RESEARCH ARTICLE

INTERNATIONAL MICROBIOLOGY
OPEN ACCESS

International Microbiology (2016) 19:109-119
doi:10.2436/20.1501.01.269. ISSN (print): 1139-6709. e-ISSN: 1618-1095
www.im.microbios.org

Spatial homogeneity of bacterial and archaeal communities in the deep eastern Mediterranean Sea surface sediments

Sabine Keuter, Baruch Rinkevich

Israel Oceanographic & Limnological Research, National Institute of Oceanography, Tel Shikmona, Haifa, Israel

Received 26 April 2016 · Accepted 26 May 2016

Summary. The diversity of microorganisms inhabiting the deep sea surface sediments was investigated in 9 stations (700-1900 m depth) in the Levantine basin by 454 massive tag sequencing of the 16S rDNA V4 region using universal primers. In total, 108,811 reads (an average of 10,088 per sample) were assigned to 5014 bacterial and 966 archaeal operational taxonomic units (OTUs; at 97% cut off). The 55% of the reads were of archaea, indicating dominance of archaea over bacteria at eight of the stations. The diversity and estimated richness values were high (e.g., H´ ranged from 5.66 to 7.41 for bacteria). The compositions of the microorganisms at all stations were remarkably similar, with Bray-Curtis similarities of 0.53–0.91 and 0.74–0.99 for bacterial and archaeal orders respectively. At two stations, very high abundances of only a few genera (*Marinobacterium*, *Bacillus*, *Vibrio*, *Photobacterium*) were accountable for the dissimilarities documented compared to the other deep sea stations. Half of the bacterial reads (51%) belonged to the phylum Proteobacteria, comprising mainly Gammaproteobacteria (41–72% of the proteobacterial reads per sample), Deltaproteobacteria (12–29%), Alphaproteobacteria (7–18%) and Betaproteobacteria (3–14%). The most abundant bacterial family was Sinobacteraceae (order Xanthomonadales) with 5–10% of total bacterial reads per sample. Most abundant reads (15.4% of all microbial reads) were affiliated with Marine Group 1 archaea, putatively capable of ammonia oxidation (213 OTUs), and bacteria involved in nitrification were found in all samples. The data point to the significant role that chemolithotrophic carbon assimilation and nitrification fill in the oligotrophic deep sea Levant sediments. [**Int Microbiol** 19(2): 109-119 (2016)]

Keywords: deep sea sediments · eastern Mediterranean · microbial communities · ammonia oxidizing Archaea (AOA) · Israel

Introduction

Most of the earth´s surface area is classified as deep sea sediments, and the microbial communities of deep sea sediments constitute a significant integral part of nutrient and organic matter recycling of the benthic food web, while bacteria can make up almost 90% of the total benthic biomass [28,48]. Despite these significant ecological roles, and especially in the case of archaea, not much is known about the microbial communities and their distribution on the seafloor (apart from some hotspots like cold seeps, hydrothermal sediments or cetacean carcasses on the ocean floors, which are high in diversity and productivity [23,31]). Yet, recent studies show that the diversity of microorganisms in deep sea sediments is higher than in fairly studied other marine ecosystems such as vents, anoxic habitats and open ocean surface waters [53,66].

*Correspondence: S. Keuter, B. Rinkevich
E-mail: S. Keuter (ostrea@gmx.de); B. Rinkevich (buki@ocean.org.il)

Deep sea sediments (>200 m depth) are generally fine-grained, and oxygenated in their upper layer, which can be up to 50 cm thick layers in oligotrophic regions [59]. They are subjected to extreme conditions such as low temperatures (–1 ° to 4 °C) and high pressures [23]. It is believed that these ecosystems are generally constant, since currents are low and the environmental conditions do not change considerably, and inhabited with heterotrophic microorganisms that depend on the organic matter descending from the euphotic surface. Since only a small fraction of the primary production at the surface reaches the deep sea floors, these ecosystems are oligotrophic and energy-limited. Nevertheless, the microorganisms in deep sea sediments abound with up to 10^9 cells/g sediment, which is comparable to the levels observed in coastal sediments [50]. Compared to other oceans and seas, the deep sea waters of the Mediterranean Sea are especially poor in nutrients, and the eastern basin is possibly one of the most oligotrophic seas in the world [46]. The Mediterranean Sea is characterized by an east-west oligotrophy due to its anti-estuarine circulation pattern in which nutrients are net-exported within the Levantine intermediate water from the easternmost Levantine basin towards the Atlantic Ocean [27,41]. Thus, the Levantine basin is an ultra-oligotrophic environment exhibiting low inorganic nutrient concentrations as well as high salinities of up to 39.5 psu and high minimum temperatures of 13.5 °C down to the deep sea floor [5,26].

Microbial distribution patterns are considered to be driven by contemporary environmental as well as historical factors, including geographical barriers and past environmental regimes [47]. Marine microbial communities have been correlated to organic carbon input from the surface [66] and their distribution in the water column is affected by the movements of water masses and currents [33,60,66]. In accordance, spatial distances explained the differences between microbial community compositions of rather stable sediments lacking physical mixing compared to more homogenous water column communities [66]. At a scale of 10–30,000 km, influences of spatial distances as well as of historical sways are likely [34]. Although species and genera have been shown to inhabit similar ecological niches around the world [15,37], a comprehensive study comparing abyssal surface sediment bacteria in samples from nine different ocean regions has found that the number of shared bacterial types decreased with geographical distances [66]. Most different to other oceanic regions, in terms of dominant bacterial phyla as well as higher taxonomic resolution, was the Mediterranean Sea in the aforementioned study. This fact that was explained by the limited exchange of Mediterranean waters with other oceans' waters and the deep sea water´s high temperatures. Within the Mediterranean Sea, however, large differences between microbial communities at all spatial scales were found using the 454 sequencing technique [53].

Most of the studies that focused on the diversity of deep water surface sediment were Proteobacteria, and especially Gammaproteobacteria. They were the most common phylum, and they seem to play a dominant role in the Mediterranean Sea as well [49,53]. Studies further revealed high abundances of Deltaproteobacteria, Chloroflexi, Acidobacteria, Actinobacteria and Planctomycetes [20,25, 42,44,45]. Archaea are also common in deep Mediterranean surface sediments, increasing in importance compared to bacteria as you move eastwards, and with higher abundances of Crenarchaeota Marine Group 1 (MG1) than Euryarchaota [13,45]. However, in some exclusive habitats like methane seeps or mud volcanos [20,49], other groups of archaea, affiliated with hydrocarbon metabolizing *Methanococcus*, *Mehtanobacterium* or *Methanosarcinales*, or archaeal groups like Thermoplasmales and Halobacteriales, were found. Further information on benthic archaea in the deep Mediterranean is very sparse [6,31].

This study aims to clarify bacterial and archaeal diversity and distribution in allegedly as of yet unaffected deep sea surface sediments in the Levantine basin, using massive parallel tag sequencing of samples from eight deep water stations in the south-eastern Mediterranean Sea Levantine basin, as well as from a single, shallower station located in an area of gas drilling activities and extraction and close to the port of Ashdod. Maximum distances between the stations were about 170 km, i.e., in a range that is likely to have a historical influence on communities. Environmental factors, including the contents of metals, PAH and PCBs were compared with sequencing data in order to further relate variations of microbial diversity and community patterns. For these, the need to study regional diversity using medium scales instead of single spots was pointed out earlier [22].

Material and methods

Sampling of sediments. Sampling was carried out at 9 stations (Fig. 1) in the Levantine basin of the south-eastern Mediterranean Sea in June and July 2013 with the RV 'SHIKMONA' (Israel Oceanographic and Limnological Research). Eight stations were ≥1200 m deep, whereas station S4_03 reached ~700 m at its deepest point (Fig. 1, Table 1). At stations G05, G09, G12, G14, G16, G26, G30, G31 and S4_03 four casts were done with a box-corer and surface sediments were scooped into plastic tubes and immediately frozen for DNA extraction. Environmental data were determined from samples of one of the casts (Table 1). Casts at every station were 144 m apart from each other on average with a range of 9–1,032 m. To check for vertical variability, additional sediment was taken from one of the cores at station G09,

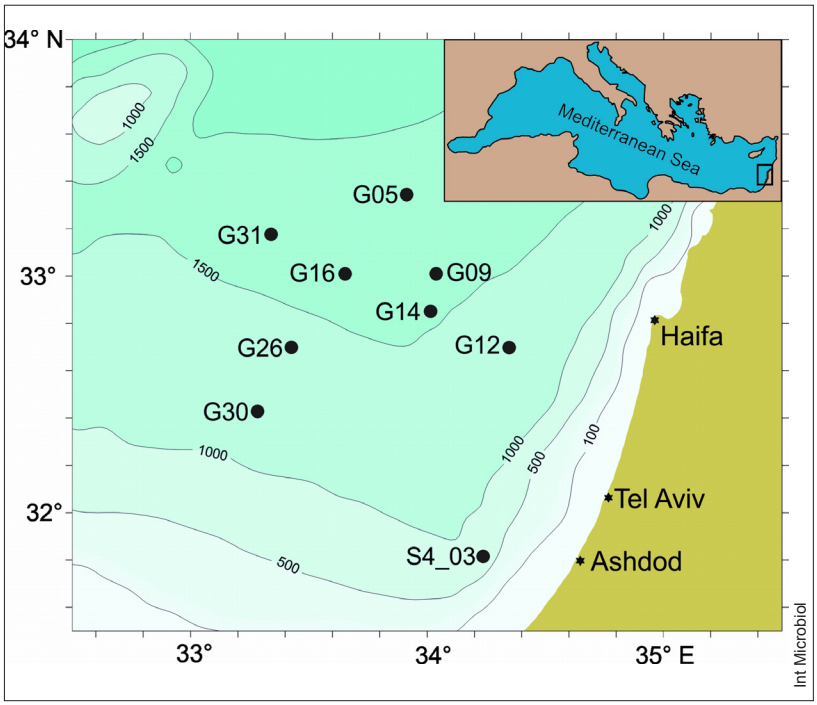

Fig. 1. Stations in the Levantine basin sampled in June and July 2013. Inserted: map of the Mediterranean Sea; the square depicts the location of the study site off the Israeli coast.

from a depth of 3– 4 cm (samples G09D). Depths and coordinates were provided by the Physical Oceanography Department, water content by the Marine Biology Department and TOC data were measured by the Marine Chemistry Department, all of the IOLR´s National Institute of Oceanography in Haifa. Grain sizes were determined by the Geological Survey of Israel (GSI), Jerusalem.

DNA extraction and 454 sequencing. DNA was extracted from 3 different casts (for samples G09D only one cast was used) per station using the MoBio Powersoil DNA isolation kit (Mo Bio Laboratories, Carlsbad, CA). DNA concentrations were measured with a nanodrop (ThermoScientific, Watham, MA) and equal amounts of each of the three extractions per station were pooled together for 16S-based tag encoded FLX amplicon pyrosequencing (bTEFAP) with the primer set bac515F (GTGCCAGCMGCCGCGGTAA) and bac806R (GGACTACVSGGGTATCTAAT) [8,9,10] at Molecular Research LP (sequencing and bioinformatics service provider; Shallowater, TX). After a single-step 30-cycle PCR using the HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA; denaturation at 94 °C for 30 s; annealing at 53 °C for 40 s and elongation at 72 °C for 1 min for 28 cycles). All amplicon products from the different samples were mixed in equal concentrations and purified using Agencourt Ampure beads (Agencourt Bioscience Corporation, MA). The samples were sequenced using 454 GS FLX titanium (Roche, Penzberg, Germany) and the data derived were processed using a proprietary analysis pipeline [3,8,9,10,11,56] at Molecular Research LP. The sequences were depleted of barcodes and primers. Sequences <200 bp, with ambiguous base calls and sequences with homopolymer runs exceeding 6 bp were removed. Sequences were denoised and chimeras removed, and operational taxonomic units (OTUs) were defined after removal of singleton sequences,

Table 1. Environmental variables and sediment characteristics of the sampling sites

Station	Latitude [°N]	Longitude [°E]	Bottom depth [m]	Water content [%]	TOC [%]	PAHs [μg kg^{-1}]	PCBs [mg kg^{-1}]	TRPH [mg kg^{-1}]	Cadmium [mg kg^{-1}]
G12	32.697	34.343	1387	21	0.87	39.96	0.2	11	0.062
G14	32.849	34.012	1587	34	0.73	12.18	0.3	11	0.087
G09	33.011	34.035	1689	34	0.89	33.16	0.8	19	0.104
G16	33.010	33.653	1653	32	0.63	16.7	0.4	18	0.068
G05	33.347	33.914	1901	30	0.73	18.97	0	18	0.062
G26	32.699	33.429	1388	34	0.63	20.78	0	17	0.064
G31	33.177	33.344	1678	42	0.59	21.66	0	17	0.066
G30	32.422	33.284	1198	35	0.58	16.13	0	14	0.062
S4_03	31.819	34.233	698	26	1.21	190.07	7.7	63	0.049

with clustering at 3% divergence (97% similarity). OTUs were then taxonomically classified using BLASTN against a curated Greengenes database [7] and compiled into each taxonomic level.

The 454 pyrosequencing data were deposited in the NCBI Sequence Read Archive under the project number PRJNA270910 (SRA numbers SRX883264-73).

Statistical analyses. Ecological diversity indices and richness estimators were calculated using OTUs (97% similarity) and cluster analyses of the community compositions, and analyses of similarity (ANOSIM) were performed using the program PAST (Paleontological Statistics, Version 2.17c) [17]. The 'envfit' function in the 'vegan' package for R was used to test how environmental variables correlated with bacterial community compositions in percentages on the family level. The significance of association was calculated by 999 random permutations [39].

Results

Environmental variables. The nine sediment sampling sites were located on two crossing transects in the Levantine basin with water column depths ranging from 1198 to 1901 m, including a single shallow station (700 m) nearer to the coast (Fig. 1). All sediments were fine-grained (58–73% clay, 26–41% silt; from O. Crouvi, personal communication) and of the same brownish color. The highest TOC contents (1.25%) were found at station S4_03, as well as the highest PCB (polychlorinated biphenyl), PAHs (polycyclic aromatic hydrocarbons) and TRPHs (total recoverable petroleum hydrocarbons) concentrations (Table 1), and the lowest TOC contents were measured at stations G30 and G31 (0.59 and 0.59%, respectively). In addition to the variables listed in Table 1, measurements of 16 (heavy) metals (Cd, Hg, Ba, Sr, Mn, As, Mo, Be, Co, Pb, U, Ni, V, Cr, Cu, Zn) and 7 metal oxides (SiO_2, Fe_2O_3, MgO, Al_2O_3, CaO, $CaCO_3$, TiO_2) were further included in the envfit analyses (data not shown), which revealed a significant relation between cadmium and the compositions of bacterial families ($P = 0.026$), while all other environmental factors were not related to the variations in the bacterial compositions detected at the different sites (see Table 1 for cadmium concentrations).

Microbial compositions in the deep sea samples. The V4 region of the 16S rRNA gene was amplified using a conserved primer pair, which was tagged with a short oligonucleotide sequence of 6 bp (i.e., barcodes). A total of

Table 2. An overview of the main results from the 454 massive tag sequencing of the 10 samples together

	All samples	Per sample (average)	Per sample (range)
Reads in total	108811	10881	3070–23213
Archaea reads	57130	5713	2092–12514
Thaumarchaeota reads	55746	5575	2062–12130
Euryarchaeota reads	1384	138	30–374
Bacteria reads	51026	5103	962–11299
Ratio B:A	0.89		
OTUs in total	6028	1807	795–2933
Archaeal OTUs	966	506	376–675
Bacterial OTUs	5014	1288	416–2501
Ubiquitous OTUs	192		
Ubiquitous arch. OTUs	141		
Ubiquitous bact. OTUs	51		
Singletons arch. OTUs	0.42 %	3	1–6.8
Singletons bact. OTUs	2.57 %	12	4.5–22.8
Genera in total	399		
Bacterial genera	355	176	98–262
Archaeal genera	40	19	10–32
Ubiquitous genera	61		
Ubiquitous archaeal genera	6		
Ubiquitous bacterial genera	55		

Table 3. General 454 sequencing statistics per samples including prokaryotic richness and diversity estimates based on 97% OTU clusters

	G05	G09	G09D	G12	G14	G16	G26	G30	G31	S4_03
Bacteria to Archaea ratio and OTUs per sample										
Ratio B:A	0.82	0.99	0.82	0.85	0.88	0.70	0.53	0.64	0.46	1.79
OTUs	2593	1774	1400	2933	1307	1291	1341	1671	795	2961
Diversity, evenness and richness indices and estimators calculated with OTUs (97% cut off)										
Archaea										
OTUs	675	518	437	674	449	461	491	530	376	448
Simpson (1-D)	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.98
Shannon (H)	5.42	5.23	5.08	5.34	5.18	5.29	5.27	5.37	5.12	4.84
Pielou Evenness (J)	0.34	0.36	0.37	0.31	0.40	0.43	0.40	0.41	0.45	0.28
Chao-1	763	653	570	786	559	583	613	650	555	533
Bacteria										
OTUs	1898	1239	949	2240	849	818	843	1131	416	2501
Simpson (1-D)	0.998	0.994	0.997	0.998	0.989	0.997	0.997	0.998	0.995	0.999
Shannon (H)	6.93	6.33	6.33	7.13	5.84	6.29	6.33	6.57	5.66	7.41
Pielou Evenness (J)	0.54	0.45	0.59	0.56	0.41	0.66	0.66	0.63	0.69	0.66
Chao-1	2290	1653	1406	2703	1218	1290	1236	1548	734.6	2916
Total observed richness/Chao-1 estimate * 100										
Total	84.4	75.8	70.0	83.3	73.0	68.4	72.5	75.5	61.3	85.6
Bacteria	82.9	75.0	67.5	82.9	69.7	63.4	68.2	73.1	56.6	85.8
Archaea	88.5	79.3	76.7	85.7	80.3	79.1	80.1	81.6	67.8	84.1
	G05	G09	G09D	G12	G14	G16	G26	G30	G31	S4_03

174,825 reads were obtained and a total of 108,811 reads were assigned to 6,028 OTUs (97% similarity) after sequence processing (Table 2). Of these reads, 57,130 were archaeal (55,746 of Crenarchaeota, 1,384 of Euryarchaeaota) and 51,026 were bacterial (of 177 families). Most of the OTUs were of bacteria (5014), 966 OTUs were of archaea, 48 OTUs were unclassified or incorrectly classified and not further included in the analyses. The bacterial OTUs were assigned to 177 families and 355 genera (Table 2).

Diversity indices were calculated with OTUs a at 97% similarity cut-off level (Table 3). The archaeal and bacterial diversity indices´ values were similar in all samples. The Shannon index values (H´) were lower for the archaea (4.84–5.42) than they were for the bacteria (5.66–7.41), and highest for the bacteria at station S4_03 (7.41). Also the evenness values (E) did not differ much between the samples and were higher for bacteria (0.4–0.69) than for archaea (0.28–0.45). The Chao richness estimator values for bacteria varied and were about 4 times higher at station S4_03 (2,916) compared to station G31 (734). As total reads of station sample G31 were lowest (3,070), the observed richness covered only 57% and 68% of the estimated total bacterial and archaeal richness, respectively, while the other samples showed a higher coverage range of 63% (G16) to 86% (S4_03) of bacterial richness and of 77% (G16) to 88% (G05) of archaeal richness (Table 3). The percentages of singletons (unique reads that occurred only once in a sample) were quite low but not constant throughout the samples (1.0–6.8% for archaea and 4.5–22.8% for bacteria; Table 2).

On average, 55% of the reads were of archaea, ranging from 35% at station S4_03 to 68% at station G31, with bacteria to archaea ratios (Table 3) correlating with the sediment TOC content (coefficient = –0.92). Averagely only 2.4% of archaeal reads were of Euryarchaeota (e.g., Archaeoglobaceacea, Thermoplasmataceae, Methanococcacea, Methansaetacea) and the vast share of Thaumarchaeota consisted mainly of a few genera in the Marine Group I, all presumably involved in ammonia oxidation [54].

Among the 5 most abundant genera (59,878 reads), 40,965 were affiliated with *Nitrosopumilus*, 10,592 with

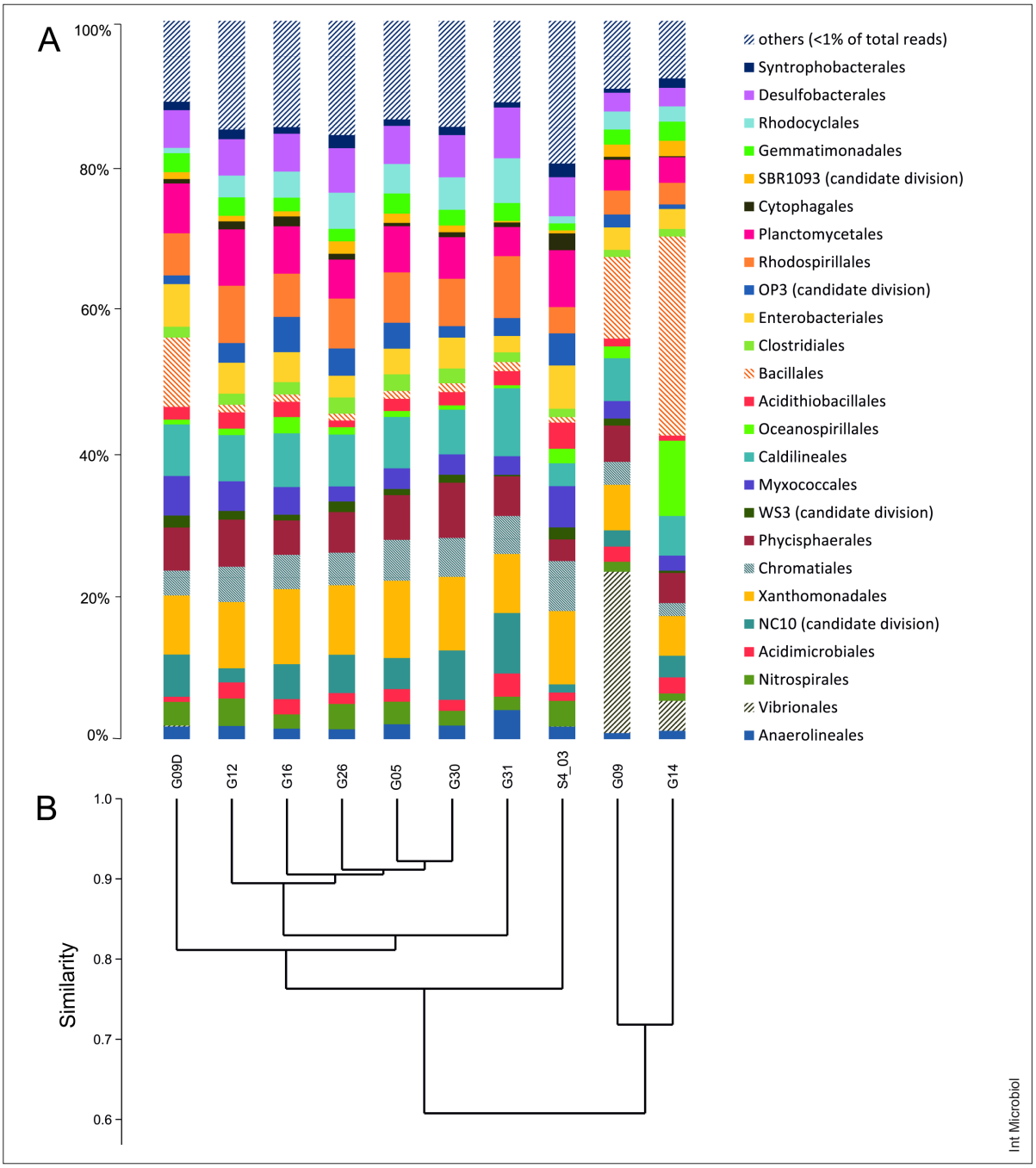

Fig. 2. (**A**) Bacterial community structure in the sediment samples (order level). (**B**) Cluster analysis of bacterial orders (Bray–Curtis similarity).

Cenarchaeum, 4,448 with Sinobacteraceae (the most abundant bacterial group at the genus level) and 3,882 reads were affiliated with *Cand*. Nitrosoarchaeum. In numbers of archaeal reads per sample, around 20% were of the genus *Cenarchaeum,* between 3% and 14% of *Nitrosoarchaeum*, and 62% to 76% of *Nitrosopumilus***.** Some archaeal reads (max. 0.8%) were affiliated with *Nitrososphaera*, an ammonia oxidizing archaeum (AOA) first isolated from a hot spring [19].

Most of the bacterial OTUs belonged to the phylum Proteobacteria (15–35% of the total reads per sample and 51% of all bacterial reads), with the most abundant classes being Gammaproteobacteria (41–72% of the bacterial reads per sample), Deltaproteobacteria (12–29%), Alphaproteobacteria

Table 4. Bray–Curtis similarities between samples calculated for archaeal (top right) and bacterial orders (bottom left)

									Archaeal orders	
Sample ID	G05	G09	G09D	G12	G14	G16	G26	G30	G31	S4_03
G05	1	0.96	0.96	0.96	0.97	0.95	0.97	0.96	0.95	0.77
G09	0.62	1	0.94	0.96	0.96	0.97	0.96	0.98	0.94	0.78
G09D	0.81	0.68	1	0.94	0.98	0.94	0.97	0.94	0.95	0.77
G12	0.90	0.62	0.82	1	0.95	0.98	0.95	0.97	0.91	0.82
G14	0.55	0.70	0.61	0.54	1	0.94	0.99	0.95	0.96	0.76
G16	0.91	0.63	0.81	0.89	0.55	1	0.95	0.98	0.92	0.81
G26	0.92	0.62	0.79	0.86	0.55	0.88	1	0.96	0.96	0.77
G30	0.92	0.62	0.82	0.88	0.54	0.88	0.89	1	0.92	0.80
G31	0.83	0.59	0.74	0.80	0.52	0.81	0.84	0.85	1	0.74
S4_03	0.76	0.53	0.73	0.80	0.47	0.80	0.74	0.77	0.64	1
	Bacterial orders									

(7–18%) and Betaproteobacteria (3–14%). Further high abundant phyla were Planctomycetes (3–7% of the bacterial reads per sample), Chloroflexi (3–4%), and Firmicutes in samples G09, G09D and G14 (13%, 12% and 30% of the bacterial reads, respectively). Further phyla that represented >1% of the total reads per sample were Gemmatimonadetes, Actinobacteria, Nitrospirae, and the candidate divisions SBR1093, OP3 and NC10 (Fig. 2A). In total, the samples comprised between 18 and 22 bacterial phyla. The most abundant bacterial order was Xanthomonadales, consisting almost exclusively of members of the family Sinobacteraceae (5–10% of the total bacterial reads, Fig. 2). 156 OTUs were of Sinobacteraceae, with up to 715 reads per OTU. Furthermore, several bacterial taxa involved in nitrification were identified in all samples: the ammonia oxidizing bacterium *Nitrosococcus* made up 1.2–3.9% of bacterial reads per sample, and the nitrite oxidizers *Nitrospina* and *Nitrospira* constituted 2–6% of the bacterial reads in the samples.

The microbial community compositions of the 10 samples taken from the stations were remarkably similar (Fig. 2A,B; Table 4). The Bray–Curtis similarity index values reached 0.98 and 0.92 when calculated with orders of archaea and bacteria, respectively, and when calculated with OTUs they still reached values as high as 0.79 for archaea (G09 and G14) and 0.59 for bacteria (G05 and G12). Main differences in community composition occurred in samples G14, S4_03 and in both samples of station G09 (Fig. 2B). Bacillales, which in the other samples amounted to ~1%, have increased to 10% and 11% in samples G09D and G09, respectively, and to 27% of all the bacterial reads at station G14, consisting almost exclusively of *Bacillus sp*. Oceanospirillales were not predominant among the ribogroups of bacteria in most of the samples (<2.5%) but accounted for 10% at station G14 (of these 91% were similar to *Marinobacterium jannaschi*). Another significant difference was the large proportion of Vibrionales at station G14 (4%; *Photobacterium*) and even more at station G09 (22.5%; *Vibrio* and *Photobacterium*), while this order was absent or rare at the remaining stations. The removal of these three orders increased the Bray–Curtis similarity index values of G09 and G14 with the other samples from an average of 0.62 and 0.56, respectively, to an average of 0.85 and 0.82, respectively. Unlike these samples, the sample of station S4_03 differed not by extreme counts of few ribotypes, but rather by the overall composition. It also revealed highest counts of reads that were low in abundance (<1% of total reads), fewer reads of Planctomycetales but highest reads of Enterobacteriales.

Discussion

In order to elucidate the spatial distribution of microorganisms in deep sea surface sediments and to reduce the general information deficiency regarding microbial community compositions in sediments of the eastern Mediterranean Sea [42,53], sequences of surface sediment samples from 9 stations located in the Levantine basin were produced by 454 massive tag sequencing and compared to each other. The results revealed high species richness and high diversity in the Levantine deep surface sediments, including 3–4 cm below the surface. The coverage of the sequencing efforts was rela-

tively high, especially for archaea, and the diversity indices´ values for archaeal OTUs were almost as high as they were for bacterial OTUs. Since a few archaeal OTUs were most abundant, evenness values were lower than those observed for bacterial OTUs. High microbial diversity was also reported for stations in the central and eastern Mediterranean Sea [30,42,43,44,53] and Gammaproteobacteria, further Deltaproteobacteria, Alphaproteobacteria and Planctomycetes were found as abundant members of the microbial communities in eastern Mediterranean sediments [42,45,49,53]. However, in a strong contrast to the aforementioned studies, the numbers of reads of Acidobacteria were very low in all of the samples in this study (<0.4 % of total reads).

Of the Gammaproteobacteria, the most abundant bacterial family was Sinobacteraceae. These microorganisms were found in high abundances in sediment of the oligotrophic South Pacific gyre (six of the most abundant 30 OTUs; [57]), in contaminated estuarine sediments [55], in manganese oxide-rich sediments [58], and they presented almost 10% of sequences in polluted beach sand samples [16]. The family Sinobacteraceae was only recently proposed, based on a single isolate, and the metabolic characteristics of this bacterial group are not yet documented [65]. The discovery of high percentages of Sinobacteracea in a variety of marine sea bottoms, pristine as well as affected by contaminations, calls for further studies concentrating on these Gammaproteobacteria.

Comparisons between the sediment samples taken from the eight deep (>1,000 m) stations located up to 130 km away from each other resulted in high similarities at the order level (Fig. 1, Table 4), including sample G09D, which was taken at 3–4cm sediment depth. These results hint at low environmental dynamics across the sampling area, as most of the stations are located at a depth between 1,200 and 1,900 m under a relatively uniform water mass, the Cretan Outflow Water. Due to the lack of physical mixing, the differences of the microbial communities might be the results of the historical effects which include limitation of dispersal and past environmental conditions which influenced the population structure of microbes [34].

Contaminants, including heavy metals, were reported to influence bacterial diversity and species distribution [55]. At station S4_03, concentrations of PCBs, PAHs and TRPHs were much higher than at the other stations, but it is also the only shallow station and the one nearest to the coast (40 km). Furthermore, the station is located at an area of gas drilling activities and extraction and close to a port (Ashdod, Israel) so that anthropogenic influences are highly expected here and might explain the higher concentration of the persistent pollutants, for examples PCBs or PAHs. Thus, the unexpected similarity between the bacterial composition at this shallow coastal station and the bacterial compositions at the deep stations is particularly remarkable, but it is still conceivable that contaminants are not necessarily associated with bacterial diversity, as reported for the case of petroleum contamination of Mediterranean Sea sediments [43].

In contrast, the envfit function of R revealed a significant correlation between cadmium and the compositions of the bacterial families, primarily in stations G14 and G09, where cadmium levels were the highest, though still in the range of the usual measured cadmium concentration in deep sea sediment [24]. Indeed, these two stations differed from the other sediment sites in terms of bacterial compositions, substantially caused by the high read abundances of a few genera, namely *Marinobacterium*, *Bacillus* and *Photobacterium* in station G14 and *Vibrio* and *Bacillus* in station G09, which also caused reduced evenness values for these samples (<0.5).

It might be worth mentioning that *Bacillus*, abundant in stations G09 and G14, was discovered to be a cadmium and mercury resistant taxon [21,32], a fact which might be related to the high abundances of *Bacillus* at these stations (mercury concentrations were also higher at station G09 with 46.3 µg/kg, compared to 23.8–36.2 µg/kg at the other stations; data not shown).

The further overrepresented genera *Marinobacterium*, *Vibrio* and *Photobacterium* at the stations G14 and G09 are known as r-strategists, able to grow fast when resources are plenty, and might have benefited from past local nutrient supplies absorbed into the sea bottom, that could not be detected by TOC measurements anymore.

The sequencing results further revealed extremely high shares of archaea in most of the samples, comparable to mesopelagic waters in the Mediterranean Sea [62]. While high archaeal abundances were assigned for some deep sea [36,57] and sandy [38] sediments, in most studies bacteria were more abundant than archaea [35 and references therein], something which was noted throughout the Mediterranean Sea, with a decreasing trend eastwards [13], where ratios decreased to <1.5. Archaeal dominance is correlated to their advantage over bacteria in oligotrophic sediments [51,52]. The negative correlation coefficient between TOC content and the percentages of archaeal reads in the samples supports these findings.

While both microbial domains include approximately half of the sequenced tags, only 966 OTUs were of archaea compared to 5,014 bacterial OTUs. On the genus level the discrepancy increases to 40 versus 355 genera. Aller´**s** [1] comprehensive study showed that in 16S rDNA analyses archaea

were seldom as diverse as bacteria in same environments. This discrepancy, which cannot be explained as of yet, might be correlated to physiological functions. It is further possible that the sequencing of different genes like the 16S-23S intergenic spacer region or longer reads, may lead to different portraits. The majority of the archaea were of the novel archaeal division of Thaumarchaeota [54], and only a minor fraction belonged to Euryarchaeota. This relatively low diversity of Archaea of mainly Marine Group 1 representatives is known for oxic sediments [40], and similar archaeal compositions were found in sediments off Acre in the Levant [49] and in surface sediments off Monterey Bay and the Antarctic shelf [2,14].

Half of the thaumarchaeal reads in our study were affiliated with the hydrothermal vent clone pIVWA5 (acc nr AB019728), a member of the MG1 group and therefore a putative ammonia oxidizer [54]. Further abundant Marine Group 1 reads were of the genera *Nitrosopumilus* and *Nitrosoarchaeum*, both ammonia oxidizing archaea (AOA), and *Cand*. Cenarchaeum, which is considered as putative AOA encoding the key enzyme AmoA. AOA are recognized as ubiquitous, exhibiting higher affinities to ammonia than ammonia oxidizing bacteria, and also preferring low oxygen concentrations [12,18]. Some AOA are also capable of assimilating organic carbon, but so far growth was obligatorily coupled to ammonia oxidation [54]. They were found in high numbers in marine ecosystems like oxygen minimum zones where they seem to provide nitrite for anaerobic ammonia oxidation to dinitrogen, and in *Beggiota* mats, where nitrification by archaea has been coupled with nitrate respiration [61]. Abundances of MG1 archaea were linked with the importance of chemolithotrophy in oligotrophic aphotic Mediterranean Sea water [64] and they have been shown to be the dominant nitrifiers in the North Sea and North Atlantic [63]. The high percentages of thaumarchaeotal reads together with some ammonia oxidizing bacteria (mainly *Nitrosococcus* with 1.2—3.9 % of bacterial reads per sample) in our samples corroborates the assumption that these microorganisms contribute significantly to carbon assimilation coupled with ammonia oxidation to nitrite in the sediments of the Levantine basin. A high proportion of autotrophic C fixation by archaea was also found in deep waters of the Thyrrenian basin [64]. Nitrite is the intermediate product of aerobic nitrification and is oxidized to nitrate by nitrite oxidizing bacteria (NOB). The genera *Nitrospina* and *Nitrospira*, the two most common marine nitrite oxidizers, constituted 2-6% of the bacterial reads in the samples. The numerical discrepancy between the AOA and the NOB reads might be due to low activity rates of AOA compared to NOB, or due to additional processes that AOA are involved in [29].

Nitrification is an integral part of the N-cycle and plays an important role in deep sea sediments coupling ammonification with denitrification, and has been shown to be a significant process in the deep sea sediment of the NW Atlantic consuming up to 35% of the oxygen in the sediments [4]. Since in oligotrophic ocean basins the oxic zone can span tens of centimeters, all the sediments samples in this study are considered well oxygenated [59]. Thus, denitrification or anaerobic ammonia oxidation (anammox) are expected here only in anaerobic microenvironments [4]. Accordingly, Planctomycetes species putatively capable of anammox were found only in negligible numbers (e.g., a total of 128 reads of *Candidatus* Brocadia), while *Phycisphaera spp.* was overrepresented in the phylum Planctomycetes, comprising 253 OTUs and 3–8% of bacterial reads in each sample.

This study elucidated the microbial diversity in and between Levantine deep sea sediment sites within distances of up to 140 km, and compared archaeal and bacterial compositions of the sequence reads of the same samples, and of a high number of samples collected from an area that is not subject to extreme environmental variables. The data showed the extent to which microbial communities located over a vast area can be similar and provided an important hint regarding the role of chemolithotrophic carbon assimilation and nitrification in oligotrophic deep sea sediments.

Acknowledgements. We thank the crew of the R/V Shikmona for their devoted work at sea, J. Silverman and A. Kerry from the Marine Biology department, Y. Gertner and N. Kress from the Marine Chemistry department and M. Kanari and T. Ketter from the Physical Oceanography department of the IOLR, and O. Crouvi from the Geological Survey Israel (GSI) for metadata, H. Bernard for graphical work and J. Douek and G. Paz for their general help. This work was supported by the Israeli Ministry of National Infrastructures, Energy and Water and by the EC PERSEUS (FP7-287600).

Competing interests. None declared.

References

1. Aller JY, Kemp PF (2008) Are Archaea inherently less diverse than Bacteria in the same environments? FEMS Microbiol Ecol 65:74-87
2. Bowman JP, McCuaig RD (2003) Biodiversity, community structural shifts, and biogeography of Prokaryotes within Antarctic Continental Shelf sediment. Appl Environ Microbiol 69:2463-2483
3. Capone KA, Dowd SE, et al. (2011) Diversity of the human skin microbiome early in life. J Invest Dermatol 131:2026-2032
4. Christensen JP, Rowe GT (1984) Nitrification and oxygen consumption in northwest Atlantic deep sea sediments. J Mar Res 42:1099-1116
5. Coll M, Piroddi C, Steenbeek J, Kaschner K, et al. (2010) The biodiver-

sity of the Mediterranean Sea: estimates, patterns, and threats. PLoS One 5:e11842
6. Danovaro R, Company JB, Corinaldesi C, D'Onghia G, Galil B, et al. (2010) Deep sea biodiversity in the Mediterranean Sea: The known, the unknown, and the unknowable. PLoS ONE 5:e11832
7. DeSantis T Z, Hugenholtz P, et al. (2006) Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol 72:5069-5072
8. Dowd SE, Callaway TR, Wolcott RD, Sun Y, McKeehan T, Hagevoort RG, Edrington TS (2008) Evaluation of the bacterial diversity in the feces of cattle using 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP). BMC Microbiol 8:125
9. Dowd SE, Sun Y, Wolcott RD, Domingo A, Carroll JA (2008) Bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) for microbiome studies: bacterial diversity in the ileum of newly weaned Salmonella-infected pigs. Foodborne Pathog Dis 5:459–472
10. Dowd SE, Wolcott RD, Sun Y, McKeehan T, Smith E, Rhoads D (2008) Polymicrobial nature of chronic diabetic foot ulcer biofilm infections determined using bacterial tag encoded FLX amplicon pyrosequencing (bTEFAP). PLoS One 3:e3326
11. Eren AM, Zozaya M, Taylor CM, Dowd SE, Martin DH, et al. (2011) Exploring the diversity of Gardnerella vaginalis in the genitourinary tract microbiota of monogamous couples through subtle nucleotide variation. PLoS ONE 6:e26732
12. Francis CA, Roberts KJ, Beman JM, Santoro AE, Oakley BB (2005) Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. Proc Natl Acad Sci USA 102:14683-14688
13. Giovannelli D, Molari M, d'Errico G, Baldrighi E, Pala C, et al. (2013) Large-scale distribution and activity of prokaryotes in deep sea surface sediments of the Mediterranean Sea and the adjacent Atlantic Ocean. PLoS ONE 8:e72996
14. Goffredi SK, Wilpiszeski R, Lee R, Orphan VJ (2008) Temporal evolution of methane cycling and phylogenetic diversity of archaea in sediments from a deep sea whale-fall in Monterey Canyon, California. ISME J 2:204-220
15. Hagström Å, Pinhassi J, Zweifel UL (2000) Biogeographical diversity among marine bacterioplankton. Aquat Microb Ecol 21:231-244
16. Halliday E, McLellan SL, Amaral-Zettler LA, Sogin ML, Gast RJ (2014) Comparison of bacterial communities in sands and water at beaches with bacterial water quality violations. PLoS ONE 9:e90815
17. Hammer Ø, Harper DAT, Ryan, PD (2001) Past: Paleontological statistics software package for education and data analysis. Palaeontol Electron 4:1-9
18. Hatzenpichler R (2012) Diversity, physiology, and niche differentiation of ammonia-oxidizing archaea. Appl Environ Microbiol 78:7501-7510
19. Hatzenpichler R, Lebedeva EV, Spieck E, Stoecker K, Richter A, Daims H, Wagner M (2008) A moderately thermophilic ammonia-oxidizing crenarchaeote from a hot spring. Proc Natl Acad Sci USA 105:2134-2139
20. Heijs SK, Laverman AM, Forney LJ, Hardoim PR, Van Elsas JD (2008) Comparison of deep sea sediment microbial communities in the Eastern Mediterranean, FEMS Microbiol Ecol 64:362-377
21. Hu Q, Dou M-N, Qi H-Y, Xie X-M, Zhuang G-Q, Yang M (2007) Detection, isolation, and identification of cadmium-resistant bacteria based on PCR-DGGE. J Environ Sci 19:1114-1119
22. Jacob M, Soltwedel T, Boetius A, Ramette A (2013) Biogeography of deep sea benthic bacteria at regional scale (Lter Hausgarten, Fram Strait, Arctic). PLoS ONE 8:e72779
23. Jørgensen BB, Boetius A (2007) Feast and famine–microbial life in the deep sea bed. Nature Microbiol Rev 5:770-781
24. Kennish MJ (2011) Practical handbook of estuarine and marine pollution. CRC Press, London
25. Kouridaki I, Polymenakou PN, Tselepides A, Mandalakis M, Smith K (2010) Phylogenetic diversity of sediment bacteria from the deep Northeastern Pacific Ocean: a comparison to the deep Eastern Mediterranean Sea. Int Microbiol 13:143-150
26. Kress N, Herut B (2001) Spatial and seasonal evolution of dissolved oxygen and nutrients in the Southern Levantine Basin (Eastern Mediterranean Sea): chemical characterization of the water masses and inferences on the N:P ratios. Deep Sea Res. Part I Oceanogr Res Pap 48:2347-2372
27. Krom MD, Woodward EMS, Herut B, Kress N, Carbo P, et al. (2005) Nutrient cycling in the south east Levantine basin of the eastern Mediterranean: Results from a phosphorus starved system. Deep sea Res Pt II 52: 2879-2896
28. Li HR, Yu Y, Luo W, Zeng YX, Chen B (2009) Bacterial diversity in surface sediments from the Pacific Arctic Ocean. Extremophiles 13:233-246
29. Lloyd KG, May MK, Kevorkian RT, Steen AD (2013) Meta-analysis of quantification methods shows that archaea and bacteria have similar abundances in the subseafloor. Appl Environ Microbiol 79:7790-7799
30. Luna GM, Dell'Anno A, Giuliano L, Danovaro R (2004) Bacterial diversity in deep sea sediments: relationship with the active bacterial fraction and substrate availability. Environ Microbiol 6:745-753
31. Luna GM, Stumm K, Pusceddu A, Danovaro R (2009) Archaeal diversity in deep sea sediments estimated by means of different terminal-restriction fragment length polymorphisms (T-RFLP) protocols. Curr Microbiol 59:356-361
32. Mahler I, Levinson HS, Wang Y, Halvorson HO (1986) Cadmium- and mercury-resistant *Bacillus* strains from a salt marsh and from Boston Harbor. Appl Environ Microbiol 52:1293-1298
33. Mapelli F, Varela MM, Barbato M, Alvariño R, Fusi M, Álvarez M, Merlino G, Daffonchio D, Borin S (2013) Biogeography of planktonic bacterial communities across the whole Mediterranean Sea. Ocean Sci 9:585-595
34. Martiny JB, Bohannan BJ, Brown JH, Colwell RK, Fuhrman JA et al (2006) Microbial biogeography: putting microorganisms on the map. Nat Rev Microbiol 4:102-112
35. Molari M, Giovannelli D, d'Errico G, Manini E (2012) Factors influencing prokaryotic community structure composition in sub-surface coastal sediments. Estuar Coast Shelf S 97:141-148
36. Molari M, Manini E (2012) Reliability of CARD-FISH procedure for enumeration of Archaea in deep sea surficial sediments. Curr Microbiol 64:242-250
37. Mullins TD, Britschgi TB, Krest RL, Giovannoni SJ (1995) Genetic comparisons reveal the same unknown bacterial lineages in Atlantic and Pacific bacterioplankton communities. Limnol Oceanogr 40:148-158
38. Nitahara S, Kato S, Urabe T, Usui A, Yamagishi A (2011) Molecular characterization of the microbial community in hydrogenetic ferromanganese crusts of the Takuyo-Daigo Seamount, northwest Pacific. FEMS Microbiol Lett 321:121-129
39. Oksanen J, Blanchet FG, Kindt R, Legendre P, O'Hara RB, Simpson GL, Solymos P, Stevens MH, Wagner H (2011) Vegan: Community Ecology Package. R package version 1.17-6. [http://CRAN.R project.org/package=vegan]
40. Orcutt BN, Sylvan JB, Knab NJ, Katrina J, Edwards KJ (2011) Microbial ecology of the dark ocean above , at, and below the seafloor microbial ecology of the dark ocean above, at, and below the seafloor. Microbiol Mol Biol Rev 75:361-422
41. Pinardi N, Masetti E (2000) Variability of the large scale general circulation of the Mediterranean Sea from observations and modelling: a review. Palaeogeogr Palaeocl 158:153-173
42. Polymenakou PN, Bertilsson S, Tselepides A, Stephanou EG (2005) Bacterial community composition in different sediments from the Eastern Mediterranean Sea: a comparison of four 16S ribosomal DNA clone libraries. Microbial Ecol 50:447-62

43. Polymenakou PN, Bertilsson S, Tselepides A, Stephanou EG (2005) Links between geographic location, environmental factors and microbial community composition in sediments of the Eastern Mediterranean Sea. Microbial Ecol 49:367-378
44. Polymenakou PN, Lampadariou N, Mandalakisc M, Tselepides A (2009) Phylogenetic diversity of sediment bacteria from the southern Cretan margin, Eastern Mediterranean Sea. Syst Appl Microbiol 32:17-26
45. Polymenakou PN, Christos A, Christakis CA, Mandalakis M, Oulas A (2015) Pyrosequencing analysis of microbial communities reveals dominant cosmopolitan phylotypes in deep sea sediments of the eastern Mediterranean Sea. Res Microbiol 166:448-457
46. Psarra S, Tselepides A, Ignatiades L (2000) Primary productivity in the oligotrophic Cretan Sea (NE Mediterranean): Seasonal and interannual variability. Progr Oceanogr 46:187-204
47. Ramette A, Tiedje JM (2007) Multiscale responses of microbial life to spatial distance and environmental heterogeneity in a patchy ecosystem. Proc Natl Acad Sci USA 104:2761-2766
48. Rowe G, Sibuet M, Deming J, Khripounoff A, Tietjen J, Macko S, Theroux R (1991) Total sediment biomass and preliminary estimates of organic-carbon residence time in deep sea benthos. Mar Ecol Prog Ser 79: 99-114
49. Rubin-Blum M, Antler G, Turchyn AV, Tsadok R, Goodman-Tchernov BN, et al. (2014) Hydrocarbon-related microbial processes in the deep sediments of the Eastern Mediterranean Levantine basin. FEMS Microbiol Ecol 87:780-796
50. Schauer R, Bienhold C, Ramette A, Harder J (2010) Bacterial diversity and biogeography in deep sea surface sediments of the South Atlantic Ocean. ISME J 4:159-170
51. Schippers A, Neretin LN, Kallmeyer J, Ferdelman TG, Cragg BA, Parkes RJ, Jørgensen BB (2005) Prokaryotic cells of the deep sub-seafloor biosphere identified as living bacteria. Nature 433:861-864
52. Schippers A, Kock D, Höft C, Köweker G, Siegert M (2012) Quantification of microbial communities in subsurface marine sediments of the Black Sea and off Namibia. Front Extreme Microbiol 3:16
53. Sevastou K, Lampadariou N, Polymenakou PN, Tselepides A (2013) Benthic communities in the deep Mediterranean Sea: exploring microbial and meiofaunal patterns in slope and basin ecosystems. Biogeosciences Discuss 9:17539-17581
54. Stahl DA, de la Torre JR (2012) Physiology and diversity of ammonia-oxidizing archaea. Annu Rev Microbiol 66:83-101
55. Sun MY, Dafforn KA, Johnston EL, Brown MV (2013) Core sediment bacteria drive community response to anthropogenic contamination over multiple environmental gradients. Environ Microbiol 15:2517-2531
56. Swanson KS, Dowd SE, Suchodolski JS, Middelbos IS, Vester BM, et al. (2011) Phylogenetic and gene-centric metagenomics of the canine intestinal microbiome reveals similarities with humans and mice. ISME J 5:639-649
57. Tully BJ, Heidelberg JF (2013) Microbial communities associated with ferromanganese nodules and the surrounding sediments. Front Microbiol 4:161
58. Vandieken V, Pester M, Finke N, Hyun J-H, Friedrich MW, Loy A, Thamdrup B (2012) Three manganese oxide-rich marine sediments harbor similar communities of acetate-oxidizing manganese-reducing bacteria. ISME J 6:2078-2090
59. Wenzhöfer F, Glud RN (2002). Benthic carbon mineralization in the Atlantic: a synthesis based on in situ data from the last decade. Deep sea Res Part I-Oceanogr Res Pap 49:1255-1279
60. Wilkins D, van Sebille E, Rintoul SR, Lauro FM, Cavicchioli R (2013) Advection shapes Southern Ocean microbial assemblages independent of distance and environment effects. Nat Comm 4:2457
61. Winkel M, de Beer D, Lavik G, Peplies J, Mußmann M (2014) Close association of active nitrifiers with Beggiatoa mats covering deep sea hydrothermal sediments. Environ Microbiol 16:1612-1626
62. Winter C, Kerros M-E, Weinbauer MG (2009) Seasonal changes of bacterial and archaeal communities in the dark ocean: Evidence from the Mediterranean Sea. Limnol Oceanogr 54:160-170
63. Wuchter C, Abbas B, Coolen MJ, Herfort L, van Bleijswijk J, et al. (2006) Archaeal nitrification in the ocean. Proc Natl Acad Sci USA 103: 12317-12322
64. Yakimov MM, Cono VL, Smedile F, DeLuca TH, Juárez S, et al. (2011) Contribution of crenarchaeal autotrophic ammonia oxidizers to the dark primary production in Tyrrhenian deep waters (central Mediterranean Sea). ISME J 5:945-961
65. Zhou Y, Zhang YQ, Zhi XY, Wang X, Dong J, Chen Y, Lai R, Li WJ (2008) Description of *Sinobacter flavus* gen. nov., sp. nov., and proposal of *Sinobacteraceae* fam. nov. Int J Syst Evol Microbiol 58:184-189
66. Zinger L, Amaral-Zettler LA, Fuhrman JA, Horner-Devine MC, Huse SM, et al. (2011) Global patterns of bacterial beta-diversity in seafloor and seawater ecosystems. PLoS ONE 6:e24570