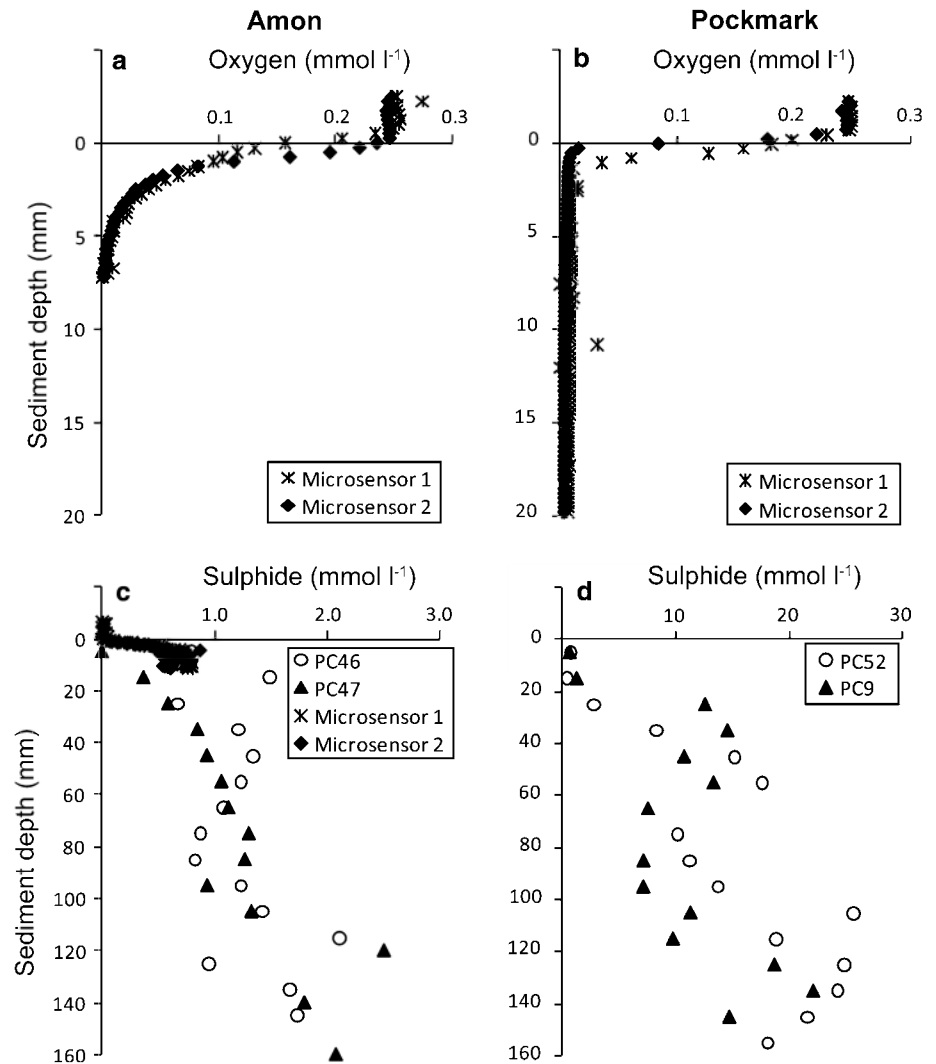
profile suggested that sulphide was oxidized completely within the first millimetres of sediment. In the Pockmark area, sulphide concentrations at 12.5 cm depth reached 25 mmol l^{-1} (Fig. 3d), but sulphide was also completely consumed within the surface sediments (Table 2). No porewater data were available for Cheops due to limitation in dive time.

At the reference site off Amon, sulphate and chloride concentrations were homogeneous along the whole length of the core with a mean of 32 ± 2.4 mmol l^{-1} for sulphate and 530 ± 49.5 mmol l^{-1} for chloride (Fig. 4a). On Amon reduced sediments, sulphate and chloride profiles were homogeneous along the length of the core reaching values similar to the reference sample with the exception of a high sulphate concentration at the top of the core (Fig. 4b). In the Pockmark area, the consumption of sulphate was visible throughout the 16 cm of the core length, whereas lower variation was observed in chloride (Fig. 4c).

Integrated sulphate reduction rates were negligible at the reference site, and low at the reduced sediment of

Amon. Intermediate rates were measured for Cheops and high rates associated with the Pockmark area (Table 2). Likewise, total benthic oxygen fluxes were very low at the reference site, and 1–2 orders of magnitude higher at all reduced sediment sites, with the highest rates associated with the black sediments of the Pockmark area (Table 2).

Macro- and meiofaunal community description

Composition, abundance, density and α-diversity patterns

Macrofaunal densities in the reduced sediments varied from 650 to 2,100 individuals m^{-2} at Amon (Table 3), 1,950–3,500 individuals m^{-2} in the Pockmark area (Table 4) and from 3,200 to 5,250 individuals m^{-2} at Cheops (Table 4). This represents approximately 3.5, 55 and 78% of the total fauna sampled at each site (Tables 5, 6). In comparison, the fully oxic reference site had from 541 to 1,081 macrofaunal individuals m^{-2} (Table 3),
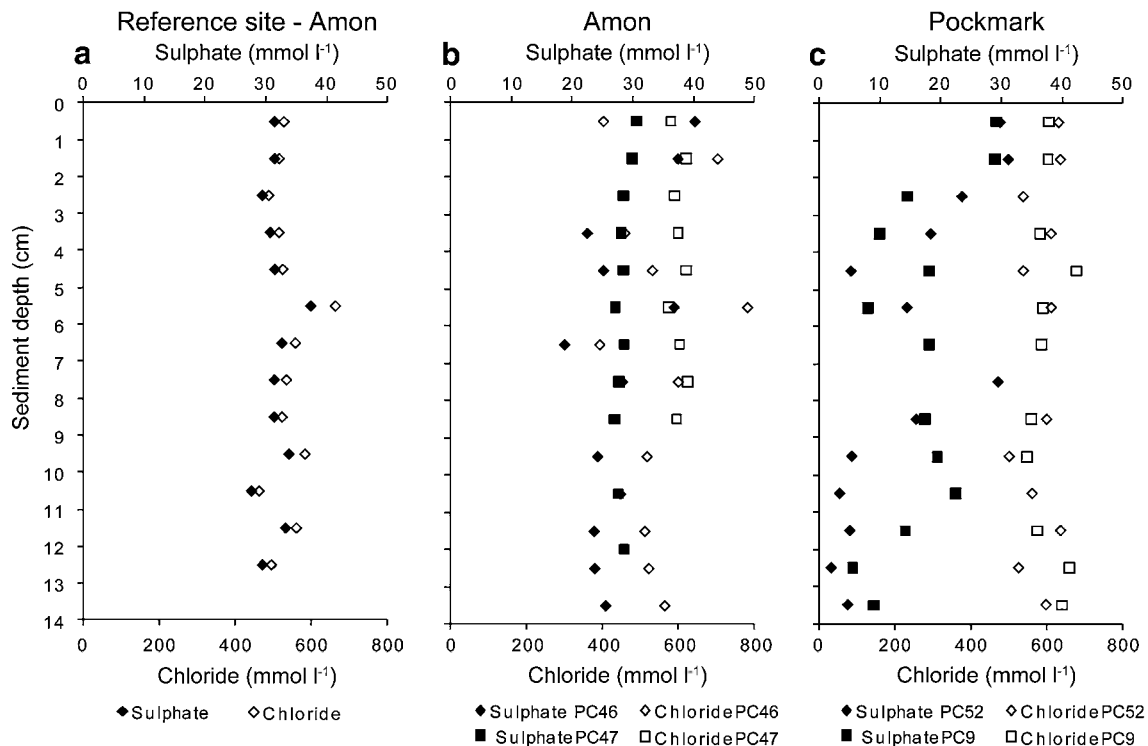
Fig. 4 Sulphate and chloride profiles measured in porewater extracted from sediment cores sampled **a** at the reference site off Amon MV, **b** in reduced sediments on the Amon MV and, **c** in bacterial mats covering reduced sediments in the Pockmark area. The measurements were performed during the BIONIL cruise (2006)

which represented 67% of the total fauna. On the carbonate crust microhabitat (CC), densities varied from 97 to 1,151 individuals m^{-2} at Amon (Table 3) and from 1,852 to 7,353 individuals m^{-2} in the Pockmark area (Table 4) representing, respectively, 100% (except CC2 where it only represents 20%) and 63% of the total fauna sampled (Tables 3, 4).

Despite the large sieve mesh size used (250 μm), many specimens corresponding to meiofaunal groups (copepods, ostracods, nematodes, mites) were found in our samples (Tables 5, 6). Densities of meiofauna >250 μm at reduced sediment sites varied from 31,800 to 75,550 individuals m^{-2} at Amon (Table 5), 1,450 to 3,950 individuals m^{-2} in the Pockmark area (Table 6) and 600 to 2,850 individuals m^{-2} at Cheops (Table 6). At Amon, meiofaunal mean density was higher in reduced sediments compared to carbonate crusts (Table 5). Overall, nematodes dominated the meiofauna >250 μm samples, varying from 95 to 100% of total abundances on the reduced sediments from the Amon and Cheops (Tables 5, 6). However, in the reduced sediments of the Pockmark area, nematodes represented only 36% of the meiofauna (Table 6). Here, harpacticoid copepods reached up to 37.5% of the meiofaunal abundance, followed by ostracods (~16%). Surprisingly, besides four nematodes, no meiofauna was sampled from

the CC microhabitat of Amon, compared to a relatively high number of nematodes found at the CC of the Pockmark area (Tables 5, 6).

Relative faunal abundances varied between microhabitats and between replicate samples of the same microhabitat, especially on carbonate crusts. Overall, the steady increase of the individual-based rarefaction curves suggests that the sampling effort was not sufficient (Fig. 5a). Only the curves of the reduced microhabitats of Cheops and the Pockmark area showed that we attained a relatively good estimation of their taxonomic richness. These curves also showed that the taxonomic richness between the different reduced sediment microhabitats was highest on Amon, followed by the Pockmark area and finally Cheops. The opposite trend was observed for the CC microhabitats, where the diversity was higher in the Pockmark area (Fig. 5a). Since the ranking of the curves would probably remain the same with additional macro-faunal samples, we can conclude with confidence that the total family richness (S) on Amon was highest on reduced sediments, intermediate on the Reference site and lowest on CC, while the CC microhabitat in the Pockmark area was richer than the reduced sediments (Tables 7, 8; Fig. 5a).

In terms of evenness (Pielou's index J', Table 8), the reference sample harboured the most even distribution

Table 3 Amon—macrofaunal (>250 μm) densities (individuals m^{-2}) per replicate and relative abundance (%) of each taxa in the microhabitats studied on the Amon mud volcano: reduced sediments (Red, $n = 3$), carbonate crusts (CC, $n = 3$) and the reference site (Ref, $n = 3$)

Taxonomic groups	Reduced sediments				Carbonate crusts				Reference samples			
	Red1	Red2	Red3	%	CC1	CC2	CC3	%	Ref1	Ref2	Ref3	%
Cnidaria (total)	**0**	**0**	**0**	**0**	**0**	**0**	**935**	**72.21**	**0**	**270**	**135**	**17.65**
Anthozoa-Zoantharia*	0	0	0	0	0	0	935	72.21	0	0	0	0
Medusozoa-Scyphozoa*	0	0	0	0	0	0	0	0	0	270	135	17.65
Polychaeta (total)	**1,900**	**400**	**1,650**	**82.29**	**119**	**0**	**144**	**16.67**	**541**	**405**	**676**	**70.59**
Capitellidae*	1,100	100	50	26.04	0	0	0	0	0	0	0	0
Dorvilleidae*	300	50	1,250	33.33	0	0	0	0	135	135	0	11.76
Hesionidae*	0	0	50	1.04	0	0	0	0	0	0	0	0
Paraonidae*	0	0	0	0	0	0	0	0	135	0	0	5.88
Pholoidae*	0	0	0	0	0	0	144	11.11	0	0	0	0
Phyllodocidae*	100	0	0	2.08	0	0	0	0	0	0	0	0
Spionidae*	100	0	50	3.13	0	0	0	0	135	135	405	29.41
Siboglinidae, Frenulata*	300	50	200	11.46	0	0	0	0	0	0	0	0
Syllidae*	0	0	0	0	119	0	0	5.56	0	0	270	11.76
Terebellida*	0	150	50	4.17	0	0	0	0	135	135	0	11.76
Larvae	0	50	0	1.04	0	0	0	0	0	0	0	0
Bivalvia (total)	**100**	**150**	**300**	**11.46**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Lucinidae*												
Lucinoma kazani	0	150	50	4.17	0	0	0	0	0	0	0	0
Mytilidae*												
Idas modiolaeformis	50	0	0	1.04	0	0	0	0	0	0	0	0
Thyasiridae*												
Thyasira striata	0	0	100	2.08	0	0	0	0	0	0	0	0
Vesicomyidae*												
Isorropodon perplexum	0	0	150	3.13	0	0	0	0	0	0	0	0
Sareptidae*												
Und. Sareptidae	50	0	0	1.04	0	0	0	0	0	0	0	0
Gastropoda (total)	**0**	**50**	**0**	**1.04**	**0**	**0**	**72**	**5.56**	**0**	**0**	**0**	**0**
Calliotropidae*												
Putzeysia wiseri	0	0	0	0	0	0	72	5.56	0	0	0	0
Und. Gastropoda*	0	50	0	1.04	0	0	0	0	0	0	0	0
Sipuncula (total)	**0**	**0**	**0**	**0**	**0**	**97**	**0**	**5.56**	**0**	**0**	**135**	**5.88**
Phascolosomatidae*												
Phascolosoma aff. granulatum	0	0	0	0	0	97	0	5.56	0	0	0	0
Golfingiidae*												
Nephasoma minutum	0	0	0	0	0	0	0	0	0	0	135	5.88
Crustacea (total)	**50**	**50**	**150**	**5.21**	**0**	**0**	**0**	**0**	**0**	**0**	**135**	**5.88**
Decapoda-Thalassinidae*	0	0	50	1.04	0	0	0	0	0	0	0	0
Cumacea-Nannastacidae*												
Cumella pygmaea	0	0	50	1.04	0	0	0	0	0	0	0	0
Isopoda*	0	0	0	0	0	0	0	0	0	0	135	5.88
Leptostraca*	50	0	0	1.04	0	0	0	0	0	0	0	0
Larvae	0	50	50	2.09	0	0	0	0	0	0	0	0
Total density (ind. m^{-2})	**2,050**	**650**	**2,100**		**119**	**97**	**1,151**		**541**	**676**	**1,081**	

Total densities and relative abundances from each taxonomic group are highlighted in bold. _Und._ undetermined individuals. (*) Taxonomic level used for alpha-diversity analyses, here mostly family level. All sampling was performed during the BIONIL cruise (2006)

Table 4 Pockmark area and Cheops—macrofaunal (>250 μm) densities (individuals m^{-2}) per replicate and relative abundance (%) of each taxa in the microhabitats studied in the Pockmark area: reduced sediments (Red, $n = 3$), carbonate crusts (CC, $n = 3$) and on the Cheops MV: reduced sediments (Red, $n = 3$)

| Taxonomic groups | Pockmark area | | | | | | | | Cheops MV | | | |
| | Reduced sediments | | | | Carbonate crusts | | | | Reduced sediments | | | |
	Red1	Red2	Red3	%	CC1	CC2	CC3	%	Red1	Red2	Red3	%
Polychaeta (total)	**2,550**	**2,650**	**1,800**	**83.83**	**1,042**	**123**	**4,412**	**39.62**	**3,800**	**5,100**	**3,000**	**95.58**
Capitellidae*	450	650	250	16.16	208	0	0	1.89	0	0	0	0
Cirratulidae*	0	0	0	0	208	0	0	1.89	0	0	0	0
Dorvilleidae*	1,350	1,500	1,350	50.30	208	0	0	1.89	0	0	50	0.40
Glyceridae*	50	0	0	0.60	0	0	0	0	0	0	0	0
Hesionidae*	500	350	200	12.57	417	0	588	7.55	950	1,050	1,050	24.50
Sabellidae*	0	0	0	0	0	0	2,941	18.87	0	0	0	0
Spionidae*	100	100	0	2.40	0	123	0	1.89	2,750	2,750	1,300	54.62
Syllidae*	0	0	0	0	0	0	294	1.89	0	0	0	0
Terebellida*	100	50	0	1.80	0	0	588	3.77	100	1,300	600	16.06
Bivalvia (total)	**350**	**500**	**50**	**10.78**	**0**	**0**	**1,176**	**7.55**	**0**	**0**	**0**	**0**
Lucinidae*												
Lucinoma kazani	0	100	0	1.20	0	0	0	0	0	0	0	0
Und. Lucinidae	0	100	0	1.20	0	0	0	0	0	0	0	0
Mytilidae*												
Idas modiolaeformis	0	0	0	0	0	0	1,176	7.55	0	0	0	0
Vesicomyidae*												
Isorropodon perplexum	100	150	0	2.99	0	0	0	0	0	0	0	0
Und. Bivalvia	150	150	50	5.39	0	0	0	0	0	0	0	0
Gastropoda (total)	**0**	**350**	**100**	**5.39**	**1,667**	**1,728**	**1,471**	**50.94**	**0**	**50**	**50**	**0.80**
Calliotropidae*												
Putzeysia wiseri	0	0	0	0	208	123	294	5.66	0	0	0	0
Trochidae*	0	0	0	0	208	123	588	7.54	0	0	0	0
Skeneidae*												
Akritogyra conspicua	0	0	0	0	0	123	0	1.9	0	0	0	0
Und. *Skeneidae*	0	100	0	1.20	0	0	0	0	0	0	0	0
Orbitestellidae*												
Lurifax vitreus	0	0	0	0	1,250	1,358	588	35.85	0	0	0	0
Und. Gastropoda	0	250	100	4.19	0	0	0	0	0	50	50	0.80
Crustacea (total)	**0**	**0**	**0**	**0**	**0**	**0**	**294**	**1.89**	**200**	**100**	**150**	**3.61**
Amphipoda-Gammaridae												
Sebidae*												
Seba sp.	0	0	0	0	0	0	294	1.89	0	0	0	0
Und. Gammaridae	0	0	0	0	0	0	0	0	50	0	0	0.40
Leptostraca												
Nebaliidae*	0	0	0	0	0	0	0	0	0	0	50	0.40
Und. Leptostraca	0	0	0	0	0	0	0	0	150	100	100	2.81
Total density (ind. m^{-2})	**2,900**	**3,500**	**1,950**		**2,708**	**1,852**	**7,353**		**4,000**	**5,250**	**3,200**	

Total densities and relative abundances from each taxonomic group are highlighted in bold. *Und.* undetermined individuals. (*) Taxonomic level used for alpha-diversity analyses, here mostly family level. All sampling was performed during the BIONIL (2006) and MEDECO (2007) cruises

followed by CC from the Pockmark area and reduced sediments from Amon (also shown in Fig. 5b). Finally, the reduced sediments from Cheops were the poorest microhabitat sampled in the NDSF. It was among the least even of all microhabitats along with CC from Amon and Red from Pockmark (Tables 7, 8). At Amon, polychaetes were the dominant taxa in the reduced habitats and reference samples, constituting respectively ∼82 and ∼71% of total faunal

Table 5 Amon—meiofaunal (>250 µm) densities (individuals m^{-2}) per replicate and relative abundance (%) of each taxa in the reduced sediment microhabitat (Red, $n = 3$) studied on the Amon and in the reference samples (Ref, $n = 3$)

Taxonomic groups	Reduced sediments				Carbonate crusts				Reference site			
	Red1	Red2	Red3	%	CC1	CC2	CC3	%	Ref1	Ref2	Ref3	%
Nematoda (total)	**74,650**	**29,900**	**28,300**	**95.15**	**0**	**388**	**0**	**100**	**405**	**135**	**405**	**77.78**
Crustacea (total)	**900**	**1,900**	**4,000**	**4.85**	**0**	**0**	**0**	**0**	**270**	**0**	**0**	**22.22**
Copepoda-Harpacticoida												
Miraciidae*												
Bulbamphiascus imus	500	1,050	200	1.24	0	0	0	0	0	0	0	0
Bulbamphiascus sp. 1	0	0	0	0	0	0	0	0	0	0	0	0
Thyphlamphiascus confusus	0	0	550	0.39	0	0	0	0	0	0	0	0
Ameiridae*												
Amphiascus sp. 2	0	0	0	0	0	0	0	0	0	0	0	0
Haifameira archibenthoica	0	400	2,400	2.00	0	0	0	0	0	0	0	0
Haifameira sp. 1	0	0	200	0.14					0	0	0	0
Agestidae*												
Eurycletodes sp.	0	0	0	0	0	0	0	0	270	0	0	22.22
Tisbidae*												
Tisbella sp.	0	0	0	0	0	0	0	0	0	0	0	0
Und. Harpacticoida	0	0	50	0.07	0	0	0	0	0	0	0	0
Copepoda-Cyclopoida												
Oncaeidae*												
Oncaea sp.	0	0	50	0.04	0	0	0	0	0	0	0	0
Copepoda-Calanoida*												
Calanoida sp. 1	0	150	450	0.43	0	0	0	0	0	0	0	0
Calanoida sp.	0	300	0	0.21	0	0	0	0	0	0	0	0
Und. Calanoida	300	0	0	0.21	0	0	0	0	0	0	0	0
Ostracoda												
Polycopidae*												
Polycope sp. 3M	50	0	0	0.04	0	0	0	0	0	0	0	0
Pontocyprididae*												
Propontocypris sp. 2M	0	0	1	0.04	0	0	0	0	0	0	0	0
Propontocypris cf. levis	50	0	0	0.04	0	0	0	0	0	0	0	0
% Meiofauna/total fauna	97.36	98.30	93.71		0	80.00	0		55.56	16.67	27.27	
Total density (ind. m^{-2})	**75,500**	**31,800**	**32,300**		**0**	**388**	**0**		**676**	**135**	**405**	

Total densities and relative abundances from each taxonomic group are highlighted in bold. *Und.* undetermined individuals. (*) Taxonomic level used for alpha-diversity analyses, here mostly family level. All sampling was performed during the BIONIL cruise (2006)

abundance. A total of 8 polychaete families or orders (since the Terebellida were not identified at the family level) were represented in the reduced sediments (Tables 3, 7). With ~11.5% of the total macrofaunal abundance, bivalves were the second dominant taxon in the reduced sediments of Amon, whereas cnidarians (~18%) ranked second in the reference sample. Gastropods and crustaceans were present in low abundance (<6%) in reduced sediments, while on reference samples, low abundances were for the crustaceans and sipunculians. On CC from Amon, cnidarians represented 72% of the total abundance, distantly followed by polychaetes (~17%; Table 8). Some gastropods and

sipunculians were also present in low abundance (<6%; Table 3). In the Pockmark area, reduced sediments were dominated by 8 polychaete taxa, reaching ~84% of the total macrofaunal abundance, followed by bivalves and gastropods (Tables 4, 7). Contrary to Amon, the fauna from CC in the Pockmark area was more evenly distributed with gastropods and polychaetes representing, respectively, ~51 and 40% of the total abundance (Table 4). Bivalves and crustaceans were also present, representing less than 8% of the total macrofaunal abundance (Table 4). The rarefaction curves and the Pielou's index confirm the higher evenness of the CC from the Pockmark area compared to CC from Amon

Table 6 Pockmark area and Cheops—meiofaunal (>250 μm) densities (individuals m^{-2}) per replicate and relative abundance (%) of each taxa in the microhabitats studied on the Pockmark area: reduced sediments (Red, $n = 3$), carbonate crusts (CC, $n = 3$) and on the Cheops mud volcano (MV): reduced sediments (Red, $n = 3$)

Taxonomic groups	Pockmark area								Cheops MV			
	Reduced sediments				Carbonate crusts				Reduced sediments			
	Red1	Red2	Red3	%	CC1	CC2	CC3	%	Red1	Red2	Red3	%
Nematoda (total)	**1,100**	**500**	**1,000**	**36.11**	**5,417**	**247**	**2,941**	**97.44**	**750**	**2,850**	**600**	**100**
Crustacea (total)	**2,850**	**1,300**	**450**	**63.89**	**0**	**123**	**0**	**2.56**	**0**	**0**	**0**	**0**
Copepoda-Harpacticoida												
Miraciidae*												
Bulbamphiascus imus	1,450	850	350	36.81	0	0	0	0	0	0	0	0
Ameiridae*												
Amphiascus sp. 2	0	0	0	0	0	123	0	2.56	0	0	0	0
Tisbidae*												
Tisbella sp.	50	0	0	0.69	0	0	0	0	0	0	0	0
Copepoda-Cyclopoida												
Cyclopina sp.	0	250	0	3.47								
Copepoda-Calaonida*												
Calanoida sp. 1	100	0	0	1.39	0	0	0	0	0	0	0	0
Calaonida sp. 2	50	0	0	0.69	0	0	0	0	0	0	0	0
Calaonida sp. 3	50	0	0	0.69	0	0	0	0	0	0	0	0
Calanoida sp.	250	0	0	3.47	0	0	0	0	0	0	0	0
Ostracoda												
Pontocyprididae*												
Propontocypris sp. 1M	50	0	0	0.69	0	0	0	0	0	0	0	0
Propontocypris cf.*levis*	700	200	100	13.89	0	0	0	0	0	0	0	0
Propontocypris cf. *setosa*	50	0	0	0.69	0	0	0	0	0	0	0	0
Argilloecia sp.	50	0	0	0.69	0	0	0	0	0	0	0	0
Chelicerata												
Und. Acarina*	50	0	0	0.69	0	0	0	0	0	0	0	0
% Meiofauna/total fauna	57.66	33.96	42.65		66.67	16.67	28.57		15.79	35.19	15.79	
Total density (ind. m^{-2})	**3,950**	**1,800**	**1,450**		**5,417**	**370**	**2,941**		**750**	**2,850**	**600**	

Total densities and relative abundances from each taxonomic group are given in bold. *Und.* undetermined individuals. (*) Taxonomic level used for alpha-diversity analyses, here mostly family level. All sampling was performed during the BIONIL (2006) and MEDECO (2007) cruises

Fig. 5 **a** Rarefaction curves and **b** right tail-sum intrinsic diversity profiles for the pooled macrofaunal abundance data at the family level and at each microhabitat from the Amon MV (3 microhabitats including the reference sample), the Pockmark area (2 microhabitats) and the Cheops MV (1 microhabitat)
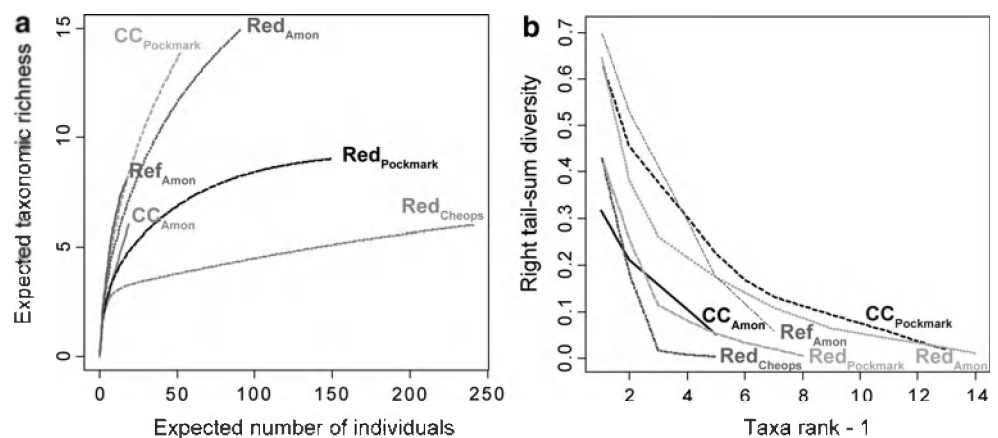
Table 7 Red—biological descriptors of the reduced sediment microhabitats sampled on the Amon MV, the Pockmark area and the Cheops MV in the Nile Deep-Sea Fan

Biological descriptors	Amon	Pockmark area	Cheops
Macrofauna-dominant	Polychaetes reaching 82.3% of total abundance (Dorvilleidae, Capitellidae)	Polychaetes reaching 83.8% of total abundance (Dorvilleidae)	Polychaetes, reaching 95.6% of total abundance (Spionidae, Hesionidae)
Macrofauna-others	Bivalves, crustaceans, gastropods	Bivalves, gastropods	Crustaceans, gastropods
Mean macrofaunal densities (individuals m^{-2})	1,600 ± 912	2,783 ± 782	**4,150 ± 1,033**
Mean Jaccard's similarity	0.32	0.47	**0.77**
Symbiont-bearing fauna	**17.1–36.4%**	0–10%	0%
Total macrofaunal biomass (kg preserved wet weight m^{-2})	**$1.2 \times 10^{-1} \pm 1 \times 10^{-1}$**	$1.2 \times 10^{-3} \pm 1 \times 10^{-3}$	$6.4 \times 10^{-3} \pm 3 \times 10^{-3}$
Macrofaunal diversity indices			
Total richness (S)	**15**	9	6
Shannon (H_e')	**1.93**	1.35	1.05
Exp (H_e')	*6.89*	*3.86*	*2.86*
Gini-Simpson (D_{GS})	**0.78**	0.63	0.59
(1/1 − D_{GS})	*4.62*	*2.68*	*2.44*
Evenness (J')	**0.71**	0.61	0.59
Meiofauna (>250 μm)-dominant	Nematodes with 95.2% of the total abundance	Copepods with 63.9% of the total abundance	100% nematodes
Meiofauna (>250 μm)-others	Copepods, ostracods (6%)	Acarina (1.3%)	–
Mean meiofaunal densities (individuals m^{-2})	**46,733 ± 25,004**	2,400 ± 1,354	1,400 ± 1,258
Mean Jaccard's similarity	0.31	0.41	**0.67**
Meiofaunal diversity indices			
Total richness (S)	**7**	**7**	1
Nematoda richness	1	1	**1**
Copepoda richness	4	3	0
Ostracoda richness	2	2	0
Acarina richness	0	1	0
Shannon (H_e')	0.24	**1.39**	0
Exp (H_e')	*1.27*	*4.01*	
Gini-Simpson (D_{GS})	0.09	**0.70**	–
(1/1 − D_{GS})	*1.10*	*3.37*	
Evenness (J')	0.12	**0.71**	–

The highest values are highlighted in bold. The meiofaunal data include only the large meiofauna >250 μm. The number equivalents of Shannon and Simpson indices are given in italics

(Tables 7, 8; Fig. 5a), the highest evenness being observed on the reference site (Table 8). Finally, on Cheops, polychaetes largely dominated reduced sediments, with a mean of 95.6% of the faunal abundance, followed by low proportions of crustaceans and gastropods (Tables 4, 7).

The RTS performed with the same dataset is difficult to interpret due to the crossing of the curves (Fig. 5b). Using this analysis, Amon reduced sediments were the richest and most even microhabitat among the three reduced sediments. In contrast, the CC from the Pockmark area was more diverse than that from Amon (Fig. 5b).

Finally, we pooled the data obtained for the different microhabitats to determine the diversity of each site, including the reference samples from Amon. For Amon and the Pockmark area, the sample-based rarefaction curves did not level-off, suggesting that sampling was insufficient to accurately estimate taxonomic richness (Fig. 6a). At Cheops, despite the low sample number ($n = 3$), the curve levelled-off, suggesting that its macrofaunal diversity was well described (Fig. 6a). Our data indicate that diversity was higher at microhabitats sampled on Amon, followed by the Pockmark area, the reference

Table 8 CC and Ref—biological descriptors of the carbonate crust microhabitats sampled on the Amon MV, in the Pockmark area and at the reference site (Amon MV) in the Nile Deep-Sea Fan

Biological descriptors	Carbonate crusts		Reference site
	Amon	Pockmark area	Amon
Macrofauna-dominant	Cnidarians (Zoantharia) reaching 72.2% of total abundance	Gastropoda (*Lurifax vitreus*) and polychaetes (Sabellidae) in various proportions, reaching 50.9, 39.6% of total abundance	Polychaetes, reaching a mean of 70.6% of total abundance (Spionidae)
Macrofauna-others	Gastropods, sipunculians	Bivalves, crustaceans	Cnidarians, sipunculians, crustaceans
Mean macrofaunal densities (individuals m^{-2})	456 ± 602	**3,971 ± 2,960**	766 ± 281
Mean Jaccard's similarity	0.0	**0.31**	0.34
Symbiont-bearing fauna	0%	**0–16%**	0%
Total macrofaunal biomass (kg preserved wet weight m^{-2})	$1.8 \times 10^{-3} \pm 5 \times 10^{-2}$	$\mathbf{3 \times 10^{-2} \pm 5 \times 10^{-2}}$	$3.2 \times 10^{-5} \pm 6 \times 10^{-5}$
Macrofaunal diversity indices			
Total richness (*S*)	5	**14**	8
Shannon (H_e')	0.96	**2.08**	1.92
Exp (H_e')	*2.61*	*8.00*	*6.82*
Gini-Simpson ($D_{GS} = 1 - \lambda$)	0.46	**0.81**	0.83
(1/λ)	*1.84*	*5.31*	*5.90*
Evenness (*J'*)	0.60	**0.79**	0.92
Meiofauna (>250 μm)-dominant	Only 4 individuals (nematodes)	Nematodes with 97.4% of the total abundance	Only 9 individuals (nematodes, copepods)
Meiofauna (>250 μm)-others	–	Copepods (11%)	–
Mean meiofaunal densities (individuals m^{-2})	129 ± 224	**2,909 ± 2,523**	–
Mean Jaccard's similarity	–	0.67	–
Meiofaunal diversity indices			
Total richness (*S*)	1	2	2
Nematoda richness	1	1	1
Copepoda richness	0	1	1
Ostracoda richness	0	0	0
Shannon (H_e')	–	0.12	–
Exp (H_e')		*1.13*	
Gini-Simpson ($D_{GS} = 1 - \lambda$)	–	0.05	–
(1/λ)		*1.05*	
Evenness (*J'*)	–	0.17	–

The highest values are highlighted in bold. The meiofauna data include only the large meiofauna >250 μm. The number equivalents of Shannon and Simpson indices are given in italics

samples from Amon and lastly, by Cheops (Fig. 6a). Overall, the distribution of macrofauna was relatively even on Amon, since the most abundant taxon only reached ~30% for both active and reference sites, whereas the most abundant taxon represented ~45 and ~55% on the Pockmark area and Cheops, respectively (Fig. 6b).

Symbiont-bearing fauna versus heterotrophic fauna

The symbiont-bearing fauna (for types of symbioses see Olu-Le Roy et al. 2004) represented 17–36% of the total

faunal abundance in the reduced sediments from Amon, while the CC and the reference sample did not harbour any (Tables 7, 8). The symbiont-bearing fauna was represented by Frenulata polychaetes as well as by four bivalve species (*Lucinoma kazani*, *Idas modiolaeformis*, *Thyasira striata* and *Isorropodon perplexum*; Table 3). The Pockmark area had between 10 and 16% of symbiont-bearing fauna both in the sediment and in the CC microhabitats, respectively (Tables 5, 7, 8). This fauna was represented by two known bivalve species (*L. kazani* and *I. perplexum*) and one unknown Lucinidae in the sediments. Only one bivalve

Fig. 6 **a** Rarefaction curves and **b** right tail-sum intrinsic diversity profiles for the pooled macrofaunal abundance data at the family level, according to the three study sites after pooling active microhabitats. Amon MV, $n = 6$, Pockmark area, $n = 6$, and Cheops MV, $n = 3$. The reference sample from Amon is reported separately, $n = 3$ (Ref)
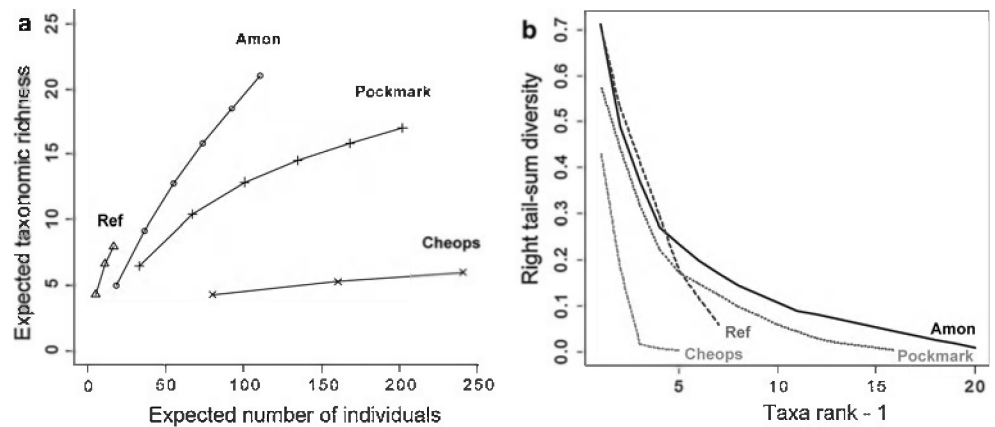
species was present in the CC microhabitat (*I. modiolaeformis*; Table 4). No symbiont-bearing fauna was sampled at Cheops (Tables 7, 8) or at the reference site.

Vertical distributions within the sediments

The distribution within the sediment layers down to 10 cm below the seafloor of the macrofaunal relative abundances differed between the three reduced sediment microhabitats (Fig. 7a). At all reduced sediment sites, oxygen did not penetrate deeper than a few millimetres, and sulphide concentration increased with sediment depth below 2 cm to $1–2$ mmol l^{-1} at Amon and to $10–20$ mmol l^{-1} in sediments of the Pockmark area. Hence, in the Pockmark area, the macrofauna was concentrated at the uppermost layer [0–1 cm] with about 85% of the total abundance (Fig. 7a). However, some capitellid polychaetes and undetermined bivalves that may be vesicomyids and lucinids were found in the [5–10 cm] layers. In the Cheops area, the macrofauna was almost evenly distributed between the [0–1 cm] and [1–3 cm] layers and only few spionid polychaetes were found in the [5–10 cm] layer. The profile obtained for the reduced sediments of Amon was different, with a more homogeneous distribution within the 10 cm layer and a relatively high abundance at [5–10 cm] (Fig. 7a) of frenulate, capitellid and dorvilleid polychaetes. At the reference site, most macrofauna was concentrated in almost equal proportions in the two first layers [0–1 cm] and [1–3 cm]. There, the distribution showed a clear decrease with depth, as no organism was found in the [5–10 cm] layer (Fig. 7a). The meiofauna >250 μm also decreased with depth at the reference site, with up to 60% of the total meiofaunal abundance found in the two first layers (Fig. 7b). The same trend was observed in reduced sediments from Amon with this time almost 60% concentrated in the first layer. This pattern was different at the Pockmark area, where the meiofaunal abundance was highest in the [3–5 cm] layer followed by the [1–3 cm] and [5–10 cm]

layers (Fig. 7b). However, the few organisms found in the [5–10 cm] layer consisted of nematodes at Amon and harpacticoid copepods at the Pockmark area.

In general, all soft sediments showed a low macrofaunal biomass, except the reduced sediments of Amon where the mean biomass reached 0.12 ± 0.1 kg pww m^{-2} (Table 7). Here, the vertical distribution showed an increase in depth from 1 to 10 cm with about 40% of the total macrofaunal biomass remaining in the [5–10 cm] layer (Fig. 7c) due to the presence of few lucinid and thyasirid bivalves. In the reduced sediments of the Pockmark area, most of the macrofaunal biomass (93%) was observed within the top layer [0–1 cm] and mainly consisted of dorvilleid polychaetes. Reduced sediments at Cheops hosted a similar biomass in the top two layers [0–1] and [1–3 cm], with 43 and 47% of the total biomass, respectively, mainly represented by hesionid, spionid, terebellid polychates and Leptostraca crustaceans. In the following layers, a sharp decrease was observed with depth (Fig. 7c). At the reference site, mean biomass was very low (Table 8) and integrally located within the [1–3 cm] layer (Fig. 7c) due to the presence of one sipunculian and a few spionid polychaetes.

Beta-diversity patterns

The first two components of the principal component analysis (PCA) on macrofaunal data accounted for 41.7% of the variance in macrofaunal distribution (Fig. 8a). Three clusters appear in reduced space: the carbonate crust samples, the reduced sediment samples except those from Cheops, and the Amon reference site and Cheops reduced sediments samples. Thus, the variability between microhabitats was higher than that within the microhabitats, but lower than that among the geostructures (Fig. 8). Polychaete taxa (Spionidae, Terebellida, Dorvilleidae, Capitellidae) and gastropods (Orbitestellidae) had the greatest impact on the variation in community structure. Focusing
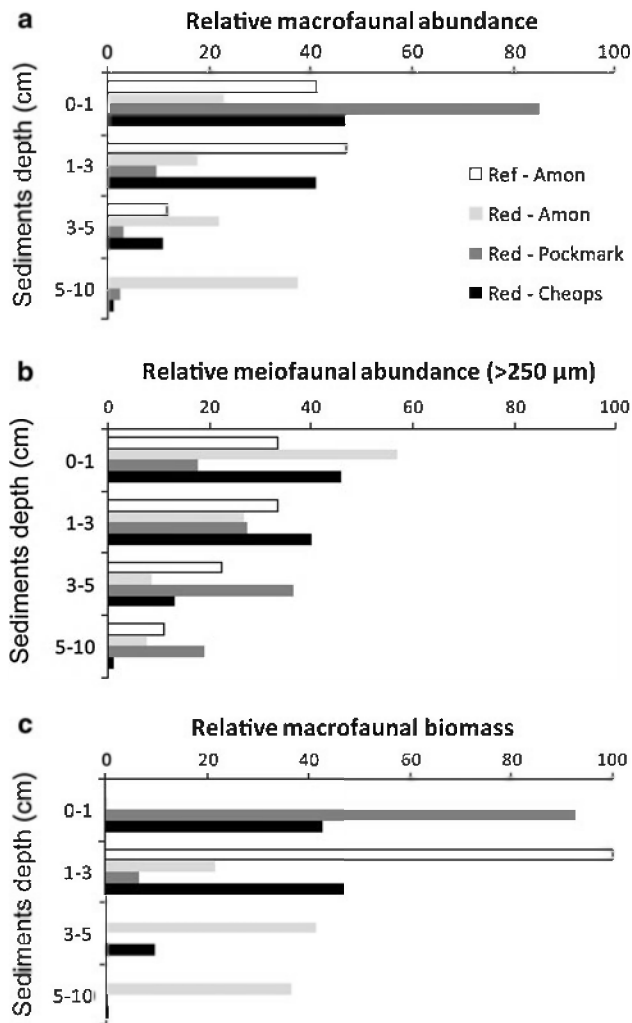
Fig. 7 Vertical distribution of **a** macrofaunal and **b** meiofaunal (>250 μm) relative abundances and **c** macrofaunal relative biomass with depth after summing the three replicates (*n* = 3) for each sediment layer of the reduced sediment microhabitats from Amon MV, Pockmark area and Cheops MV. The reference site from Amon is also included

on these taxa, they appeared to be indicative of different groups, representing different types of microhabitats rather than different sampling locations. In the first group, Spionidae and Terebellida highly contributed to the positioning of the reduced sediments from Cheops and reference samples from Amon. In the second group, Orbitestellidae highly contributed to the positioning of both carbonate crust microhabitats (Pockmark and Amon). Finally Capitellidae and Dorvilleidae presented high contributions in reduced sediments from Amon and Pockmark area (Fig. 8a). These three groups were also distinguished on the Ward's cluster (Fig. 8b). According to the datasets, the pooling of both carbonate crust microhabitats seemed to be due to their low number of individuals (*n* = 18 and 53) rather than to the presence of shared taxa (Tables 3, 4). The

Ward's cluster also showed that the reduced sediments from Cheops had higher similarities with the carbonate crust microhabitats than with the other reduced sediment 'soft' microhabitats (Fig. 8b).

A PCA with Hellinger-transformed meiofaunal >250 μm data only (not shown) exhibited the same general distance patterns as the one with macrofauna sensu stricto (Procruste test stat = 0.53, *p* = 0.013, 1,000 permutations). However, meiofaunal sampling was very incomplete, especially on hard substratum, and these results might reflect this paucity of observations.

Discussion

This study investigated the differences in faunal diversity in relation to different habitats and their biogeochemical conditions at three cold seep sites of the NDSF. Due to inevitably limited submersible time, sampling effort was relatively low given the high heterogeneity of cold seep sites. Nevertheless, our results provide the first insights into seep faunal composition and diversity and their relationships with environmental conditions in the NDSF area.

Difference between the reference site and cold seep habitats at Amon MV

The reference site did not show any evidence of seepage whether direct (i.e. no detection of methane and sulphide fluxes) or indirect (i.e. absence of symbiont-bearing fauna). The fauna found at the reference site was different from that of the reduced habitats and carbonate crusts from Amon. Densities of macro- and meiofauna were twofold lower than in reduced sediments, and total benthic oxygen consumption was an order of magnitude lower. Accordingly, biomass was 1–4 orders of magnitude lower than at all other active sites from Amon. The reference site showed a relatively high evenness, but a lower diversity than the reduced sediment sites of Amon, therefore, refuting our initial hypothesis. The reduced sediment microhabitats sampled within the present study support an overall higher faunal diversity compared to close-by oxygenated habitats. This pattern has already been observed on Eel River at about 500 m depth where diversity on clam beds was similar or higher than on non-seep sites (Levin et al. 2003, 2010). However, this is in contradiction with other studies from 770 to 3,200 m depth where diversity appears to be lower at active seep sites (Sahling et al. 2002; Levin et al. 2010; Menot et al. 2010).

Overall, the relationship between seepage activity and diversity at our sites could best explained by a hump-shaped curve. We suggest that at the relatively low sulphide concentrations encountered at Amon, the reduced
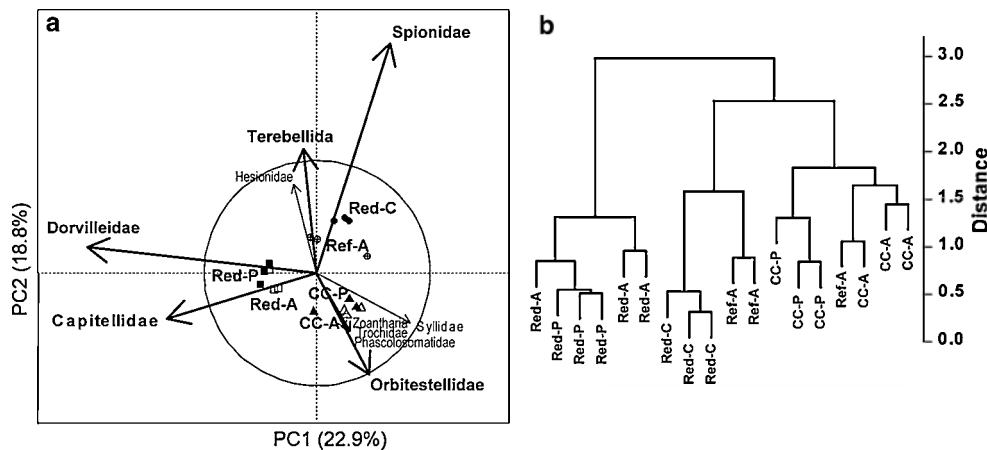
Fig. 8 a Principal component analysis (*PCA*, scaling type 1) of Hellinger-transformed macrofaunal abundances on the Amon MV (*A*), Pockmark area (*P*) and the Cheops MV (*C*). The first two axes represent 41.7% of the total variance in macrofaunal abundance. The *circle* of equilibrium indicates the significant contribution of five taxa (radius = 0.65). Vectors shorter than 0.34 were removed. **b** Ward's hierarchical clustering was performed with Hellinger-transformed macrofaunal abundances for each microhabitat types. *Red* reduced sediments, *CC* carbonate crusts and *Ref* reference sample

sediments could be considered as an 'ecotone' enhancing diversity by presenting a high variability of chemical environment and ecological niches, favouring the establishment of symbiont-bearing fauna but not limiting heterotrophic fauna by toxic concentrations of sulphide (Cordes et al. 2010). In contrast, the Pockmark area or similarly active seeps such as Hydrate ridge (Sahling et al. 2002) appear to show declined diversity, despite the high abundances/biomasses observed, potentially selecting for specialists able to withstand high sulphide concentrations and fluxes. On the other hand, the reference site would harbour regular deep-sea organisms but limit colonization by symbiont-bearing species.

Reduced sediment microhabitats

The reduced sediment microhabitats were influenced by fluid emissions as attested by biogeochemical conditions. Sulphide concentrations at 12 cm below the seafloor in the Pockmark area were 10 times higher than what was observed on Amon for the same sediment depth. This flux was associated with a higher oxygen uptake and faster consumption at the water–sediment interface, suggesting higher microbial activity within reduced sediments of the Pockmark area compared to all other sites. According to a previous study (Girnth et al. 2011), some evidences of brine overflowing the sediments were recorded at Amon reduced sediments. However, the homogeneity of the chloride profiles at both sites does not support the presence of upward seepage of saline fluids as observed in some areas of Cheops. Biogeochemical measurements indicate a ranking of activity with the Pockmark reduced sediments as the most sulphidic habitat, followed by Amon and Cheops.

Accordingly, the reduced sediment microhabitats at Amon and the Pockmark area were colonized by symbiont-bearing species, including siboglinid polychaetes and bivalves (Vesicomyidae, Lucinidae, Mytilidae and Thyasiridae) as well as by dorvilleid and capitellid polychaetes. The presence of these two families provides indirect evidence for fluid emissions as they are usually associated with areas rich in organic matter and with sulphidic environments (Rouse and Fauchald 1997). Dorvilleid polychaetes also dominate reduced sediment microhabitats from other seep sites at 1,100 m depth in the Marmara Sea (Ritt et al. 2010), at 2,020 m depth in the Mediterranean Sea (Amsterdam MV, B. Ritt et al., in preparation) and at 500 m depth at the Eel River off California (Levin et al. 2003). Large nematodes were abundant in the reduced sediments of Amon, as previously observed on other mud volcanoes at 1,220 m (Van Gaever et al. 2006) and 5,000 m depth (Olu et al. 1997).

The fauna in the reduced sediment microhabitat from Amon hosted the highest abundance of symbiont-bearing species, and its macrofaunal taxonomic richness was two times higher than in the Pockmark area. The bivalve species observed in the Pockmark area either harboured sulphide-oxidizing endosymbionts such as *Lucinoma kazani* and *Isorropodon perplexum* (Salas and Woodside 2002; Olu-Le Roy et al. 2004) or different types of symbionts as in the undetermined species *Idas* sp. Med (Duperron et al. 2008).

On Cheops, the reduced sediments sampled were located about 250 m away from a brine lake which features extreme physico-chemical conditions. Methane concentrations at the surface of the lake varied from 2.4 to 3.7 mmol l^{-1} with temperatures of 20–40°C and salinities 210–244 g l^{-1} (V. Mastalerz, F. O. Harmegnies, personal

communication). Sediment cores revealed a dark layer covering a layer of brown hemipelagic sediments, suggesting the occurrence of a relatively recent sulphidic mud flow. Sulphate reduction rates and oxygen consumption rates were considerably lower than at the other sites, indicating a low sulphide flux, as confirmed by porewater measurements. Accordingly, despite the detection of elevated methane concentrations in the bottom waters of Cheops above the reduced sediments, no symbiont-bearing fauna was observed. This microhabitat was dominated by spionid (mean of 2,267 individuals m^{-2}), hesionid (mean of 1,017 individuals m^{-2}) or terebellid (mean of 667 individuals m^{-2}) polychaetes, commonly found in sandy or muddy environments, from intertidal habitats to abyssal depths (Rouse and Pleijel 2001). Dense Terebellida beds, especially the Ampharetidae family have been observed at about 1,100 m depth on the New Zealand margin (Sommer et al. 2009) and in the Marmara Sea (Ritt et al. 2010), as well as at 770 m at the Hydrate Ridge off Oregon (Levin et al. 2010) where methane concentrations in the bottom water reached up to 2 mmol l^{-1} and 0.7 μmol l^{-1}, respectively, for the first two sites. At Cheops, methane concentrations varied between 4 and 8 μmol l^{-1}. This suggests that terebellids are able to inhabit a wide range of environmental conditions. Further chemical measurements are needed to have a better understanding of the intriguing processes occurring within reduced sediments on Cheops.

Carbonate crust microhabitats

As expected, the difference in substratum between the soft sediment and carbonate crust microhabitats appears to influence the composition and distribution of the seep fauna in the NDSF. Nevertheless, the clustering of the CC microhabitats from Amon and the Pockmark area appears to be due to the low number of sampled individuals rather than to the presence of common taxa. Sampling efficiency on hard substrata does not appear to be ideal as suggested by the high heterogeneity between replicates and the low Jaccard's similarity coefficients obtained. However, these results may also reflect the real heterogeneity of this habitat in terms of faunal composition and spatial distribution. Visual observations showed that the carbonate crusts sampled in the Pockmark area were associated with reduced sediments, whereas those from Amon did not appear to be located in the vicinity of an active area. In addition, the crusts showed different facies and colour that may reflect different fluid intensities and evolution stages (Bayon et al. 2009a). For example, the carbonates from the south-western part of Amon are thick, dark and cemented, and can reach 1 m of thickness. They may have been formed in the distant past, during a period of intense fluid emissions (Dupré et al. 2007). The absence of symbiont-bearing fauna and the dark colour of crusts—due to their exposure to

oxygen-rich bottom water and their iron and manganese oxide cover—corroborate the hypothesis that these carbonates may be relatively old. Fluid emissions may be very low on this part of Amon, explaining the absence of symbiont-bearing fauna. The lowest faunal densities, biomass and taxonomic richness than in the Pockmark area further support the fact that this environment on Amon is not favourable, neither to symbiont-bearing fauna, nor to heterotrophic fauna.

The light-coloured and crumbly carbonates from the Pockmark area differed morphologically from the 'inactive' crusts found on Amon as they seem to be relatively young. The precipitation of authigenic carbonates is controlled by high alkalinity of porewaters (Aloisi et al. 2002; Michaelis et al. 2002) due to the activity of archaea and bacteria consortia involved in the anaerobic oxidation of methane (AOM) coupled to sulphate reduction (Boetius et al. 2000). Another by-product of AOM is sulphide which may support sulphide-oxidizing symbionts of chemosynthetic fauna such as the siboglinid polychaetes found beneath the crusts. In addition, the methane concentrations were high, reaching >5 μmol l^{-1}. Siboglinids may contribute to bio-irrigation and favour advection and diffusion processes that play a significant role in carbonate precipitation (Bayon et al. 2009a).

Comparison between Amon, the Pockmark area and Cheops

The reduced sediment microhabitats appeared to be distributed along a gradient related to seepage intensity and thus, to methane, oxygen and sulphide fluxes, with the Pockmark area as the most intense seepage site and Cheops as the most instable with suspected brine seepage activity. The fauna from the reduced sediment microhabitats of the Pockmark area and Amon was rather similar and highly influenced by the presence of symbiotic organisms as well as dorvilleid and capitellid polychaetes, usually associated with organically enriched, reduced environments (Rouse and Pleijel 2001). Both communities showed relatively similar oxygen consumption rates. The taxonomic diversity was higher on Amon followed by the Pockmark area and Cheops. However, the reduced sediments, associated with the debris-flow of the Pockmark area, were more sulphidic and probably also more stable than the fresh Amon mud flow. The lower diversity and biomass observed in the reduced sediments of the Pockmark than of Amon could be due to the high level of sulphide flux limiting the survival of most benthic species. The reduced sediments of the Pockmark have an intermediate position between Amon and Cheops in terms of faunal diversities and densities. At the time of sampling, the mud flow at Cheops was probably recent as only a very thin sulphidic horizon was observed on top of hemipelagic sediments. These conditions may

explain the lowest diversity and the lack of symbiont-bearing fauna on the sampling site located 250 m away from the central brine lake. The sedimentary instability on Cheops induced by the brine seepage seemed to be a limiting factor for the establishment of fauna. According to video observations, instability seems to decrease from the summit towards the periphery as suggested by the presence of carbonate crusts with symbiont-bearing mytilids at about 700 m away from the lake.

Comparison at a larger scale

In terms of seepage activity, Amon is comparable to the Håkon Mosby MV (1,200 m depth) from the Norwegian margin and the Amsterdam MV (2,020 m depth) located on the Mediterranean Ridge. These MVs discharge mud, fluids and gases from their summits, which undergo episodic mud eruptions (Zitter et al. 2005; Dupré et al. 2007; Feseker et al. 2008). This activity induces instability and chemical gradients that influence the distribution of fauna, which is concentrically distributed around a chaotic summit (Zitter et al. 2005; Jerosch et al. 2007). The reduced site sampled at Amon was located on the base of the mud volcano, and influenced by lateral brine and mud flows (Girnth et al. 2011). Interestingly, the dimensions of the reduced sediment site was rather small (ca. 250 m) and the next larger reduced sediment patches were >2 km away.

The carbonate crusts and reduced sediments of the Pockmark area from the NDSF showed similarities with a more recently discovered site, the giant REGAB pockmark in the Gulf of Guinea at 3,160 m depth. There, carbonate crusts are colonized by dense mussel beds and siboglinid assemblages (Olu-Le Roy et al. 2007). Pockmark and carbonate crust areas have also been observed at 740 m depth on the Storegga slide and the Nyegga area located on the north-west of the Norway margin which have undergone slide events. There, benthic fauna is represented by Siboglinidae, Crinoidae, Pycnogonidae and microbial mats (Hovland et al. 2005; Hovland and Svensen 2006; Paull et al. 2008). However, these features are taller than those observed in the central province of the NDSF as they reach 190 m long and 40 m wide, while pockmarks do not exceed a few metres in diameter at the NDSF study site.

The Cheops MV share similarities with the Napoli MV located on the Mediterranean Ridge at 1,900 m depth, as they both harbour brine seepages (Charlou et al. 2003). However, faunal biomass and taxonomic richness on Cheops are lower than those observed on Napoli (B. Ritt et al., in preparation). Faunal composition and distribution may be strongly linked to the activity of the MV. Mud flows may initiate higher sedimentary and chemical instabilities on Cheops, limiting the colonization of benthic species. Although considered as an extreme habitat, the vicinity of brine lakes can be colonized by dense colonies of Porifera as observed on Napoli (B. Ritt, personal observation) or by dense mussel communities, such as those found on the shoreline of brine pools in the Gulf of Mexico at 650 m depth (Macdonald et al. 1990; Smith et al. 2000). The absence of dense assemblages of symbiont-bearing fauna close to the brines on Cheops was thus unexpected and may be explained by the effect of recent disturbances.

In conclusion, our comparative investigation of active cold seeps on the NDSF suggested that seepage activity substantially enhanced benthic activity, biomass and diversity compared to the surrounding oxygenated and oligotrophic deep-sea environments. The biomass and biogeochemical activity of seep associated communities is 1–2 orders of magnitude higher than the surrounding and diversity was increased by a factor of 1.5. Reduced sediment microhabitats exhibited highest biomasses and diversity in comparison with surrounding oxygenated areas (reference site). As expected, reduced sediments and carbonate crusts were characterized by distinct faunal composition, and faunal similarities were observed within each microhabitat type regardless of the site and geological structure (i.e. mud volcano, pockmark). However, our heterogeneous results on carbonate crusts require more investigation and emphasize the importance of developing a specific sampling tool dedicated to hard substratum in the deep sea. No simple relationship was detected between chemical conditions, sediment instability, fluid intensity and community structure although there seems to be a gradient related to seepage intensity between the different sites (Pockmark > Amon > Cheops). Further investigations with an appropriate sampling strategy, especially regarding the meiofauna, may help in highlighting the links between faunal distribution and environmental conditions in the NDSF. Most likely, temporal dynamics in such active geological systems may be very important in structuring community diversity. It could be studied in the future by deploying autonomous video cameras and sensors as well as by regularly monitoring a same site. Time-series data would help determine the response of the fauna to instabilities and disturbances induced by the occurrence of mud flows or brine seepage, and in which ways local diversity is related to temporal fluctuations and stability of habitats.

References

Aloisi G, Bouloubassi I, Heijs SK, Pancost RD, Pierre C, Sinninghe Damste JS, Gottschal JC, Forney LJ, Rouchy JM (2002) CH_4-consuming microorganisms and the formation of carbonate crusts at cold seeps. Earth Planet Sci Lett 203:195–203

Baker MC, Ramirez-Llodra EZ, Tyler P, German CR, Boetius A, Cordes EE, Dubilier N, Fisher CR, Levin LA, Metaxas A, Rowden AA, Santos RS, Shank TM, Van Dover CL, Young CM, Warén A (2010) Biogeography, ecology, and vulnerability of chemosynthetic ecosystems in the deep sea. In: McIntyre A (ed) Life in the World's Oceans: Diversity, Distribution, and Abundance. Wiley-Blackwell, Hoboken, p 384

Bayon G, Henderson GM, Bohn M (2009a) U-Th stratigraphy of a cold seep carbonate crust. Chem Geol 260:47–56

Bayon G, Loncke L, Dupre S, Ducassou E, Duperron S, Etoubleau J, Foucher JP, Fouquet Y, Gontharet S, Huguen C, Klaucke I, Mascle J, Olu-Le Roy K, Ondreas H, Pierre C, Sibuet M, Stadnitskaia A, Woodside J (2009b) Multi-disciplinary investigations of fluid seepage on an unstable margin: the case of the central Nile deep sea fan. Mar Geol 261:92–104

Bellaiche G, Loncke L, Gaullier V, Mascle J, Courp T, Moreau A, Radan S, Sardou O (2001) The Nile cone and its channel system: new results after the Fanil cruise. Comptes Rendus De L'Academie Des Sciences Série II Fascicule a-Sciences De La Terre Et Des Planètes 333:399–404

Boetius A, Ravenschlag K, Schubert CJ, Rickert D, Widdel F, Gieseke A, Amann R, Jorgensen BB, Witte U, Pfannkuche O (2000) A marine microbial consortium apparently mediating anaerobic oxidation of methane. Nature 407:623–626

Campbell KA (2006) Hydrocarbon seep and hydrothermal vent paleoenvironments and paleontology: past developments and future research directions. Palaeogeogr Palaeoclimatol Palaeoecol 232:362–407

Charlou JL, Donval JP, Zitter T, Roy N, Jean-Baptiste P, Foucher JP, Woodside J (2003) Evidence of methane venting and geochemistry of brines on mud volcanoes of the eastern Mediterranean Sea. Deep Sea Res Part I Oceanogr Res Pap 50:941–958

Cita MB, Ryan WBF, Paggi L (1981) Prometheus mud breccia. An example of shale diapirism in the western Mediterranean Ridge. Ann geol Des Pays Hell 13:37–49

Cline JD (1969) Spectrophotometric determination of hydrogen sulfide in natural waters. Limnol Oceanogr 14:454–458

Cordes EE, Becker EL, Hourdez S, Fisher CR (2010) Influence of foundation species, depth, and location on diversity and community composition at Gulf of Mexico lower-slope cold seeps. Deep Sea Res Part II Top Stud Oceanogr 57:1870–1881

de Beer D, Sauter E, Niemann H, Kaul N, Foucher JP, Witte U, Schlüter M, Boetius A (2006) In situ fluxes and zonation of microbial activity in surface sediments of the Håkon Mosby mud volcano. Limnol Oceanogr 51:1315–1331

Dimitrov LI (2002) Mud volcanoes—the most important pathway for degassing deeply buried sediments. Earth Sci Rev 59:49–76

Duperron S, Halary S, Lorion J, Sibuet M, Gaill F (2008) Unexpected co-occurrence of six bacterial symbionts in the gills of the cold seep mussel Idas sp. (Bivalvia: Mytilidae). Environ Microbiol 10:433–445

Dupré S, Woodside J, Foucher JP, de Lange G, Mascle J, Boetius A, Mastalerz V, Stadnitskaia A, Ondreas H, Huguen C, Harmegnies FO, Gontharet S, Loncke L, Deville E, Niemann H, Omoregie E, Roy KOL, Fiala-Medioni A, Dahlmann A, Caprais JC, Prinzhofer A, Sibuet M, Pierre C, Sinninghe Damste JS (2007) Seafloor geological studies above active gas chimneys off Egypt (Central Nile deep sea fan). Deep Sea Res Part I Oceanogr Res Pap 54:1146–1172

Dupré S, Buffet G, Mascle J, Foucher JP, Gauger S, Boetius A, Marfia C (2008) High-resolution mapping of large gas emitting mud volcanoes on the Egyptian continental margin (Nile deep sea fan) by AUV surveys. Mar Geophys Res 29:275–290

Feseker T, Foucher JP, Harmegnies F (2008) Fluid flow or mud eruptions? Sediment temperature distributions on Håkon Mosby mud volcano, SW Barents Sea slope. Mar Geol 247:194–207

Foucher JP, Westbrook GK, Boetius A, Ceramicola S, Dupre S, Mascle J, Mienert J, Pfannkuche O, Pierre C, Praeg D (2009) Structure and drivers of cold seep ecosystems. Oceanography 22:92–109

Gauthier O, Sarrazin J, Desbruyères D (2010) Measure and mismeasure of species diversity in deep-sea chemosynthetic communities. Mar Ecol Prog Ser 402:285–302

Gini C (1912) Variabilità e Mutabilità. Tipographia di Paolo Cuppini, Bologna

Girnth A-C, Grünke S, Lichtschlag A, Felden J, Knittel K, Wenzhöfer F, de Beer D, Boetius A (2011) A novel, mat-forming Thiomargarita population associated with a sulfidic fluid flow from a deep-sea mud volcano. Environ Microbiol 13:495–505

Gontharet S, Pierre C, Blanc-Valleron MM, Rouchy JM, Fouquet Y, Bayon G, Foucher JP, Woodside J, Mascle J (2007) Nature and origin of diagenetic carbonate crusts and concretions from mud volcanoes and pockmarks of the Nile deep-sea fan (eastern Mediterranean Sea). Deep Sea Res Part II Top Stud Oceanogr 54:1292–1311

Gotelli NJ, Colwell RK (2001) Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. Ecol Lett 4:379–391

Gray JS (2000) The measurement of marine species diversity, with an application to the benthic fauna of the Norwegian continental shelf. J Exp Mar Biol Ecol 250:23–49

Grünke S, Felden J, Lichtschlag A, Girnth AC, Wenzhöfer F, de Beer D, Boetius A (in press) Niche differentiation among mat-forming, sulfide-oxidizing bacteria at cold-seeps of the Nile deep sea fan (Eastern Mediterranean Sea). Geobiology 9

Hessler RR, Jumars PA (1974) Abyssal community analysis from replicate box cores in the central North Pacific. Deep Sea Res 21:185–209

Hill MO (1973) Diversity and evenness: a unifying notation and its consequences. Ecology 54:427–432

Hovland M, Svensen H (2006) Submarine pingoes: indicators of shallow gas hydrates in a pockmark at Nyegga, Norwegian Sea. Mar Geol 228:15–23

Hovland M, Gardner JV, Judd AG (2002) The significance of pockmarks to understanding fluid flow processes and geohazards. Geofluids 2:127–136

Hovland M, Svensen H, Forsberg CF, Johansen H, Fichler C, Fossa JH, Jonsson R, Rueslatten H (2005) Complex pockmarks with carbonate-ridges off mid-Norway: products of sediment degassing. Mar Geol 218:191–206

Hsu KJ, Montadert L, Bernoulli D, Cita MB, Erickson A, Garrison RE, Kidd RB, Melieres F, Muller C, Wright R (1977) History of Mediterranean salinity crisis. Nature 267:399–403

Huguen C, Mascle J, Woodside J, Zitter T, Foucher JP (2005) Mud volcanoes and mud domes of the Central Mediterranean Ridge: near-bottom and in situ observations. Deep Sea Res Part I Oceanogr Res Pap 52:1911

Huguen C, Foucher JP, Mascle J, Ondreas H, Thouement M, Stadnitskaia A, Pierre C, Bayon G, Loncke L, Boetius A, Bouloubassi I, De Lange G, Caprais JC, Fouquet Y, Woodside J, Dupre S, the NAUTINIL Scientific Party (2009) Menes Caldera, a highly active site of brine seepage in the Eastern Mediterranean Sea: "in situ" observations from the NAUTINIL expedition. Mar Geol 261:138–152

Hurlbert SH (1971) The nonconcept of species diversity: a critique and alternative parameters. Ecology 52:577–586

Jaccard P (1901) Distribution de la flore alpine dans le Bassin des Dranses et dans quelques régions voisines. Bulletin de la société vaudoise des sciences naturelles

Jackson DA (1995) PORTEST: a PROcustean randomization TEST of community environment concordance. Ecosciences 2:297–303

Jerosch K, Schluter M, Foucher JP, Allais AG, Klages M, Edy C (2007) Spatial distribution of mud flows, chemoautotrophic communities, and biogeochemical habitats at Hakon Mosby Mud Volcano. Mar Geol 243:1–17

Jost L (2006) Entropy and diversity. Oikos 113:363–375

Jost G (2007) Partitioning diversity into independent alpha and beta components. Ecology 88:2427–2439

Judd AG, Hovland M (2007) Seabed fluid flow—the impact on geology, biology and the marine environment

Kindt R, Coe R (2005) Tree diversity analysis. A manual and software for common statistical methods for ecological and biodiversity studies. World Agroforestry Centre (ICRAF), Nairobi

Kopf AJ (2002) Significance of mud volcanism. Rev Geophys 40:52

Legendre P, Gallagher ED (2001) Ecologically meaningful transformations for ordination of species data. Oecologia 129:271–280

Legendre P, Legendre L (1998) Numerical ecology, 2nd English edn

Levin LA (2005) Ecology of cold seep sediments: interactions of fauna with flow, chemistry and microbes oceanography and marine biology—an annual review, vol 43. Crc Press/Taylor & Francis Group, Boca Raton, pp 1–46

Levin LA, Mendoza GF (2007) Community structure and nutrition of deep methane-seep macrobenthos from the North Pacific (Aleutian) Margin and the Gulf of Mexico (Florida Escarpment). Mar Ecol Evol Perspect 28:131–151

Levin LA, Ziebis W, Mendoza GF, Growney VA, Tryon MD, Mahn C, Gieskes JM, Rathburn AE (2003) Spatial heterogeneity of macrofauna at northern California methane seeps: influence of sulfide concentration and fluid flow. MEPS 265:123–139

Levin LA, Mendoza GF, Gonzalez JP, Thurber AR, Cordes EE (2010) Diversity of bathyal macrofauna on the northeastern Pacific margin: the influence of methane seeps and oxygen minimum zones. Mar Ecol 31:94–110

Liu C, Whittaker RJ, Ma K, Malcolm JR (2007) Unifying and distinguishing diversity ordering methods for comparing communities. Popul Ecol 49:89–100

Loncke L, Mascle J, the Fanil Scientific Party (2004) Mud volcanoes, gas chimneys, pockmarks and mounds in the Nile deep-sea fan (Eastern Mediterranean): geophysical evidences. Mar Pet Geol 21:669

Macdonald IR, Reilly JF, Guinasso NL, Brooks JM, Carney RS, Bryant WA, Bright TJ (1990) Chemosynthetic mussels at a brine-filled pockmark in the Northern Gulf of Mexico. Science 248:1096–1099

Mascle J, Zitter T, Bellaiche G, Droz L, Gaullier V, Loncke L (2001) The Nile deep sea fan: preliminary results from a swath bathymetry survey. Mar Pet Geol 18:471–477

Mascle J, Sardou O, Loncke L, Migeon S, Camera L, Gaullier V (2006) Morphostructure of the Egyptian continental margin: Insights from swath bathymetry surveys. Mar Geophys Res 27:49–59

Menot L, Galéron J, Olu K, Caprais JC, Crassous P, Khripounoff A, Sibuet M (2010) Spatial heterogeneity of macrofaunal communities in and near a giant pockmark area in the deep Gulf of Guinea. Mar Ecol 31:78–93

Michaelis W, Seifert R, Nauhaus K, Treude T, Thiel V, Blumenberg M, Knittel K, Gieseke A, Peterknecht K, Pape T, Boetius A, Amann R, Jorgensen BB, Widdel F, Peckmann JR, Pimenov NV, Gulin MB (2002) Microbial reefs in the Black Sea fueled by anaerobic oxidation of methane. Science 297:1013–1015

Milkov AV (2000) Worldwide distribution of submarine mud volcanoes and associated gas hydrates. Mar Geol 167:29–42

Niemann H, Losekann T, de Beer D, Elvert M, Nadalig T, Knittel K, Amann R, Sauter EJ, Schluter M, Klages M, Foucher JP, Boetius A (2006) Novel microbial communities of the Haakon Mosby mud volcano and their role as a methane sink. Nature 443:854–858

Oksanen J, Kindt R, Legendre P, O'Hara B, Simpson GL, Solymos P, Henry M, Stevens H, Wagner H (2008) Vegan: community ecology package. R package version 1.15-1. http://cran.r-project.org/, http://vegan.r-forge.r-project.org/

Olu K, Lance S, Sibuet M, Henry P, Fiala-Medioni A, Dinet A (1997) Cold seep communities as indicators of fluid expulsion patterns through mud volcanoes seaward of the Barbados accretionary prism. Deep Sea Res Part I Oceanogr Res Pap 44:811

Olu-Le Roy K, Sibuet M, Fiala-Medioni A, Gofas S, Salas C, Mariotti A, Foucher J-P, Woodside J (2004) Cold seep communities in the deep eastern Mediterranean Sea: composition, symbiosis and spatial distribution on mud volcanoes. Deep Sea Res Part I Oceanogr Res Pap 51:1915

Olu-Le Roy K, Caprais JC, Fifis A, Fabri MC, Galeron J, Budzinsky H, Le Menach K, Khripounoff A, Ondreas H, Sibuet M (2007) Cold-seep assemblages on a giant pockmark off West Africa: spatial patterns and environmental control. Mar Ecol Evol Perspect 28:115–130

Omoregie EO, Mastalerz V, de Lange G, Straub KL, Kappler A, Roy H, Stadnitskaia A, Foucher JP, Boetius A (2008) Biogeochemistry and community composition of iron- and sulfur-precipitating microbial mats at the Chefren mud volcano (Nile deep sea fan, Eastern Mediterranean). Appl Environ Microbiol 74:3198–3215

Patil GP, Taillie C (1982) Diversity as a concept and its measurement. J Am Stat Assoc 77:548–561

Paull CK, Hecker B, Commeau R, Freeman-Lynde RP, Neumann C, Corso WP, Golubic S, Hook JE, Sikes E, Curray J (1984) Biological communities at the Florida Escarpment resemble hydrothermal vent taxa. Science 226:965–967

Paull CK, Ussler W, Holbrook WS, Hill TM, Keaten R, Mienert J, Haflidason H, Johnson JE, Winters WJ, Lorenson TD (2008) Origin of pockmarks and chimney structures on the flanks of the Storegga Slide, offshore Norway. Geo-Mar Lett 28:43–51

Pielou EC (1969) An introduction to mathematical ecology, New York

R Development Core Team (2009) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Ritt B, Sarrazin J, Caprais J-C, Noël P, Gauthier O, Pierre C, Henry P, Desbruyères D (2010) First insights into the structure and environmental setting of cold-seep communities in the Marmara Sea. Deep Sea Res Part I Oceanogr Res Pap 57:1120–1136

Ritt B, Desbruyeres D, Caprais JC, Khripounoff A, Le Gall C, Gauthier O, Buscail R, Olu K, Sarrazin J (in preparation) Cold seep communities in the deep eastern Mediterranean Sea: composition, spatial patters and environmental control on the Mediterranean Ridge mud volcanoes

Rouse GW, Fauchald K (1997) Cladistics and polychaetes. Zoologica Scripta 26:139–204

Rouse GW, Pleijel F (2001) Polychaetes. Oxford University Press, Oxford

Sahling H, Rickert D, Lee RW, Linke P, Suess E (2002) Macrofaunal community structure and sulfide flux at gas hydrate deposits from the Cascadia convergent margin, NE Pacific. Mar Ecol Prog Ser 231:121–138

Salas C, Woodside J (2002) *Lucinoma kazani* n. sp. (Mollusca: Bivalvia): evidence of a living benthic community associated with a cold seep in the Eastern Mediterranean Sea. Deep Sea Res Part I Oceanogr Res Pap 49:991–1005

Sanders HL (1968) Marine benthic diversity: a comparative study. Am Nat 102:243–282

Sarradin PM, Caprais JC (1996) Analysis of dissolved gases by headspace sampling gas chromatography with column and detector switching. Preliminary results. Anal Commun 33:371–373

Sarradin PM, Waeles M, Bernagout S, Le Gall C, Sarrazin J, Riso R (2009) Speciation of dissolved copper within an active hydrothermal edifice on the Lucky Strike vent field (MAR, 37° N). Sci Total Environ 407:869–878

Sarrazin J, Juniper SK (1999) Biological characteristics of a hydrothermal edifice mosaic community. Mar Ecol Prog Ser 185:1–19

Shannon CE (1948) A mathematical theory of communication. Bell Syst Tech J 27:379–423

Sibuet M, Olu K (1998) Biogeography, biodiversity and fluid dependence of deep-sea cold-seep communities at active and passive margins. Deep Sea Res Part II Top Stud Oceanogr 45:517

Simpson EH (1949) Measurement of diversity. Nature 163:688

Smith EB, Scott KM, Nix ER, Korte C, Fisher CR (2000) Growth and condition of seep mussels (*Bathymodiolus childressi*) at a Gulf of Mexico Brine Pool. Ecology 81:2392–2403

Sommer S, Linke P, Pfannkuche O, Niemann H, Treude T (2009) Benthic respiration in a seep habitat dominated by dense beds of ampharetid polychaetes at the Hikurangi Maring (new Zealand). Mar Geol. doi:10.1016/j.margo.2009.06.003

Southward EC, Andersen AC, Hourdez S (in press) *Lamellibrachia anaximandri* n. sp., a new vestimentifera tubeworm from the Mediterranean (Annelida). Zoosystema

Thistle D (2003) The deep-sea floor: an overview ecosystems of the deep ocean. Elsevier Science Bv, Amsterdam, pp 5–37

Tothmérész B (1998) On the characterization of scale-dependant diversity. Abstracta Bonatica 22:149–156

Treude T, Smith CR, Wenzhöfer F, Carney E, Bernardino AF, Hannides AK, Krüger M, Boetius A (2009) Biogeochemistry of a deep-sea whale-fall: sulfate reduction, sulfide efflux and methanogenesis. Mar Ecol Prog Ser 382:1–21

Van Gaever S, Moodley L, de Beer D, Vanreusel A (2006) Meiobenthos at the Arctic Håkon Mosby Mud Volcano, with a parental-caring nematode thriving in sulphide-rich sediments. Mar Ecol Prog Ser 321:143–155

Werne JP, Haese RR, Zitter T, Aloisi G, Bouloubassi I, Heijs S, Fiala-Medioni A, Pancost RD, Sinninghe Damste JS, de Lange G (2004) Life at cold seeps: a synthesis of biogeochemical and ecological data from Kazan mud volcano, eastern Mediterranean Sea. Chem Geol 205:367

Whittaker RH (1960) Vegetation of the Siskiyou Mountains, Oregon and California. Ecol Monogr 30:279

Zitter TAC, Woodside JM, Mascle J (2003) The Anaximander Mountains: a clue to the tectonics of southwest Anatolia. Geol J 38:375–394

Zitter TAC, Huguen C, Woodside JM (2005) Geology of mud volcanoes in the eastern Mediterranean from combined sidescan sonar and submersible surveys. Deep Sea Res Part I Oceanogr Res Pap 52:457–475