RESEARCH ARTICLE

Advancing the understanding of biogeography–diversity relationships of benthic microorganisms in the North Sea

Melanie Sapp[1], Eleanor Ruth Parker[1], Lorna Rachel Teal[2] & Michaela Schratzberger[1]

[1]Lowestoft Laboratory, Centre for Environment, Fisheries and Aquaculture Science (CEFAS), Suffolk, UK; and [2]Oceanlab, University of Aberdeen, Aberdeenshire, UK

Correspondence: Melanie Sapp, Lowestoft Laboratory, Centre for Environment, Fisheries and Aquaculture Science (CEFAS), Pakefield Road, Lowestoft, Suffolk NR33 0HT, UK. Tel.: +44 1502 524 518; fax: +44 1502 513 865; e-mail: melanie.sapp@cefas.co.uk

Present address: Lorna Rachel Teal, IMARES, Postbus 68, 1970AB, IJmuiden, the Netherlands.

Received 7 May 2009; revised 24 May 2010; accepted 5 July 2010.
Final version published online 23 August 2010.

DOI:10.1111/j.1574-6941.2010.00957.x

Editor: Patricia Sobecky

Keywords
microbial community; archaea; bacteria; sediment; DGGE.

Abstract

Knowledge on the spatial distribution of prokaryotic taxa is an essential basis to understand microbial diversity and the factors shaping its patterns. Large-scale patterns of faunal distribution are thought to be influenced by physical environmental factors, whereas smaller scale spatial heterogeneity is maintained by species-specific life-history characteristics, the quantity and quality of food sources and local disturbances including both natural and man-induced events. However, it is still not clear which environmental parameters control the diversity and community structure of sedimentary microorganisms mediating important ecosystem processes. In this study, multiscale patterns were elucidated at seven stations in the Oyster Ground, North Sea (54°4′N/4°E), 100 m to 11 km apart. These were related to biotic (e.g. multicellular organisms) and abiotic parameters (e.g. organic carbon content in the sediment) to establish the relationship between the distribution of both bacterial and archaeal communities and their environment. A relatively high variability was detected at all scales for bacterial and archaeal communities, both of which were controlled by different suites of biotic and abiotic environmental variables. The bacterial community consisted mainly of members belonging to the *Gammaproteobacteria* and the *Fibrobacteres/Acidobacteria* group. Members of the *Deltaproteobacteria*, *Bacteroidetes* and *Actinobacteria* also contributed to the bacterial community. *Euryarchaeota* formed the majority of archaeal phylotypes together with three phylotypes belonging to the *Crenarchaeota*.

Introduction

Organisms are generally influenced by complex and interacting sets of physical and biological processes, leading to a variation in their distribution at different spatial scales. An appreciation of this spatial scaling, which is an essential basis for understanding the scales at which organisms interact with one another and with their environment, is central to many aspects of ecology, including the maintenance of species diversity and the identification of their different ecological roles (Staley & Gosink, 1999; Ramette & Tiedje, 2007).

In general, spatial scaling of microbial diversity can have various sources (Green & Bohannan, 2006). One of these is the distance–decay relationship. It describes a random primary spatial distribution with population growth in nonrandom spatial niches, resulting in spatial patterns. These are controlled by habitat heterogeneity, whereas changes in the community composition occur with geographic distance. Additionally, the taxa–area relationship characterizes the coherence of species richness and sampled area, whereas the local : global taxa richness ratio refers to the global number of species decreasing with the mean body size so that small organisms tend to have a cosmopolitan distribution. The latter has been shown for protist freshwater species (Green & Bohannan, 2006), but is not supported for bacteria (Martiny *et al.*, 2006; Pommier *et al.*, 2007).

Generally, the distribution of communities has attracted much theory and counter-theory to explain the evolutionary processes in the sea. These determine the diversity of the species pool within a geographic area and the ecological processes determining how many of those species will be represented at any one station and time. Baas-Becking

© 2010 CEFAS
Journal compilation © 2010 Federation of European Microbiological Societies.
Published by Blackwell Publishing Ltd.

Downloaded from https://academic.oup.com/femsec/article-abstract/74/2/410/589506 by University of Zurich user on 11 December 2019

(1934) hypothesized that everything is everywhere and the environment selects. Recent research has applied mesocosm or *in situ* studies to examine whether this hypothesis can be supported. One of these studies found a taxa–area relationship in a salt marsh resulting from similarity in the composition of bacterial communities related to environmental heterogeneity (Horner-Devine *et al.*, 2004b), emphasizing that the environment can have a strong selecting influence on microbial community structure. A selection for certain taxa by the environment is also supported by a high degree of endemism, but few cosmopolitan phylotypes in different clone libraries of marine planktonic microorganisms (Pommier *et al.*, 2007). Biogeographical patterns were also detected for free-living microbiota of different habitats, confirming a strong environmental influence (Martiny *et al.*, 2006). Several important factors were summarized by Horner-Devine *et al.* (2004a, b) including temperature, nutrient status, salinity and contamination with pollutants, among others. These might explain differences in the bacterial diversity and include habitat type and heterogeneity, primary productivity, response of bacterial taxa to gradients of disturbance and biogeographic location. Interestingly, the variation in community composition along a gradient of primary productivity revealed different responses of different bacterial groups, whereas the overall bacterial richness did not display a significant relationship with primary productivity in freshwater mesocosms (Horner-Devine *et al.*, 2003).

For sedimentary systems, it could be shown that habitat characteristics including physico-chemical conditions, sediment chlorophyll *a* and organic carbon content, particle size as well as benthic fauna can influence microbial communities (Liu *et al.*, 2003; Polymenakou *et al.*, 2005; Quéric & Soltwedel, 2007; Sørensen *et al.*, 2007; Jackson & Weeks, 2008; Wu *et al.*, 2008). Despite the recent findings, benthic microbial biogeography is still largely understudied, especially considering smaller spatial scales and macrofaunal influence (Hewson *et al.*, 2007). Furthermore, the North Sea is an interesting ecosystem to study as nutrient budgets suggest that it is a sink for nutrients through burial and denitrification (Seitzinger & Giblin, 1996; Brion *et al.*, 2004).

The aim of the present study was to elucidate multiscale patterns within the prokaryotic benthic community at the Oyster Ground, North Sea, and to establish the extent to which a subset of biotic and abiotic parameters can explain spatial variation in the structure of the microbial community.

Materials and methods

Sample collection, geochemical and macrofaunal analysis of sediments

Multiscale variation was studied at seven randomly selected stations at the Oyster Ground, which forms a large circular depression in the central North Sea (54°4′N/4°E) (Fig. 1). It reaches a maximum depth of > 50 m at its centre and is bordered by the shallower bathymetry of the Dogger Bank, German Bight and Southern Bight. Samples were taken at different scales ranging from 1 to 11 km distance between stations (Fig. 1) and 100 m distance between replicate cores within each station.

Four replicate samples (a–d) were taken at each station with a NIOZ corer (cylinder $\varnothing$ 30 cm) from within a 100-m bull ring (random drift) in February 2007. From each successive NIOZ deployment, three to four vertical core ($\varnothing$ 5.5 cm) subsamples were collected for particle size, porosity and organic carbon content analysis. These were sliced at 0.5–1 cm intervals to a depth of 10 cm and stored at -20 °C. Additional subsample cores were taken for chlorophyll *a* and phaeophytin analysis and for oxygen microprofiling using microelectrodes (two profiles per subsample core). These analyses were carried out immediately post-collection. At each station, three intact NIOZ cores were sampled for pore-water nutrient profiles using a methodology described by Sivyer (1999). Samples were filtered through a 0.2-μm filter (Sartorius, Goettingen, Germany) before preservation with mercuric chloride (Fisher Scientific, Loughborough, UK).

Infauna was obtained from four replicate intact NIOZ cores via sieving through 5 and 1 mm meshes. Individuals from beam trawls were identified to the species level and weighed on-board. All infauna individuals were picked by hand from the NIOZ samples onboard and preserved in

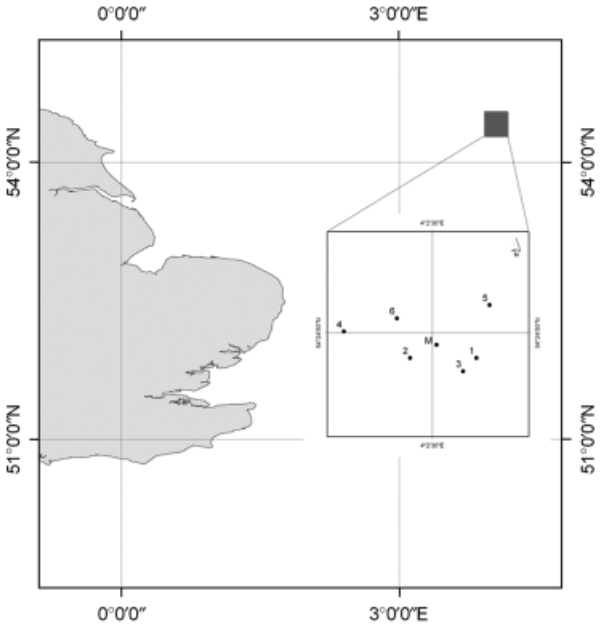

Fig. 1. Geographic location of the Oyster Ground, North Sea and positions of studied sites.

Downloaded from https://academic.oup.com/femsec/article-abstract/74/2/410/589506 by University of Zurich user on 11 December 2019

© 2010 CEFAS
Journal compilation © 2010 Federation of European Microbiological Societies.
Published by Blackwell Publishing Ltd.

10% formalin. With the exception of the polychaetes (family level), infauna were identified to the species level. The total biomass (blotted wet weight) per species and sample was determined in the laboratory. Fragments of organisms were identified to and weighed at the family level, but no counts were made. A fragment is considered an organism where the head or significant parts of the shell were missing.

For the study of the microbial community, the top 2 cm of replicate intact NIOZ cores were subsampled by scraping sterile 50-mL polypropylene tubes (Fisher Scientific) across the sediment surface (Scala & Kerkhof, 2000; Asami *et al.*, 2005; Liang *et al.*, 2007; Santmire & Leff, 2007). All microbial samples were kept at $-20\,^{\circ}C$ until further analysis.

Sediment analysis

After thawing, sediment samples were wet sieved through a 1-mm sieve, and the fraction >1 mm was oven dried at 80 °C for 24 h. This fraction was then dry sieved at 0.5 phi intervals, down to 4 phi (63 μm). A subsample of the <1 mm fraction was analysed on a Malvern Mastersizer 2000 laser sizer (Herrenberg, Germany). The laser sizer results were combined with the dry sieve results to yield the full particle size distribution. The mean particle size diameter (hereafter referred to as grain size) was calculated from these results (Dyer, 1986).

Porosity was calculated using the dry weights and wet weights of known volumes of sediment slices assuming a sediment particle density of $2.7\,g\,cm^{-3}$ and a seawater density of $1.035\,g\,cm^{-3}$ (Berner, 1971).

For the analysis of sediment organic carbon and total nitrogen, the samples were defrosted and freeze dried. They were then homogenized and subsamples of 100 mg from each replicate and sampling depth were transferred into preweight and acid-washed wide-mouth glass vials. Further sample preparation was carried out according to Hedges & Stern (1984). In brief, the samples were subjected to subsequent aqueous acidification with HCl (Fisher Scientific) and redried. Subsamples of treated sediment (10–20 mg) were then weighted into tin cups (Elemental Microanalyis, Devon, UK) for analysis. Organic carbon and nitrogen contents were determined using an elemental analyser (Flash EA 1112, Thermo-Finnigan, Bremen, Germany) interfaced (ConFlo III Interface, Thermo-Finnigan) with a continuous flow isotope mass spectrometer (Finnigan MAT Delta^{Plus}, Thermo-Finnigan) calibrated against known quantities of urea.

Pigments were extracted in 90% buffered acetone (Fisher Scientific) and refrigerated before analysis. A Turner Designs Model 10AU filter fluorometer (Sunnyvale) was used to measure extracted chlorophyll *a* and phaeopigment fluorescence before and after acidification following the method described by Tett (1987). The fluorometer was calibrated using a solution of pure chlorophyll *a* (Sigma-Aldrich, St. Louis) with the concentration being determined spectrophotometrically. The percentage error of chlorophyll *a* analyses was $<2\%$ relative to Turner-certified reference material.

Nitrate, nitrite, phosphate, silicate and ammonium were determined using a Skalar autoanalyser (Breda, the Netherlands) according to Kirkwood (1996) within 1 month after collection. The detection limits for nitrate, nitrite, ammonium, phosphate and silicate analyses were 0.1, 0.01, 0.2, 0.02 and $0.1\,\mu mol\,L^{-1}$, respectively.

Sediment oxygen profiles were taken in cores from consecutive NIOZ corer deployments using Clark-type oxygen microelectrodes (Unisense OX500μm, Aarhus, Denmark) described by Revsbech (1989) and a custom-made profiling stand combined with a manual micromanipulator MM33 (Unisense). Zero per cent oxygen calibration readings were recorded in ascorbate solution (0.2 M NaOH, 0.1 M ascorbic acid, BHD, Poole, UK) and ranged from 2 to 7 pA. Hundred per cent readings were obtained in aerated water overlying the core at ambient temperature and salinity for each core and replicate profile. The profiles were recorded continuously, using PICOLOG technology recorder software (Pico Technology, St. Neots, UK). The oxygen penetration depth (OPD) was taken to be the depth at which oxygen saturation above 0% was last observed using a method adapted from Rabouille *et al.* (2003).

Overall, comparable measurements were used relevant to the microbial sampling so that, carbon, pigment and nutrient concentrations from the top 2 cm of sediment were averaged for multivariate analyses.

Extraction of nucleic acids

DNA from attached and free-living microbial cells was extracted from 1 g of sediment using the Power Soil extraction kit (MO BIO Laboratories, Carlsbad) according to the instructions of the manufacturer. This method included a bead-beating step, which was performed for 5 min. All DNA extracts were eluted with 100 μL of Tris buffer (10 mM) and stored at $-20\,^{\circ}C$ until further analyses.

Microbial community analyses

Generally, denaturing gradient gel electrophoresis (DGGE) was used to study the variation in the archaeal and bacterial community based on specific phylotypes.

Amplification of bacterial 16S rRNA genes and DGGE

PCR amplification of 16S rRNA gene fragments was performed as described earlier (Teske *et al.*, 1996; Sapp *et al.*, 2007a, b) using the primers BAC341f with a 40-bp GC-rich

© 2010 CEFAS
Journal compilation © 2010 Federation of European Microbiological Societies.
Published by Blackwell Publishing Ltd.

Downloaded from https://academic.oup.com/femsec/article-abstract/74/2/410/589506 by University of Zurich user on 11 December 2019

sequence and BAC907rm. PCR mixtures with a volume of 100 μL contained 10 μL of 10 × CoralLoad Taq buffer, 20 μL of 5 × Q-solution, 1 mM $MgCl_2$, 300 μM of each dNTP (all Qiagen), 0.2 μM of each primer, 2 U of Taq DNA Polymerase (Qiagen) and 2–30 μL DNA of sediment samples. The 'touchdown' PCR started with a denaturing step at 94 °C for 5 min. Every cycle consisted of three steps, each for 1 min: 94 °C, annealing temperature and 72 °C. The initial annealing temperature of 65 °C was decreased by 0.5 °C per cycle until a touchdown of 55 °C, at which temperature, 12 additional cycles were carried out. Final primer extension was performed at 72 °C for 10 min, and this was followed by 22 cycles starting at 71 °C and decreasing by 1 °C per cycle. PCRs were performed in a BioRAD DNA Engine. PCR products were inspected on 1.2% (w/v) agarose gels. Additionally, PCR products were obtained from DNA of *Polaribacter filamentus*, *Sulfitobacter mediterraneus*, *Arthrobacter agilis* and *Leifsonia aquatica* (cultures from DSMZ, Braunschweig, Germany), which were used as standard patterns in DGGE. PCR products were purified using the NucleoSpin Extract II PCR purification kit (Macherey–Nagel) following the manufacturer's protocol. After measurement of the DNA concentration, fragments of 300 ng DNA per sample were resolved on 6% (w/v) polyacrylamide gels in 0.5 × TAE buffer with denaturing gradients of 25–60% urea/formamide (100% denaturant contains 7 M urea and 40% formamide). DGGE analyses were performed using a DCode system (BioRAD). Electrophoresis was run at 60 °C and 160 V for 10 h (Sigler *et al.*, 2004). DGGE gels were stained with SYBRGold as recommended by Molecular Probes (Invitrogen). Imaging was performed using a GBox System (Syngene).

Amplification of archaeal 16S rRNA genes and DGGE

PCR amplification of 16S rRNA gene fragments was performed using the primers ARC344f with a 40-bp GC-rich sequence at the 5′ end (5′-CGC CCG CCG CGC CCC GCG CCC GGC CCG CCG CCC CCG CCC GAC GGG GTG CAG CAG GCG CGA-3′) (Perreault *et al.*, 2007) and ARC915r (5′-GTG CTC CCC CGC CAA TTC CT-3′) (Raskin *et al.*, 1994). PCR mixtures with a volume of 100 μL contained 10 μL of 10 × CoralLoad Taq buffer, 20 μL of 5 × Q-solution, 1 mM $MgCl_2$, 300 μM of each dNTP (all Qiagen), 0.2 μM of each primer, 2 U of Taq DNA Polymerase (Qiagen) and 2–30 μL DNA of sediment samples. The 'touchdown' PCR started with a denaturing step at 94 °C for 5 min. Every cycle consisted of three steps, 1 min at 94 °C, 1 min of annealing temperature and 3 min at 72 °C. The initial annealing temperature of 71 °C was decreased by 0.5 °C per cycle until a touchdown of 61 °C, at which temperature 15 additional cycles were carried out. The final primer extension was performed at 72 °C for 10 min (Casamayor *et al.*, 2000). PCRs were performed in a BioRAD DNA Engine. PCR products were inspected on 1.2% (w/v) agarose gels. PCR products were purified using the NucleoSpin Extract II PCR purification kit (Macherey–Nagel) following the manufacturer's protocol. After measurement of the DNA concentration, fragments of 150 ng DNA per sample were resolved on 6% (w/v) polyacrylamide gels in 0.5 × TAE buffer with denaturing gradients of 20–50% urea/formamide (100% denaturant contains 7 M urea and 40% formamide) including the standard pattern used in bacterial DGGE fingerprints. DGGE analyses were performed using a DCode system (BioRAD) at 60 °C and 200 V for 3.5 h (Casamayor *et al.*, 2000, 2001). DGGE gels were stained with SYBRGold (Invitrogen) while imaging was performed using a GBox System (Syngene).

DNA sequencing

Prominent DGGE bands that connected or separated samples were excised and eluted by incubation at 37 °C for 1 h in molecular-grade water. Bands were subjected to a new amplification to rerun the respective DNA fragments on additional DGGE gels. DNA of single bands was reamplified using the respective primers. After reamplification, the fragments were purified using the NucleoSpin Extract II PCR purification kit (Macherey–Nagel) following the manufacturer's protocol and inspected on 1.2% (w/v) agarose gels.

Sequencing was performed by the John Innes Centre Genome Laboratory (Norwich, UK) using an ABI PRISM 3730 XL or a 3730 DNA Analyzer (Applied Biosystems, Foster City, CA). Sequencing primers were BAC341f (5′-ACG GGA GGC AGC AG-3′) and BAC907rm for bacterial DGGE bands or ARC344f (5′-AC GGG GTG CAG CAG GCG CGA-3′) and ARC915r for archaeal DGGE bands. The nearest relatives were searched for using BLAST (http://www.ncbi.nlm.nih.gov).

Phylogenetic analysis

The ARB software package (http://www.arb-home.de) was used for phylogenetic analysis (Ludwig *et al.*, 2004). The sequence database was based on a reference file established by SILVA (released July 2008, Pruesse *et al.*, 2007). After the addition of sequences, alignment was carried out using the FAST ALIGNER integrated in the program and refined by a comparison of the closest relatives retrieved by BLAST. Sequences with > 1300 nucleotides were used to construct phylogenetic trees. The ARB 'parsimony interactive' tool was used to add partial sequences to respective trees. Phylogenetic relationships were deduced using the neighbour-joining method with the correction algorithm of Felsenstein (1993).

© 2010 CEFAS
Journal compilation © 2010 Federation of European Microbiological Societies.
Published by Blackwell Publishing Ltd.

Downloaded from https://academic.oup.com/femsec/article-abstract/74/2/410/589506 by University of Zurich user on 11 December 2019

Nucleotide sequence accession numbers

The sequences obtained in this study are available from GenBank under accession numbers FJ756419–FJ756438 (archaeal sequences) and FJ824885–FJ824908 (bacterial sequences).

Statistical analysis of DGGE profiles

Ordination techniques based on DGGE fingerprints were used to analyse the bacterial and archaeal community and to identify the factors affecting specific phylotypes.

Analyses of DGGE fingerprints were carried out using the GELCOMPAR II 5.1 software package (Applied Maths NV, Sint-Martens-Latem, Belgium).

Normalization of bacterial and archaeal DGGE gels was performed using four specific bacterial strains as a standard in addition to internal reference positions covering a broad area of positions. For sample comparison, band-matching analysis was performed. Bands were assigned to classes of common bands within all profiles. The procedure resulted in band-matching tables that included densitometric values of fingerprints for all three community analyses (Muylaert *et al.*, 2002). These band-matching tables formed the basis for community ordination analysis.

Multivariate analysis of fingerprints was performed using the subroutines nonmetric multidimensional scaling (nMDS), analysis of similarities (ANOSIM) and distance-based redundancy analysis (RDA) of the BRODGAR software suite (Highland Statistics Ltd, Newburgh, UK). Ordination of Bray–Curtis similarities was performed by nMDS (Yannarell & Triplett, 2005; Sapp *et al.*, 2007a, b). For clarity, all the plots presented are two-dimensional, although three-dimensional plots usually displayed lower stress levels.

To test the hypothesis that the within-group DGGE profile similarity was greater than between groups, an ANOSIM was conducted by the subroutine ANOSIM of the BRODGAR software (Zuur *et al.*, 2007).

Furthermore, we performed RDA to compare intersample distances based on Euclidean distance as well as sample–environment and species–environment correlations. RDA was performed as given in Zuur *et al.* (2007) based on untransformed data.

Explanatory variables included water depth, sediment type, specifically the sand and silt fraction, mean particle diameter, sorting, skewness and kurtosis of sediment particles, mean OPD, organic carbon content, total nitrogen, phosphate, ammonia, mean porosity, mean chlorophyll *a* and phaeophytin content as well as biomass of macrofaunal species. Biomass and abundance of macrofauna were chosen as explanatory variables for structuring microbial communities in separate analyses.

To differentiate the replicates of a station from those of other stations, we introduced a categorical variable in the ordinations (nominal, 'station').

For the analyses of intersample distances by RDA, we used distance biplots as distances between observations represent Euclidean distances, which is not the case for correlation biplots (Zuur *et al.*, 2007). The respective correlation biplots were used for the analyses of species–environment relationships.

An automated forward selection was carried out to analyse sample or species–environment correlations for DGGE profiles. First, the variance inflation factor (VIF) of environmental variables was calculated. Variables displaying a value > 50 were excluded from RDA analyses, assuming collinearity of the respective variable with other variables included in the dataset. Because of collinearity, we did not include the values for the sediment gravel fraction in our analyses.

The marginal effects of environmental variables were calculated to identify the variable that best explained the variation in the dataset if only one variable was used in RDA. The null hypothesis that species composition is independent of the measured variables was tested using automated forward selection and a permutation test. The Monte-Carlo permutation test provided the conditional effect of each variable.

Microbial diversity

For a direct overview of microbial diversity, we calculated the Shannon index H' (Schauer *et al.*, 2000) on the basis of DGGE community fingerprint data for both archaeal and bacterial assemblages.

Results

Abiotic parameters

The bottom water temperature for all stations was 6.9 °C, while salinity measurements revealed an average value of 34.9‰ in the sampled area. The abiotic parameters determined for this study are listed in Table 1.

The particle sediment analysis revealed that the stations consisted mainly of muddy sand, with silt contents ranging from 11% to 28%. The sediment was sorted around the 90 μm diameter fraction (range from 31 to 250 μm). The within-station variance in this fraction was small, except for some single replicate outliers at several stations. The fine fractions (0.49 to ~22 μm) were more variant across the Oyster Ground. Again, this variance was largely due to within-station replicate variability. The main station as well as station 1 showed a slightly lower percentage of silt (~13/14%) and correspondingly, different summary statistics for sorting (1.52 ± 0.125), skewness (2.35 ± 0.32) and kurtosis

© 2010 CEFAS
Journal compilation © 2010 Federation of European Microbiological Societies.
Published by Blackwell Publishing Ltd.

Downloaded from https://academic.oup.com/femsec/article-abstract/74/2/410/589506 by University of Zurich user on 11 December 2019

Table 1. Abiotic parameters and microbial diversity of studied samples

Sample	Lat.	Long.	Depth (m)	Gravel (%)	Sand (%)	Silt/clay (%)	PD (mm)	S	Skew	K	OPD (cm)	OC (%)	Total N (μmol mg^{-1})	PO_4 (μmol L^{-1})	NH_4 (μmol L^{-1})	P	Chl (mg m^{-2})	Phaeo (mg m^{-2})	Bands bacteria	*H*′ bacteria	Bands archaea	*H*′ archaea
Main-A	54°413′	4°042′	46.4	0.01	86.84	13.15	0.079	1.51	2.41	8.28	1.333	0.266	5.90	2.33	2.37	0.491	7.96	29.31	21	2.96	19	2.88
Main-B				0.00	88.00	12.00	0.081	1.45	2.57	9.25	1.333	0.266	5.90	2.33	2.37	0.491	7.96	29.31	22	3.02	20	2.96
Main-C				0.01	82.91	17.08	0.071	1.65	1.99	6.10	1.333	0.266	5.90	2.33	2.37	0.491	7.96	29.31	22	3.05	15	2.67
Main-D				0.01	89.07	10.92	0.084	1.38	2.70	10.14	1.333	0.266	5.90	2.33	2.37	0.491	7.96	29.31	21	3.01	15	2.66
1-a	54°412′	4°045′	45.7	0.00	87.09	12.91	0.080	1.46	2.44	8.51	0.650	0.208	8.57	2.50	4.85	0.475	6.38	26.70	24	3.15	21	2.99
1-b				0.03	80.40	19.57	0.065	1.75	1.78	5.17	0.650	0.208	8.57	2.50	4.85	0.475	6.38	26.70	24	3.15	17	2.77
1-c				0.12	86.64	13.25	0.079	1.53	2.30	8.07	0.650	0.208	8.57	2.50	4.85	0.475	6.38	26.70	24	3.15	19	2.9
1-d				0.04	88.57	11.38	0.083	1.41	2.62	9.85	0.650	0.208	8.57	2.50	4.85	0.475	6.38	26.70	24	3.15	17	2.91
2-a	54°412′	4°040′	ND	0.01	80.63	19.36	0.065	1.77	1.82	5.26	1.367	0.278	5.54	1.87	3.64	0.475	5.97	35.28	24	3.14	14	2.62
2-b				1.27	78.62	20.11	0.067	1.94	0.90	5.88	1.367	0.278	5.54	1.87	3.64	0.475	5.97	35.28	23	3.1	15	2.7
2-c				0.03	80.38	19.59	0.065	1.76	1.79	5.24	1.367	0.278	5.54	1.87	3.64	0.475	5.97	35.28	22	3.05	20	2.71
2-d				0.03	79.68	20.29	0.064	1.79	1.73	4.95	1.367	0.278	5.54	1.87	3.64	0.475	5.97	35.28	24	3.13	15	2.74
3-a	54°411′	4°044′	46.2	0.01	75.50	24.49	0.058	1.87	1.48	4.06	0.833	0.178	3.86	0.98	1.58	0.462	7.39	49.63	21	3.02	13	2.5
3-b				0.02	79.16	20.82	0.063	1.80	1.69	4.75	0.833	0.178	3.86	0.98	1.58	0.462	7.39	49.63	20	2.96	16	2.48
3-c				0.00	82.02	17.98	0.068	1.70	1.94	5.81	0.833	0.178	3.86	0.98	1.58	0.462	7.39	49.63	25	3.14	14	2.41
3-d				0.01	85.37	14.62	0.075	1.59	2.26	7.42	0.833	0.178	3.86	0.98	1.58	0.462	7.39	49.63	24	3.15	12	2.72
4-a	54°414′	4°035′	45.4	0.01	80.10	19.89	0.065	1.76	1.76	5.04	0.950	0.227	4.29	1.73	4.08	0.521	16.46	60.74	24	3.1	16	2.68
4-b				0.01	87.26	12.73	0.080	1.49	2.44	8.47	0.950	0.227	4.29	1.73	4.08	0.521	16.46	60.74	22	3	16	2.57
4-c				0.01	83.41	16.59	0.072	1.66	2.03	6.26	0.950	0.227	4.29	1.73	4.08	0.521	16.46	60.74	24	3.11	13	2.79
4-d				0.01	74.31	25.67	0.056	1.93	1.42	3.83	0.950	0.227	4.29	1.73	4.08	0.521	16.46	60.74	26	3.17	8	2.57
5-a	54°416′	4°046′	46.5	0.04	83.78	16.18	0.072	1.63	2.07	6.52	0.833	0.276	5.60	1.46	2.47	0.524	20.37	71.14	24	3.14	11	2.69
5-b				0.27	82.12	17.61	0.070	1.71	1.73	6.17	0.833	0.276	5.60	1.46	2.47	0.524	20.37	71.14	25	3.18	13	2.53
5-c				0.03	71.75	28.22	0.052	1.96	1.27	3.40	0.833	0.276	5.60	1.46	2.47	0.524	20.37	71.14	20	2.93	13	2.59
5-d				0.01	80.60	19.39	0.066	1.74	1.81	5.25	0.833	0.276	5.60	1.46	2.47	0.524	20.37	71.14	20	2.96	12	2.45
6-a	54°415′	4°039′	46.2	0.06	74.38	25.56	0.057	1.90	1.39	3.81	0.667	0.297	5.87	2.00	3.74	0.532	20.86	30.37	24	3.09	16	2.06
6-b				0.01	81.31	18.68	0.069	1.67	1.86	5.54	0.667	0.297	5.87	2.00	3.74	0.532	20.86	30.37	22	3.06	15	2.35
6-c				0.00	84.19	15.81	0.074	1.58	2.11	6.72	0.667	0.297	5.87	2.00	3.74	0.532	20.86	30.37	21	3	14	2.53
6-d				0.01	84.24	15.75	0.074	1.59	2.12	6.77	0.667	0.297	5.87	2.00	3.74	0.532	20.86	30.37	23	3.1	12	2.44

Lat, latitude North; Long, longitude East; PD, mean particle diameter; S, sorting; Skew, skewness; K, kurtosis; OC, organic carbon content; P, mean porosity; Chl, chlorophyll *a*; Phaeo, phaeophytin; H′, Shannon index; ND, not determined.

Journal compilation © 2010 Federation of European Microbiological Societies.
Published by Blackwell Publishing Ltd.
© 2010 CEFAS

Downloaded from https://academic.oup.com/femsec/article-abstract/74/2/410/589506 by University of Zurich user on 11 December 2019

(8.17 ± 1.74). This difference at the main station and station 1 was not reflected in the porosity measurements, which ranged evenly between 0.46 and 0.53 across stations.

Carbon fractions were low at all stations, but there was considerable variance between stations. The percentage of organic carbon ranged from 0.17 to 0.29 across the stations. The total nitrogen levels were very low, as would be expected from carbon fractions that have undergone a full winter of microbial degradation. These ranged from 3.86 to 8.57 μmol N mg^{-1} sediment. The pigment levels showed a similar trend for sediments in winter, with phaeophytin dominating (44 ± 18 mg m^{-2}) in comparison with chlorophyll *a* (13 ± 7 mg m^{-2}). Stations 4, 5 and 6 had higher pigment levels overall.

OPD ranged from 0.650 to 1.37 cm. This is typical for diffusional sediments, which show an OPD of approximately 1 cm (Revsbech *et al.*, 1980). The deeper values illustrated the low oxygen demand in the sediment at this time of the year.

The sediment pore-water nutrients phosphate and ammonium were comparatively low (< 5 μmol L^{-1}). This was consistent with the oxic part of the sediment, which kept phosphate oxidized, and a lack of ammonium generation from carbon breakdown at depth. There was still considerable variability between the stations for both phosphate (~1 to 2.5 μmol L^{-1}) and ammonium (~1.58 to 4.85 μmol L^{-1}).

Macrofaunal community

Infaunal species richness (SR) was the lowest at stations 5 (SR = 13) and 3 (SR = 14), intermediate at station 2 (SR = 18) and slightly higher at stations 1 (SR = 21), 4 (SR = 22), 6 (SR = 22) and the main station (SR = 25).

The brittlestar *Amphiura filiformis* was consistently the most abundant species across all stations, with counts ranging from 45.75 ± 20.66 (station 3, $n = 3$) to 111.25 ± 12.88 (station 4, $n = 4$) animals per NIOZ core. The bivalve, *Corbula gibba*, the crustacean, *Callianassa subterranea*, and the polychaete family *Nephtyidae* were also found consistently across all stations, albeit at much lower abundances (the counts ranged from 1 to 10, 1 to 3 and 1 to 2 per core, respectively). The main station as well as stations 3 and 4 were characterized by higher abundances of *Saxicavella jaffreysii* (2.25, 2.75 and 5.75 per core, respectively).

In terms of biomass, *A. filiformis* was again found to be one of the dominant five species at all stations, with biomass estimates ranging from 0.83 ± 0.56 g per core (station 5, $n = 3$) to 2.26 ± 0.43 g per core (station 4, $n = 4$). Despite low abundances (< 1 per core), the two echinoderms *Echinocardium caudatum* and *Brissopsis lyrifera*, due to their larger body size, dominated the biomass at all stations, except station 1, where neither were found. The highest average species biomass was found at station 4 (27.58 g per core), due primarily to *B. lyrifera* and *Echiurus echiurus*. The lowest average biomass was found at station 1 (5.13 g per core), where neither of the large echinoderms were present in the samples.

Microbial community

Bacterial diversity was stable within stations, ranging from 2.93 to 3.18 (Table 1). Slightly less stable was the diversity of archaeal communities, with the Shannon index ranging from 2.06 to 2.99, thereby displaying a slightly lower diversity than the bacterial assemblages (Table 1).

Bacteria

Ordination I

In general, 36 phylotypes were identified at different positions on the DGGE gels of the bacterial community (Fig. 2). In total, 13 phylotypes (36%) were predominant in the area sampled. These belonged mainly to members of the *Gammaproteobacteria* and *Acidobacteria*, but also included members of the *Flavobacteria*, *Actinobacteria* and *Deltaproteobacteria*, respectively. Band classes II, VI, IX and XXII were excluded from ordination, as they appeared only once or twice in the dataset.

The differences in the bacterial community structure based on DGGE fingerprints between different stations were analysed by nMDS and RDA. Both ordination methods showed a strong separation, especially for the bacterial communities of stations 1, 3 and 6 (Fig. 3a and b). This is supported by the ANOSIM analysis, revealing a strong spatial separation of the bacterial communities ($R = 0.588$, $P = 0.001$). However, the replicates of stations 2 and 4 appeared to be more scattered (Fig. 3a and b).

Ordination II: influence by environmental variables

For the analysis of bacterial DGGE fingerprints, the explanatory variable sorting of sediment particles, OPD, organic carbon content, ammonia, porosity, the biomass of the bivalve *C. gibba*, the biomass of the decapod *C. subterranea* as well as of the polychaetes *Aphroditidae*, *Glyceridae*, *Capitellidae*, the ribbon worm *Nemertea* and the nominal variable station were used.

The eigenvalues of the ordination analyses based on DGGE fingerprints are presented in Table 2. With an overall variance of 1.00, the sum of all canonical eigenvalues is 0.62, indicating that all the explanatory variables explain 62% of the variation in the species data. Concerning the variance of species data, the first axis explained 30.2% of the total variation, the first and the second axes explained 37.4% and all four axes explained 49.4% of the total explained variation.

© 2010 CEFAS
Journal compilation © 2010 Federation of European Microbiological Societies.
Published by Blackwell Publishing Ltd.

Downloaded from https://academic.oup.com/femsec/article-abstract/74/2/410/589506 by University of Zurich user on 11 December 2019

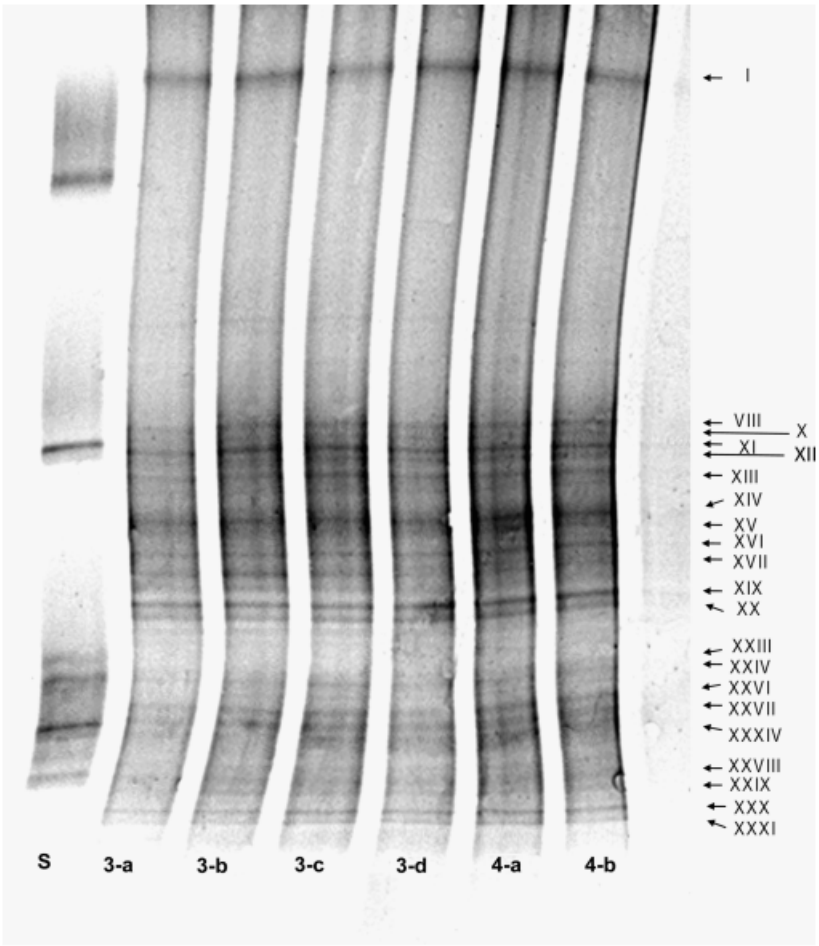

Fig. 2. DGGE analysis of bacterial 16S rRNA gene fragments amplified from DNA extracted from Oyster Ground sediment. Lanes: S, standard lanes; 3-a, 3-b, 3-c, 3-d, replicates of site 3; 4-a and 4-b, replicates of site 4. Arrows (right) indicate prominent band classes referring to sequenced DGGE bands in Table 4.

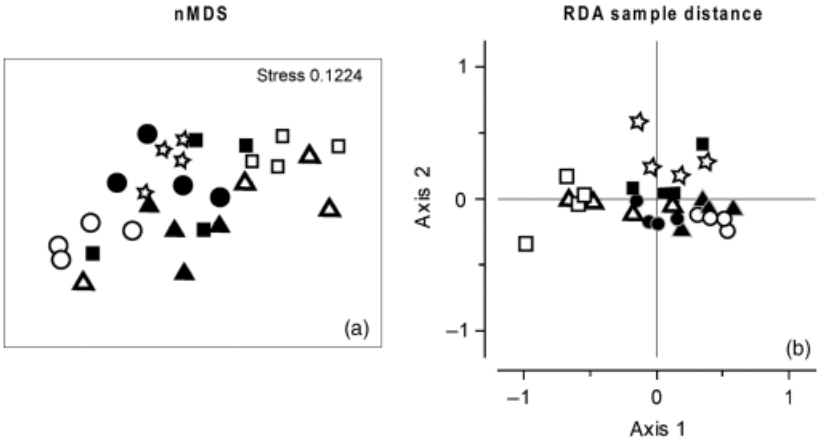

Fig. 3. nMDS plots based on Bray–Curtis similarities and RDA distance biplots of DGGE fingerprints of bacterial communities. Samples are indicated by the following symbols: main station (●), station 1 (□), station 2 (△), station 3 (☆), station 4 (■), station 5 (▲), station 6 (○) including four replicates per station. Stress level of nMDS in 3D, 0.0687 for DGGE fingerprints. (a) nMDS plot; (b) RDA distance biplot.

An overall relationship between bacterial phylotypes and environmental variables is indicated (axis 1–4: 79.6%). Biplot scaling of RDA is shown in Fig. 4, demonstrating the influence of different variables on different bacterial communities and phylotypes. Porosity strongly influenced the communities at station 6 and three replicates at station 5 (Fig. 4a). Organic carbon content showed a strong correlation with replicates at the main station and stations 2 and 5, whereas ammonia and the biomass of *Capitellidae* influenced replicates of the main station and station 1. Additionally, OPD showed a strong correlation with a replicate of station 2, while the biomass of *Glyceridae* and *C. gibba*

© 2010 CEFAS
Journal compilation © 2010 Federation of European Microbiological Societies.
Published by Blackwell Publishing Ltd.

Downloaded from https://academic.oup.com/femsec/article-abstract/74/2/410/589506 by University of Zurich user on 11 December 2019

Table 2. Eigenvalues and variance decomposition for RDA

	Axes	Eigenvalues	Cumulative percentage variance of species data	Cumulative percentage variance of species–environment correlation
Bacteria				
DGGE intersample and interspecies distances	Axis 1	0.302	30.242	48.715
	Axis 2	0.071	37.374	60.203
	Axis 3	0.063	43.670	70.344
	Axis 4	0.057	49.411	79.592
Archaea				
DGGE intersample and interspecies distances	Axis 1	0.189	18.944	28.197
	Axis 2	0.110	29.922	44.538
	Axis 3	0.077	37.660	56.055
	Axis 4	0.057	43.394	64.589

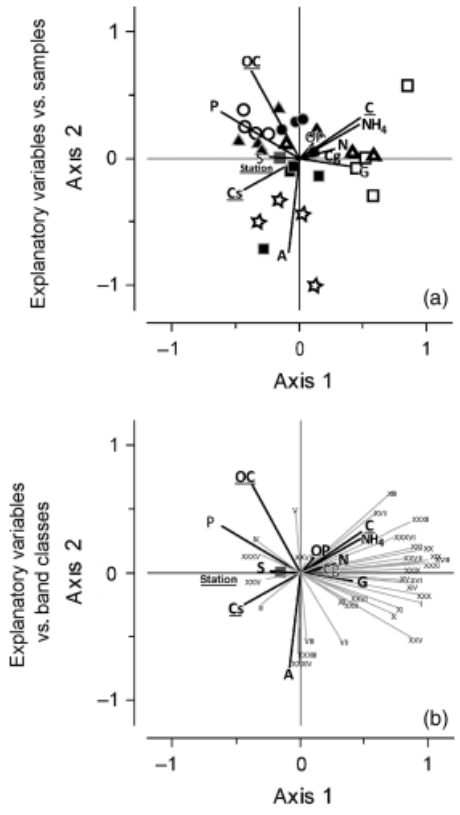

Fig. 4. RDA correlation biplots of DGGE fingerprints of the bacterial community using biomass of *Corbula gibba* (Cg), *Callianassa subterranea* (Cs), *Aphroditidae* (A), *Glyceridae* (G), *Nemertea* (N) and *Capitellidae* (C) as well as habitat characteristics including oxygen penetration depth (OP), organic carbon content (OC), ammonia (NH_4), porosity (P), sorting (S) and the nominal variable station (■). Samples are indicated by the following symbols: main station (●), station 1 (□), station 2 (△), station 3 (☆), station 4 (■), station 5 (▲), station 6 (○) while band classes are displayed by Roman numbers from I to XXXVI. Significant variables are indicated by underlined abbreviations. (a) RDA correlation biplot of samples; (b) RDA correlation biplot of phylotypes.

displayed a correlation with communities of stations 1, 2 and 4. A strong influence on the community at station 3 and a replicate of station 4 is shown for the biomass of the polychaete *Aphroditidae*. The biomass of *C. subterranea* showed a slight influence on replicates of station 4. The nominal variable station displayed a correlation with most observations, except some replicates of the main station and stations 1, 2, 3 and 4.

Applying automated forward selection and a permutation test (Table 3), it was elucidated that the nominal variable station, the biomass of *C. subterranea*, the *Capitellidae* and organic carbon content displayed the highest marginal and significant conditional effects, with the variable station having the strongest effect. Despite strong marginal effects, statistical significance at $P < 0.05$ was not achieved for the variables sediment porosity, *Glyceridaea* and ammonia (Table 3). However, especially sediment porosity contributed to the environmental variables explaining microbial variation, as shown by the length of the associated arrow (Fig. 4a).

Regarding a specific influence of environmental variables on bacterial phylotypes, significant environmental parameters showed strong correlations with different band classes (Fig. 4b). The nominal variable station was strongly correlated with the DGGE band classes III, IV, V, XXIV and XXXV. Strong correlations were also displayed for the biomass of *C. subterranea* and the band classes II and XXIV. Additionally, the biomass of *Capitellidae* was directly correlated with the band classes XIII and XVII. There was a negative correlation of organic carbon content with the band class VII, while the band classes IV and XXXV seemed to be correlated with sediment porosity.

Generally, analyses including infaunal SR and abundance (data not shown) revealed results comparable to those obtained for infaunal biomass. For the bacterial community, overall infaunal SR showed a strong and significant effect in RDA.

Archaea

Ordination I

The variation in the archaeal community was studied on the basis of DGGE fingerprints. Twenty-five phylotypes were identified at different positions in the DGGE profiles, including three predominant phylotypes (band classes I, XV, XXIV), which were detected in all samples (Fig. 5). These belonged to the *Euryarchaeota* (I, XV) and a member of the *Crenarchaeota* (XXIV).

As shown in the nMDS plot (Fig. 6a), the archaeal communities were slightly separated with certain replicates of the same station grouping together. However, replicates of respective stations did not display clearly separated groups.

Downloaded from https://academic.oup.com/femsec/article-abstract/74/2/410/589506 by University of Zurich user on 11 December 2019

© 2010 CEFAS
Journal compilation © 2010 Federation of European Microbiological Societies.
Published by Blackwell Publishing Ltd.

Table 3. Marginal and conditional effects of forwardly selected environmental variables produced by RDA

	Marginal effects		Conditional effects		
Environmental variable	*E*	*E*%	Increase in variation	*F* statistic	*P*-value
Bacteria					
Station	**0.16**	**25.66**	**0.16**	**4.927**	**0.001**
Callianassa subterranea	**0.08**	**12.42**	**0.09**	**3.085**	**0.004**
Capetellidae	**0.10**	**16.47**	**0.07**	**2.565**	**0.016**
Organic carbon content	**0.08**	**13.65**	**0.06**	**2.384**	**0.019**
Glyceridae	0.09	14.18	0.05	2.151	0.057
Oxygen penetration depth	0.04	6.84	0.04	1.458	0.148
Ammonia	0.09	14.68	0.04	1.445	0.165
Sorting	0.04	6.31	0.03	1.163	0.289
Porosity	0.14	22.19	0.03	1.146	0.309
Nemertea	0.05	8.41	0.03	1.122	0.307
Corbula gibba	0.03	5.25	0.01	0.535	0.825
Aphroditidae	0.05	7.65	0.01	0.480	0.867
Archaea					
Phosphate	**0.11**	**16.30**	**0.11**	**3.197**	**0.003**
Porosity	**0.07**	**10.04**	**0.07**	**2.177**	**0.022**
Amphiura filiformis	**0.04**	**6.52**	**0.05**	**1.746**	**0.048**
Ammonia	0.05	6.96	0.06	1.801	0.072
Eurydice pulchra	0.05	8.04	0.05	1.559	0.111
Total nitrogen	0.08	12.32	0.04	1.342	0.213
Nephtyidae	0.04	5.30	0.04	1.317	0.178
Kurtosis	0.09	13.55	0.04	1.271	0.258
Chamelea gallina	0.04	5.75	0.04	1.263	0.241
Skewness	0.08	12.65	0.04	1.245	0.238
Organic carbon content	0.03	3.94	0.03	1.096	0.337
Oxygen penetration depth	0.04	6.36	0.03	1.052	0.378
Nemertea	0.04	5.95	0.03	0.982	0.443
Polycheate fragments	0.03	3.87	0.02	0.756	0.617
Sediment type	0.08	12.35	0.02	0.702	0.644
Cheatopteridae	0.04	5.89	0.02	0.552	0.775

E, eigenvalue; *E*%, eigenvalue as percentage of sum all eigenvalues using only one explanatory variable.
Values in bold relate to significance level of 0.05.

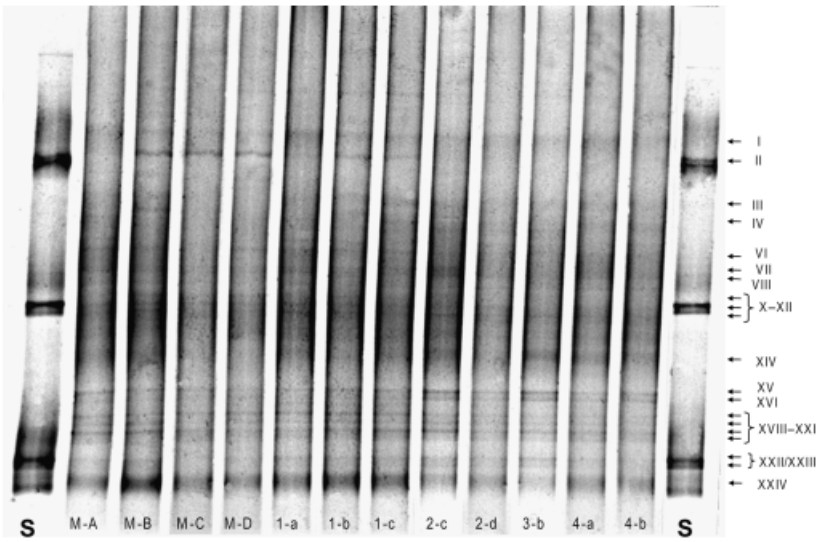

Fig. 5. DGGE analysis of archaeal 16S rRNA gene fragments amplified from DNA extracted from Oyster Ground sediment. Lanes: S, standard lanes; M-A to M-D, replicates of the main site; 1-a to 1-c, replicates of site 1; 2-c and 2-d, replicates of site 2; 3-b, replicate of site 3; 4-a and 4-b, replicates of site 4. Arrows (right) indicate prominent band classes referring to sequenced DGGE bands in Table 5.

Downloaded from https://academic.oup.com/femsec/article-abstract/74/2/410/589506 by University of Zurich user on 11 December 2019

© 2010 CEFAS
Journal compilation © 2010 Federation of European Microbiological Societies.
Published by Blackwell Publishing Ltd.

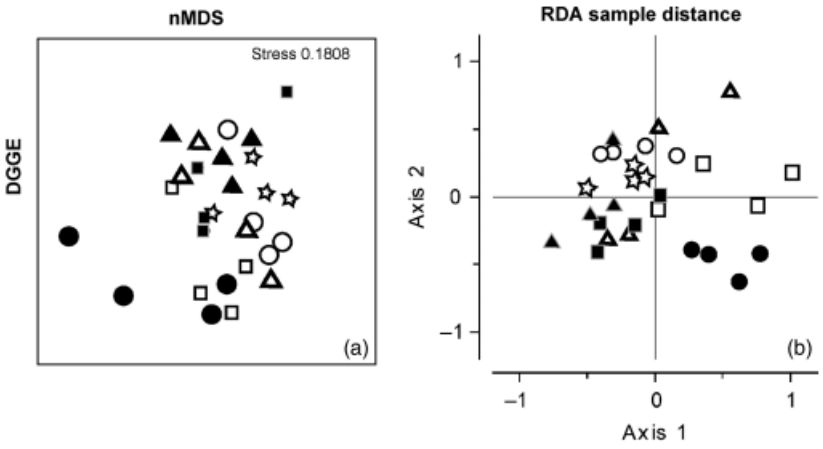

Fig. 6. nMDS plot based on Bray–Curtis similarities and RDA distance biplot of DGGE fingerprints of archaeal communities. Samples are indicated by the following symbols: main station (●), station 1 (□), station 2 (△), station 3 (☆), station 4 (■), station 5 (▲) and station 6 (○), including four replicates per station. Stress level of nMDS in 3D, 0.133. (a) nMDS plot and (b) RDA distance biplot.

This is supported by the ANOSIM analysis, revealing that archaeal communities were not strongly separated from each other, as confirmed by a low *R*-value ($R = 0.213$, $P = 0.01$). This is partly supported by the RDA intersample distance biplot (Fig. 6b), where replicates retrieved from stations 2, 4 and 5 showed no separation due to station, whereas communities collected at the main station and stations 1 and 3 displayed a slight spatial separation.

Ordination II – influence by environmental variables

In this analysis based on DGGE fingerprints, the explanatory variables sediment silt/clay fraction, skewness, kurtosis, OPD, organic carbon content, total nitrogen, phosphate, ammonia, porosity and the biomass of the brittlestar *A. filiformis*, the sea louse *Eurydice pulchra*, the catworms *Nephtyidae*, the venus clam *Chamelea gallina*, polychaete fragments and the annelids *Cheatopteridae* and *Nemertea* were used following the assessment of collinearity using VIFs. In detail, the ordinations revealed high VIF values for the biomass of certain macrofaunal species, the water depth of the samples, different sediment parameters and the pigment concentrations of chlorophyll *a* and phaeophytin, which indicated collinearity with other variables. These factors were excluded from the final RDA.

The eigenvalues of the ordination analyses based on archaeal fingerprints are presented in Table 2. The sum of all canonical eigenvalues was 0.67, meaning that all the explanatory variables explained 67% of the variation in the species data. Concerning the variance of species data, the first axis explained 18.9% of the total variation, the first and the second axes explained 29.9% and all four axes explained 43.4% (Table 2) of the total explained variation.

A relatively weak relationship between archaeal phylotypes and environmental variables was shown (axis 1–4: 64.6%), indicating that the relationship between species and measured environmental variables was less strong than for bacterial communities. Biplot scaling of RDA is shown in Fig. 8, demonstrating the influence of different variables on different archaeal communities and phylotypes. In detail, porosity influenced the communities at stations 3 and 5, while the silt/clay fraction of the sediment influenced communities at stations 3, 5 and 6 (Fig. 8a). Ammonia and phosphate showed positive correlations with communities retrieved from station 1. OPD influenced a replicate of station 1 and three replicates of the main station while one replicate of the main station was correlated with both skewness and kurtosis of sediment particles. Additionally, the biomass of *Nemertea* influenced replicates of stations 2 and 6, while the biomass of *A. filiformis* showed a strong correlation with replicates of stations 2 and 4.

Phosphate, sediment porosity and the biomass of *A. filiformis* showed the highest marginal and significant conditional effects (Table 3). Despite strong marginal effects, statistical significance at $P < 0.05$ was not achieved for the variables total nitrogen, kurtosis, skewness and sediment type (silt/clay fraction) (Table 3).

Porosity showed an influence on the band classes X and XXI, while the significant factors phosphate and the biomass of the brittlestar *A. filiformis* showed a negative correlation with some band classes (Fig. 8b). However, skewness and kurtosis of sediment affected the band classes VI, VII and XVIII (all *Euryarchaeota*) as well as class IX, while the biomass of *Nemertea* was positively correlated with some *Euryarchaeota*, namely band classes IV, XV and XVI. Total nitrogen influenced phylotypes belonging to the *Euryarchaeota* (band classes II, III), the *Crenarchaeota* (band classes XXII, XXIV) and an unknown phylotype (band class XIII).

Infaunal species did not show a strong link with archaeal community data in general, except for a strong correlation with the abundance of *A. filiformis*.

Phylogenetic analysis

For the study of the bacterial community, sequence data of 101 excised bands could be retrieved, representing 25

© 2010 CEFAS
Journal compilation © 2010 Federation of European Microbiological Societies.
Published by Blackwell Publishing Ltd.

Downloaded from https://academic.oup.com/femsec/article-abstract/74/2/410/589506 by University of Zurich user on 11 December 2019

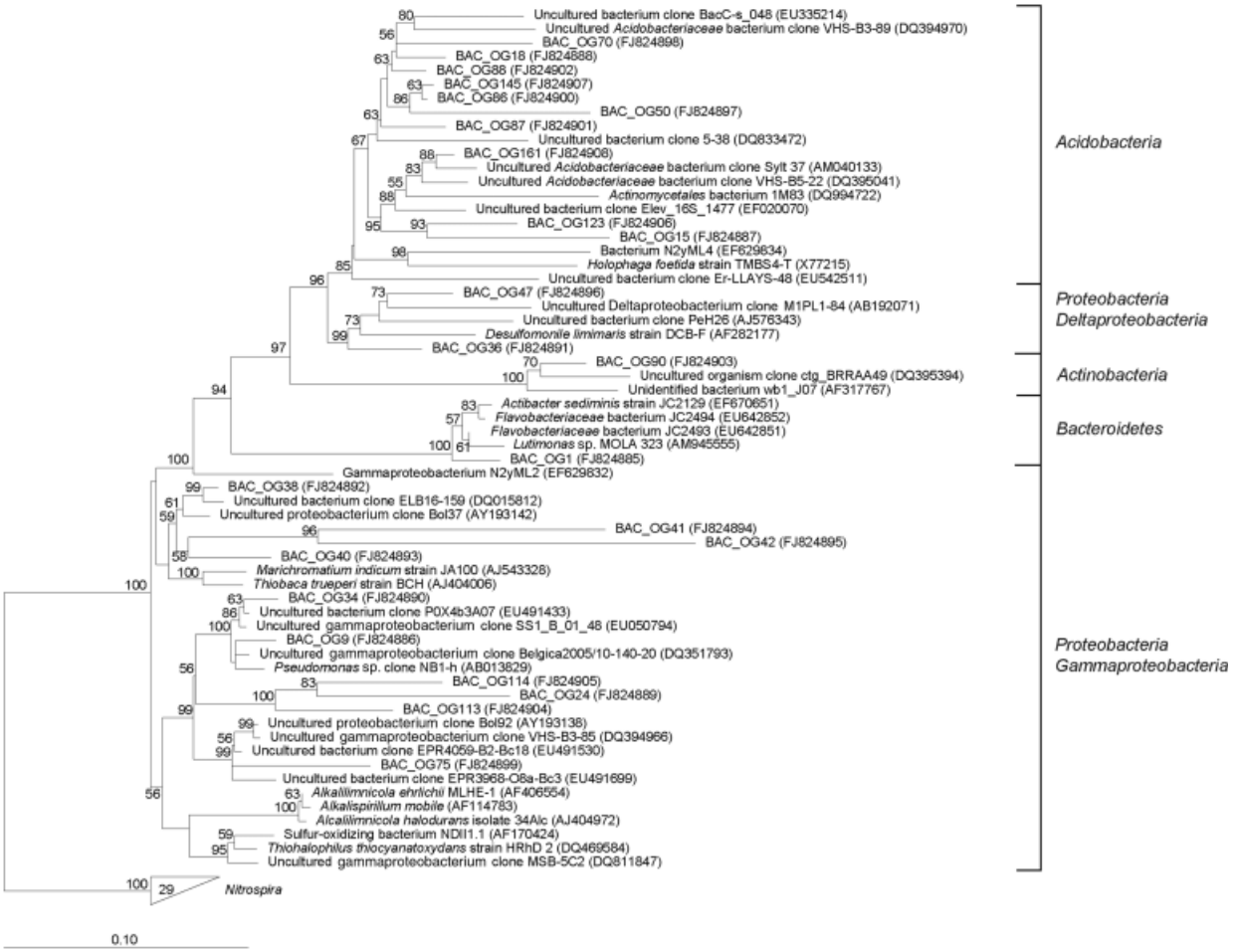

Fig. 7. Phylogenetic tree of *Proteobacteria*, *Acidobacteria* and *Bacteroidetes*. GenBank accession numbers are given in parentheses. Bootstrap values above 50% are displayed.

different phylotypes. Sequence data revealed the presence of four phyla of the Bacteria (Fig. 7). Most sequences were related to members of the *Proteobacteria* and members of the *Fibrobacteres/Acidobacteria* group.

Within the *Proteobacteria*, most phylotypes belonged to the *Gammaproteobacteria*. Additionally, we found one member of the *Deltaproteobacteria*, one member of the *Bacteroidetes* and a member of the *Actinobacteria* (Fig. 7). The closest relatives of the sequenced bands derived from BLAST analyses are listed in Table 4. The results revealed some close matches with 95–99% sequence identity. However, the majority of bands did not reveal a close match to sequences in the database.

No sequencing data could be retrieved for DGGE band classes II, III, IV, VI, IX, XVIII, XXI, XXII, XXIV, XXXIII, XXXV and XXXVI. Furthermore, the phylotype BAC_OG_45 showed a very difficult alignment in ARB and was excluded from phylogenetic analysis.

Archaeal sequence data of 63 excised bands revealed 20 different phylotypes. It could be shown that archaeal phylotypes clustered mainly into the phylum of the *Euryarcheota* (Fig. 9). We could also detect three members belonging to the *Crenarcheota*. The closest relatives of the sequenced bands derived from BLAST analyses are listed in Table 5. The results revealed some close matches with 99% and 94%. No sequencing data could be retrieved for archaeal DGGE band classes V, XIII, IX, XVII and XXV.

Discussion

The benthic environment hosts high biodiversity and plays a key role in biogeochemical cycling, especially for the carbon, sulphur and nitrogen cycles (Snelgrove, 1999; Madigan *et al.*, 2003; Paerl & Steppe, 2003). Nevertheless, spatial scaling of benthic microorganisms as well as the link between benthic microbial diversity and habitat characteristics are not well understood. In our study, we detected a fairly high small-scale variability for bacterial and archaeal communities (i.e. within a 100 m station ring). We could not

Downloaded from https://academic.oup.com/femsec/article-abstract/74/2/410/589506 by University of Zurich user on 11 December 2019

© 2010 CEFAS
Journal compilation © 2010 Federation of European Microbiological Societies.
Published by Blackwell Publishing Ltd.

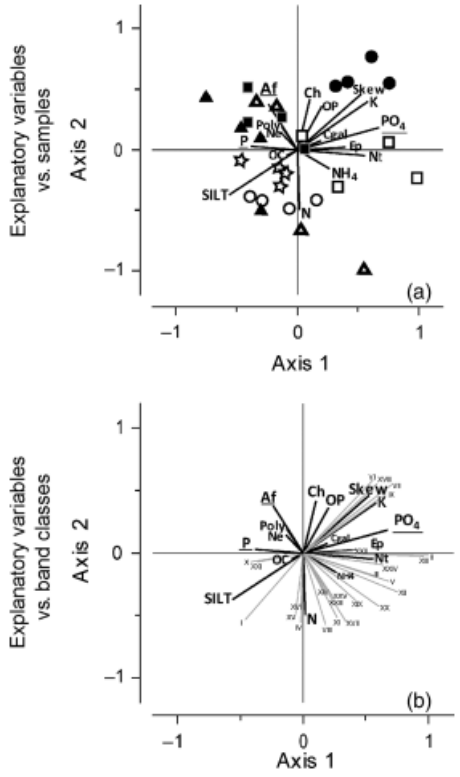

Fig. 8. RDA correlation biplots of DGGE fingerprints of the archaeal community using biomass of *Amphiura filiformis* (Af), *Nemertea* (N), *Eurodice pulchra* (Ep), *Chamelea gallina* (Cgal), *Chaetopteridae* (Ch), Polychaetes (Poly) and *Nephtyidae* (Ne) as well as habitat characteristics including oxygen penetration depth (OP), organic carbon content (OC), ammonia (NH_4), phosphate (PO_4), total nitrogen (Nt), porosity (P), silt-clay fraction (SILT) and skewness (Skew) of the sediment and kurtosis (K). Samples are indicated by the following symbols: main station (●), station 1 (□), station 2 (△), station 3 (☆), station 4 (■), station 5 (▲) and station 6 (○) while band classes are displayed by Roman numbers from I to XXV. Significant variables are indicated by underlined abbreviations. (a) RDA correlation biplot of samples and (b) RDA correlation biplot of phylotypes.

find a clear separation of bacterial or archaeal communities between stations, 1–11 km apart, despite the analysis of the bacterial community being based on single phylotypes. However, we found small spatial differences in the bacterial community within the Oyster Ground for certain stations. Our results indicate a relatively heterogeneous distribution of the majority of specific bacterial phylotypes collected in the studied area. It is suggested that in this study, DGGE was suitable to display fine-scale differences in the bacterial community. We could not detect a distance–decay or a taxa–area relationship in this study, probably due to the rather homogeneous habitat characteristics. However, it could be elucidated that environmental parameters control sedimentary microbial communities (Liu *et al.*, 2003; Polymenakou *et al.*, 2005; Quéric & Soltwedel, 2007; Sørensen *et al.*, 2007; Jackson & Weeks, 2008; Wu *et al.*, 2008). Our analyses showed that the bacterial and the archaeal communities were influenced by various parameters. These included sediment characteristics such as organic carbon content, sediment type and porosity, kurtosis and skewness of sediment particles and porewater nutrients as well as the macrofaunal community, especially the biomass of polychaetes such as *Glyceridae* or *Capitellidae*, the decapod *C. subterranea* and the ribbon worm *Nemertea*.

Overall, we detected robust relationships of variability in communities and environmental parameters, taking into account that we studied the total microbial community including dormant cells and extracellular DNA, rather than active communities based on RNA analyses. Although different methods such as rRNA gene fingerprinting or the removal of free extracellular DNA by ethidium or propidium monoazide exist to overcome this problem, it was not possible to apply those due to the practical constraints associated with offshore sampling. However, it is generally known that extracellular DNA is an important part of the sedimentary DNA pool and that degradation rates are slower than in the water column (Luna *et al.*, 2002; Dell'Anno & Corinaldesi, 2004). These degradation rates can differ considerably. As the amount of dissolved DNA deriving from the water column is probably low (Paul *et al.*, 1987), we assumed that extracellular DNA amplified and reflected in the fingerprints accounted for the total microbial community in the studied sediments. The environmental variables included in our study explained over 60% of the variation despite this methodological limitation. However, a study of the active benthic communities might be expected to reveal stronger species–environment correlations.

Furthermore, it has to be taken into account that the fingerprinting methods used are based on PCR amplification, which has potential biases (Suzuki & Giovannoni, 1996; von Wintzingerode *et al.*, 1997; Bidle & Azam, 2001). An additional problem occurs for the analysis of sedimentary communities as species with a low abundance might not appear in distinct bands, but unresolved background blur so that community variation might not be sufficiently resolved by DGGE (Bowman *et al.*, 2003).

Bacterial communities showed strong correlations with the organic carbon content and porosity, while archaeal communities were also correlated with porosity, but did not seem to be influenced by the organic carbon content. It is assumed that this finding indicates an autotrophic lifestyle of present archaeal taxa. However, it is likely that our results reflect different environmental adaptations to the respective habitat as the archaeal communities also showed strong correlations with sediment characteristics such as the sediment type and specific properties of sediment particles determined by kurtosis (bowing) and skewness (finer or coarser particles). This was not the case for the bacterial

Downloaded from https://academic.oup.com/femsec/article-abstract/74/2/410/589506 by University of Zurich user on 11 December 2019

© 2010 CEFAS
Journal compilation © 2010 Federation of European Microbiological Societies.
Published by Blackwell Publishing Ltd.

Table 4. Relatedness of bacterial phylotypes to known organisms

DGGE band class	DGGE band	Phylogenetic group	Closest relative	Similarity (%)	Base positions compared	Accession number of closest relative
I	BAC_OG_1	*Flavobacteria*	*Flavobacteriaceae* bacterium T15	96	541	AY177723
V	BAC_OG_113	*Gammaproteobacteria*	Gammaproteobacterium 79 EDB1	90	503	AM882524
VII	BAC_OG_38	*Gammaproteobacteria*	Gammaproteobacterium Sva0318	99	585	AJ240989
VIII	BAC_OG_114	*Beta/Gammaproteobacteria*	Uncultured bacterium TfP20L28	86	522	EU362266
X	BAC_OG_40	*Gammaproteobacteria*	Uncultured bacterium isolate MGP DGGE Band 13	91	533	AF530109
XI	BAC_OG_41	*Gammaproteobacteria*	Gammaproteobacterium 79 EDB1	80	548	AM882524
XII	BAC_OG_75	*Gammaproteobacteria*	Uncultured bacterium Tomm05_1274_3_Bac51	94	580	FM179882
XIII	BAC_OG_24	*Beta/Gammaproteobacteria*	Uncultured bacterium clone P0X3b5H10	86	500	EU491400
XIV	BAC_OG_42	*Gammaproteobacteria*	Uncultured Gammaproteobacterium clone JTB255	84	492	AB015254
XV	BAC_OG_9	*Gammaproteobacteria*	Uncultured bacterium clone P0X3b5H10	96	517	EU491400
XVI	BAC_OG_45	Difficult alignment ARB	Uncultured Gammaproteobacterium clone Y182	81	497	AB116435
XVII	BAC_OG_34	*Gammaproteobacteria*	Uncultured bacterium clone P0X3b5H10	96	505	EU491400
XIX	BAC_OG_47	*Deltaproteobacteria*	Uncultured bacterium MGP 17A	91	541	AF530114
XX	BAC_OG_36	*Deltaproteobacteria*	Uncultured *Acidobacterium* HCM3MC78_11E_FL	90	517	EU373925
XXIII	BAC_OG_50	*Acidobacteria*	Uncultured bacterium clone P9X2b2A03	87	494	EU491274
XXIV	BAC_OG_145	*Acidobacteria*	Uncultured bacterium clone P9X2b8B05	91	508	EU491320
XXV	BAC_OG_15	*Acidobacteria*	Uncultured *Acidobacteriaceae* bacterium Sylt 37	85	492	AM040133
XXVI	BAC_OG_123	*Acidobacteria*	Uncultured *Acidobacteriaceae* bacterium Sylt 37	92	525	AM040133
XXVII	BAC_OG_161	*Acidobacteria*	Uncultured *Acidobacteriaceae* bacterium Sylt 37	95	487	AM040133
XXXII	BAC_OG_18	*Acidobacteria*	Uncultured bacterium 112D36	92	531	EU735016
XXXIV	BAC_OG_86	*Acidobacteria*	Uncultured bacterium 105G48	95	526	EU925891
XXVIII	BAC_OG_87	*Acidobacteria*	Uncultured *Holophaga/Acidobacterium* Sva0515	93	501	AJ241004
XXIX	BAC_OG_88	*Acidobacteria*	Uncultured *Holophaga/Acidobacterium* Sva0515	93	514	AJ241004
XXX	BAC_OG_70	*Acidobacteria*	Uncultured *Acidobacteriaceae* bacterium AT-s3-24	88	517	AY225641
XXXI	BAC_OG_90	*Actinobacteria*	Uncultured bacterium P0X3b5C01	95	563	EU491386

communities. It is assumed that archaea in the studied area are controlled by microscale habitat characteristics as already hypothesized (Aller & Kemp, 2008).

The correlation of macrofaunal biomass with the bacterial community could be shown, revealing a strong influence of the burrowing mud shrimp *C. subterranea*, possibly due to its capacity to introduce high levels of oxygen and nutrients from the overlying seawater deep into the sediment (Forster & Graf, 1992; Ziebis *et al.*, 1996; Howe *et al.*, 2004). Generally, burrowing of these types of infauna can create high levels of biogeochemical heterogeneity on small scales (less than 1 cm) either vertically or horizontally (Aller, 1982, 1988, 1990; Lohrer *et al.*, 2004). It is likely that this influenced the differences and heterogeneity in the bacterial community within a station.

A similar influence on the bacterial community is assumed for the polychaetes *Capitellidae* and *Glyceridae* (Méndez *et al.*, 2001; Hietanen *et al.*, 2007; Quintana *et al.*, 2007; Pischedda *et al.*, 2008).

For the composition of the archaeal community, macrofaunal biomass seemed to be less important, as could be seen by the marginal effects of environmental variables. However, the biomass of the brittle star *A. filiformis* was strongly correlated with some archaeal communities and this could be ascribed to its sediment disturbing activity as it lives buried in the sediment (Solan & Kennedy, 2002; Vopel *et al.*, 2003). This finding corresponds with the negative correlations shown for archaeal phylotypes and the biomass of *A. filiformis*. However, a direct negative effect of this organism on certain archaeal phylotypes would need to be addressed using experimental approaches.

Furthermore, it is supposable that the molecular techniques applied detected symbionts of macrofauna. However, the effect on the community profiles is thought to be minor as it appears unlikely that the symbiont signals were strong enough to override signals from the overall benthic (free-living) community, but rather contributed. Our data indicated that some infauna correlated alongside with the chemicals measured; see for example the correlation of ammonia and *Capitellidae* with the community structure as shown in Fig. 4. The separation of macrofaunal symbionts could be certainly achieved by sieving the samples directly on board in a sterile way, which was not feasible for this study.

It is further assumed that the absence of a correlation of pigment concentration and microbial communities resulted from the physiological status the prokaryotes might have been in as the sampling took place before the first phytoplankton bloom, which would have brought fresh organic carbon to the seabed.

It also needs to be considered that we did not study the influence of meiofaunal species, ciliates and viral particles that has been shown previously (Alkemade *et al.*, 1992; Jensen, 1996; Hoschitz *et al.*, 1999; Riemann & Helmke,

© 2010 CEFAS
Journal compilation © 2010 Federation of European Microbiological Societies.
Published by Blackwell Publishing Ltd.

Downloaded from https://academic.oup.com/femsec/article-abstract/74/2/410/589506 by University of Zurich user on 11 December 2019

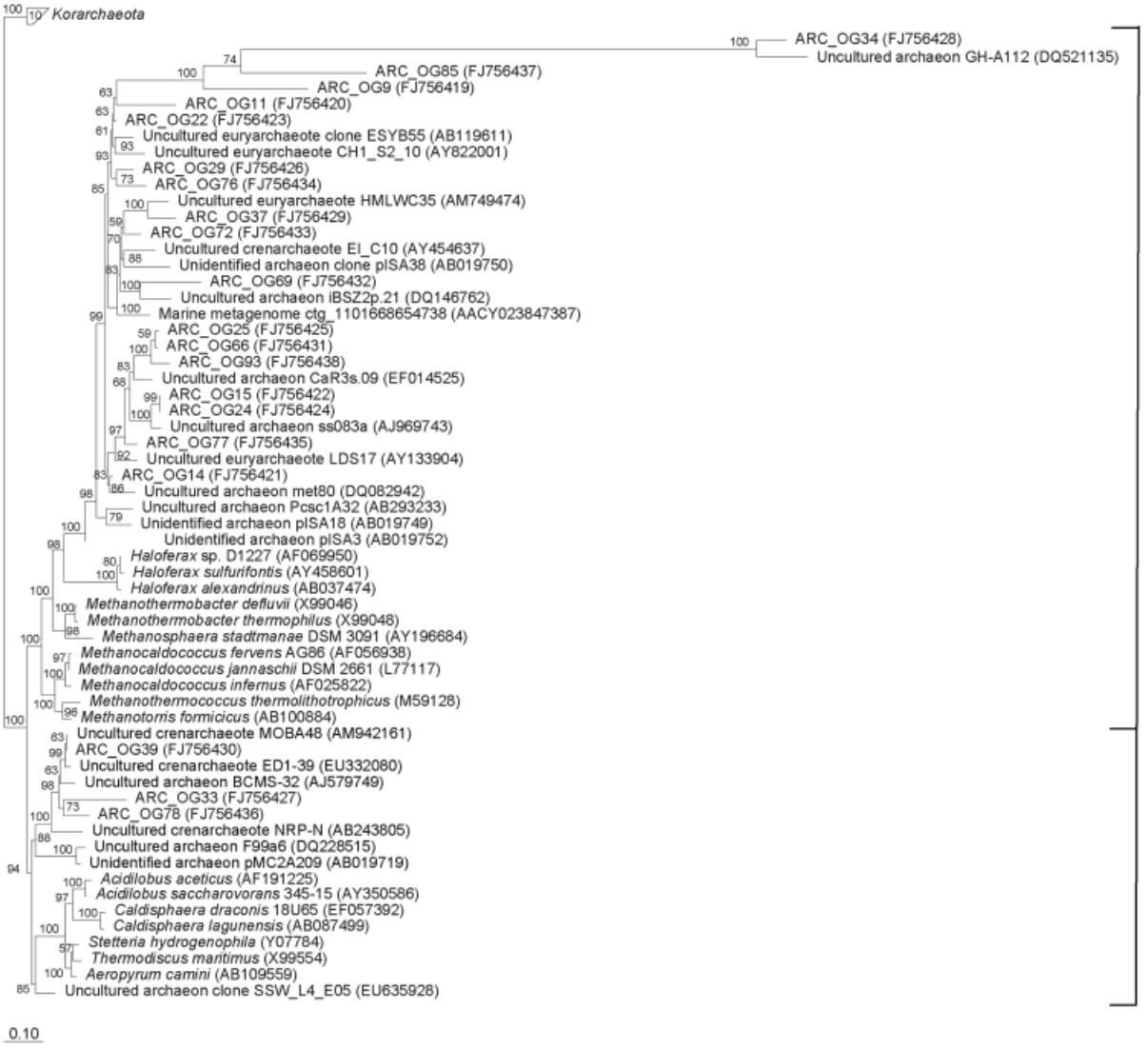

Fig. 9. Phylogenetic tree of *Crenarchaeota* and *Euryarchaeota*. GenBank accession numbers are given in parentheses. Bootstrap values above 50% are displayed.

2002; Glud & Middelboe, 2004; Nussbaumer *et al.*, 2004; Moens *et al.*, 2005; Standing *et al.*, 2006; Prast *et al.*, 2007). These biotic factors might also contribute considerably to the variation in the microbial communities.

In this study, most bacterial sequences were related to members of the *Gammaproteobacteria* and members of the *Fibrobacteres*/*Acidobacteria* group. Also, two members of the *Deltaproteobacteria*, and one member of the *Flavobacteria* and the *Actinobacteria* were detected. This general composition of the sedimentary bacterial community is confirmed by other studies (Llobet-Brossa *et al.*, 1998; Ravenschlag *et al.*, 1999; Urakawa *et al.*, 1999; Bowman *et al.*, 2003; Mußmann *et al.*, 2005; Stevens *et al.*, 2005; Musat *et al.*, 2006; Liang *et al.*, 2007). Generally, most phylotypes belonging to the *Acidobacteria* clustered with sequences retrieved from marine sequences. In detail, the phylotype BAC_OG70 clustered with the sequence VHS-B3-89 (Zhang *et al.*, 2008) retrieved from harbour sediment. Within the *Acidobacteria*, some phylotypes inter alia BAC_OG88 and BAC_OG87 formed a group clustering with the clone 5-38 (DQ833472) found in sediment in Asia. Another cluster was formed with the marine sponge isolate 1M83 (Jiang *et al.*, 2007) and the phylotypes BAC_OG161, BAC_OG123 and BAC_OG15. Within the *Acidobacteria*, phylotypes displayed some relatedness to *Holophaga foetida* (X77215, Liesack *et al.*, 1994), a homoacetogenic bacterium isolated from freshwater sediment. Two phylotypes namely BAC_OG47 and BAC_OG36 clustered with the anaerobic dehalogenating Deltaproteobacterium *Desulfomonile limimaris* (AF282177, Sun *et al.*, 2001). Within the *Gammaproteobacteria*, the phylotype

© 2010 CEFAS
Journal compilation © 2010 Federation of European Microbiological Societies.
Published by Blackwell Publishing Ltd.

Downloaded from https://academic.oup.com/femsec/article-abstract/74/2/410/589506 by University of Zurich user on 11 December 2019

Table 5. Relatedness of archaeal phylotypes to known organisms

DGGE band class	DGGE band	Phylogenetic group	Closest relative	Similarity (%)	Base positions compared	Accession number of closest relative
I	ARC_OG_37	*Euryarchaeota*	Uncultured euryarchaeote HMLWC35	86	504	AM749474
II	ARC_OG_69	*Euryarchaeota*	Uncultured archaeon iBSZ2p.21	95	442	DQ146762
III	ARC_OG_11	*Euryarchaeota*	Uncultured euryarchaeote, ESYB55	83	480	AB119611
IV	ARC_OG_29	*Euryarchaeota*	Uncultured archaeon GH-A112	87	423	DQ521135
VI	ARC_OG_72	*Euryarchaeota*	Uncultured crenarchaeote EI_C10	89	498	AY454637
VII	ARC_OG_85	*Euryarchaeota*	Uncultured archaeon oBS65f.72	86	489	DQ146745
VIII	ARC_OG_22	*Euryarchaeota*	Uncultured archaeon GH-A112	90	505	DQ521135
X	ARC_OG_9	*Euryarchaeota*	Uncultured archaeon GH-A112	92	518	DQ521135
XI	ARC_OG_34	*Euryarchaeota*	Uncultured euryarchaeote LDS17	90	529	AY133904
XII	ARC_OG_76	*Euryarchaeota*	Uncultured euryarchaeote LDS17	85	477	AY133904
XIV	ARC_OG_14	*Euryarchaeota*	Uncultured archaeon met80	92	553	DQ082942
XV	ARC_OG_15	*Euryarchaeota*	Uncultured archaeon, ss083a	94	555	AJ969743
XVI	ARC_OG_24	*Euryarchaeota*	Uncultured archaeon, ss083a	94	546	AJ969743
XVIII	ARC_OG_77	*Euryarchaeota*	Uncultured archaeon CaR3s.09	92	533	EF014525
XIX	ARC_OG_93	*Euryarchaeota*	Uncultured archaeon CaR3s.09	88	475	EF014525
XX	ARC_OG_25	*Euryarchaeota*	Uncultured archaeon CaR3s.09	91	515	EF014525
XXI	ARC_OG_66	*Euryarchaeota*	Uncultured archaeon CaR3s.09	92	546	EF014525
XXII	ARC_OG_78	*Crenarchaeota*	Uncultured archaeon, BCMS-32	87	496	AJ579749
XXIII	ARC_OG_33	*Crenarchaeota*	Uncultured crenarchaeote MOBA48	95	481	AM942161
XXIV	ARC_OG_39	*Crenarchaeota*	Uncultured crenarchaeote ED1-39	99	534	EU332080

BAC_OG38 clustered with the sequence ELB16-159 (DQ015812) found in Antarctic lake water. It formed a larger cluster with a group of sequences containing BAC_OG40 and the purple sulphur *Gammaproteobacteria Marichromatium indicum* JA100 (Arunasri *et al.*, 2005) and *Thiobaca trueperi* BCH (Rees *et al.*, 2002). Additionally, the phylotypes BAC_OG34 and BAC_OG9 clustered with sequences retrieved from seafloor lavas (EU491433, Santelli *et al.*, 2008) or sediment (DQ351793, Gillan & Pernet, 2007), respectively. With these, the phylotypes BAC_OG114, BAC_OG24 and BAC_OG113 clustered distantly, whereas the phylotype BAC_OG75 was linked to the sequence EPR3968-O8a-Bc3 (EU491699, Santelli *et al.*, 2008) retrieved from seafloor lavas. Additionally, the phylotype BAC_OG90 clustered with *Actinobacteria*, especially with the sequences ctg_BRRAA49 (DQ395394) found in a deep-sea coral and wb1_J07 (AF317767, Holmes *et al.*, 2001) retrieved from Australian caves. The phylotype BAC_OG1 clustered with members of the *Bacteroidetes* isolated from seawater or sediment namely *Lutimonas* sp. MOLA 323 (Ribalet *et al.*, 2008) and *Actibacter sediminis* JC2129 (Kim *et al.*, 2008). Overall, the bacterial sequences detected in this study seemed to largely represent benthic bacterial phylotypes.

The archaeal phylotypes retrieved in this study belonged mainly to the *Euryarchaeota*. Generally, the dominance of *Euryarcheota* in marine sediments is supported by other authors (Munson *et al.*, 1997; Purdy *et al.*, 2002; Parkes *et al.*, 2007). Within this phylum, phylotypes recorded in the Oyster Ground have been found in many other environments for example the phylotypes ARC_OG25, ARC_OG66 as well as ARC_OG93 clustered with the sequence CaR3s.09 found in Arctic freshwater (Galand *et al.*, 2008). The phylotypes ARC_OG77, ARC_OG15 and ARC_OG24 appeared to be closely related, the latter clustering with the archaeal sequence ss083a extracted from a soil salinity gradient (Walsh *et al.*, 2005). Furthermore, the phylotype ARC_OG14 clustered with the sequence met80 retrieved from polychaete tubes in a hydrothermal vent (Moussard *et al.*, 2006). Within the *Euryarchaeota*, some phylotypes clustered together inter alia ARC_OG29 and ARC_OG76, but could not be directly related to sequences in the ARB database, whereas the phylotypes ARC_OG37 and ARC_OG72 clustered with the sequence HMLWC35, found in freshwater archaeoplankton (Auguet & Casamayor, 2008). Furthermore, the sequence GH-A112 retrieved from Arctic sediment (Perreault *et al.*, 2007) clustered with ARC_OG34, which was also distantly related to ARC_OG85, ARC_OG9, ARC_OG11 and ARC_OG22 clustering with the sequence ESYB55 (Kaku *et al.*, 2005) retrieved from estuarine sediment. Additionally, the phylotype ARC_OG69 clustered with the sequence IBSZ2p.21 found in the coastal Arctic Ocean (Galand *et al.*, 2006). We could also detect three phylotypes belonging to the *Crenarchaeota*. Here, the phylotype ARC_OG39 clustered with the sequence ED1-39 found in the surface layer of marine sediment (Park *et al.*, 2008), whereas the phylotypes ARC_OG78 and ARC_OG33 showed some clustering with the sequence NRP-N retrieved from rice paddy fields (Sakai *et al.*, 2007).

Downloaded from https://academic.oup.com/femsec/article-abstract/74/2/410/589506 by University of Zurich user on 11 December 2019

© 2010 CEFAS
Journal compilation © 2010 Federation of European Microbiological Societies.
Published by Blackwell Publishing Ltd.

Conclusion

Generally, our study provides valuable and novel insights into the composition, spatial variation and environmental control of the abundant microbial phylotypes at the Oyster Ground. We detected a fairly high variability for bacterial and archaeal communities within a 100 m station ring and could not find a clear separation of bacterial or archaeal communities between different stations within the Oyster Ground, although the analysis of the bacterial community based on single phylotypes revealed spatial differences for certain stations.

Because of the high variability between replicates, it is essential for future studies to look at spatial scaling of benthic microbial communities with a representative level of replication at various scales including small scales from millimetres to kilometres. Furthermore, we could show that sedimentary bacteria and archaea display different environmental adaptations to the respective habitat as the archaeal communities showed strong correlations with small-scale sediment characteristics, which was not the case for bacterial communities.

Acknowledgements

The officers and crew of RV 'Endeavour' kindly assisted in the collection of samples. We would like to thank Karema Warr, Helen Lees, Veronique Créach, Jim R. Ellis, Vanessa Stelzenmueller and Clare Mason for their support. We are grateful for lab assistance by Freya Goodsir. This initiative was financed by Cefas Research and Development funding (research project DP210). We are indebted to the reviewers providing helpful suggestions for the manuscript. Sampling was supported by the Department for Environment, Food and Rural Affairs (research project ME3205). L.R.T. was jointly supported by a University of Aberdeen 6th century scholarship and Cefas Research and development funding (research project DP204). The authors are grateful for support by the EU-Network MARBEF, which is funded in the Community's Sixth Framework Program (contract no. GOCE-CT-2003-505446).

References

Alkemade R, Wielemaker A, De Jong SA & Sandee AJJ (1992) Experimental evidence for the role of bioturbation by the marine nematode *Diplolaimella dievengatensis* in stimulating the mineralization of *Spartina anglica* detritus. *Mar Ecol-Prog Ser* **90**: 149–155.

Aller JY & Kemp PF (2008) Are Archaea inherently less diverse than Bacteria in the same environments? *FEMS Microbiol Ecol* **65**: 74–87.

Aller R (1982) The effects of macrobenthos on chemical properties of marine sediments and overlying water. *Animal-Sediment Relations, the Biogenic Alteration of Sediments* (Call P & Tevesz M, eds), pp. 53–102. Plenum Press, New York.

Aller R (1988) Benthic fauna and biogeochemical processes in marine sediments: the role of burrowing structures. *Nitrogen Cycling in Coastal Marine Environments* (Blackburn T & Sørensen J, eds), pp. 301–338. John Wiley & Sons, Hoboken, NJ.

Aller RC (1990) Bioturbation and manganese cycling in Hemipelagic sediments. *Philos T R Soc A* **331**: 51–68.

Arunasri K, Sasikala C, Ramana CV, Suling J & Imhoff JF (2005) *Marichromatium indicum* sp. nov., a novel purple sulfur gammaproteobacterium from mangrove soil of Goa, India. *Int J Syst Evol Micr* **55**: 673–679.

Asami H, Aida M & Watanabe K (2005) Accelerated sulfur cycle in coastal marine sediment beneath areas of intensive shellfish aquaculture. *Appl Environ Microb* **71**: 2925–2933.

Auguet JC & Casamayor EO (2008) A hotspot for cold Crenarchaeota in the neuston of high mountain lakes. *Environ Microbiol* **10**: 1080–1086.

Baas-Becking LGM (1934) *Geobiologie of inleiding tot de milieukunde*. Van Stokkum & Zoon, The Hague.

Berner RA (1971) *Principles of Chemical Sedimentology*. McGraw-Hill Companies, New York.

Bidle KD & Azam F (2001) Bacterial control of silicon regeneration from diatom detritus: significance of bacterial ectohydrolases and species identity. *Limnol Oceanogr* **46**: 1606–1623.

Bowman JP, McCammon SA, Gibson JAE, Robertson L & Nichols PD (2003) Prokaryotic metabolic activity and community structure in Antarctic continental shelf sediments. *Appl Environ Microb* **69**: 2448–2462.

Brion N, Baeyens W, De Galan S, Elskens M & Laane R (2004) The North Sea: source or sink for nitrogen and phosphorus to the Atlantic Ocean? *Biogeochemistry* **68**: 277–296.

Casamayor EO, Schäfer H, Baòeras L, Pedrós-Alió C & Muyzer G (2000) Identification of and spatio-temporal differences between microbial assemblages from two neighboring sulfurous lakes: comparison by microscopy and denaturing gradient gel electrophoresis. *Appl Environ Microb* **66**: 499–508.

Casamayor EO, Muyzer G & Pedrós-Alió C (2001) Composition and temporal dynamics of planktonic archaeal assemblages from anaerobic sulfurous environments studied by 16S rDNA denaturing gradient gel electrophoresis and sequencing. *Aquat Microb Ecol* **25**: 237–246.

Dell'Anno A & Corinaldesi C (2004) Degradation and turnover of extracellular DNA in marine sediments: ecological and methodological considerations. *Appl Environ Microb* **70**: 4384–4386.

Dyer K (1986) *Coastal and Estuarine Sediment Dynamics*. John Wiley and Sons, Chichester, UK.

Felsenstein J (1993) *PHYLIP (Phylogeny Inference Package), Version 3.5c*. Department of Genetics, University of Washington, Seattle, WA.

Forster S & Graf G (1992) Continuously measured changes in redox potential influenced by oxygen penetrating from

© 2010 CEFAS
Journal compilation © 2010 Federation of European Microbiological Societies.
Published by Blackwell Publishing Ltd.

Downloaded from https://academic.oup.com/femsec/article-abstract/74/2/410/589506 by University of Zurich user on 11 December 2019

burrows of *Callianassa subterranea*. *Hydrobiologia* **235–236**: 527–532.

Galand PE, Lovejoy C & Vincent WF (2006) Remarkably diverse and contrasting archaeal communities in a large arctic river and the coastal Arctic Ocean. *Aquat Microb Ecol* **44**: 115–126.

Galand PE, Lovejoy C, Pouliot J, Garneau ME & Vincent WF (2008) Microbial community diversity and heterotrophic production in a coastal Arctic ecosystem: a stamukhi lake and its source waters. *Limnol Oceanogr* **53**: 813–823.

Gillan DC & Pernet P (2007) Adherent bacteria in heavy metal contaminated marine sediments. *Biofouling* **23**: 1–13.

Glud RN & Middelboe M (2004) Virus and bacteria dynamics of a coastal sediment: implication for benthic carbon cycling. *Limnol Oceanogr* **49**: 2073–2081.

Green J & Bohannan BJM (2006) Spatial scaling of microbial biodiversity. *Trends Ecol Evol* **21**: 501–507.

Hedges J & Stern J (1984) Carbon and nitrogen determinations of carbonate-containing solids. *Limnol Oceanogr* **28**: 657–663.

Hewson I, Jacobson-Meyers ME & Fuhrman JA (2007) Diversity and biogeography of bacterial assemblages in surface sediments across the San Pedro Basin, Southern California Borderlands. *Environ Microbiol* **9**: 923–933.

Hietanen S, Laine AO & Lukkari K (2007) The complex effects of the invasive polychaetes *Marenzelleria* spp. on benthic nutrient dynamics. *J Exp Mar Biol Ecol* **352**: 89–102.

Holmes A, Tujula N, Holley M, Contos A, James J, Rogers P & Gillings R (2001) Phylogenetic structure of unusual aquatic microbial formations in Nullarbor caves, Australia. *Environ Microbiol* **3**: 256–264.

Horner-Devine MC, Leibold MA, Smith VH & Bohannan BJM (2003) Bacterial diversity patterns along a gradient of primary productivity. *Ecol Lett* **6**: 613–622.

Horner-Devine MC, Carney KM & Bohannan BJM (2004a) An ecological perspective on bacterial biodiversity. *P Roy Soc Lond B Bio* **271**: 113–122.

Horner-Devine MC, Lage M, Hughes JB & Bohannan BJM (2004b) A taxa–area relationship for bacteria. *Nature* **432**: 750–753.

Hoschitz M, Buchholz TG & Ott JA (1999) *Leptonemella juliae* sp.n. and *Leptonemella vestari* sp.n. (*Stilbonematinae*), two new free-living marine nematodes from a subtidal sand bottom. *Ann Naturhist Mus Wien* **101 B**: 423–435.

Howe RL, Rees AP & Widdicombe S (2004) The impact of two species of bioturbating shrimp (*Callianassa subterranea* and *Upogebia deltaura*) on sediment denitrification. *J Mar Biol Assoc UK* **84**: 629–632.

Jackson CR & Weeks AQ (2008) Influence of particle size on bacterial community structure in aquatic sediments as revealed by 16S rRNA gene sequence analysis. *Appl Environ Microb* **74**: 5237–5240.

Jensen P (1996) Burrows of marine nematodes as centres for microbial growth. *Nematologica* **42**: 320–329.

Jiang S, Sun W, Chen M *et al.* (2007) Diversity of culturable actinobacteria isolated from marine sponge *Haliclona* sp. *Antonie van Leeuwenhoek* **92**: 405–416.

Kaku N, Ueki A, Ueki K & Watanabe K (2005) Methanogenesis as an important terminal electron accepting process in estuarine sediment at the mouth of Orikasa river. *Microbes Environ* **20**: 41–52.

Kim J-H, Kim K-Y, Hahm Y-T, Kim B-S, Chun J & Cha C-J (2008) *Actibacter sediminis* gen. nov., sp. nov., a marine bacterium of the family *Flavobacteriaceae* isolated from tidal flat sediment. *Int J Syst Evol Micr* **58**: 139–143.

Kirkwood D (1996) Nutrients: practical notes on their determination in seawater. *ICES Techniques in Marine Environmental Sciences No. 17*. International Council for the Exploration of the Seas, Copenhagen.

Liang JB, Chen YQ, Lan CY, Tam NFY, Zan QJ & Huang LN (2007) Recovery of novel bacterial diversity from mangrove sediment. *Mar Biol* **150**: 739–747.

Liesack W, Bak F, Kreft J-U & Stackebrandt E (1994) *Holophaga foetida* gen. nov., sp. nov., a new, homoacetogenic bacterium degrading methoxylated aromatic compounds. *Arch Microbiol* **162**: 85–90.

Liu X, Tiquia SM, Holguin G *et al.* (2003) Molecular diversity of denitrifying genes in continental margin sediments within the oxygen-deficient zone off the Pacific coast of Mexico. *Appl Environ Microb* **69**: 3549–3560.

Llobet-Brossa E, Rosselló-Mora R & Amann R (1998) Microbial community composition of Wadden sea sediments as revealed by fluorescence *in situ* hybridization. *Appl Environ Microb* **64**: 2691–2696.

Lohrer AM, Thrush SF & Gibbs MM (2004) Bioturbators enhance ecosystem function through complex biogeochemical interactions. *Nature* **431**: 1092–1095.

Ludwig W, Strunk O, Westram R *et al.* (2004) A software environment for sequence data. *Nucleic Acids Res* **32**: 1363–1371.

Luna GM, Manini E & Danovaro R (2002) Large fraction of dead and inactive bacteria in coastal marine sediments: comparison of protocols for determination and ecological significance. *Appl Environ Microb* **68**: 3509–3513.

Madigan MT, Martinko JM & Parker J (2003) Mikrobielle Oekologie. *Brock Mikrobiologie*, *Vol. 9* (Goebel W, ed), pp. 763–767. Spektrum Akademischer Verlag GmbH, Berlin.

Martiny JBH, Bohannan BJM, Brown JH *et al.* (2006) Microbial biogeography: putting microorganisms on the map. *Nat Rev Microbiol* **4**: 102–112.

Méndez N, Linke-Gamenick I, Forbes VE & Baird DJ (2001) Sediment processing in *Capitella* spp. (Polychaeta: *Capitellidae*): strain-specific differences and effects of the organic toxicant fluoranthene. *Mar Biol* **138**: 311–319.

Moens T, Dos Santos GAP, Thompson F, Swings J, Fonsêca-Genevois V, Vincx M & De Mesel I (2005) Do nematode mucus secretions affect bacterial growth? *Aquat Microb Ecol* **40**: 77–83.

Moussard H, Moreira D, Cambon-Bonavita MA, López-García P & Jeanthon C (2006) Uncultured Archaea in a hydrothermal microbial assemblage: phylogenetic diversity and

© 2010 CEFAS
Journal compilation © 2010 Federation of European Microbiological Societies.
Published by Blackwell Publishing Ltd.

Downloaded from https://academic.oup.com/femsec/article-abstract/74/2/410/589506 by University of Zurich user on 11 December 2019

characterization of a genome fragment from a euryarchaeote. *FEMS Microbiol Ecol* **57**: 452–469.

Mußmann M, Ishii K, Rabus R & Amann R (2005) Diversity and vertical distribution of cultured and uncultured *Deltaproteobacteria* in an intertidal mud flat of the Wadden Sea. *Environ Microbiol* **7**: 405–418.

Munson MA, Nedwell DB & Embley TM (1997) Phylogenetic diversity of Archaea in sediment samples from a coastal salt marsh. *Appl Environ Microb* **63**: 4729–4733.

Musat N, Werner U, Knittel K *et al.* (2006) Microbial community structure of sandy intertidal sediments in the North Sea, Sylt-Rømø Basin, Wadden Sea. *Syst Appl Microbiol* **29**: 333–348.

Muylaert K, Van der Gucht K, Vloemans N, De Meester L, Gillis M & Vyverman W (2002) Relationship between bacterial community composition and bottom-up versus top-down variables in four eutrophic shallow lakes. *Appl Environ Microb* **68**: 4740–4750.

Nussbaumer AD, Bright M, Baranyi C, Beisser CJ & Ott JA (2004) Attachment mechanism in a highly specific association between ectosymbiotic bacteria and marine nematodes. *Aquat Microb Ecol* **34**: 239–246.

Paerl HW & Steppe TF (2003) Scaling up: the next challenge in environmental microbiology. *Environ Microbiol* **5**: 1025–1038.

Park SJ, Park BJ & Rhee SK (2008) Comparative analysis of archaeal 16S rRNA and amoA genes to estimate the abundance and diversity of ammonia-oxidizing archaea in marine sediments. *Extremophiles* **12**: 605–615.

Parkes RJ, Cragg BA, Banning N *et al.* (2007) Biogeochemistry and biodiversity of methane cycling in subsurface marine sediments (Skagerrak, Denmark). *Environ Microbiol* **9**: 1146–1161.

Paul JH, Jeffrey WH & DeFlaun MF (1987) Dynamics of extracellular DNA in the marine environment. *Appl Environ Microb* **53**: 170–179.

Perreault NN, Andersen DT, Pollard WH, Greer CW & Whyte LG (2007) Characterization of the prokaryotic diversity in cold saline perennial springs of the Canadian high arctic. *Appl Environ Microb* **73**: 1532–1543.

Pischedda L, Poggiale JC, Cuny P & Gilbert F (2008) Imaging oxygen distribution in marine sediments. The importance of bioturbation and sediment heterogeneity. *Acta Biotheor* **56**: 123–135.

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. *Microb Ecol* **49**: 367–378.

Pommier T, Canback B, Riemann L *et al.* (2007) Global patterns of diversity and community structure in marine bacterioplankton. *Mol Ecol* **16**: 867–880.

Prast M, Bischoff AA, Waller U, Amann R & Berninger UG (2007) Effect of ciliates on nitrification and nitrifying bacteria in Baltic Sea sediments. *Mar Ecol-Prog Ser* **350**: 55–61.

Pruesse E, Quast C, Knittel K, Fuchs BM, Ludwig W, Peplies J & Glockner FO (2007) SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res* **35**: 7188–7196.

Purdy KJ, Munson MA, Nedwell DB & Embley TM (2002) Comparison of the molecular diversity of the methanogenic community at the brackish and marine ends of a UK estuary. *FEMS Microbiol Ecol* **39**: 17–21.

Quéric NV & Soltwedel T (2007) Impact of small-scale biogenic sediment structures on bacterial distribution and activity in Arctic deep-sea sediments. *Mar Ecol* **28**: 66–74.

Quintana CO, Tang M & Kristensen E (2007) Simultaneous study of particle reworking, irrigation transport and reaction rates in sediment bioturbated by the polychaetes *Heteromastus* and *Marenzelleria*. *J Exp Mar Biol Ecol* **352**: 392–406.

Rabouille C, Denis L, Dedieu K, Stora G, Lansard B & Grenz C (2003) Oxygen demand in coastal marine sediments: comparing *in situ* microelectrodes and laboratory core incubations. *J Exp Mar Biol Ecol* **285–286**: 49–69.

Ramette A & Tiedje JM (2007) Biogeography: an emerging cornerstone for understanding prokaryotic diversity, ecology, and evolution. *Microb Ecol* **53**: 197–207.

Raskin L, Stromley JM, Rittmann BE & Stahl DA (1994) Group-specific 16S rRNA hybridization probes to describe natural communities of methanogens. *Appl Environ Microb* **60**: 1232–1240.

Ravenschlag K, Sahm K, Pernthaler J & Amann R (1999) High bacterial diversity in permanently cold marine sediments. *Appl Environ Microb* **65**: 3982–3989.

Rees GN, Harfoot CG, Janssen PH, Schoenborn L, Kuever J & Lunsdorf H (2002) *Thiobaca trueperi* gen. nov., sp. nov., a phototrophic purple sulfur bacterium isolated from freshwater lake sediment. *Int J Syst Evol Micr* **52**: 671–678.

Revsbech N (1989) An oxygen microsensor with a guard cathode. *Limnol Oceanogr* **34**: 474–478.

Revsbech NP, Sørensen J, Blackburn TH & Lomholt JP (1980) Distribution of oxygen in marine sediments measured with microelectrodes. *Limnol Oceanogr* **25**: 403–411.

Ribalet F, Intertaglia L, Lebaron P & Casotti R (2008) Differential effect of three polyunsaturated aldehydes on marine bacterial isolates. *Aquat Toxicol* **86**: 249–255.

Riemann F & Helmke E (2002) Symbiotic relations of sediment-agglutinating nematodes and bacteria in detrital habitats: the enzyme-sharing concept. *Mar Ecol* **23**: 93–113.

Sakai S, Imachi H, Sekiguchi Y, Ohashi A, Harada H & Kamagata Y (2007) Isolation of key methanogens for global methane emission from rice paddy fields: a novel isolate affiliated with the clone cluster rice cluster. *Appl Environ Microb* **73**: 4326–4331.

Santelli CM, Orcutt BN, Banning E *et al.* (2008) Abundance and diversity of microbial life in ocean crust. *Nature* **453**: 653–656.

Santmire JA & Leff LG (2007) The influence of stream sediment particle size on bacterial abundance and community composition. *Aquat Ecol* **41**: 153–160.

Sapp M, Schwaderer AS, Wiltshire KH, Hoppe H-G, Gerdts G & Wichels A (2007a) Species-specific bacterial communities in the phycosphere of microalgae? *Microb Ecol* **53**: 683–699.

Downloaded from https://academic.oup.com/femsec/article-abstract/74/2/410/589506 by University of Zurich user on 11 December 2019

© 2010 CEFAS
Journal compilation © 2010 Federation of European Microbiological Societies.
Published by Blackwell Publishing Ltd.

Sapp M, Wichels A, Wiltshire KH & Gerdts G (2007b) Bacterial community dynamics during the winter–spring transition in the North Sea. *FEMS Microbiol Ecol* **59**: 622–637.

Scala DJ & Kerkhof LJ (2000) Horizontal heterogeneity of denitrifying bacterial communities in marine sediments by terminal restriction fragment length polymorphism analysis. *Appl Environ Microb* **66**: 1980–1986.

Schauer M, Massana R & Pedrós-Alió C (2000) Spatial differences in bacterioplankton composition along the Catalan coast (NW Mediterranean) assessed by molecular fingerprinting. *FEMS Microbiol Ecol* **33**: 51–59.

Seitzinger S & Giblin A (1996) Estimating denitrification in North Atlantic continental shelf sediments. *Biogeochemistry* **35**: 235–260.

Sigler WV, Miniaci C & Zeyer J (2004) Electrophoresis time impacts the denaturing gradient gel electrophoresis-based assessment of bacterial community structure. *J Microbiol Meth* **57**: 17–22.

Sivyer D (1999) Nitrogen cycling in intertidal sediments of the Wash, UK. Research Thesis, University of East Anglia, Norwich.

Snelgrove PVR (1999) Getting to the bottom of marine biodiversity: sedimentary habitats: ocean bottoms are the most widespread habitat on earth and support high biodiversity and key ecosystem services. *BioScience* **49**: 129–130.

Solan M & Kennedy R (2002) Observation and quantification of *in situ* animal-sediment relations using time-lapse sediment profile imagery (t-SPI). *Mar Ecol-Prog Ser* **228**: 179–191.

Sørensen KB, Glazer B, Hannides A & Gaidos E (2007) Spatial structure of the microbial community in sandy carbonate sediment. *Mar Ecol-Prog Ser* **346**: 61–74.

Staley JT & Gosink JJ (1999) Poles apart: biodiversity and biogeography of sea ice bacteria. *Annu Rev Microbiol* **53**: 189–215.

Standing D, Knox OGG, Mullins CE, Killham KK & Wilson MJ (2006) Influence of nematodes on resource utilization by bacteria – an *in vitro* study. *Microb Ecol* **52**: 444–450.

Stevens H, Brinkhoff T & Simon M (2005) Composition of free-living, aggregate-associated and sediment surface-associated bacterial communities in the German Wadden Sea. *Aquat Microb Ecol* **38**: 15–30.

Sun B, Cole JR & Tiedje JM (2001) *Desulfomonile limimaris* sp. nov., an anaerobic dehalogenating bacterium from marine sediments. *Int J Syst Evol Micr* **51**: 365–371.

Suzuki MT & Giovannoni SJ (1996) Bias caused by template annealing in the amplification of mixtures of 16S rRNA genes by PCR. *Appl Environ Microbiol* **62**: 625–630.

Teske A, Wawer C, Muyzer G & Ramsing NB (1996) Distribution of sulfate-reducing bacteria in a stratified Fjord (Mariager Fjord, Denmark) as evaluated by most-probable-number counts and denaturing gradient gel electrophoresis of PCR-amplified ribosomal DNA fragments. *Appl Environ Microb* **62**: 1405–1415.

Tett P (1987) Plankton. *Biological Surveys of Estuaries and Coasts* (Baker J & Wolff JE, eds), pp. 280–341. Cambridge University Press, Cambridge.

Urakawa H, Kita-Tsukamoto K & Ohwada K (1999) Microbial diversity in marine sediments from Sagami Bay and Tokyo Bay, Japan, as determined by 16S rRNA gene analysis. *Microbiology* **145**: 3305–3315.

von Wintzingerode F, Gobel U & Stackebrandt E (1997) Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. *FEMS Microbiol Rev* **21**: 213–229.

Vopel K, Thistle D & Rosenberg R (2003) Effect of the brittle star *Amphiura filiformis* (Amphiuridae, Echinodermata) on oxygen flux into the sediment. *Limnol Oceanogr* **48**: 2034–2045.

Walsh DA, Papke RT & Doolittle WF (2005) Archaeal diversity along a soil salinity gradient prone to disturbance. *Environ Microbiol* **7**: 1655–1666.

Wu L, Kellogg L, Devol AH, Tiedje JM & Zhou J (2008) Microarray-based characterization of microbial community functional structure and heterogeneity in marine sediments from the Gulf of Mexico. *Appl Environ Microb* **74**: 4516–4529.

Yannarell AC & Triplett EW (2005) Geographic and environmental sources of variation in lake bacterial community composition. *Appl Environ Microb* **71**: 227–239.

Zhang W, Ki JS & Qian PY (2008) Microbial diversity in polluted harbor sediments I: bacterial community assessment based on four clone libraries of 16S rDNA. *Estuar Coast Shelf S* **76**: 668–681.

Ziebis W, Forster S, Huettel M & Jørgensen BB (1996) Complex burrows of the mud shrimp *Callianassa truncata* and their geochemical impact in the sea bed. *Nature* **382**: 619–622.

Zuur A, Ieno E & Smith G (2007) *Analysing Ecological Data*. Springer, Berlin.

Downloaded from https://academic.oup.com/femsec/article-abstract/74/2/410/589506 by University of Zurich user on 11 December 2019

© 2010 CEFAS
Journal compilation © 2010 Federation of European Microbiological Societies.
Published by Blackwell Publishing Ltd.