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Permeability shapes bacterial communities in sublittoral surface sediments

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Summary

The first interaction of water column-derived organic matter with benthic microbial communities takes place in surface sediments which are acting as biological filters catalyzing central steps of elemental cycling. Here we analyzed the bacterial diversity and community structure of sediment top layers at seven sites in the North Sea where sediment properties ranged from coarse-grained and highly permeable to fine-grained and impermeable. Bacterial communities in surface sediments were richer, more even and significantly different from communities in bottom waters as revealed by Illumina tag sequencing of 16S rRNA genes. Sediment permeability had a clear influence on community composition which was confirmed by CARD-FISH. Sulfate-reducing *Desulfobacteraceae* (2–5% of total cells), *Flavobacteriaceae* (3–5%) were more abundant in impermeable than in highly permeable sediments where acidobacterial Sva0725 dominated (11–15%). Myxobacterial *Sandaracinaceae* were most abundant in medium permeable sediments (3–7%). *Woeseiaceae*/JTB255 and *Planctomycetes* were major groups in all sediments (4–6%, 8–22%). *Planctomycetes* were highly diverse and branched throughout the phylum. We propose *Planctomycetes* as key bacteria for degradation of high molecular weight compounds and recalcitrant material entering surface sediments from the water column. Benthic *Flavobacteriaceae* likely have restricted capabilities for macromolecule degradation and might profit with *Sandaracinaceae* and *Acidobacteria* from low molecular weight compounds.

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Introduction

In the oceans, about 20% of primary production takes places at continental shelves (Jahnke, 2010), making them a hot spot for global carbon cycling. The majority of sublittoral continental shelf sediments are permeable sands (Huettel *et al*., 2014). These allow for an advective transport of oxygen as well as particulate and dissolved organic matter into surface sediments (Huettel *et al*., 1996; Huettel *et al*., 2007; Chipman *et al*., 2012), where the organic matter is readily oxidized by the heterotrophic benthic community. Thus, planktonic primary production is tightly linked to benthic remineralization. Moreover, the hydrodynamic forcing of porewater advection leads to transient redox conditions in the upper layers of permeable sediments. In impermeable sediments advection is of minor relevance and mainly observed in the presence of local bioirrigation or bioturbation. Instead, they are largely diffusion-limited and exhibit rather stable redox conditions. They are less efficiently supplied with water column-derived oxygen and organic matter.

Strong phytoplankton blooms in the North Sea provide large amounts of photosynthetically fixed organic carbon to the microbial community (Boon *et al*., 1998). Part of the algal biomass is rapidly degraded in the water column by distinct taxa of *Flavobacteriia* (*Formosa*, *Polaribacter*, NS3a marine group, *Tenacibaculum* and *Ulvibacter*) and *Gammaproteobacteria* (clade SAR92, *Reinekea*), that possess specialized carbohydrate active enzymes (CAZymes; Teeling *et al*., 2012; 2016). Nevertheless, due to the shallow water depth of the North Sea (most parts $<$100 m), 15% to 50% of organic carbon produced by primary production in surface waters can evade this planktonic degradation and reaches surface sediments (Joiris *et al*., 1982; Jørgensen *et al*., 1990; Wollast, 1998). While the diversity and function of the planktonic microbial community is well studied, still little is known about benthic microbial communities in coastal surface sediments. Benthic microbial communities are more diverse than those in the overlaying water column (Zinger *et al*., 2011), harbor anaerobic taxa (Gobet *et al*., 2012) and are nearly exclusively found attached to sediment grain

surfaces (Rusch *et al*., 2003). Only few studies investigated the bacterial community in the top few centimeters of sublittoral surface shelf sediments by next generation sequencing of 16S rRNA genes (Newton *et al*., 2013; Tait *et al*., 2015; O'Reilly *et al*., 2016). Gammaproteobacterial family *Woeseiaceae*/JTB255 marine benthic group (Du et al., 2016; Mußmann et al., 2017), *Planctomycetaceae*, *Bacteroidetes* families *Saprospiraceae* and *Flavobacteriaceae*, alphaproteobacterial family *Rhodobacteraceae* and order *Rhodospirillales* and *Firmicutes* family *Clostridiaceae* were particularly abundant. Interestingly, in productive regions abundance of benthic flavobacterial 16S rRNA genes were positively correlated with grain size (i.e. permeability), potentially reflecting the dominant aerobic organoheterotrophic lifestyle known for planktonic *Flavobacteriales* (O'Reilly *et al*., 2016).

The understanding of abundant bacterial clades and their distribution pattern in surface shelf sediments of different permeability is still sparse, in particular at high phylogenetic and vertical resolution. This study, aims at the identification, localization and quantification of dominant bacterial clades in sublittoral surface sediments in the North Sea. The following questions were addressed: (1) How similar are bacterial communities in oxygenated surface sediments and the overlaying bottom water? (2) Does the uppermost sediment layer exhibit a microbial community different to underlying sediment layers? (3) Do diversity and community composition differ between sediments of different permeability? (4) Are there seasonal changes in benthic bacterial community composition, and, if yes, do these have a greater influence than sediment permeability? To address these questions, we examined North Sea sediments of different permeability at high vertical resolution considering oxygen penetration depths and redox conditions (Ahmerkamp, 2016). Dominant bacterial taxa were identified by tag sequencing of 16S rRNA genes and quantified by catalyzed reporter deposition fluorescence in situ hybridization (CARD-FISH) using newly designed and established oligonucleotide probes.

Results

Bacterial diversity was studied in sublittoral surface sediments (0–2 cm depth sampled in September 2014 in 0.5 to 1 cm intervals) and corresponding bottom waters from seven different sites in the North Sea (Fig. 1). Based on sediment median grain size and permeability (Supporting Information Table S1), sites were assigned to three classes, i.e. coarse-grained sediments with high permeability (site CCP-D and CCP-G), medium-grained sediments with medium permeability (sites NOAH-B, NOAH-E and NOAH-I) and sediments with fine grain size which are impermeable (sites NOAH-H and CCP-J). Using the Illumina platform for sequencing of 16S rRNA gene fragments, a total of 918,493 high quality reads with a median length of 460 bp passed our quality routine (Supporting Information Table S2). On species level ($OTU_{0.97}$), total bacterial richness in North Sea sediments was $7{,}026 \pm 3{,}697$ as estimated by Chao1 (Table1).

Vertical structuring of the bacterial community

To resolve fine scale vertical structuring of the bacterial community, we compared the largely oxygenated uppermost sediment depth layers (0–1 cm for permeable and 0–0.5 cm for impermeable sites) with the subjacent sediment depth layers (1–2 cm and 0.5–2 cm respectively). Bacterial richness ($Chao1_{0.97}$: $5{,}267 \pm 3{,}407$ and $7{,}494 \pm 3{,}995$, Table 1) and evenness (inverse $Simpson_{0.97}$: 169 ± 46 and 180 ± 53) were similar for uppermost and subjacent sediment depth layers. Similar bacterial alpha diversity in different sediment depth layers was further supported by beta diversity analysis (ANOSIM, $R = -0.06$, $p = 0.74$). A negative R value indicated that dissimilarity was greater between different sites than between different depth layers of a specific site. Hierarchical clustering of individual sediment samples based on Bray-Curtis dissimilarity ($OTU_{0.97}$) supported these findings. Samples clustered according to sampling sites (local clustering) instead of depth layers (global clustering; Fig. 2).

Influence of sediment permeability on bacterial diversity

Local bacterial richness at highly permeable sites CCP-D and CCP-G ($Chao1_{0.97}$: $3{,}503 \pm 340$) was comparable to richness in medium permeable sites NOAH-E and NOAH-B ($Chao1_{0.97}$: $3{,}320 \pm 594$). However, richness at the third medium permeable site NOAH-I ($Chao1_{0.97}$: $11{,}290 \pm 847$) as well as impermeable sites NOAH-H and CCP-J ($Chao1_{0.97}$: $8{,}679 \pm 3{,}554$) was distinctly higher. Hierarchical clustering of sediment samples based on Bray Curtis dissimilarity matrix ($OTU_{0.97}$) resulted in three major clusters (Fig. 2). The first cluster comprised all samples from impermeable sediments, the second cluster comprised all samples for medium permeable sediments and the third cluster comprised all samples from highly permeable sediments. The evenness in samples of different permeability was in the same range (inverse $Simpson_{0.97}$: 187 ± 84, 148 ± 51 and 191 ± 21, respectively). The communities within these clusters were significantly different (ANOSIM, $R = 0.76$–1, $p < 0.03$) indicating a strong influence of permeability on the bacterial community structure.

Bacterial diversity in top sediment layers and bottom waters

Bacterial communities in uppermost sediments (0–0.5/1–2 cm) are more rich and more even than in the water

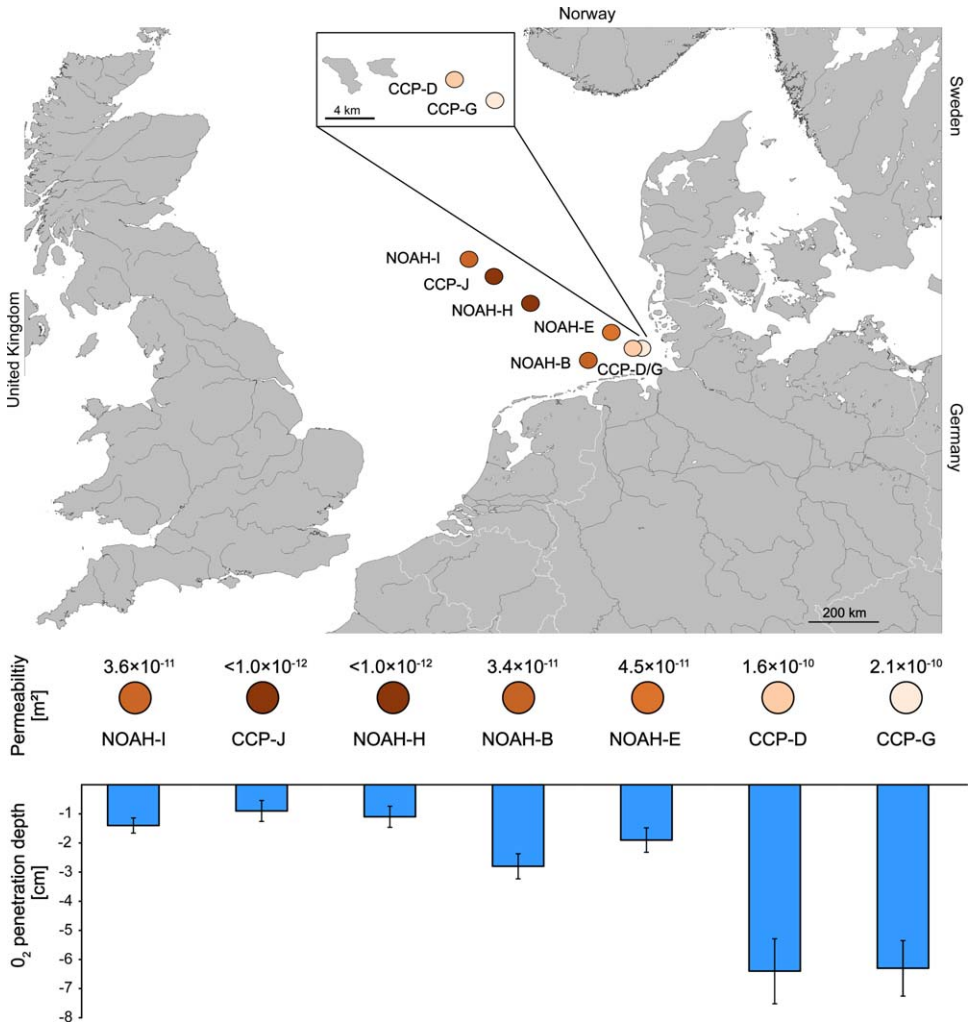

Fig. 1. Geographic location, sediment permeability and oxygen penetration depth of sampling sites in the North Sea. The close-up shows the location of sites CCP-D and CCP-G off the island of Helgoland. The sediment sites were classified as (1) fine grained and low to impermeable (CCP-J, NOAH-H), (2) medium grained and medium permeable (NOAH-I, NOAH-B, NOAH-E), and (3) coarse grained and highly permeable (CCP-D, CCP-G). Color coding reflects normalized (log10) sediment permeability. Error bars for oxygen penetration depths give the standard deviation over a transect of 60 cm. All sites were sampled in September 2014. In addition, site NOAH-B was sampled in March 2014 and February 2015 to study seasonal effects. Data for sediment permeability and oxygen penetration depth taken from Ahmerkamp (2016).

column as indicated by higher $Chao1_{0.97}$ (5,267 ± 3,407 vs. 2,687 ± 569) and higher inverse $Simpson_{0.97}$ (169 ± 46 vs. 26 ± 20). The bacterial communities were significantly different as tested by analysis of similarity (ANOSIM, $R = 0.92$, $p = 0.003$).

Community composition in surface sediments and bottom waters

Phylogenetic analysis of 16S rRNA gene sequences showed clear differences between bacterial communities in surface sediments and in corresponding bottom waters on family level (Fig. 3A). In sediments, most sequence-abundant were gammaproteobacterial *Woeseiaceae*/JTB255 (7% ± 3 of total sequences), *Ectothiorhodospiraceae* (4% ± 2), and *Alteromonadaceae* (4% ± 2), deltaproteobacterial Sh765B-TzT-29 (2% ± 1), *Sandaracinaceae* (3% ± 2), *Desulfobulbaceae* (4% ± 3) and *Desulfobacteraceae* (3% ± 2), *Flavobacteriaceae* (6% ± 4), *Planctomycetaceae* (7% ± 4) and *Phycisphaeraceae* (2% ± 1). In contrast, bacterial community in bottom waters was dominated by alphaproteobacterial *Pelagibacterales*/SAR11 surface clade 1 (11% ± 6), SAR11 surface clade 2 (2% ± 1), and *Rhodobacteraceae* (9% ± 2), gammaproteobacterial clade SAR86 (5% ± 2) and *Alteromonadaceae* (3% ± 1), *Flavobacteriaceae* (9% ± 3) and actinobacterial clade OM1 (18% ± 4). These groups contributed 54% ± 0.1 to total sequences in bottom waters while they accounted for only 14% ± 0 in the sediment. These differences were most pronounced for SAR11 clade surface 1 and SAR11 clade surface 2, which both made up 14% ± 5 in bottom waters but only 0.2% ± 0 of 16S rRNA gene sequences retrieved from sediments.

Although relative sequence abundances of *Flavobacteriaceae* were comparable in sediments and bottom waters, different subclades beyond the family level became evident for benthic and pelagic communities. For example, members of clades NS4-, NS5-, and NS2b marine group accounted for 60% ± 16 of *Flavobacteriaceae* sequences in bottom waters but only for 0.5% ± 0.7 in the sediments.

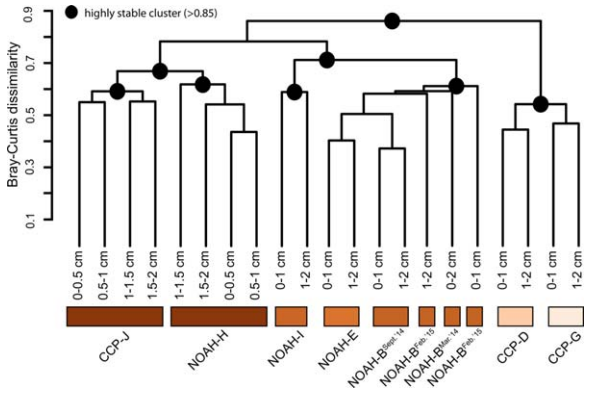

Fig. 2. Hierarchical clustering of individual sediment depth layers per sampling site based on subsampled Bray-Curtis dissimilarity matrix ($OTU_{0.97}$) and the complete linkage method. Stability of clusters was tested by bootstrapping 100 times. Color coding according to Fig. 1.

Instead, benthic *Flavobacteriaceae* were dominated by *Lutibacter* in impermeable sediments and *Eudoraea* in medium and highly permeable sediments. Within actinobacterial OM1 clade (Rappé *et al.*, 1997), genus '*Cd.* Actinomarina' contributed 96% ± 6 of total OM1-related sequences in bottom waters but only 1% ± 1 in sediments. Benthic OM1 comprised other, diverse and uncultured genera.

Sequence-abundant clades in North Sea sediments of different permeability

The phylogenetic community composition of the individual samples was investigated in the context of sediment permeability (Fig. 3A). Relative 16S rRNA gene sequence abundances of gammaproteobacterial *Alteromonadaceae* and *Ectothiorhodospiraceae*, deltaproteobacterial *Desulfobulbaceae* and *Desulfobacteraceae*, epsilonproteobacterial *Helicobacteraceae* as well as *Flavobacteriaceae* were approximately fivefold lower in sediments of high permeability than in impermeable sediments. For example, *Alteromonadaceae* and *Flavobacteriaceae* made up 5% ± 1 and 9% ± 2 of total sequences in impermeable sediments but only 1% ± 0.3 and 2% ± 2 in highly permeable sediments respectively. *Desulfobulbaceae* (*Desulfobulbus* spp. and uncultured relatives) and *Desulfobacteraceae* (clade Sva0081 and uncultured relatives) accounted for 6% ± 4 and 4% ± 1 of total sequences in impermeable sediments while they accounted only for 0.6% ± 0.2 and 0.5% ± 0.1 in highly permeable sediments. *Helicobacteraceae* (mainly *Sulfurimonas* spp., *Sulfurovum* spp.) were detected at abundances of 1% ± 1 of total sequences at impermeable sediments but only rarely in highly permeable sediments (<0.1%). In contrast to the taxa described above, relative 16S rRNA gene sequence abundances of alphaproteobacterial *Rhodospirillaceae*, actinobacterial clade OM1, acidobacterial clades Sva0725 and subgroup22 as well as gammaproteobacterial ammonia-oxidizing *Chromatiaceae* (mainly *Nitrosoccocus* spp.) and nitrite-oxidizing *Nitrospiraceae* (mainly *Nitrospira* spp.) were 2 to 10-fold lower in impermeable sediments than in highly permeable sediments.

Relative sequence abundances of deltaproteobacterial *Sandaracinaceae* (2% to 5%) and gammaproteobacterial *Woeseiaceae*/JTB255 (3% to 12%) were high at all sites. However, diversity within both families was low. Only two $OTU_{0.97}$ were found contributing 80% to 96% of *Sandaracinaceae*-related and one $OTU_{0.97}$ contributing 50% to 82% of *Woeseiaceae*/JTB255-related sequences, representing the most abundant benthic $OTU_{0.97}$ in the whole dataset. *Sandaracinaceae* $OTU_{0.97}$#5 and $OTU_{0.97}$#110 were affiliated with a cluster solely composed of sequences of marine origin (Supporting Information Fig. S1A and B). Most closely related sequences were retrieved from sublittoral German Bight sediments (99% identity, Brinkhoff *et al.*, 2012) and Arctic surface sediments (99% identity; Li *et al.*, 2009). The marine *Sandaracinaceae* cluster exhibited 16S rRNA gene similarities of 91% to

Table 1. Estimated bacterial diversity in North Sea surface sediments. Chao1 and inverse Simpson indices were calculated based on operational taxonomic units (OTU) clustered at 97% similarity of bacterial 16S rRNA gene sequences.

Category	Data set	Chao1[a]	Inverse Simpson[a]
Sediments	Depth 0-2 cm	7,026 ± 3,697	170 ± 48
Vertical	Bottom water	2,687 ± 569	26 ± 20
	Sediments 0-1 cm	5,267 ± 3,407	169 ± 46
	Sediments 1-2 cm	7,494 ± 3,995	180 ± 53
Spatial	CCP-J	10,580 ± 9,109	203 ± 30
	NOAH-H	6,774 ± 4,355	172 ± 72
	NOAH-I	11,290 ± 847	206 ± 16
	NOAH-B$^{Sept.\ '14}$	3,357 ± 1,020	93 ± 5
	NOAH-E	3,283 ± 102	148 ± 87
	CCP-D	3,242 ± 270	173 ± 2
	CCP-G	3,763 ± 48	209 ± 3
Seasonal	NOAH-B$^{Mar.\ '14}$	10,882	119
	NOAH-B$^{Sept.\ '14}$	3,357 ± 1,020	93 ± 5
	NOAH-B$^{Feb.\ '15}$	8,773 ± 757	149 ± 42

[a]Based on subsampling within a category without replacements. Four categories were formed for analysis of bacterial diversity that are (i) 'Sediments' (datasets from all different sediments and depth layers pooled), (ii) 'Vertical' (bottom water datasets pooled, 0–1 cm depth layers as well as 1–2 cm depth layers pooled from all sites), (iii) 'Spatial' (all depths layers (0–2 cm) pooled for each site sampled in September 2014), and (iv) 'Seasonal' (all depths layers (0–2 cm) pooled for site NOAH-B for one sampling point). Given values are the mean and standard deviation of the averaged values of 25 independent calculations for each separately sequenced sample. Values for the individual samples are provided as supplementary information (Supporting Information Table S2).

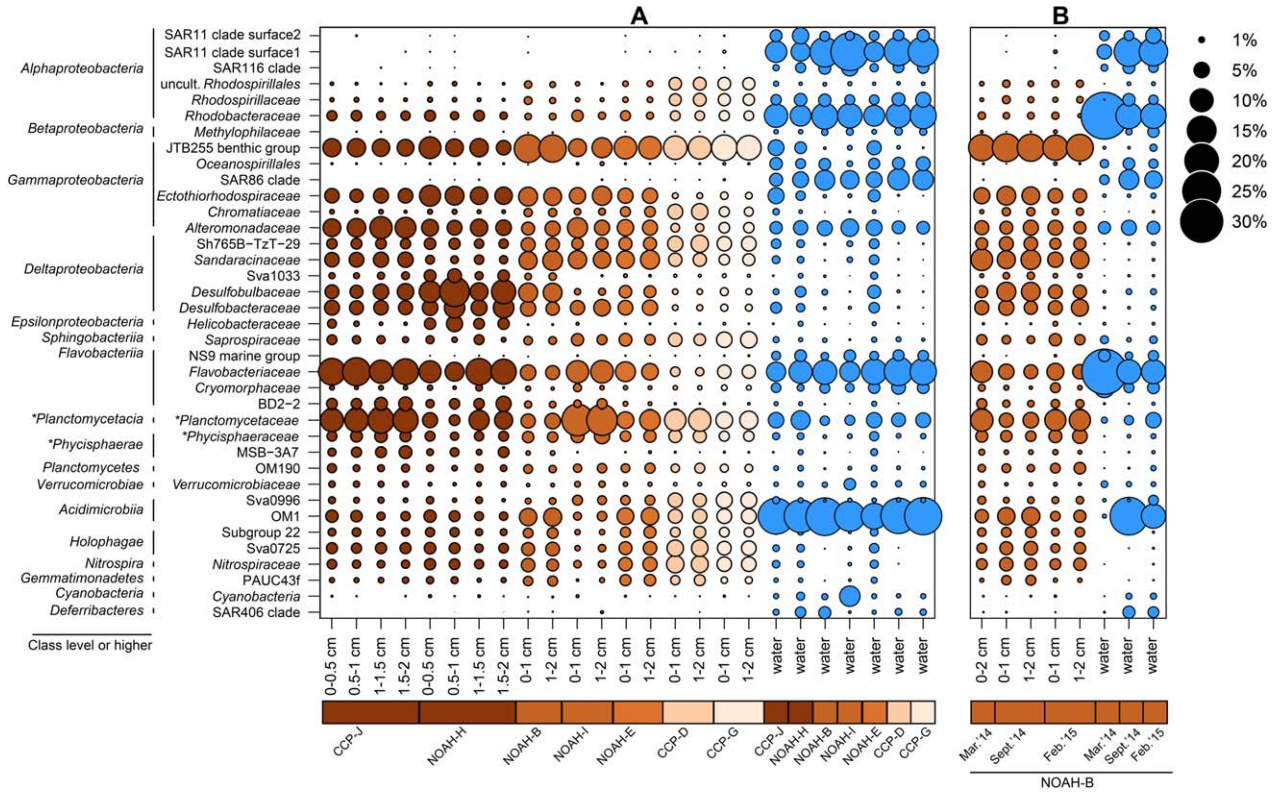

Fig. 3. Relative abundances of bacterial families and uncultivated clades in sediments (brown) and corresponding bottom waters (blue) based on sequencing of 16S RNA genes (V3-V4 region). Only taxa that made up >1% of total sequences in any given sample are listed. In total, depicted taxa contributed 62% ± 3 of total sequences in sediments and 81% ± 5 in bottom waters. Taxonomy based on SILVA SSU NR Ref database, release 119. *: in contrast to existing taxonomy, our analysis suggested the rank of a phylum for these clades.
A. Sampling sites are ordered from impermeable sediments (left) to highly permeable sediments (right).
B. Data for site NOAH-B are ordered according to sampling date to visualize seasonal changes.

100%, while similarity between marine and terrestrial clusters was <92%.

Phylogenetic diversity of Planctomycetes in the North Sea

Members of the phylum *Planctomycetes* were particular abundant based on 16S rRNA gene sequencing. Independent of sediment permeability, sequences affiliated with the classes *Planctomycetia* and *Phycisphaerae* made up 2% to 15% and 1% to 4% of total sequences in all sediment sites and depth layers respectively. In the water column, relative abundance was lower with 2% to 6% and 0.2% to 1% of sequences affiliated with *Planctomycetia* and *Phycisphaerae. Planctomycetes* phylogeny was studied in greater detail to identify possible overlaps between $OTU_{0.97}$ dominating the water column with those detected in sediments of different permeability. The diversity within *Planctomycetes* was huge in impermeable (6,305 species-level *Planctomycetes* $OTU_{0.97}$), medium permeable (3,012 $OTU_{0.97}$) and highly permeable sediments (3,201 $OTU_{0.97}$) as classified by SILVAngs pipeline. In corresponding bottom water samples, *Planctomycetes* diversity was two- to fivefold lower. Representative sequences were widely distributed within the phylum (Fig. 4). We did not detect differences in the phylogenetic distributions of water column- and sediment-derived sequences. Furthermore, no remarkable differences were observed for the distribution of sequences from highly permeable, medium permeable and impermeable sediments. Neither bottom water-specific nor sediment-specific *Planctomycetes* $OTU_{0.97}$ were detected.

About one-fourth of retrieved benthic and planktonic *Planctomycetes* sequences (>2% of total sequences) affiliated with clade DDSe3017 (Fig. 4). This clade is named after strain DDSe3017 that was isolated from Moreton Bay sediments, Australia, using *N*-acetyl-glucosamine as carbon source (Izumi *et al.*, 2013). The cluster comprises sequences of mainly marine sediments and sea water. All sequences within clade DDSe3017 showed ≤ 92% 16S rRNA gene sequence similarity to any other *Planctomycetes* thus likely representing a new family within the class *Planctomycetia* according to the established taxonomic thresholds (Yarza *et al.*, 2014). The second most abundant

Fig. 4. Phylogenetic tree showing the distribution of 16S rRNA gene sequences retrieved from the North Sea within the phylum *Planctomycetes*. The tree was calculated using the maximum-likelihood (PhyML) algorithm with filters considering only 40% conserved positions. A selection of candidate division OP3 sequences was used as outgroup. Different calculations of phylogenetic trees did not result in a stable branching order for some subgroups. Consequently, the phylogenetic affiliations of these subgroups are shown as multifurcations. Datasets with 16S rRNA gene sequences retrieved from individual North Sea samples were assigned to six categories: impermeable sediments (brown circle), medium permeable sediments (brown square) and highly permeable sediments (brown triangle) and their corresponding bottom water samples (blue circle, blue square, blue triangle). OTU clustering was done using the SILVAngs pipeline (database release 128). Partial sequence North Sea $OTU_{0.97}$ that were represented by at least 10 sequences were added to the reconstructed tree under parsimony criteria. The sizes of the symbols represent the relative abundance of the sequences for each category within a cluster. Gray-colored branches indicate clusters comprising North Sea sequences. Probe coverage is indicated by gray-colored boxes. *, clade assigned to *Blastopirellula* in SILVA SSU Ref NR 128; $, 23 sequences (= 15%) of this cluster were targeted by probes PLA445ab. §, clusters comprising only North Sea sequences did not contain sequence information at probe target site. Scale bar represents 10% estimated sequence divergence.

clade of the class *Planctomycetia* was *Thermogutta/Thermostilla* and relatives comprising almost one-fifth of the *Planctomycetes* sequences. This clade was particular sequence-abundant in sea water overlying impermeable sediments (2% of total sequences). In addition to numerous sequences of terrestrial origin, this clade comprises a

Table 2. In situ abundance of bacterial clades in surface sediments (0–2 cm depth) as determined by CARD-FISH. Detection rates of *Bacteria* using the general probes EUB338 I-III were between 81% and 96% of total cells. Only for sites CCP-D and CCP-G detection rate was lower with 75%. Relative abundance is given in parenthesis. Abundances <0.5% were close to detection limit and not further quantified.

Site	Total cells [10^7 cm^{-3}]	*Gammaproteob.* [10^7 cm^{-3}]	*Woeseiaceae* [10^7 cm^{-3}]	*Desulfobulbaceae* [10^7 cm^{-3}]	*Desulfobacteraceae* [10^7 cm^{-3}]	*Sandaracinaceae* [10^7 cm^{-3}]
CCP-J	57	18.2 (32%)	2.3 (4%)	(<0.5%)	3.9 (7%)	0.6 (1%)
NOAH-H	37	14.4 (39%)	2.2 (6%)	0.4 (1%)	2.2 (6%)	0.8 (2%)
NOAH-I	50	16.0 (32%)	2.0 (4%)	(<0.5%)	3.0 (6%)	2.5 (5%)
NOAH-B$^{Sept.\ '14}$	77	23.9 (31%)	0.7 (1%)	(<0.5%)	5.4 (7%)	5.4 (7%)
NOAH-E	47	14.1 (30%)	2.8 (6%)	(<0.5%)	1.0 (2%)	1.4 (3%)
CCP-D	54	8.6 (16%)	1.6 (3%)	(<0.5%)	(<0.5%)	0.5 (1%)
CCP-G	30	5.4 (18%)	1.2 (4%)	(<0.5%)	(<0.5%)	0.3 (1%)
NOAH-B$^{Mar.\ '14}$	32	7.7 (24%)	0.9 (3%)	n.a.	n.a.	1.2 (4%)
NOAH-B$^{Sept.\ '14}$	77	23.9 (31%)	0.7 (1%)	(<0.5%)	5.4 (7%)	5.4 (7%)
NOAH-B$^{Feb.\ '15}$	21	5.0 (24%)	0.6 (3%)	n.a.	n.a.	1.3 (6%)

Site	*Acidobacteria* [10^7 cm^{-3}]	*Bacteroidetes* [10^7 cm^{-3}]	*Planctomycetes*[a] [10^7 cm^{-3}]	*Planctomycetia*[b] [10^7 cm^{-3}]	Clade DDSe3017 [10^7 cm^{-3}]	*Phycisphaerae* [10^7 cm^{-3}]
CCP-J	3.4 (6%)	2.3 (4%)	8.0 (14%)	6.3 (11%)	(<0.5%)	1.7 (3%)
NOAH-H	1.5 (4%)	1.9 (5%)	3.3 (9%)	2.2 (6%)	(<0.5%)	1.1 (3%)
NOAH-I	3.0 (6%)	2.5 (5%)	11.0 (22%)	9.0 (18%)	1.0 (2%)	2.0 (4%)
NOAH-B$^{Sept.\ '14}$	6.9 (9%)	2.3 (3%)	6.1 (8%)	4.6 (6%)	0.5 (0.6%)	1.5 (2%)
NOAH-E	5.2 (11%)	1.9 (4%)	7.0 (15%)	5.6 (12%)	0.5 (1%)	1.4 (3%)
CCP-D	5.9 (11%)	(<0.5%)	11.9 (22%)	10.3 (19%)	0.6 (1%)	1.6 (3%)
CCP-G	4.5 (15%)	(<0.5%)	3.6 (12%)	2.7 (9%)	0.6 (2%)	0.9 (3%)
NOAH-B$^{Mar.\ '14}$	2.6 (8%)	1.0 (3%)	3.5 (11%)	2.9 (9%)	0.2 (0.5%)	0.6 (2%)
NOAH-B$^{Sept.\ '14}$	6.9 (9%)	2.3 (3%)	6.1 (8%)	4.6 (6%)	0.5 (0.6%)	1.5 (2%)
NOAH-B$^{Feb.\ '15}$	2.3 (11%)	0.4 (2%)	3.2 (15%)	2.1 (10%)	0.4 (2%)	1.1 (5%)

n.a., not analyzed.
[a]Sum of probes PLA46 and PHYC309.
[b]*Planctomycetia* as well as '*Cd.* Brocadiae', Pla3, Pla4, and OM190.

marine subclade with sequences from East Pacific Rise vents (e.g. JN874313, Sylvan *et al.*, database release), or from surface waters in the Northern Bering Sea (Zeng *et al.*, 2012) or in the Pacific Ocean off Oregon (Morris *et al.*, 2006). Within the class *Phycisphaerae*, North Sea sequences formed separate clusters or were affiliated mainly with uncultivated clades such as Urania-1B-19 (Fig. 4).

Planctomycetia as well as *Phycisphaerae* revealed a maximum 16S rRNA gene sequence similarity of only 72% and 76% to each other, to class '*Cd.* Brocadiae' or to any other *Planctomycetes* clade such as OM190, VadinHA49 or Pla3. According to the taxonomic threshold values of 75% for a phylum (Yarza *et al.*, 2014), *Planctomycetia*, *Phycisphaerae*, and '*Cd.* Brocadiae' likely represent different phyla.

Visualization and in situ quantification of abundant benthic clades

Quantification of sequence-abundant clades in surface sediments (0–2 cm depth) was done by CARD-FISH (Table 2). Absolute prokaryotic cell numbers varied between samples and were in the range of 2.1 to 7.7 $\times$ 10^8 cells cm^{-3}. Between 75% and 96% of prokaryotic cells were identified as *Bacteria* as detected by probe mix EUB338 I-III. A major part of the detected community were *Gammaproteobacteria*, which made up between 16% and 39% of total cells with highest values at impermeable sites and lowest values at highly permeable sites. *Woeseiaceae*/JTB255 accounted for 1% to 6% of total cells. We likely have missed a major part of *Woeseiaceae*/JTB255, since probe JTB1270 (Supporting Information Table S3) only covers 24% of the target group (SILVA database SSU Ref NR, release 128). Relative cell abundances of *Desulfobacteraceae* as detected by probe DSS658 showed a similar trend as observed for *Gammaproteobacteria*: cell abundances were highest in impermeable (6% to 7% of total cells) sediments and medium permeable sediments from sites NOAH-B (7%) and NOAH-I (6%) but lowest in highly permeable sites (<0.5%). Interestingly, relative cell abundances at medium permeable site NOAH-E were considerably lower (2%) compared to remaining sites of medium permeability (Table 2). Relative cell abundances of *Acidobacteria* as detected by probe HOAC1402 were lowest in impermeable sediments at sites NOAH-H and CCP-J and medium permeable site NOAH-I with 4% to 6% of total cells. *Acidobacteria* were significantly more abundant (two sample *t*-test: $p < 0.009$) in sediments with highest permeability accounting for 11% to 15% of total cells.

Acidobacteria cells showed a nearly homogenous morphology of elongated rods; as reported for isolated marine *Acidobacteria* (Green *et al*., 2015). Using probe CF319a that covers parts of the phylum *Bacteroidetes* (mainly *Flavobacteriales* and *Bacteroidales)* we detected an opposite trend. Relative cell numbers were highest at impermeable sites NOAH-H and CCP-J (4% and 5% of total cells) but lowest at highly permeable sites CCP-D and CCP-G (<0.5%).

To detect *Sandaracinaceae*-related cells, probe SAND653 was developed. It covers 84% of the marine *Sandaracinaceae* cluster (Supporting Information Fig. S1B) with only one outgroup hit (SILVA database SSU Ref NR, release 128). Probe signals were bright and showed single cells or small clusters of cocci of 1–1.5 μm diameter (Supporting Information Fig. S1C). Relative abundance of *Sandaracinaceae* ranged between 1% and 2% of total cells at impermeable and highly permeable sites to 3% to 7% in sediments of medium permeability.

Planctomycetes cell abundances were exceptionally high with 6% to 19% of total cells as detected by probe PLA46 (Table 2). Probe PLA46 targets class *Planctomycetia* as well as '*Cd*. Brocadiae', clades Pla3 lineage, Pla4 lineage and OM190. Members of the *Phycisphaerae* are not targeted by this probe. For quantification of the most sequence-abundant *Planctomycetia* subclade DDSe3017, we designed and tested a set of new probes. Probe mix PLA445ab covers 89% of sequences within clade DDSe3017 (SILVA database SSU Ref NR, release 128) and 15% of an uncultured clade (e.g. HM598258, Fig. 4). Hybridization of sediment samples with probe mix PLA445ab was successful and resulted in bright signals of cocci-like cells. Clade DDSe3017 abundance was 0.5% to 2% of total cells (Table 2). For *Phycisphaerae* we developed probe PHYC309 that covers 57% of *Phycisphaerae* (Fig. 4). Coverage of subclade Urania-1B-19 was 100% while coverage of subclade SM1A02 was only 38%. For *Phycisphaerae* subclades comprising only North Sea sequences no sequence information at the probe target site was available. Therefore, these clades were not considered for probe coverage calculation. Dual hybridization of sediment samples with probes PHYC309 and PLA46 confirmed the specificity of each probe (Supporting Information Fig. S2). Detection rates for *Phycisphaerae* ranged between 2% and 5% of total cells. In total we detected 8% to 22% *Planctomycetes* of total cells in surface sediments (sum of counts by probes PLA46 and PHYC309).

Seasonal changes of bacterial diversity and community composition at station NOAH-B

To assess annual dynamics of bacterial diversity and community composition, site NOAH-B was sampled in March 2014, in September 2014 and February 2015, reflecting early spring, late summer and late winter settings. Both, estimated richness and evenness were lowest in September 2014 as shown by a $Chao1_{0.97}$ of 3,357 ± 1,020 and inverse Simpson index of 93 ± 5 (Table 1). In March 2014 and February 2015 bacterial richness was comparably high ($Chao1_{0.97}$: 10,882 and 8,773 ± 757). For late summer samples, hierarchical clustering based on Bray-Curtis dissimilarity matrix for $OTU_{0.97}$ showed that the bacterial community at NOAH-B was most closely related to the community at NOAH-E (Fig. 2). Nevertheless, NOAH-B late winter and early spring samples were more similar to NOAH-B late summer samples than to any other sample.

The community composition at NOAH-B was stable during the year on family level, except for sequences affiliated with *Planctomycetaceae* and *Desulfobulbaceae* (Fig. 3B). *Planctomycetaceae* were found only half as frequent in September 2014 samples (3% ± 0.4) compared to March 2014 (8%) and February 2015 (7% ± 0.1) samples. Vice versa, *Desulfobulbaceae* were twice as high in September 2014 (5% ± 0.1) compared with March 2014 (2%) and February 2015 (3% ± 0.1). Absolute cell numbers at site NOAH-B were 3.7 and 2.4-fold lower in late winter (February 2015) and early spring (March 2014) than in late summer (Table 2). No seasonal effects on the bacterial community structure were detected by CARD-FISH with the probe set applied in this study. In summary, seasonality likely had only minor influence on bacterial diversity and community composition. More frequent sampling could reveal whether we might have missed short-lived responses of specialized clades as it has been described for benthic *Flavobacteriia* in the English Channel (Tait *et al*., 2015).

Discussion

Marine sands are highly dynamic systems that are regularly flushed with bottom waters pumping organic matter and oxygen into the sediment (Huettel *et al*., 2014). Moreover, the sands themselves are frequently mobilized, thus affecting the advective inflow of reactive compounds (Ahmerkamp *et al*., 2015) and leading to very dynamic redox conditions which contrast the stable redox conditions in diffusion-controlled impermeable sediments. In the top sediment layer the first interaction of water column-derived organic matter with the benthic microbial community takes place. Despite the frequent flushing with bottom waters, the microbial community in tidal sediments was shown to be quite different to the community in the water column (Gobet *et al*., 2012; Newton *et al*., 2013). Our fine-scale vertical analysis of the microbial community goes beyond previous studies. Here we showed that already the first 5 mm of the sediments are populated by microbial communities remarkably different from those in the bottom waters. In particular, the much lower abundance of alphaproteobacterial SAR11, gammaproteobacterial SAR86 and '*Cd*. Actinomarina'

(clade OM1) in surface sediments illustrate the minor relevance of these groups in the benthos and indicate that other groups are responsible for element cycling.

Influence of sediment depth and permeability on bacterial diversity

Both alpha- and beta-diversity patterns of benthic bacterial species ($OTU_{0.97}$) did not show any vertical structuring at a resolution of 0.5 to 1 cm depth intervals in surface sediments. The influence of local factors was greater than the influence of global factors for structuring the community in the top sediment layers. North Sea sediments are highly dynamic systems influenced by tidal currents and waves increasing the temporal and spatial variability of prevailing redox conditions (Ahmerkamp *et al*., 2015). Furthermore tidal currents and waves cause a constant resuspension and deposition of particulate organic matter as well as sediment grains together with their colonizing microbial community (Kösters and Winter, 2014; Ahmerkamp, 2016). Consequently microbial community members are exposed to oxic bottom waters in the top sediment layer with access to fresh substrate, but are buried in deeper anoxic sediment layers shortly after. The environmental settings favored the growth of a highly diverse and metabolically versatile bacterial community, supporting the function of surface sediments as efficient biological filters.

Sediment permeability (and grain size) had a strong influence on estimated local species richness which was highest at impermeable sites while it was lowest at highly permeable sites. Other studies reported species richness in surface sediments to be negatively correlated with grain size which was attributed to more efficient retention of organic matter that supplies a more diverse community (Franco *et al*., 2007; O'Reilly *et al*., 2016). Estimated $OTU_{0.97}$ richness at sites of medium permeability did not follow a trend: it was low at NOAH-B ($Chao1_{0.97}$: 3,357) and NOAH-E ($Chao1_{0.97}$: 3,283) but exceptionally high at NOAH-I ($Chao1_{0.97}$: 11,290), suggesting that other local factors than permeability likely had an additional influence on $OTU_{0.97}$ richness. The three sediment sites of highest estimated species richness CCP-J, NOAH-H (both impermeable sediments) and NOAH-I (medium permeable) only had in common the furthest location from shore. Interestingly species richness of benthic infauna in these regions has also been described to be three times higher compared to sites in the coastal regions (Neumann *et al*., 2013). Less frequent pulses of organic matter, for example through phytoplankton blooms, into the sediment in regions furthest offshore could explain the high estimated species richness in these regions. According to the intermediate disturbance hypothesis (Connell, 1978), not too frequent but regular disturbances (e.g. by substrate input) might allow for the coexistence of a specialized and an established community, thus increasing species richness.

Despite differences in species richness in sediments of different permeability, overall estimated benthic richness ($Chao1_{0.97}$: 2,635–11,889, Supporting Information Table S2) was comparable with estimated richness in deep sea surface sediments (29 sites; $Chao1_{0.97}$: ~3,000 to ~7,700; Bienhold *et al*., 2016), in surface sediments of the Western English Channel ($Chao1_{0.97}$: 4,947–7,726; Tait *et al*., 2015) and in impermeable Chinese sublittoral surface sediments (12 sites; $Chao1_{0.97}$: 6,504–10,476; Liu *et al*., 2015).

Sulfur cycling

In situ quantification revealed up to 15-fold higher relative abundances of sulfate-reducing *Desulfobacteraceae* in medium permeable and impermeable sediments than in highly permeable sediments (Table 2). Despite frequent penetration of oxygen into medium permeable and impermeable sediments (Ahmerkamp, 2016), these data clearly indicate sulfur cycling and suggest the presence of anoxic niches in surface sediments. In North Sea surface sediments, three-quarters of *Desulfobacteraceae* sequences were assigned to clade Sva0081 which was initially described by Ravenschlag *et al*. (2000). Single cell genomics and metatranscriptomics indicated that clade Sva0081 uses diverse energy sources including organic acids, aromatic compounds and hydrogen (Mußmann et al., 2015). Several defense mechanisms against oxidative stress are encoded in the genome enabling clade Sva0081 to thrive in surface sediments with fluctuating redox conditions, such as North Sea sublittoral sediments studied here.

Based on higher relative 16S rRNA gene frequencies of sulfur oxidizers, the oxidative part of the sulfur cycle seemed to be most pronounced in impermeable and medium permeable sediments, likely caused by the higher biogenic production of reduced sulfur compounds. Candidates for sulfur oxidation are epsilonproteobacterial *Sulfurimonas* spp. and *Sulfurovum* spp. which were significantly more abundant in impermeable ($1.4\% \pm 1.2$) compared to highly permeable (0.1%) sediments (two sample *t*-test: $p < 0.0162$). Previously, *Sulfurimonas* and *Sulfurovum* have been detected in intertidal sediments in the North Sea (Pjevac *et al*., 2014). Isolates of these genera are facultative or strict anaerobs and oxidize reduced sulfur species with nitrate or oxygen (Inagaki *et al*., 2003; Inagaki *et al*., 2004; Takai *et al*., 2006; Mino *et al*., 2014). Their dominance at impermeable sites suggests prevalent suboxic to anoxic niches even in the top 5 mm where sufficient sulfide is produced. Other candidates for sulfur oxidation were gammaproteobacterial chemolithoautotrophic *Acidiferrobacter* (*Ectothiorhodospiraceae*) and

Woeseiaceae/JTB255. *Acidiferrobacter* was significantly higher abundant in impermeable and medium permeable than in highly permeable sediments (two sample *t*-test: $p < 0.0001$ and $p < 0.0163$; Fig. 3A). Members of this genus were highly abundant constituting up to 8% of total cells in tidal sediments in the North Sea (Lenk *et al*., 2011) where they oxidize thiosulfate and sulfite, likely using oxygen as electron acceptor (Dyksma *et al*., 2016). *Woeseiaceae*/JTB255 were present in all sublittoral North Sea sediments (1% to 6%) and comparably abundant as previously reported for European tidal sediments (3% to 6%). Based on ^{14}C-bicarbonate tracer experiments, mixotrophic members of *Woeseiaceae*/JTB255 can also grow as chemolithoautotrophic sulfur oxidizers (Dyksma *et al*., 2016).

High abundance of non-predatory Sandaracinaceae and Acidobacteria

High cell numbers of deltaproteobacterial family *Sandaracinaceae* were found in medium permeable sediments (3% to 7% of total cells) but lower numbers in impermeable and highly permeable sediments (1% to 2%). A previous study based on qPCR found similar high relative numbers of *Sandaracinaceae*-related 16S rRNA gene copies in fine (8% of total 16S rRNA gene copies) compared to coarse (2%) and muddy (2%) sediments (Brinkhoff *et al*., 2012). The only cultured representative of this family was isolated from soil (Mohr *et al*., 2012) and exhibits similar physiological and morphological characteristics as known for terrestrial aerobic *Myxobacteria*. This includes the formation of fruiting bodies, swarming motility, predatory behavior and resistance to antibiotics (Shimkets *et al*., 2006**;** Zusman *et al*., 2007). *Sandaracinaceae* detected in this study belonged to a phylogenetic cluster comprising only sequences from marine habitats (Supporting Information Fig. S1B). Based on microscopic observations, we neither found evidence for formation of fruiting bodies nor for intracellular predatory behaviour as observed for *Bdellovibrio* (Sockett, 2009). Thus, marine *Sandaracinaceae* likely exhibit a less complex lifestyle than their terrestrial counterparts. This is supported by metagenomic data that suggested marine benthic *Myxobacteria* as organoheterotrophic consumers of low molecular weight (LMW) organic matter such as the fermentation products ethanol, hydrogen, butyrate and acetate (Baker *et al*., 2015).

Another major clade of the benthic bacterial community in the North Sea was *Acidobacteria* with 9% to 15% of total cells at medium permeable and highly permeable sites. Phylogenetically, detected *Acidobacteria* belonged to subgroup 22 and clade Sva0725. Clade Sva0725 had originally been detected in permanently cold shelf sediments off Svalbard (Ravenschlag *et al*., 1999) but was also found in deep sea (Zeng *et al*., 2011; Polymenakou *et al*., 2015) and coastal surface sediments (Acosta-González *et al*., 2013; Edgcomb *et al*., 2013). Most cultured *Acidobacteria* are slowly growing aerobic heterotrophs isolated from soil habitats (Kielak *et al*., 2016 and references therein). Most acidobacterial genomes possess only few genes involved in complex carbohydrate degradation but several high affinity transporters for uptake of amino acids and peptides (Eichorst *et al*., 2007; Kielak *et al*., 2016). In contrast to soil *Acidobacteria*, little is known about marine species. The only cultured aerobic marine strains, i.e. *Acanthopleuribacter pedis* (Fukunaga *et al*., 2008), *A*. sp. str. N2yML4 (Mohamed *et al*., 2008), and *A*. sp. str. DG1540 (Green *et al*., 2015), are living attached to algal and sponge surfaces. They are strictly aerobic and only distantly related to North Sea abundant clade Sva0725 (<80% 16S rRNA identity). Like soil isolates, *Acanthopleuribacter pedis* also utilizes several amino acids.

Benthic and planktonic Flavobacteriaceae differ

Bacteroidetes are the main degraders of high molecular weight (HMW) organic matter in the water column (Teeling *et al*., 2012; 2016). In North Sea sediments, *Bacteroidetes* cell numbers were significantly higher in medium permeable and impermeable sediments (3% to 5% of total cells) than in highly permeable sediments (<0.5%; two sample *t*-test: $p < 0.0262$). Since highly permeable sediments are more efficiently flushed with sea water containing substrates from the water column, this distribution of *Bacteroidetes* was unexpected. Our observed trend is in contrast to reports by O'Reilly and colleagues (2016) who found *Bacteroidetes* sequence abundance positively correlated with grain size, but is comparable to cell distributions in tidal muddy vs. sandy sediments (Llobet-Brossa *et al*., 1998).

In bottom waters, *Flavobacteriaceae* were affiliated with uncultured clades NS4-, NS5-, and NS2b-marine group. They were clearly different from benthic *Flavobacteriaceae* which were dominated by uncultured clades as well as *Eudoraea* and *Lutibacter*. Benthic isolates of *Lutibacter* are aerobic and facultative anaerobic organoheterotrophs respiring carbohydrates. Only a few macromolecules (starch, Tween80, DNA, casein) were hydrolyzed depicting a limited spectrum of macromolecule degradation (Choi and Cho, 2006; Park *et al*., 2010; Lee *et al*., 2012; Choi *et al*., 2013). Similar to *Lutibacter*, marine *Eudoraea* species grow on aesculin, sugars, organic and amino acids (Alain *et al*., 2008). Fermentation by *Lutibacter* and *Eudoraea* was observed for some strains. These taxa were abundant in all North Sea sediments independent of seasonality suggesting a minor role of *Flavobacteriaceae* in particulate organic matter degradation. Benthic flavobacterial *Psychroserpens*, *Flaviramulus*, *Aestuariicola*, *Algibacter* and *Sufflavibacter*, previously assumed to

degrade fresh organic material (Ruff *et al*., 2014; Tait *et al*., 2015), were not found.

Planctomycetes are likely the key organisms for complex organic matter degradation in North Sea surface sediments

Based on high abundances of up to 14×10^7 cells cm^{-3} (22% of total cells), *Planctomycetes* of the classes *Phycisphaerae* and *Planctomycetia* likely represent the key bacteria in North Sea surface sediments for the degradation of particulate organic matter (POM), HMW organic matter and complex recalcitrant organic matter settled down from the water column. In surface sediments *Planctomycetes* were >1000-fold more abundant than in the North Sea water column (Pizzetti *et al*., 2011). Furthermore, in surface sediments *Planctomycetes* were 1.7 to 40-fold more abundant than *Bacteroidetes* which are the key degraders of HMW organic matter in the water column (Teeling *et al*., 2012). Cell numbers were comparable with those obtained previously for tidal surface sediments off Sylt (up to 19% of total cells, probe PLA886; Musat *et al*., 2006) or with those for sublittoral sediments of the South Atlantic Bight (up to 19%, Rusch *et al*., 2003).

Marine *Planctomycetes* that are related to abundant $OTU_{0.97}$ detected in this study (e.g. *Rhodopirellula, Blastopirellula*, *Planctomyces, Phycisphaera*) live attached to surfaces of aggregates and macroalgae (Schlesner *et al*., 2004; Lee *et al*., 2013), where they reach up to 50% of total cells (Bengtsson and Øvreås, 2010, Pizzetti *et al*., 2011). We observed identical $OTU_{0.97}$ in bottom waters and surface sediments. In sediments, these *Planctomycetes* live attached to sediment grains. This allows a direct access to POM and HMW organic matter and makes *Planctomycetes* in sediments as successful as in biofilms on algal surfaces and planktonic aggregates. Marine algae are rich in sulfated polysaccharides and in storage compounds (e.g. starch) which can be used by *Planctomycetes* (Schlesner *et al*., 2004; Jeske *et al*., 2013; Lee *et al*., 2013). Notably, the high number of sulfatases in *Planctomycetes* genomes of *Rhodopirellula* spp., *Blastopirellula marina, Pirellula staleyi* and other strains (Glöckner *et al*., 2003; Kim *et al*., 2016) and their distinct expression when grown on chondroitin sulfate, λ-carrageenan and fucoidan (Wegner *et al*., 2013), support the specialization of *Planctomycetes* in the degradation of diverse recalcitrant sulfated material.

Within the class *Phycisphaerae*, most sequences were affiliated with uncultivated clade Urania-1B-19. As indicated by DNA stable-isotope probing, relatives of this group colonize marine aggregates and readily utilize proteins (Orsi *et al*., 2016). *Phycisphaera mikurensis* is additionally capable of nitrate reduction or fermentation suggesting an adaptation to periodically anoxic conditions in marine surface sediments (Fukunaga *et al*., 2009).

In the following we summarize our hypotheses on potential functions of major taxa in the degradation of organic matter in sandy surface sediments. Compared to the water column, marine sandy sediments are poorer in fresh and easily degradable substances. Organic matter reaching the surface of the sediments can be recalcitrant and is composed of POM as well as dissolved HMW and LMW organic matter.

Benthic *Planctomycetes* likely initiate the breakdown of POM and HMW organic matter. Benthic *Flavobacteriaceae* are taxonomically different from planktonic *Flavobacteriaceae*. Unlike planktonic flavobacteria, cultivated benthic species have no or only limited capabilities to degrade macromolecules. They rather use simple carbohydrates or are fermentative bacteria. Fermentation products, such as ethanol and fatty acids, might be used by myxobacterial *Sandaracinaceae*. *Acidobacteria* were among the most abundant phyla (up to 15% of total cells), however, their role is still unclear. Periods of anoxia regularly occur in highly dynamic permeable surface sediments. In the absence of oxygen, remineralization of organic matter is mediated through dissimilatory reduction of sulfur compounds by *Desulfobacteraceae* and *Desulfobulbaceae*. Biogenically formed reduced sulfur species feed sulfide-oxidizing autotrophic *Acidiferrobacter* and *Sulfurovum/Sulfurimonas*. Members of the *Woeseiaceae*/JTB255 are metabolically diverse and likely also involved in sulfide oxidation in North Sea sediments.

Experimental procedures

Sampling

Surface sediments and bottom waters were retrieved from seven stations (Fig. 1) in the North Sea in March 2014 (cruise RV He417), September 2014 (cruise RV He432) and February 2015 (cruise RV Uthoern). Bottom waters were sampled with a rosette or a Niskin bottle three to five meters above sediment surfaces always prior to sediment retrieval. Between 200 ml and 1000 ml were immediately filtered onto 0.2 μm pore size polycarbonate filters and stored at −20°C until further processing. Undisturbed surface sediment was retrieved with a box corer, a Van Veen grab sampler or a multicorer. Sediment was sampled with PVC cores and immediately subsampled in 0.5 cm to 1 cm depth intervals. Sediment subsamples were frozen at −20°C or fixed for CARD-FISH.

Sediment properties and oxygen penetration depth measurements

Subsamples for sediment grain size distribution were measured using a laser diffraction particle size analyzer (Beckman Coulter, LS 200) for 92 size classes ranging from 0:4 to 2000 μm. The sediment permeability was calculated based on

median grain size (d_g) following the empirical relation by Gangi (1985):

$$k = Dar \times 735 \times 10^6 \times {d_g}^2$$

where k is the permeability in m^2 and *Dar* is the conversion factor for unit Darcy into m^2 ($=9.869 \times 10^{-13}$). Based on median grain size and permeability, the sediment was classified as (1) fine grained and low to impermeable ($d_g < 165$ μm, $k < 2 \times 10^{-11} m^2$), (2) medium grained and medium permeable ($165\ \mu m < d_g < 370\ \mu m$, $2 \times 10^{-11}\ m^2 < k < 1 \times 10^{-10}\ m^2$), and (3) coarse grained and highly permeable ($370\ \mu m < d_g$, $1 \times 10^{-10}\ m^2 < k$).

Oxygen penetration depths were measured in situ by an automated benthic observatory using 10 cm long needle shaped oxygen optodes (Pyroscience, Germany). At each station 12 oxygen profiles were measured along a 60 cm transect at least eight times over a tidal cycle to cover the spatial and temporal variation of oxygen penetration depths. For details see Ahmerkamp (2016).Despite oxygen penetration depths of >2 cm, 40% (sites CCP-G, NOAH-B) to 50% of penetration depth (site NOAH-E) regularly changed between oxic and anoxic conditions. Supporting Information Table S1 summarizes information on sediment site characteristics.

DNA extraction, sequencing and sequence processing

Total nucleic acids were extracted from 2 g to 5 g of homogenized surface sediments or, for water samples, from 0.5 to 1 polycarbonate filter according to Zhou and colleagues (Zhou *et al.*, 1996). For each sample, six replicate PCR reactions were set up using a master mix containing 1x high fidelity buffer, 0.02U/μL high-fidelity Phusion polymerase (Thermo Fisher Scientific Inc., USA), 1.5 mM $MgCl_2$, 5% DMSO, 0.2 mM dNTPs, and 0.4 μM of each primer 341f and 785rev (Herlemann *et al.*, 2011; Klindworth *et al.*, 2013). The PCR program started with an initial denaturation step for 30s at 98°C, followed by 25 cycles of 98°C for 10s, 55°C for 30s, 72°C for 22s and a final extension for 10 min at 72°C. Each forward primer carried a unique 6 nucleotide identifier, which allowed parallel multiplex sequencing of 26 different samples. PCR products were combined into a single pool at equal molarity and sequenced (2x300 bases, paired-end) on an Illumina MiSeq instrument (Illumina Inc., USA). Reads were quality trimmed (>q21, minlength = 250) and merged (strict, overlap >60) using software package BBmap v4.3. Reads were further processed with a modified MiSeq SOP (Kozich *et al.*, 2013) using mothur v1.22.2 (Schloss *et al.*, 2009), classified with (SILVA database SSU Ref NR, release 119 (Quast *et al.*, 2013) and globally clustered in operational taxonomic units (OTU) at 97% sequence divergence. To reduce any sequencing- and/or PCR-derived biases we included a preclustering step (99% sequence identity) and uchime chimera removal (Edgar *et al.*, 2011) removing 70% of unique sequences. Finally, all 16S rRNA gene sequences classified as mitochondria, chloroplasts, *Archaea* and 'unclassified' were removed from the dataset. Illumina sequencing raw data have been stored in the

European Nucleotide Archive (ENA) under study accession number PRJEB18774 (http://www.ebi.ac.uk/ena/data/view/PRJEB18774).

Statistical analysis

Statistical tests were performed using the R package vegan (Oksanen *et al.*, 2013) and customized R-scripts with $OTU_{0.97}$. Sequence abundance tables were randomly subsampled to the lowest number of sequences in the dataset. Alpha diversity values of inverse Simpson and Chao1 (Chao, 1984), were calculated independently 25 times and are displayed as a mean value. Beta diversity of community dissimilarities are based on Bray-Curtis dissimilarity coefficient (Bray and Curtis, 1957) and was used for hierarchical clustering. To test whether differences in community structure between clusters of sediment samples are significant, analysis of similarity (ANOSIM) was performed with 999 permutations at equal group size treatment. ANOSIM values of R close to 1 indicate that dissimilarity of community structure between groups is high. Low *R* values indicate that groups cannot be separated based on community dissimilarity. Negative *R*-values indicate that variability between samples within groups was high (Chapman and Underwood, 1999).

Phylogenetic analysis

Phylogenetic analysis was done using the software package ARB (Ludwig *et al.*, 2004) based on the SILVA database SSU Ref NR (release123 for *Sandaracinaceae* and release 128 for *Planctomycetes*). For phylogenetic reconstruction of *Sandaracinaceae* the tree was calculated by maximum-likelihood (PhyML) without filter using 224 high quality (pintail > 90, seq_quality > 91, align-quality > 92) nearly full-length sequences (>1300 bp). $OTU_{0.97}$ representative sequences (clustered using mothur) were added under parsimony criteria. Phylogenetic trees for *Planctomycetes* were done based on ca. 2700 nearly full length (>1400 bp) and high quality (pintail > 90) sequences. Prior to tree reconstruction, the aligment was manually curated. Trees were calculated by Maximum-likelihood (PhyML), FastTree, and neighbor-joining analysis using filters considering only positions that are conserved in 25%, 40% or 50% of the sequences (*E.coli position* 64-1388). For phylogenetic analysis, subsets of North Sea sequences were pooled and assigned to one of the following six categories: 'bottom waters above highly permeable sediments',' highly permeable sediments', 'bottom waters above medium permeable sediments', 'medium permeable sediments', 'bottom waters above impermeable sediments', and 'impermeable sediments'. These 6 datasets were classified using the SILVAngs pipeline (Quast *et al.*, 2013). Resulting *Planctomycetes* $OTU_{0.97}$ that comprised >10 sequences per category were added to the reconstructed trees under parsimony criteria with local/global optimization.

Oligonucleotide probe design

Probes were developed for *Sandaracinaceae* (probe SAND653), *Phycisphaerae* (probe PHYC309) and *Planctomycetia* clade DDSe3017 (probe Pla445ab) based on (SILVA database SSU Ref NR, release 123 (Supporting Information Table S3). Probe design was performed with the probe design tool implemented in ARB. Probes were tested on environmental samples at varying formamide concentrations from 0% to 60% at 46°C hybridization temperature. The highest possible

formamide concentration at which signals were still bright enough for detection was selected for subsequent hybridizations.

Catalyzed reporter deposition fluorescence in situ hybridization (CARD-FISH)

Sediment samples were fixed in 1.5% formaldehyde at room temperature for 1.5 h. Cells were dislodged using a HD70 ultrasonication probe at 20% for 5 × 30s (Bandelin, Berlin, Germany), transferred on polycarbonate membrane filter (0.2 μm pore size) and embedded in 0.1% low gelling temperature agarose. Prior to hybridization, filters were inactivated in methanol with 0.15% H_2O_2 for 20 min, washed and permeabilized in lysozyme solution (10 mg/ml) at 37°C for 45–60 min. To enhance the permeability of *Planctomycetes* cell walls, filter pieces for hybridization with probes EUB338 I-III, PLA46a, PLA445ab and PHYC309 were additionally permeabilized with achromopeptidase (60 U/ml) at 37°C for 30 min. Hybridization and CARD step was performed as described using Alexa488-labeled tyramides (Pernthaler *et al.*, 2002). Horseradish peroxidase (HRP)-labeled probes were ordered from Biomers.net (Ulm, Germany). Probes used and formamide concentrations are given in Supporting Information Table S3. For dual hybridizations, HRP from the first probe were inactivated by 3% H_2O_2 in MilliQ water for three minutes and second CARD amplification was performed with Alexa594-labeled tyramides.

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Conflict of Interest

The authors declare no conflict of interest.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. *Sandaracinaceae* in North Sea surface sediments. Panel A: Phylogenetic tree based on 16S rRNA gene sequences showing the affiliation of the family *Sandaracinaceae* within the *Deltaproteobacteria*. The tree was calculated by maximum-likelihood analysis with a filter considering only positions which are 50% conserved. Panel B: Phylogenetic tree showing the affiliation of most abundant North Sea $OTU_{0.97}$ (printed in green) to selected reference sequences within *Sandaracinaceae*. The tree was calculated by maximum likelihood analysis using nearly full length (>1300 bp) sequences without filter. Representative $OTU_{0.97}$ sequences were added under parsimony criteria. Probe coverage is indicated by grey colored box. Scale bar in panel A and B gives 10% estimated sequence divergence. Panel C: Confocal laser scanning micrograph of typical coccoid *Sandaracinaceae*-cells as detected by CARD-FISH using probe SAND653 (green). Total cells were stained with DAPI (blue). Scale bar, 1 μm.

Fig. S2. Micrographs showing *Phycisphaerae* in North Sea surface sediments as detected by CARD-FISH. Panel A: Typical particle-attached *Phycisphaerae*-cells visualized with the general DNA stain DAPI (printed in blue) and CARD-FISH probe PHYC309 targeting *Phycisphaerae* (printed in green), aquired with confocal laser scanning microscopy. Panel B: Dual hybridization with probe PLA46 (in green) targeting *Planctomycetes* (except *Phycisphaerae*) and PHYC309 (in red), counterstained with DAPI (blue), acquired with epifluoresence microscopy. Scale bar, 1 μm.

Table S1. Sediment characteristics. Except for CCP-D and NOAH-B (March 2014, February 2015), data were reprinted from Ahmerkamp, 2016[$].

Table S2. Diversity parameters of southern North Sea samples. Depicted values are a mean of 25 independently calculated diversity values using R-package vegan.

Table S3. Oligonucleotide probes used for CARD-FISH.