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Interconnectivity vs. isolation of prokaryotic communities in European deep-sea mud volcanoes

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Abstract

By exploiting the available data on 16S rRNA gene sequences – spanning over a sampling period of more than 10 yr – retrieved from sediments of the Haakon Mosby mud volcano (HMMV), Gulf of Cadiz (GoC) and eastern Mediterranean (Amsterdam and Kazan mud volcanoes; AMSMV, KZNMV) mud volcanoes/pockmarks, we investigated whether these systems are characterized by high (interconnectivity) or low (isolation) connection degree based on shared bacterial and archaeal phylotypes. We found only two archaeal and two bacterial phylotypes to occur in all three sites and a few more that were found in two of the three sites. Although the number of shared species depends a lot on the analysis depth of each sample, the majority of the common phylotypes were related mostly to cold seep deep-sea habitats, while for some of them their relative abundance was high enough to be considered as key-species for the habitat they were found. As new tools, like next generation sequencing platforms, are more appropriate for revealing greater depth of diversity but also allow sample replication and uniform sampling protocols, and gain wider recognition and usage, future attempts are more realistic now for fully elucidating the degree of specificity in deep-sea mud volcanoes and pockmarks microbial communities.

1 Introduction

Cold seeps support an enormous biomass of free-living and symbiotic microbial life that is nourished by the oxidation of methane, higher hydrocarbons and sulphide. In fact, methane-fuelled microbial communities in anoxic sediments above gas hydrates and gas vents have the highest biomass that is known to occur in marine ecosystems, with up to 10^{12} cells cm^3 (Boetius et al., 2000). Because of their distinct biogeochemistry dominated by fluid flow and hydrocarbon transport, methanotrophs, hydrocarbon degraders, sulphate-reducing and sulphide-oxidizing bacteria are the key functional groups at cold-seep ecosystems. During the European Commission's Framework Six

Programme HERMES and Framework Seven Programme HERMIONE many marine sites have been investigated along the European margin, which are characterized by the presence of various seep-related structures with different intensity of activity and biological diversity. Unraveling the associated biodiversity and understanding the driving forces and function of the seep-associated ecosystems by means of an integrated approach comprised one of the research topics of the projects (Vanreusel et al., 2009). Through the research activities of the above-mentioned programs, three main areas harbouring prominent seep ecosystems have been recognised (Fig. 1): (1) the Nordic margin with the Haakon Mosby mud volcano (HMMV) and many pockmarks, (2) the Gulf of Cádiz (GoC), and (3) the eastern Mediterranean Sea with ample mud volcanoes and brine pool structures.

Along the Nordic margin, the highly active HMMV is situated at the Norwegian-Barents-Spitzbergen continental margin at 1250 m water depth and was first located in 1989 during a side scan survey. An expedition in 1995 revealed very steep temperature gradients in the sediments, recovered methane hydrate from 2 m below sea floor (b.s.f.) while siboglinid tubeworms were also found, suggesting active chemosynthesis (Vogt et al., 1997). The concentric structure of the HMMV can be divided in several sub-habitats characterized by significantly different biogeochemical sediment conditions (Niemann et al., 2006; De Beer et al., 2006).

Mud volcanism in the GoC is characterized by a wide diversity of processes and environmental settings, such as different types of fluid migration pathways, tectonic activity and/or salt diapirism, migration velocity, fluid composition and alteration processes, depth, sea bottom temperature (from 4 °C to 13 °C if under the influence of the Mediterranean outflow water), and the presence of gas hydrates. The migrating fluid and mud reaching the surface is of very deep origin (Hensen et al., 2007). In the Gulf of Cádiz, overall activity for the anaerobic oxidation of methane (AOM) is typical for low to moderately active seeps (Maignien et al., 2012; Niemann et al., 2006). Discrete AOM hotspots, exhibiting activity one order higher than the typical sites, have been observed. There, the development of white bacterial mats is sustained. In contrast, salt diapir-driven mud volcanoes characterized by hypersaline pore water show low microbial activity, although methane and sulfate are present in large amounts. Interestingly, these environmental setting variations and AOM activity are reflected by diverse microbial community compositions.

In the eastern Mediterranean Sea, mud volcanoes (MV) and cold seep areas were discovered during the late 1970s (e.g. Cita et al., 1981) while ongoing research has revealed several such formations on the accretionary prism of the Hellenic Arc (Mediterranean Ridge) and within the Anaximander Mountains (Woodside et al., 1998; Lykousis et al., 2004), as well as the seafloor of the Nile deep sea fan (NDSF) (Loncke and Mascle, 2004). The Anaximander area, located at depths between 1700 m and 2000 m, is characterized by strong compressional tectonic processes superimposed by faulting. It harbors large conical MV of several km across but only a few hundred meters high. Fresh mud flows, brines, clasts and carbonate crusts may be observed on their surface, depending on the current activity of the volcano (Zitter et al., 2005). Two of the biggest and more active volcanoes of the area are Amsterdam MV (AMSMV) and Kazan MV (KZNMV). The Nile deep turbidic system displays many fluid-releasing structures on the seafloor including mud volcanoes, mud pies and pockmarks (Loncke and Mascle, 2004). The mud volcanoes of NDSF (Amon and Isis of the Eastern, North Alex of the Central and Chefren and Mykerinos of the Western NDSF) are circular features (1–3 km in diameter) in water depths of 500–3000 m with elevations of up to 100 m above the seabed. They are located above well-developed feeder channels, clearly seen on the seismic data (Loncke and Mascle, 2004; Dupré et al., 2007). The presence of brine mud basins within the volcano craters has also been reported (Omoregie et al., 2008).

Following one of the key objectives of HERMIONE, i.e. the distribution and interconnection of deep-sea ecosystems, in this paper we exploit the available data on prokaryotic occurrence in the sediments of the HMMV, Gulf of Cadiz and eastern Mediterranean MV/pockmarks and explore whether these systems are characterized by high (interconnectivity) or low (isolation) degree of shared bacterial and archaeal species. Such comparisons among distant ecosystems but with similar dominant microbial processes can provide meaningful information about the spatial and temporal scales of variability. Towards this, we investigated the occurrence of published 16S rRNA gene sequences in these habitats. In particular we aimed at elucidating the shared bacterial and archaeal phylotypes between the three sites, as it is hypothesized that such species are best candidates for having a predominant role in ecosystem functioning (Konopka, 2009).

2 Materials and methods

In this study, the analyses were performed on 16 data sets consisting of 16S rRNA gene sequences (Table 1) originating from eight individual projects. The data sets were retrieved from GenBank (http://www.ncbi.nlm.nih.gov/) from the 15 June 2012 release. Prokaryotic SSU rRNA sequences were identified as records that had any of the terms: “16S”, “16S SSU”, or “small subunit’ and “prokaryotic”/“archaeal”/“bacterial” “ribosomal RNA”, together with terms used to select for location: “Haakon Mosby”, “Gulf of Cadiz”, “Nile Fan”, “Amsterdam mud volcano”, “Kazan mud volcano”, “East Mediterranean mud volcanoes”, Data sets that contained only short sequences (less than 700 bp) were excluded from the analysis. Moreover, only datasets corresponding to environmental samples (i.e. not enrichment cultures) were used. For each data set, clusters of Operational Taxinomic Units (OTUs) or phylotypes were generated based on ≥98 % rRNA gene sequence similarity levels by multiple sequence alignment using ClustalW2 (Larkin et al., 2007). The generated data sets with the unique OTUs were compared to one another and the number of common OTUs between each analysed pair of dataset was noted. Similarity matrices (Table 2), one for Archaea and one for Bacteria, based on the number of common OTUs retrieved, were created. Network visualisations were performed by using the Cytoscape software, version 2.8.2 (Smoot et al., 2011).

The phylogenetic affiliation of the common OTUs was identified by their placement into phylogenetic trees. The trees were constructed from aligned sequences using the

SILVA alignment utility (Pruesse et al., 2007) (http://www.arb-silva.de/aligner/) by the neighbour-joining method using the Kimura 2-parameter. Bootstrap analyses for 1000 replicates were performed to assign confidence levels to the tree topology using the MEGA5 software (Tamura et al., 2011). The dispersal (or presence in a global scale) of each one of the common OTUs was investigated using the Basic Local Alignment Search Tool (http://blast.ncbi.nlm.nih.gov/).

3 Results and discussion

Disentangling the different temporal and spatial scales of deep-sea microbial communities can distinguish diversity deserts from hotspots and relate these microorganisms and/or their metabolic traits to the prevailing environmental factors. In addition, meaningful working hypotheses can be securely set on the interconnectivity or isolation of such communities (Gonzalez et al., 2012). Regarding deep-sea MVs, the benefit from such an approach lies mostly in the spatial scale, since their unpredictable eruptions frequency and/or changes in fluids flow, along with the sampling limitations due to the depth they occur, renders the investigation of their microbial communities temporal changes not feasible for the time being. Thus, in this paper we attempt to recognize shared bacterial and archaeal phylotypes among three major methane-related sites of the European margin. Such common phylotypes can be considered as fundamental species for these habitats (Konopka, 2009; Magurran and McGill, 2010), based on their global distribution and inferred metabolic traits. Our study did not aim to relate OTU occurrence with prevailing chemical conditions because not all sites have been studied for this in the same detail/parameters. We consider methane and other hydrocarbons flow as their most characteristic feature.

The percentage of shared archaeal OTUs between the three major sites we studied was 12.2 %, 40.0 % and 46.1 % for the East Mediterranean, the Gulf of Cadiz and the HMMV, respectively, while the corresponding values for Bacteria were 3.0 %, 21.5 % and 8.3 % (Fig. 2). In a recent synthesis study, by comparing 9.6 million bacterial

V6-rRNA amplicons from 509 samples spanning from the global ocean's surface to the deep-sea floor, it was considered that the 10 % of shared bacterial types between the pelagic and benthic communities implies greatly different communities (Zinger et al., 2011). Despite the much smaller datasets we used and the technical discrepancies between the individual studies, we observed higher percentages of shared OTUs, especially for the Archaea, suggesting that these habitats are somewhat interconnected via these phylotypes. Pairwise comparisons of the specific studies showed even higher percentages between certain studies (Table 2, Fig. 3). Whether these common phylotypes could be key-stone species for these habitats remains to be investigated with ecophysiological approaches, but it seems that the occurrence of these microorganisms is rather restricted to very similar habitats i.e. methane-dominated sediments (see below). This endemic character of these microorganisms consists a clear demonstration of biogeographic pattern of methane-dominated deep-sea sediments, as it happens in other highly specific or extreme environments like hydrothermal vents (Hanson et al., 2012). The differences in the percentage of shared phylotypes – for both Bacteria and Archaea – could be partially attributed to the different depth of analysis and/or different sampling effort. For example, the datasets of Pachiadaki et al. (2010, 2011), had the highest spatial resolution (i.e. vertical sediment sampling) and it was between these two sites that the highest number of common OTUs were found. As it is usually indicated with rarefaction curves, deeper or fine-scaled analysis results in more OTUs, and, thus, it is more possible to find such shared phylotypes between different samples.

3.1 Archaea

In all studies considered here, the number of retrieved archaeal OTUs was lower than the bacterial. The lower archaeal vs. bacterial species' richness seems to apply to hydrate-bearing sediments, cold seeps as well as MVs and is possibly a general trend in methane-related environments (Pachiadaki et al., 2010 and references therein).

The OTU comparison among the different data sets (Table 2) revealed that the number of shared OTUs is high. Considering the low number of retrieved total archaeal

OTUs (less than 30 in all but one datasets), the shared OTUs have a substantial relative abundance. The highest number of shared OTUs was found between AMSMV Pac and KZNMV Pac databases, having 12 OTUs (48.0 % and 63.1 % of their total archaeal OTUs, respectively) in common.

To further examine the key players in this microbial network (Fig. 4) the identity and the potential role of the internal nodes with the highest number of edges, namely the more common OTUs was examined. The most common one – representative sequence HQ588641 –, retrieved in seven data sets and in all examined sites, was affiliated with the anaerobic methane oxidizing euryarchaeotal group, ANME-3. ANME-3 group has been found to dominate in sediments of the Haakon Mosby MV and believed to perform AOM either without obligatory physical association with a sulfate reducing partner or with alternative partners (Lösekann et al., 2007). This group is related only with methane-rich environments, but it seems that within these environments is more widely spread that initially assumed. This specific OTU, HQ588641, was reported from a variety of locations, such as methane seep of Yung-An ridge [GU553624] and subseafloor gas-hydrate sediments at the Kaoping Canyon [JQ817531] in SW Taiwan, deep-sea methane seep sediment from Nankai Trough Japan [AB461393]; methane seeps of Hikurangi Margin, New Zealand [JF268331]; Mississippi canyon Gulf of Mexico [GU190976]; Hydrate Ridge Cascadia Margin [AM229243] and Eel River Basin [AF354136]. Abundance data that could be retrieved from some of the studies indicate that this OTU dominated specific habitats in those locations, such as the sediment below *Beggiatoa* mats (Lösekann et al., 2007). The vertical distribution of the archaeal communities in the Amsterdam and Kazan MVs (Pachiadaki et al., 2010; Pachiadaki et al., 2011) revealed that this OTU is present in all sediment layers reaching up to a relative abundance of ~ 70 % at 30 cm b.s.f. in Amsterdam MV.

Three OTUs, HQ588637, HQ588644 and HQ588639 were present in six of the data sets and in four (out of the total five) locations. They all were identified as anaerobic methane oxidizers belonging to the groups ANME-2c, ANME-2a and ANME-1, respectively. These OTUs have a world wide distribution, but their presence, as before, is restricted in habitats that AOM is a key process, from the cold sediments of Skan Bay, Alaska [DQ640143; DQ5229339] and the methane seeps, Northern North Sea [FM179890; FM179862]; to the gas hydrate sediments of the Hydrate Ridge [AM229230; AM229254; AM713449], the hydrocarbon seeps of the Gulf of Mexico [GU190995; GU190981; DQ521780] and the hydrothermal sediments in the Guaymas Basin [F419650; FP565147]. The OTUs of ANME-1 and ANME-2 groups were found to co-occur in all examined data sets, which is a rather common finding in AOM related studies (Dhillon et al., 2005; Knittel et al., 2005; Mills et al., 2005; Orphan et al., 2002) since it seems that, although phylogenetically distant, these groups perform AOM in a wide range of environmental settings. Our data further revealed that actually there is a co-occurrence of all three anaerobic methane oxidizing groups, ANME-1; ANME-2 (both 2a and 2c subgroups) and ANME-3. The shared ANME-1 OTU [HQ5886439] was also retrieved in AOM performing microbial mat from the Back Sea [FP565147] (Meyerdierks et al., 2011), which is a habitat dominated by the ANME-1 Archaea. All three phylotypes were found in high abundances in East Mediterranean MVs. The ANME-2c OTU [HQ588637] dominated the surface clone library of Kazan MV (~ 87 % of all archaeal clones) and the 25 cm b.s.f. layer of Amsterdam MV (~ 39 %), the ANME-2a [HQ588644] appeared at Amsterdam MV with a relative abundance reaching ~ 55 % in the 10 cm b.s.f. clone library and at Kazan MV ~ 40 % in the 30 cm b.s.f. one. The ANME-1 [HQ588639] was also abundant with ~ 31 % and 22 % for 5 cm b.s.f. Amsterdam MV and 10 cm b.s.f. Kazan MV, respectively.

The next common OTU [HQ588645] shared among four of the data sets was affiliated to MBG-D, Marine Benthic Group-D, (synonymous with marine group III, MG-III, a lineage within the Thermoplasmatales). For this group is difficult to suggest potential ecophysiological roles, other than its association with the deep subsurface (Teske and Sørensen, 2008) and especially with methane rich environments (Biddle et al., 2011). This OTU seems to be present in several AOM hot-spots. The OTUs HQ588636, HQ588640 and FN820369 (each common among three of the data sets) also belong to the MBG-D. All of them are exclusively found in methane rich habitats indicating that this group together with the ANME is probably playing an important role in the process of AOM.

One crenarchaeotal OTU, HQ588636, belonging to MBG-B, Marine Benthic Group-B (synonymous with Deep Sea Archaeal Group) is present in three of the data bases. This group of Archaea is thought to represent one of the dominant archaeal lineages in clone libraries of archaeal 16S rRNA and occurs in a wide range of sampling sites and sediment types (Teske and Sørensen, 2008) but perhaps that is not the case for cold seeps and mud volcanoes (Lösekann et al., 2007; Pachiadaki et al., 2010, 2011), where it was probably outcompeted by the ANMEs. Still within this group, there are branches consisting of phylotypes occurring in cold seeps and carbonates (Teske, 2006).

Finally, two OTUs, HQ588635 and AJ704644 (each of which is present in three of the data sets) were affiliated to Thaumarchaeota, MG-I, Marine Group-I. This group seems to account for a major portion of all prokaryotic picoplankton in seawater (Karner et al., 2001) and is also found in the marine sedimentary subsurface (Teske, 2006). The OTUs which were retrieved form the studied data bases were also found in a variety of habitats including upwelling systems (EF645847); wetlands (EU420691) and water above seafloor reflecting the cosmopolitan, not methane related distribution of those phylotypes.

3.2 Bacteria

The bacterial data sets appear more diverse in terms of species richness since all but one (GoC Nie) contain a high number of OTUs. Again the highest absolute number of shared OTUs was observed between AMSMV Pac and KZNMV Pac (Table 2b). The 37 common OTUs constitute 17.4 and 21.0 % of their total bacterial OTUs, respectively. The two different data sets of Amsterdam MV, AMSMV Pac and AMSMV Heij, share 22 OTUs corresponding to 10.3 and 23.9 % of their total bacterial OTUs, respectively. In all other dataset comparisons the relative abundance of shared OTUs is >10 %. Strangely, the two different data sets from the Gulf of Cadiz, GoC Nie and GoC Mai, appeared not to have any common OTU, but this might be caused by the fact that the GoC Nie clone libraries were probably not thoroughly sampled.

The most common OTU is the δ-Proteobacterium HQ588562, which is present in six of the data sets and in all five locations. Interestingly, apart from the five studied locations this phylotype is only found in two other sites, the gas hydrate sediments of Hydrate Ridge, Cascadia Margin [AJ535240] and the marine methane seep of Santa Barbara Basin [AF354160]. All these sequences, forming a single OTU, cluster into a distinct clade within the SEEP-SRB1f subgroup of the δ-Proteobacteria (Schreiber et al., 2010). The role of this subgroup in the process of AOM is still unclear.

Another common OTU, HQ588530, belonging to δ-Proteobacteria was found in six of the data sets but only in four of the examined locations (absent from HMMV) has also a limited distribution. Apart from the studied European sites, this phylotype was present in clone libraries from enrichments of Hydrate Ridge sediments, Cascadia Margin [FN549955], as well as clones from direct cell capture of Eel River Basin methane seeps [EU622295]. This OTU was affiliated in the SEEP-SRB1a subgroup, the members of which are believed to be the syntrophic partners of ANME-2 (a and c subgroups) Archaea (Pernthaler et al., 2008; Schreiber et al., 2010), as well as partners for the ANME-3 (Schreiber et al., 2010). In the same subgroups belongs one more common OTU, HQ588491, present in five of the examined data sets and four locations (not retrieved from KZNMV).

To the SEEP-SRB1e subgroup was affiliated OTU HQ588370, which is shared among four of the data sets and again not present in HMMV. This OTU is more widely spread and is found, apart from enrichments performing AOM, in Hydrate Ridge sediments, Cascadia Margin [AM229187]; Gullfaks oil and gas fields sediment, North Sea [FM179902]); Gulf of Mexico cold-seep sediment [AM746087]; oil-polluted sediments [JQ580337]; subseafloor sediment at the Good Weather Ridge, gas hydrate potential area, SW Taiwan [JQ816985]; Arctic marine surface sediment [FN396770]; Sylt sediment, Wadden Sea [AM040136]. Although this subgroup of δ-Proteobacteria is associated with the AOM aggregates, the exact ecophysiological role still remains unknown.

Three more OTU HQ588451, HQ588544 and HQ588410 (each shared among four data sets) belong to δ-Proteobacteria, pointing out the importance of this group in the AOM hot spots in the European margins.

A member of the candidate division JS1 [HQ588424] and a member of Bacteriodetes [HQ588428] were among of the common OTUs. There are both wide spread subsurface groups. Sulfate reducing δ-proteobacteria, which are very abundant in the studied locations, can utilize a wide spectrum of substrates which are being oxidized completely or incompletely to acetate, supplying their habitat with different forms of organic material, as well as reduced sulfur compounds (Webster et al., 2006). Members of JS1 and Bacteriodetes have been associated with acetate incorporation in sulfate reducing environments and that could explain the high abundance of these groups in sediments where AOM driven by sulfate reduction takes place (Pachiadaki et al., 2011).

Data regarding abundance of the JS1 OTU [HQ588424] revealed that it might be a central player in the East Mediterranean MVs, especially in Amsterdam MV. This OTU had a persistent appearance in all sediment layers studied with relative abundances that increased from ~ 2 % at the surface layer to ~ 34 % at 15 cm b.s.f. and decreased again to ~ 12 % at the deepest studied layer, 30 cm b.s.f.

Two γ-Proteobacterial OTUs, HQ588450 and HQ588378, seem to be important for the studied system. Each one of them is present in four data sets. Both of them were affiliated to sulfur and sulfide oxidizers or to environmental sequences originating from habitats where sulfur and sulfide oxidation occurred or was predicted. Such microorganisms are energetically fuelled by the sulfide produced by the sulfate reduction of the AOM process, and refuel AOM.

Twenty OTUs were found to be shared among three of the investigated sites. They belong to γ-Proteobacteria, δ-Proteobacteria, Chloroflexi, α-Proteobacteria, Actinobacteria and Acidobacteria. Members of the last four groups are often found in close association with the ANME-SRB consortia (Pernthaler et al., 2008; Zhang et al., 2011) and believed to be using the by-products of AOM. Fifty two OTUs were common between two of the examined datasets.

Community comparisons between analogue/similar habitats are difficult to be made due to different methodological approaches used by each research team, from sampling and nucleic acid extraction to amplification conditions, primers used and analysis of clone libraries. An underestimation of the shared OTUs is assumed, except from studies conducted by the same team and the same protocols. Despite the biases, these comparisons can provide useful information and network analysis of similar environments can unveil the “key players” within complex microbial communities.

It has been stated (Gonzalez et al., 2012 and references there in) that under similar environmental conditions, microbial communities can have strikingly different composition and function; conversely, different community configurations can function similarly. It seems that in some cases, such as the MVs of the European margins, microbial community composition and structure can vary but the major microbiological processes are similar. The presence of few “core” phylotypes in all (or most) of the studied sites implies that these play a central role on the ecosystem functioning.

It is known that AOM, the key process in MVs, is mediated by a consortium of methane oxidizing Archaea and sulphate reducing Bacteria. The importance of this partnership is captured in the created network, since ANME and SRB are the most connected nodes. Other highly-edged notes are the sulphide and sulphur reducers, extremely important for AOM refuelling. Estimations of β-diversity showed highly dissimilar communities (especially in the case of bacteria) and measurements of rates different activities but these different communities in similar, but geographically distant, enviroments seem to favor the presence of few specific OTUs among many phylogenetically close others. Core OTUs are required for the core processes: oxidation of methane, sulfate reduction, sulfide oxidation. Secondary processes in the same environments, such as the acetate utilization and degradation of complex organic matter, can be probably performed by different OTUs or different groups. Surprisingly, the majority of the core OTUs from the European marine MVs seem to be present exclusively in methane-rich marine environments, demonstrating the endemic character of these microorganisms. AOM is a widespread process, that takes place in anaerobic environments worldwide, from rice pads to swamps and marine sediments, but the key OTUs retrieved from the studied databases never clustered with OTUs from terrestrial or fresh-water origin.

This study provides an overview of all the existing data based on cloning/sequencing of 16S rRNA gene data. With the advent of the next-generation sequencing (NGS) technologies, and their astonishing pace of improvement, it is expected that in the near future only such approaches will be applied in order to provide new insights in the MV prokaryotic diversity. These older approaches, compared to the the NGS ones, should be considered as for a “quick and dirty” snapshot of the communities (Zinger et al., 2012).

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References

Biddle, J. F., Cardman, Z., Mendlovitz, H., Albert, D. B., Lloyd, K. G., Boetius, A., and Teske, A.: Anaerobic oxidation of methane at different temperature regimes in Guaymas Basin hydrothermal sediments, ISME J., 6, 1018–1031, 2011.

Boetius, A., Ravenschlag, K., Schubert, C. J., Rickert, D., Widdel, F., Gleseke, A., Amann, R., Jørgensen, B. B., Witte, U., and Pfannkuche, O.: A marine microbial consortium apparently mediating anaerobic oxidation methane, Nature, 407, 623–626, 2000.

Cita, M. B., Ryan, W. B. F., and Paggi, L.: Prometheus mud-breccia: An example of shale diapirism in the Western Mediterranean Ridge, Ann. Geol. Pays Hellen., 30, 543–570, 1981.

De Beer, D., Sauter, E., Niemann, H., Kaul, N., Foucher, J. P., Witte, U., Schüter, M., and Boetius, A.: In situ fluxes and zonation of microbial activity in surface sediments of the Haakon Mosby Mud Volcano, Limnol. Oceanogr., 51, 1315–1331, 2006.

Dhillon, A., Lever, M., Lloyd, K. G., Albert, D. B., Sogin, M. L., and Teske, A.: Methanogen diversity evidenced by molecular characterization of methyl coenzyme M reductase A (mcrA) genes in hydrothermal sediments of the Guaymas Basin, Appl. Environ. Microb., 71, 4592–4601, 2005.

Dupré, S., Woodside, J., Foucher, J.-P., de Lange, G., Mascle, J., Boetius, A., Mastalerz, V., Stadnitskaia, A., Ondréas, H., Huguen, C., Harmègnies, F., Gontharet, S., Loncke, L., Deville, E., Niemann, H., Omoregie, E., Olu-Le Roy, K., Fiala-Medioni, A., Dählmann, A., Caprais, J.-C., Prinzhofer, A., Sibuet, M., Pierre, C., and Damstè, J. S.: Seafloor geological studies above active gas chimneys off Egypt (Central Nile Deep Sea Fan), Deep-Sea Res. Pt. I, 54, 1146–1172, 2007.

Gonzalez, A., King, A., Robeson II, M. S., Song, S., Shade, A. Metcalf, J. L., Knight, R.: Characterizing microbial communities through space and time, Curr. Opin. Biotech., 23, 431–436, 2012.

Hanson, C. A., Fuhrman, J. A., Horner-Devine, M. C., and Martiny, J. B. H.: Beyond biogeographic patterns: processes shaping the microbial landscape, Nat. Rev. Micro., 10, 497–506, 2012.

Heijs, S. K., Haese, R. R., Van Der Wielen, P. W. J. J., Forney, L. J., and Van Elsas, J. D.: Use of 16S rRNA gene based clone libraries to assess microbial communities potentially involved in anaerobic methane oxidation in a Mediterranean cold seep, Microbial. Ecol., 53, 384–398, 2007.

Hensen, C., Nuzzo, M., Hornibrook, E., Pinheiro, L. M., Bock, B., Magalhães, V. H., and Brückmann, W.: Sources of mud volcano fluids in the Gulf of Cadiz-indications for hydrothermal imprint, Geochim. Cosmochim. Ac., 71, 1232–1248, 2007.

Karner, M. B., DeLong, E. F., and Karl, D. M.: Archaeal dominance in the mesopelagic zone of the Pacific Ocean, Nature, 409, 507–510, 2001.

Knittel, K., Lösekann, T., Boetius, A., Kort, R., and Amann, R.: Diversity and distribution of methanotrophic archaea at cold seeps, Appl. Environ. Microb., 71, 467–479, 2005.

Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. J., and Higgins, D. G.: Clustal W and Clustal X version 2.0, Bioinformatics, 23, 2947–2948, doi:10.1093/bioinformatics/btm404, 2007.

Loncke, L. and Mascle, J.: Mud volcanoes, gas chimneys, pockmarks and mounds in the Nile deep-sea fan (Eastern Mediterranean): Geophysical evidences, Mar. Petrol. Geol., 21, 669–689, 2004.

Lösekann, T., Knittel, K., Nadalig, T., Fuchs, B., Niemann, H., Boetius, A., and Amann, R.: Diversity and abundance of aerobic and anaerobic methane oxidizers at the Haakon Mosby Mud Volcano, Barents Sea, Appl. Environ. Microb., 73, 3348–3362, 2007.

Magurran, A. E. and McGill, B. J.: Biological diversity: Frontiers in measurement and assessment. Oxford University Press, Oxford, 368 pp., 2010.

Maignien, L., Parkes, R. J., Cragg, B., Niemann, H., Knittel, K., Coulon, S., Akhmetzhanov, A., and Boon, N.: Anaerobic oxidation of methane in hypersaline cold seep sediments, FEMS Microbiol. Ecol., doi:10.1111/j.1574-6941.2012.01466.x, 2012.

Meyerdierks, A., Kube, M., Kostadinov, I., Teeling, H., Glöckner, F. O., Reinhardt, R., and Amann, R.: Metagenome and mRNA expression analyses of anaerobic methanotrophic archaea of the ANME-1 group, Environ. Microbiol., 12, 422–439, 2010.

Mills, H. J., Martinez, R. J., Story, S., and Sobecky, P. A.: Characterization of microbial community structure in Gulf of Mexico gas hydrates: Comparative analysis of DNA- and RNA-derived clone libraries, Appl. Environ. Microb., 71, 3235–3247, 2005.

Niemann, H., Duarte, J., Hensen, C., Omoregie, E., Magalhães, V. H., Elvert, M., Pinheiro, L. M., Kopf, A., and Boetius, A.: Microbial methane turnover at mud volcanoes of the Gulf of Cadiz, Geochim. Cosmochim. Ac., 70, 5336–5355, 2006.

Omoregie, E. O., Mastalerz, V., De Lange, G., Straub, K. L., Kappler, A., Røy, H., Stadnitskaia, A., Foucher, J. P., and Boetius, A.: 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, 2008.

Orphan, V. J., House, C. H., Hinrichs, K. U., McKeegan, K. D., and DeLong, E. F.: Multiple archaeal groups mediate methane oxidation in anoxic cold seep sediments, P. Natl Acad. Sci. USA, 99, 7663–7668, 2002.

Pachiadaki, M. G., Lykousis, V., Stefanou, E. G., and Kormas, K. A.: Prokaryotic community structure and diversity in the sediments of an active submarine mud volcano (Kazan mud volcano, East Mediterranean Sea), FEMS Microbiol. Ecol., 72, 429–444, 2010.

Pachiadaki, M., Kallionaki, A., Dählmann, A., De Lange, G., and Kormas, K.: Diversity and Spatial Distribution of Prokaryotic Communities Along A Sediment Vertical Profile of A Deep-Sea Mud Volcano, Microbiol. Ecol., 62, 655–666, 2012.

Pernthaler, A., Dekas, A. E., Brown, C. T., Goffredi, S. K., Embaye, T., and Orphan, V. J.: Diverse syntrophic partnerships from deep-sea methane vents revealed by direct cell capture and metagenomics, P. Natl Acad. Sci. USA, 105, 7052–7057, 2008.

Pruesse, E., Quast, C., Knittel, K., Fuchs, B. M., Ludwig, W., Peplies, J., and Glöckner, F. O.: SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB, Nucleic Acids Res., 35, 7188–7196, 2007.

Schreiber, L., Holler, T., Knittel, K., Meyerdierks, A., and Amann, R.: Identification of the dominant sulfate-reducing bacterial partner of anaerobic methanotrophs of the ANME-2 clade, Environ. Microbiol., 12, 2327–2340, doi:10.1111/j.1462-2920.2010.02275.x, 2010.

Smoot, M. E., Ono, K., Ruscheinski, J., Wang, P.-L., and Ideker, T.: Cytoscape 2.8: new features for data integration and network visualization, Bioinformatics, 27, 431–432, 2011.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S.: MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods, Mol. Biol. Evol., 28, 2731–2739, 2011.

Teske, A. P.: Microbial communities of deep marine subsurface sediments: Molecular and cultivation surveys, Geomicrobiol. J., 23, 357–368,, 2006.

Teske, A. and Sørensen, K. B.: Uncultured archaea in deep marine subsurface sediments: Have we caught them all?, ISME J., 2, 3–18, 2008.

Vanreusel, A., Andersen, A. C., Boetius, A., Connelly, D., Cunha, M. R., Decker, C., Hilario, A., Kormas, K. A., Maignien, L., Olu, K., Pachiadaki, M., Ritt, B., Rodrigues, C., Sarrazin, J., Tyler, P., Van Gaever, S., and Vanneste, H.: Biodiversity of cold seep ecosystems along the European margins, Oceanography, 22, 110–127, 2009.

Vogt, P. R., Cherkashev, A., Ginsburg, G. D., Ivanov, G. I., Crane, K., Lein, A. Y., Sundvor, E., Pimenov, N. V., and Egorov, A. V.: Haakon Mosby mud volcano: a warm methane seep with seafloor hydrates and chemosynthesis-based ecosystem in late Quanternary Slide Valley, Bear Island Fan, Barents Sea passive margin, EOS T. Am. Geophysical Union Supplement, 78, 187–189, 1997.

Webster, G., Watt, L. C., Rinna, J., Fry, J. C., Evershed, R. P., Parkes, R. J., and Weightman, A. J.: A comparison of stable-isotope probing of DNA and phospholipid fatty acids to study prokaryotic functional diversity in sulfate-reducing marine sediment enrichment slurries, Environ. Microbiol., 8, 1575–1589, 2006.

Zhang, Y., Maignien, L., Zhao, X., Wang, F., and Boon, N.: Enrichment of a microbial community performing anaerobic oxidation of methane in a continuous high-pressure bioreactor, BMC Microbiol., 11, 137, doi:10.1186/1471-2180-11-137, 2011.

Zinger, L., Amaral-Zettler, L. A., Fuhrman, J. A., Horner-Devine, M. C., Huse, S. M., Welch, D. B. M., Martiny, J. B. H., Sogin, M., Boetius, A., and Ramette, A.: Global Patterns of Bacterial Beta-Diversity in Seafloor and Seawater Ecosystems, PLoS ONE 6, e24570, doi:10.1371/journal.pone.0024570, 2011.

Zinger, L., Gobet, A., and Pommier, T.: Two decades of describing the unseen majority of aquatic microbial diversity, Mol. Ecol., 21, 1878–1896, 2012.

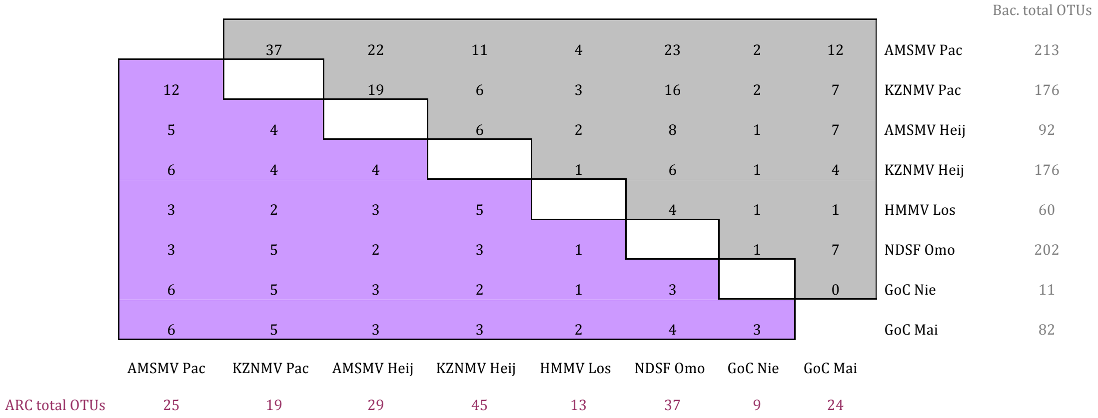
Zitter, T. A. C., Huguen, C., and Woodside, J. M.: Geology of mud volcanoes in the eastern Mediterranean from combined sidescan sonar and submersible surveys, Deep-Sea Res. Pt. I, 52, 457–475, 2005.

Table 1. Data sets of 16S rRNA gene sequences used in this study.

Code	Site	Site	Domain	# of unique OTUs	Reference
AMSMV Pac Bac	Amsterdan MV	35°20.0′ N 30°16.30′ E	Bacteria	213	(Pachiadaki et al., 2011)
AMSMV Pac Arc	Amsterdan MV	35°20.03′ N 30°16.30′ E	Archaea	25	(Pachiadaki et al., 2011)
AMSMV Heij Bac	Amsterdan MV	35°20.00′ N 30°16.00′ E	Bacteria	92	(Heijs et al., 2008)
AMSMV Heij Arc	Amsterdan MV	35°20.00′ N 30°16.00′ E	Archaea	29	(Heijs et al., 2008)
KZNMV Pac Bac	Kazan MV	35°25.92′ N 30°33.70′ E	Bacteria	176	(Kormas et al., 2008; Pachiadaki et al., 2010)
KZNMV Pac Arc	Kazan MV	35°25.92′ N 30°33.70′ E	Archaea	19	(Kormas et al., 2008; Pachiadaki et al., 2010)
KZNMV Heij Bac	Kazan MV	35°25.90′ N 30°33.70′ E	Bacteria	117	(Heijs et al., 2007)
KZNMV Heij Arc	Kazan MV	35°25.90′ N 30°33.70′ E	Archaea	45	(Heijs et al., 2007)
HMMV Los Bac	Haakon Mosby MV	72°00.25′N 14°43.50′ E	Bacteria	60	(Lösekann et al., 2008)
HMMV Los Arc	Haakon Mosby MV	72°00.25′N 14°43.50′ E	Archaea	13	(Lösekann et al., 2008)
NDSF Omo Bac	Nile deep sea fan	32°06.74′ N 28°10.35′ E	Bacteria	202	(Omoregie et al., 2008)
NDSF Omo Arc	Nile deep sea fan	32°06.74′ N 28°10.35′ E	Archaea	37	(Omoregie et al., 2008)
GoC Mai Bac	Gulf of Cadiz		Bacteria	82	Unpublished*
GoC Mai Arc	Gulf of Cadiz		Archaea	24	Unpublished*
GoC Nie Bac	Gulf of Cadiz	35°39.72′ N 07°19.98′ E	Bacteria	11	(Niemann et al., 2006)
GoC Nie Arc	Gulf of Cadiz	35°39.72′ N 07°19.98′ E	Archaea	9	(Niemann et al., 2006)

* Maignien, L. and Boon, N.: Activity and distribution of anaerobic methananotrophs in mud volcanos from the Gulf of Cadiz, unpublished, Archaea accession numbers: FN820355-FN820437, Bacteria accession numbers: FN820294-FN820354, GenBank, submitted on 26-04-2010.

Table 2. Number of shared and total bacterial (BAC, grey) and archaeal (ARC, purple) operational taxonomic units (OTUs) between the different sites.



	AMSMV Pac	KZNMV Pac	AMSMV Heij	KZNMV Heij	HMMV Los	NDSF Omo	GoC Nie	GoC Mai		Bac. total OTUs
		37	22	11	4	23	2	12	AMSMV Pac	213
	12		19	6	3	16	2	7	KZNMV Pac	176
	5	4		6	2	8	1	7	AMSMV Heij	92
	6	4	4		1	6	1	4	KZNMV Heij	176
	3	2	3	5		4	1	1	HMMV Los	60
	3	5	2	3	1		1	7	NDSF Omo	202
	6	5	3	2	1	3		0	GoC Nie	11
	6	5	3	3	2	4	3		GoC Mai	82
ARC total OTUs	25	19	29	45	13	37	9	24		

Fig. 1. Map of the European marginal seas. HMMV: Haakon Mosby mud volcano, GoC: Gulf of Cadiz, AM: Anaximander Mountains, NDSF: Nile Deep-Sea Fan.

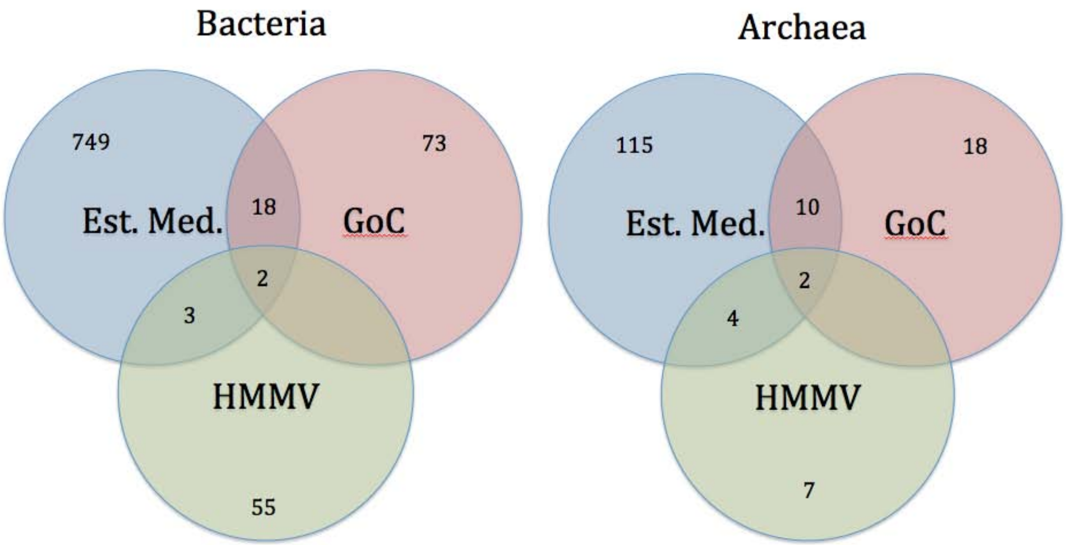


Fig. 2. Venn diagram of the shared phylotypes between the East Mediterranean (Est. Med.), the Gulf of Cadiz (GoC) and the Haakon Mosby (HMMV) mud volcanoes.

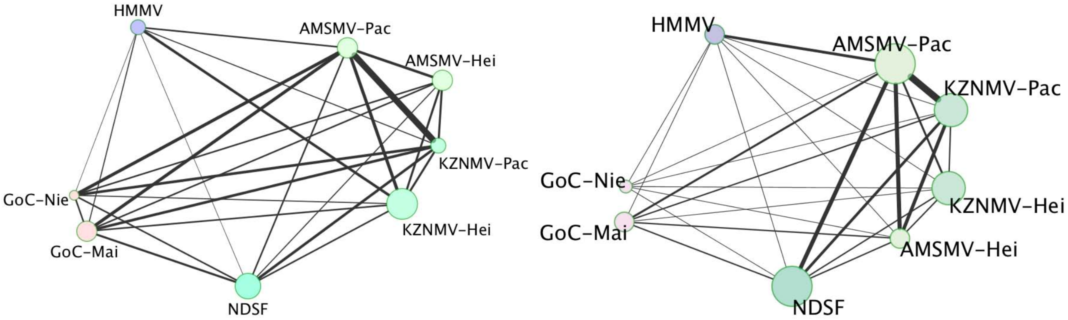


Fig. 3. Degree of connectivity between the 16 data sets analysed based on the archaeal (left) and bacterial (right) shared OTUs. The thickness of the links is relative to the number of shared OTUs and the size of the each node is relative to the number of unique OTUs found in each site.

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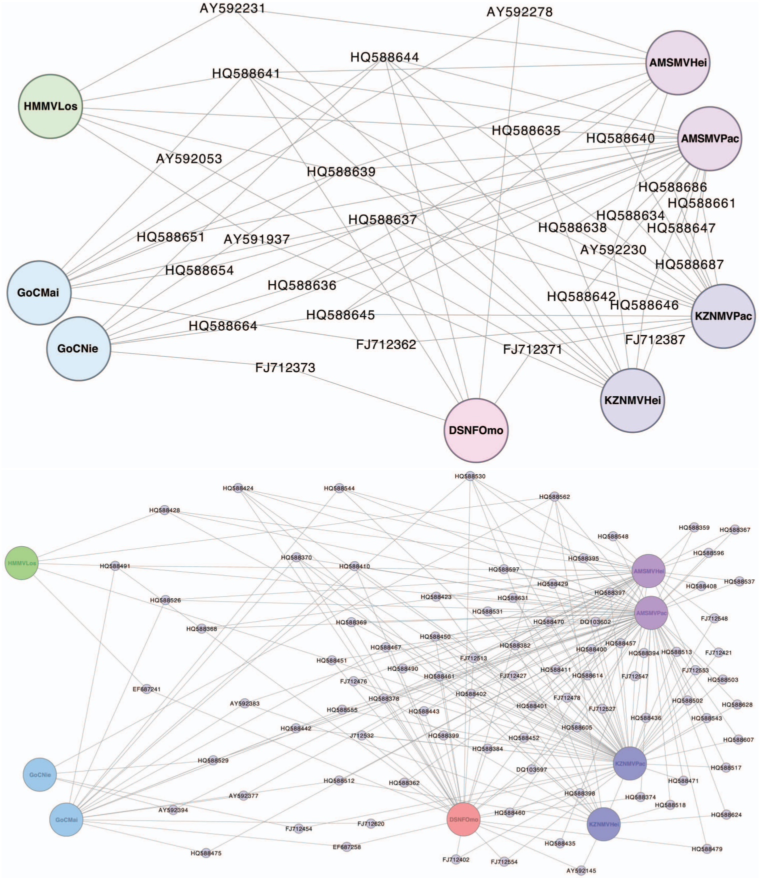

Fig. 4. GenBank accession numbers of the shared archaeal (top) and bacterial (bottom) OTUs between the 16 datasers analysed.