Bacterial community structure and functional profiling of high Arctic fjord sediments

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Abstract

Kongsfjorden, an Arctic fjord is significantly affected by the glacier melt and Atlantification, both the processes driven by accelerated warming in the Arctic. This has led to changes in primary production, carbon pool and microbial communities, especially that in the sediment. In this study, we have examined the bacterial community structure of surface (0–2 cm) and subsurface (3–9 cm) sediments of Kongsfjorden using the high throughput sequencing analysis. Results revealed that bacterial community structure of Kongsfjorden sediments were dominated by phylum *Proteobacteria* followed by *Bacteroidetes* and *Epsilonbacteraeota*. While α - and γ - *Proteobacteria* class were dominant in surface sediments; δ - *Proteobacteria* were found to be predominant in subsurface sediments. The bacterial community structure in the surface and subsurface sediments showed significant variations ($p \leq 0.05$). Total organic carbon could be one of the major parameters controlling the bacterial diversity in the surface and subsurface sediments. Functional prediction analysis indicated that the bacterial community could be involved in the degradation of complex organic compounds such as glycans, glycosaminoglycans, polycyclic aromatic hydrocarbons and also in the biosynthesis of secondary metabolites.

1. Introduction

Marine sediment depicts most complex microbial habitats on earth. Benthic bacterial communities in the ocean play a significant role in remineralization of organic matter (Ravenschlag et al. 2000). Marine microorganisms hydrolyse high molecular weight organic matter to sufficiently small constituents for their cellular intake and also make them easily available for higher trophic levels. Bacteria and archaea are the dominant microbial communities in marine sediments. These sediment communities are impacted by several physical and chemical parameters (Nguyen and Landfald, 2015; Jorgensen et al. 2012) and can be sensitive to environmental changes and are also affected by geographic distance and ocean currents (Hamdan et al. 2013; Xiong et al. 2014). Hence microbial community composition analysis is important to understand the benthic ecosystem processes and thereby to study about the effect of climate change.

Kongsfjorden, located on the northwest coast of Spitsbergen in the Svalbard Archipelago is a tide water glacial fjord in the Arctic. Atlantic water inflow as well as several glaciers influence the water column in the Kongsfjorden (Hop et al. 2002). The fjord is now identified as an ideal environment to study the impacts of climate change in the Arctic, indicated by the decrease in ice cover and a rise in melting. Physical gradients of salinity and temperature induced by the glacier melting, which generate turbidity and also sedimentation along the fjord system (Hop et al. 2002; Kotwicki et al. 2004) that ultimately affects the distribution of pelagic and benthic organisms (Kotwicki et al. 2004; Piquet et al. 2014). At the head of the fjord, tidal glaciers discharge fresh water and suspended loads, which generate steep environmental gradients (Węsławski et al. 2000), biomass and diversity of inner fjord benthic community are also affected (Hop et al. 2002). Thus, marine sediment microbial structure and function are greatly influenced by the changes in the physico-chemical properties of water such as temperature, nutrient availability, oxygen saturation, hydrodynamics and light patterns, being linked to climate change.

Bacterial diversity in an environment is usually discovered by both culture dependent and independent techniques. Culture dependent approaches enable to characterize the cultivated bacteria and their ecological and physiological role. Nevertheless, through culture dependent approaches only a small part ($< 1\%$) of the bacteria can be cultivated (Cole et al. 2014). Culture independent methods detect the bacterial diversity which can be cultured as well as that are yet to be cultured. Previous studies have mentioned that there are complex microbial groups present in marine sediments, although most of the sequences were distantly related to the culturable bacteria. Moreover, presence of a significant fraction of bacteria that do not belong to any known taxonomic group indicates the presence of unique species which warrants further study of bacterial communities in the marine sediments (Xuezheng et al. 2014; Zhang et al. 2015; Sinha et al. 2019). Culture independent methods, besides providing insights in to the uncultured diversity, also help us to access the functional genes and biochemical pathways within these uncultured microorganisms (Simon and Daniel 2011).

Advancement of culture independent methods has revolutionised the studies of bacterial communities in Arctic fjords. In Arctic sediments, various culture independent bacterial diversity studies have been conducted (Li et al. 2009; Zeng et al. 2011, 2017; Fang et al. 2019). The primary objective of this study was to explore the bacterial diversity of surface (0–2 cm) and subsurface (3–9 cm) sediments from various stations of Kongsfjorden, Arctic. Metagenomic analysis of V_3 - V_4 amplicon regions of 16S rRNA genes were carried out in this study by using Illumina HiSeq 2500 platform. This study allows broad understanding of bacterial diversity in surface and subsurface sediments of Kongsfjorden.

2. Materials And Methods

2.1. Site description and sample collection

Kongsfjorden is situated on the western side of Spitsbergen in Svalbard archipelago (79° N, 12° E), which is influenced by both Arctic and Atlantic water. The sediment samples were collected on board research vessel R V Teisten from four locations of the Kongsfjorden (Fig. 1) using a Van Veen's grab during Arctic expedition in October 2016. Using a sterile glass corer of one-inch diameter, surface (0–2 cm) and subsurface sediments (3–9 cm) from four stations were subsampled aseptically from the undisturbed sediments collected in the grab. Physico-chemical variables of the sediment samples are presented in Table 1. The sediment samples were frozen at -80°C and brought to the laboratory at Cochin University of Science and Technology (CUSAT) for metagenomic analysis.

Table 1 Details of geological description of sampling sites and various environmental parameters measured in the sediment samples collected from the four stations of Kongsfjorden.														
Samples	Depth (m)	TC (%)	TOC (%)	TN (%)	TS (%)	Mg (%)	P (%)	Ca (%)	Fe (%)	Mo (ppm)	Mn (ppm)	Co (ppm)	Cu (ppm)	Zn (ppm)
St-1 (0-2cm)	230	3.38	2.86	0.3	0.12	2.806	0.481	0.842	2.444	1.19	297.56	13.92	29.43	82.02
St-2 (0-2cm)	230	3.24	1.72	0.24	0.12	3.42	0.209	1.091	2.815	1.22	530.81	16.2	26.53	97.48
St-3 (0-2cm)	274	2.71	0.94	0.08	ND	4.958	0.6	1.457	2.103	1.32	705.47	22.1	29.89	116.09
St-4 (0-2cm)	230	2.17	0.98	0.06	ND	2.627	0.496	1.03	2.445	0.99	516.58	18.44	29.74	90.92
St-1 (3-9cm)	230	3.15	1.68	0.25	0.2	3.828	0.523	1.684	2.757	2.72	410.61	13.64	27.45	201.2
St-2 (3-9cm)	230	3.11	1.20	0.2	ND	3.238	0.918	1.215	2.512	1.83	443.92	16.38	28.85	228.86
St-3 (3-9cm)	274	2.70	0.39	0.08	ND	3.4	0.281	1.546	2.981	1.12	642.06	22.6	35.44	174.11
St-4 (3-9cm)	230	1.75	0.94	0.05	ND	3.171	0.225	1.075	1.727	0.73	456.47	15.1	26.62	150.62

2.2. Geochemical analysis

Geochemical characteristics of eight sediment samples from four stations were carried out. Total carbon, nitrogen and sulphur analysis of sediments using CHNS analyzer (ELEMENTAR Vario EL111) and analysis of trace elements by inductively coupled plasma mass spectrometry (Icap RQ ICP-MS) were done at Sophisticated Test and Instrumentation Centre (STIC), Cochin University of Science and Technology (CUSAT). Total organic carbon was detected by using Walkley- Black titration method (Walkley and Black, 1947). Statistical analysis (ANOVA) was conducted to find out the significant relationship between environmental parameters.

2.3. DNA extraction and PCR amplification

As per manufacturer’s instructions, total genomic DNA of eight sediment samples from four different stations was extracted using Ultra Clean soil DNA isolation kit (MO BIO, CA, USA). DNA concentration was determined using Qubit™ 4 Fluorometer and quality of the isolated DNA samples were checked by agarose gel electrophoresis using 1% agarose gel. Isolated DNA from the surface sediment samples (0–2 cm) and sub-surface sediment samples (3–9 cm) of four stations were pooled separately and sent for 16S V₃-V₄ amplicon sequencing (Macrogen Inc, Republic of Korea). The universal primer set Pro341F/Pro805R was used for amplification of V₃-V₄ regions of 16S rRNA (Takahashi et al. 2014).

2.4. 16S rRNA library preparation and sequencing

In order to generate amplicon library, a unique pair of barcoded primers with Illumina adaptors was used. By using Qubit 2.0 Fluorometer and Agilent Bioanalyzer 2100 system quality of the libraries was analysed. Library was loaded on a flow cell for the cluster generation,

these fragments are occupied on the surface of oligos which are complementary to the adapters in the library. Discrete clonal clusters were generated by using the fragments through bridge amplification. Subsequent to cluster generation, sequencing of templates was performed on an Illumina HiSeq 2500 platform (2 x 300 bp size). Afterwards, DNA sequence data was submitted in the NCBI Sequencing Read Archive with accession number PRJNA678587.

2.5. Quality filtering, operational taxonomic units (OTUs) picking and annotation

After sequencing on an Illumina HiSeq platform raw fastq reads were created. The 16S rRNA primers and barcodes were removed to produce paired-end reads using in-house PERL script. Quality of the reads was detected using the FastQC program. Trimmed paired-end reads with Phred score quality > Q20 was taken for consensus generation. The paired-end reads were combined using FLASH tool (latest version. 1.2.11). By using UCLUST program, operational taxonomic units (OTUs) were generated by clustering the merged reads on the basis of their sequence similarity (similarity cut off = 0.97). In this step, singleton OTUs were removed. Then UCHIME method is applied for chimera removal using VSEARCH tool. The OTUs were processed using QIIME programme to generate a characteristic sequence for every OTU, then the sequence is aligned into SILVA rRNA 132 rRNA reference database. By using QIIME software OTUs depicting chloroplasts and mitochondrial sequences were cleared (Caporaso et al. 2010).

2.6. Diversity analysis

For the analysis of alpha diversity, OTU numbers were rarefied. Shannon, Chao1 and observed species metrics were used to determine alpha diversity indices. Venny 2.1 software was used to create a Venn diagram to indicate the number of OTUs in surface and subsurface sediments and the common OTUs shared between the surface and subsurface sediments. ANOVA was performed to find out the difference in bacterial community structure in the surface and subsurface samples.

2.7. Prediction of functional profiles

Prediction of functional gene profiles of bacterial community composition in the surface and subsurface sediments were generated on the basis of 16S rRNA gene sequences data using PICRUSt2 (Langille et al. 2013; Douglas et al. 2020). Greengenes database was used to compile the data for PICRUSt analysis. By using the database, OTUs were picked on the basis of 97% sequence similarity. By using KEGG Orthology (KO), estimate of the number of predicted gene families were obtained from metagenomic predictions. These counts were introduced into KEGG pathways and relative abundance for KO and KEGG pathways were calculated. NSTI (nearest sequenced taxon index) values were used to analyse the accuracy of functional prediction.

3. Results

3.1. Characteristics of sediment samples

The sampling sites and geochemical characteristics of the 8 sediment samples (both surface and subsurface sediments) from four different stations were analysed and summarized (Table 1). Total carbon and total organic carbon in the surface sediments varied from 2.17 to 3.38% and 0.94 to 2.86% respectively while that in subsurface sediments varied from 1.75 to 3.15% and from 0.39 to 1.68% respectively. Total nitrogen in the surface and subsurface sediment samples ranged from 0.06 to 0.3% and from 0.05 to 0.25% respectively. TS (Total sulphur) was detected only in sediments from two stations. Subsurface sediment of station-1 had higher amount of TS (0.2%) than the surface sediments of station-1 and station-2, where both the stations had 0.12% TS. The Mg, P, Ca and Fe content of surface sediment samples were found to vary from 2.62 to 4.95%, 0.20 to 0.49%, 0.84 to 1.45% and 2.10 to 2.81% respectively, while that in subsurface sediments varied from 3.17 to 3.82%, 0.22 to 0.91%, 1.07 to 1.68% and 1.72 to 2.98% respectively. Mo, Mn, Co, Cu and Zn content of surface sediments varied from 0.99 to 1.32 ppm, 297.56 to 705.47 ppm, 13.92 to 22.1 ppm, 26.53 to 29.89 ppm and 82.02 to 116.09 ppm respectively and in subsurface sediments their concentration varied from 0.73 to 2.72 ppm, 410.61 to 642.06 ppm, 13.64 to 22.6 ppm, 26.62 to 35.44 ppm and 150.62 to 228.86 ppm respectively. TC, TOC and TN contents were found to be higher in the surface sediment of station-1. Mg (Magnesium) and Mn (Manganese) content were found to be higher in surface sediment of station-3. Amount of P (Phosphorus) was higher in subsurface sediment of station-2. Compared to other stations Ca (Calcium) content was lower in surface sediment of station-1 and Fe (Iron) content was lower in subsurface sediment of station-4. Amount of Mo (Molybdenum) was higher in subsurface sediment of station-1. Amount of Co (Cobalt) was found to be comparatively lower in both surface and subsurface sediments of station-1 and higher in both surface and subsurface sediments of station-3. Cu (Copper) content was higher in subsurface sediment of station-3. Compared to surface sediments, subsurface sediments had higher content of Zn (Zinc).

Statistical analyses indicated that there were no significant statistical variation in the environmental parameters (TC, TOC, Fe, Mg, Ca, Mo and TOC:TP ratio) among the four stations of Kongsfjorden (Supplementary table 1), however, the parameters such as TC, TOC, TN, TC:TN showed significant difference in surface and subsurface sediments ($p \leq 0.05$). It clearly indicates that TC, TOC and TN are important parameters controlling the geochemistry and bacterial community structure in the surface and subsurface sediments.

3.2. Diversity of bacteria in the sediments of Kongsfjorden

A diverse group of microbial community have been reported from Arctic sediments as well as in the water column. In this study, total of 2129 OTUs from surface sediments and 2203 OTUs from subsurface sediments were obtained. In total 4332 OTU and 158021 reads were identified among surface and subsurface sediments. The identified OTUs belonged to thirty six known phyla, one unknown phylum, thirteen candidate phyla and an uncultured phylum. In addition, 156 sequences remained unclassified and are not found in reference databases (Supplementary Table 2). Top 25 phyla with higher relative abundance is shown in Table 2.

Table 2
List of bacterial phyla observed in the surface (V-A) and subsurface (V-B) sediment samples collected from Kongsfjorden. Number of OTUs observed and number of reads (N) are also detailed. Relative abundance of top 25 phyla shown in the table and complete list is provided as supplementary table 1.

	Sr. No.	Phylum	V-A		V-B	
			Total OTUs	% of reads	Total OTUs	% of reads
Abundant Phyla	1	<i>Proteobacteria</i>	859	31794	673	26041
	2	<i>Bacteroidetes</i>	382	18749	239	6723
	3	<i>Planctomycetes</i>	117	994	151	1585
	4	<i>Acidobacteria</i>	73	551	110	2009
	5	<i>Verrucomicrobia</i>	72	2717	44	685
	6	<i>Unknown</i>	67	10202	89	2703
	7	<i>Kiritimatiellaeota</i>	55	985	59	854
	8	<i>Chloroflexi</i>	53	354	157	3855
Moderate Abundant Phyla	9	<i>Epsilonbacteraeota</i>	47	6117	55	25230
	10	<i>Actinobacteria</i>	46	751	49	1249
	11	<i>Spirochaetes</i>	45	828	62	1094
	12	<i>Patescibacteria</i>	36	210	66	498
	13	<i>Latescibacteria</i>	36	267	63	833
	14	<i>Firmicutes</i>	35	1150	29	430
	15	<i>Gemmatimonadetes</i>	26	218	22	491
	16	<i>Lentisphaerae</i>	22	296	16	128
Rare Phyla	17	<i>Fibrobacteres</i>	19	290	23	229
	18	<i>Nitrospirae</i>	18	202	18	310
	19	<i>Zixibacteria</i>	16	89	34	318
	20	<i>Tenericutes</i>	16	600	15	195
	21	<i>BRC1</i>	13	63	24	260
	22	<i>Hydrogenedentes</i>	12	104	14	82
	23	<i>Calditrichaeota</i>	9	77	19	456
	24	<i>Elusimicrobia</i>	8	28	18	125
	25	<i>Nanoarchaeota</i>	7	21	32	242

Top OTUs from surface and subsurface sediments with relative abundance have been listed in Supplementary Table 3. OTU 11974 *Sulfurovum* was the most dominant OTU in subsurface sediments (23.35%). In surface sediments, OTU 21841 *Lutibacter* (4.84%) was the dominant and OTU 11974 *Sulfurovum*, OTU 4005 *Sva1033* (3.29%) were the second and third most dominant OTUs. OTU 22317 *Desulfobulbaceae* (4.14%), OTU 22621 *Sandaracinaceae* (1.83%) were found to be second and third most dominant OTUs in subsurface sediments. Sequences affiliated to OTU 20948 *Marinifilum* (3.16%), OTU 876 *Psychrilyobacter* (1.92%), OTU 24245 *Woeseia* (1.69%) and OTU 17018 *Desulforhopalus* (1.44%) were more abundant in surface sediments. OTU 23206 and OTU 18534 which were affiliated to the sequences of *Sulfurimonas* were more dominant in subsurface sediments. OTU 33074, 18062 and 7091 *Desulfobacteraceae* were more predominant in subsurface sediments. Shannon diversity index was found to be higher in the surface sediments (5.97) than in the subsurface sediments (5.24).

3.3. Bacterial community analysis

In surface sediments, the identified OTUs belonged to 46 phyla, 98 classes, 192 orders, 252 families, 330 genera, while in the subsurface sediments we could observe 51 phyla, 104 classes, 207 orders, 243 families and 290 genera. At phylum level, 46 phyla were common in surface and subsurface sediments. In the identified phyla, *Proteobacteria*, *Bacteroidetes*, *Planctomycetes*, *Chloroflexi* and *Acidobacteria* had highest number of unique OTUs and *Proteobacteria*, *Epsilonbacteraeota* and *Bacteroidetes* comprising high number of sequences. Phylum level composition revealed that, *Proteobacteria* (36.56%) were most abundant followed by *Epsilonbacteraeota* (19.87%), *Bacteroidetes* (16.04%), *Chloroflexi* (2.68%), *Verrucomicrobia* (2.14%), *Planctomycetes* (1.65%), *Acidobacteria* (1.63%), *Fusobacteria* (1.33%) and *Actinobacteria* (1.27%) accounting for 83.17% of total reads.

ANOVA analysis indicated that surface and subsurface sample have significant difference in bacterial diversity in various clade level ($p \leq 0.05$) (Supplementary table 4). Irrespective of the sediment core depth, *Proteobacteria* was the dominant phylum in surface (39.87%) and subsurface sediments (33.25%). As compared to surface sediments, the relative abundance of *Epsilonbacteraeota* was high in subsurface sediments (32.09%). *Bacteroidetes* was higher in surface (23.47%) compared to subsurface sediments (8.61%). While *Chloroflexi* (4.9%), *Planctomycetes* (2.02%), *Acidobacteria* (2.56%) and *Actinobacteria* (1.59%) were higher in subsurface sediments, *Verrucomicrobia* (3.4%) and *Fusobacteria* (2.01%) were higher in surface sediments (Supplementary Table 5 & Fig. 2).

Of the 105 classes identified, 97 were detected in both surface and subsurface sediments. Based on the average relative abundance, *Deltaproteobacteria* (21.28%) were most abundant followed by *Campylobacteria* (19.87%), *Bacteroidia* (15.58%), *Gammaproteobacteria* (12.92%), *Alphaproteobacteria* (2.3%) and *Verrucomicrobiae* (2.14%) were the six broad classes of bacteria, accounting for 74.09% of the total taxonomy. *Bacteroidia* was the largest class in surface sediments (23.19%); *Campylobacteria* was the largest class in subsurface sediments (32.09%). In the phylum *Proteobacteria*, *Gammaproteobacteria* (18.09%) was predominant in surface sediments followed by *Deltaproteobacteria* (17.99%) and *Alphaproteobacteria* (3.72%). *Deltaproteobacteria* (24.58%) exhibited higher abundance than *Gammaproteobacteria* (7.76%) in subsurface sediments, while presence of *Alphaproteobacteria* was less abundant (0.88%). *Verrucomicrobiae* and *Fusobacteria* were more abundant in surface than in subsurface sediments; *Anaerolineae* and *Dehalococcoidia* exhibited higher abundance in subsurface sediments compared to surface sediments (Supplementary Table 6 & Fig. 3a).

The dominant order in the fjord sediments were *Campylobacteriales* (19.87%), *Desulfobacteriales* (11.8%), *Flavobacteriales* (8.22%), *Bacteroidales* (5.64%), *Desulfuromonadales* (4.6%) and *Alteromonadales* (3.33%) accounting for 53.46% of total reads. *Flavobacteriales* (14.08%) was the largest order in surface sediments while in subsurface sediments was dominated by *Campylobacteriales* (32.09%). In surface sediments, *Desulfobacteriales* (8.38%) was the second most dominant followed by *Campylobacteriales* (7.65%), *Desulfuromonadales* (6.72%), *Bacteroidales* (6.44%) and *Alteromonadales* (5.15%) while in subsurface sediments, *Desulfobacteriales* (15.23%) and *Bacteroidales* (4.84%) (Supplementary Table 7 & Fig. 3b) followed the dominant order *Campylobacteriales*. At the Family level, *Flavobacteriaceae* (13.27%), *Desulfobulbaceae* (5.75%), *Sva1033* (4.54%), *Marinifilaceae* (4.03%) and *Sulfurovaceae* (3.63%) were dominant in surface sediments whereas *Sulfurovaceae* (24.51%), *Desulfobulbaceae* (7.8%), *Desulfobacteraceae* (7.43%) and *Thiovulaceae* (7.38%) were abundant in subsurface sediments (Supplementary Table 8 & Fig. 3c).

At the genus level, *Lutibacter* (6.43%), *Marinifilum* (3.63%), *Sulfurovum* (3.63%), *Sulfurimonas* (2.10%), *Psychromonas* (2.80%) and *Woeseia* (2.52%) were found to be dominant in surface sediments and genera *Sulfurovum* (24.51%) and *Sulfurimonas* (7.38%) followed by *SEEP-SRB1* (3.25%) which was under the phylum *Deltaproteobacteria* were seen predominant in subsurface sediments (Supplementary Table 9 & Fig. 3d). Venn diagram showed that surface sediments and subsurface sediment shared 1435 OTUs (Fig. 4).

3.4. Functional potentials of bacterial diversity

Functional diversity of bacterial community composition in surface and subsurface sediments were revealed by PICRUSt2 assignment of KO's to KEGG pathways. In surface sediments (V-A), total of 6627 different KO's which containing 88924551 counts were predicted. Total

of 6549 KO's with 70697628 counts were predicted in subsurface sediments (V-B). In surface sediments, these counts were introduced to 164 KEGG pathways and indicate NSTI score of 0.21, which shows reliability of functional prediction. In subsurface sediments, counts were assigned to 160 KEGG pathways and NSTI score of 0.29 was indicated. Functional prediction showed significant variations in the surface and subsurface sediments ($p \leq 0.05$). KEGG pathways which having important role in cellular homeostasis such as, carbohydrate metabolism (12.89 and 13.21%), amino acid metabolism (12.66 and 12.57%), energy metabolism (6.3 and 6.21%), replication and repair (5.17 and 5.44%), xenobiotic degradation and metabolism (4.45 and 3.77%), translation (3.02 and 3.34%), biosynthesis of other secondary metabolites (2.07 and 2.15%) and membrane transport (1.58 and 1.71%) were identified in the surface and subsurface sediments respectively (Fig. 5). In carbohydrate metabolism, predicted gene clusters for complex polysaccharide degradation such as β -glucosidase (0.08 and 0.04%) and endoglucanase (0.03%) was observed in surface and subsurface bacterial community. Genes for endo-1,4-beta xylanase, D-Inositol-3-phosphate glycosyl transferase, glycan degradation, glycosaminoglycan degradation, polycyclic aromatic hydrocarbon degradation were also observed in low abundance. Gene clusters for glycogen phosphorylase was observed in bacterial community of subsurface sediments (0.07%). KEGG pathways for xenobiotic degradation consisted of toluene degradation (0.52 and 0.62%), chloroalkane and chloroalkene degradation (0.42 and 0.32%), nitrotoluene degradation (0.35 to 0.61%), caprolactam degradation (0.28 and 0.20%) and benzoate degradation (0.23%); similarly, pathways for biosynthesis of other secondary metabolites indicated by streptomycin biosynthesis (1.51 and 1.58%) were also observed at level 2 in surface and subsurface sediment bacterial community respectively.

4. Discussion

The deposition and long-term burial of organic carbon in marine sediments has played a significant role in controlling atmospheric oxygen and carbon dioxide concentrations over the past 500 million years (Berner, 1982). Carbon burial in polar environments represents the dominant natural mechanism of long-term organic carbon sequestration with respect to climate change (Hedges et al., 1997). Fjords have been considered as hotspots of organic carbon burial as they received large amounts of carbon through annual productivity cycle and glacial melt. For instance, Kongsfjorden receives 5–10% of the organic carbon from terrestrial origin by melting of glaciers and about 90–95% of the organic carbon through primary production primarily fuelled by intrusion of Atlantic water (Kuliński et al. 2014). Buchholz and Wiencke (2016) reported that macroalgae, in particular Kelps contribute significantly to the pool of organic carbon in the Kongsfjorden. The sampling was conducted at the onset of winter; as the season is ideal for studying the microbial communities associated with organic carbon degradation in the Kongsfjorden. We found that spatially there was no significant difference in the TC and TOC content among the stations. Further, Sinha et al. (2017) have reported that seasonal variability in community is much more profound than spatial variability within a particular season. However, it is pertinent to note that vertical variability in distribution of organic carbon between the surface and subsurface sediment showed significant variation ($p \leq 0.05$).

Considering the above, the strategy adopted in this study with respect to sampling, extraction of DNA and sequencing could be adequate to delineate the diversity of bacteria in the surface and subsurface sediments during the onset of winter. We pooled the DNA sample in the surface stations together and subsurface stations together for bacterial diversity analysis because no significant variation in the organic matter content in the different stations. Our study delineated the observations that organic matter content may have influences the structuring the bacterial communities in the surface and subsurface sediments of Kongsfjorden. The functional profiling indicated that the bacteria were involved in the various remineralization processes of organic carbon such as glycan degradation, glycosaminoglycan degradation, polycyclic aromatic hydrocarbon degradation, etc.

In this study, bacterial community structure of surface and subsurface sediments of Kongsfjorden revealed through Illumina sequencing is consistent with several previous studies on bacterial community pattern of Arctic sediments (Ravenschlag et al. 1999, 2001; Li et al. 2009; Teske et al. 2011; Xuezheng et al. 2014; Zhang et al. 2014; Li et al. 2015; Zeng et al. 2017; Fang et al. 2019). The dominant phyla identified in our study was known to be widely seen in deep sea sediments (Zhang et al. 2015; Sinha et al. 2019), Crustal fluids of a deep sea hydrothermal field (Kato et al. 2013) and deep sea vents (Sylvan et al. 2012). Studies of Tian et al. (2009) disclosed that the 16S rRNA gene clone sequences retrieved from Kongsfjorden sediments having similarity to the bacterial sequences obtained from several hydrothermal vent sites. This could be very likely due to the seepage of deep ocean sediments into fjord basins by the action of bottom water currents.

In our study, *Proteobacteria* was the most dominant phyla comprising 39.87% and 33.25% of the total bacterial community structure in the surface and subsurface sediments respectively. Within this phylum, γ -*Proteobacteria* and δ -*Proteobacteria* were the two main classes encountered. In the surface sediments, the γ -*Proteobacteria* became the largest group (18.09%) followed by δ -*Proteobacteria* (17.99%), whereas the α -*Proteobacteria* contributed only 3.72%. In the subsurface sediments, δ -*Proteobacteria* was the dominant class of phylum *Proteobacteria* (24.58%), whereas the γ -*Proteobacteria* made up 7.76%. In contrast, to the abundance of γ and δ -*Proteobacteria*,

abundance of *α-Proteobacteria* (0.88%) was lower in subsurface sediments. Similar findings was also reported in a study of Teske et al. (2011) on microbial community composition of surface and subsurface sediments from a fjord of Svalbard by using 16S rRNA gene clone library analysis. They have observed dominance of *γ-Proteobacteria* in surface sediments and *δ-Proteobacteria* in subsurface sediments. Several studies have revealed that the *Proteobacteria* was the most cosmopolitan group in surface sediments (Ravenschlag et al. 2001; Bowman et al. 2003) and *γ-Proteobacteria* was the most significant group of bacteria in majority of the marine sediments (Li et al. 1999; Oni et al. 2015). Similar observations were also reported in sediments of diverse locations as shown in Table 3. In several studies, *δ-Proteobacteria* comprise a significant fraction of bacterial communities in marine surface sediments (Park et al. 2011; Bienhold et al. 2016), a finding similar to our observations, where we have got significant fraction of *δ-Proteobacteria* in surface sediments (17.99%). However, the dominance of *δ-Proteobacteria* was slightly less than *γ-Proteobacteria* (18.09%) in surface sediments. The *γ* and *δ-Proteobacteria* are the predominant group in various sediments of diverse locations listed in Table 3.

Table 3

List of bacterial groups reported in diverse locations in several studies similar to the dominant bacterial groups identified in this study.

Dominant bacteria and reported study locations	Reference
<p><i>Proteobacteria</i> were most abundant followed by <i>Epsilonbacteraeota</i>, <i>Bacteroidetes</i>, <i>Chloroflexi</i>, <i>Verrucomicrobia</i>, <i>Planctomycetes</i>, <i>Acidobacteria</i>, <i>Fusobacteria</i> and <i>Actinobacteria</i>.</p> <p>In the phylum <i>Proteobacteria</i>, <i>Gammaproteobacteria</i> was more abundant in surface sediments followed by <i>Deltaproteobacteria</i>; <i>Deltaproteobacteria</i> exhibited higher abundance than <i>Gammaproteobacteria</i> in subsurface sediments.</p> <p>Genera <i>Sulfurovum</i> and <i>Sulfurimonas</i> found to be dominant in surface and subsurface sediments</p>	This study
<p>γ-Proteobacteria as the most significant group</p> <p>Western Pacific warm pool</p> <p>Eastern Mediterranean Sea</p> <p>Polar marine sediments</p>	<p>Zeng et al. 2005</p> <p>Polymenakou et al. 2005a</p> <p>Ravenschlag et al. 2001</p> <p>Bowman et al. 2005</p>
<p>γ and δ-Proteobacteria are the predominant group in various sediments</p> <p>Antarctic continental shelf</p> <p>Yamada Bay in Japan</p> <p>Helgoland Mud Area North Sea</p> <p>Pacific Arctic ocean</p> <p>Arctic Spitsbergen</p>	<p>Bowman and McCuaig 2003</p> <p>Asami et al. 2005</p> <p>Oni et al. 2015</p> <p>Hamdan et al. 2013</p> <p>Li et al. 2009</p> <p>Ravenschlag et al. 1999</p>
<p>Sulfurovum and Sulfurimonas</p> <p>Okinawa Trough hydrothermal sediments</p> <p>Deep sea hydrothermal vent ecosystem</p> <p>Arctic sediment</p>	<p>Inagaki et al. 2004</p> <p>Takai et al. 2006</p> <p>Akerman et al. 2013</p> <p>Xuezheng et al. 2014</p>
<p>Bacteroidetes</p> <p>Sediments of Arctic fjord</p> <p>North Sea</p> <p>Northern Bering Sea</p>	<p>Cardman et al. 2014</p> <p>Xuezheng et al. 2014</p> <p>Teske et al. 2011</p> <p>Oni et al. 2015</p> <p>Zeng et al. 2011</p>

Dominant bacteria and reported study locations	Reference
Epsilonbacteraeota Hydrothermal vent field Marina back arc venting fluid Sub-seafloor crustal aquifer	Fortunato et al. 2018 Storesund et al. 2018 Trembath-Reichert et al. 2019 Tully et al. 2018
Chloroflexi Deep marine sediments Metal rich deposits in western Pacific Ocean Deep sea hydrothermal sediments	Teske 2006 Oni et al. 2015 Zhang et al. 2015 Liao et al. 2011 Flores et al. 2012
Actinobacteria Higher abundance in inner fjord of Kongsfjorden Arctic marine sediment	Zeng et al. 2013 Sinha et al. 2017 Zhang et al. 2014
Acidobacteria Sediments from Southern Cretan margin Sediments from South Ionian Sea Northern Bering Sea Svalbard sediments	Polymenakou et al. 2009 Polymenakou et al. 2005b Zeng et al. 2011 Teske et al. 2011
Planctomycetes Svalbard sediments Northern Bering Sea	Ravenschlag et al. 2001 Tian et al. 2009 Zeng et al. 2011
Verrucomicrobia Smeerenburgfjorden in Arctic Kongsfjorden Arctic marine water column	Cardman et al. 2014 Tian et al. 2009 Bano and Hollibaugh 2002 Sinha et al. 2017
Firmicutes Arctic marine sediments	Muller et al. 2014

Other dominant groups of bacteria detected in our study comprise members of phyla *Epsilonbacteraeota*, *Bacteroidetes*, *Chloroflexi*, *Verrucomicrobia*, *Planctomycetes*, *Acidobacteria*, *Fusobacteria*, *Actinobacteria*, *Spirochaetes*, *Kiritimatiellaeota*, *Firmicutes* and *Latescibacteria* (Supplementary Table 10). Similar bacterial phyla reported in other studies is detailed in Table 4. The microbial community composition has slight differences in surface and subsurface sediments. *Bacteroidetes* was second most dominant phyla (23.47%) in surficial sediments, while in subsurface sediments, *Epsilonbacteraeota* (32.09%) was the second most dominant. In surface sediments phylum *Epsilonbacteraeota* decreased to 7.65% and in subsurface sediments phylum *Bacteroidetes* decreased to 8.61%. Phylum *Chloroflexi*, *Acidobacteria*, *Actinobacteria*, *Latescibacteria* and *Planctomycetes* were more dominant in subsurface sediments and predominance of phylum *Verrucomicrobia*, *Fusobacteria* and *Firmicutes* were also have been observed in surface sediments. In our study, *Chloroflexi* was more abundant in subsurface sediments which is agreement with the study of Teske et al. (2011). Several molecular methods and studies have discovered that the *Actinobacteria* constitute a small fraction in marine bacterial communities, however perform significant ecological function in marine environments (Venter et al. 2004; Ward and Bora 2006; Polymenakou et al. 2009). Phylum *Planctomycetes* is represented by a broad distribution in most of the marine sediments (Fuerst 1995; Polymenakou et al. 2009; Oni et al. 2015) and involved in the global carbon and nitrogen cycles (Glöckner et al. 2003). We found that *Verrucomicrobia* more abundant in surface sediments (3.40%) in our study. Several studies also reported phylum *Bacteroidetes*, *Epsilonbacteraeota*, *Chloroflexi*, *Acidobacteria*, *Actinobacteria*, *Planctomycetes*, *Verrucomicrobia* and *Firmicutes* at diverse locations listed in Table 3.

Table 4

List of bacterial groups reported in other studies by different methods resembling with the dominant bacteria identified in the surface and subsurface sediments of this study.

Method of study	Major findings	References
Illumina MiSeq sequencing	<ul style="list-style-type: none"> • <i>Proteobacteria</i>, <i>Epsilonbacteraeota</i>, <i>Bacteroidetes</i>, <i>Chloroflexi</i>, <i>Verrucomicrobia</i>, <i>Planctomycetes</i>, <i>Acidobacteria</i>, <i>Fusobacteria</i> and <i>Actinobacteria</i> were the dominant phyla. • Within the phylum <i>Proteobacteria</i>, <i>Gammaproteobacteria</i> was more abundant in surface sediments followed by <i>Deltaproteobacteria</i>; <i>Deltaproteobacteria</i> was more abundant in subsurface sediments than <i>Gammaproteobacteria</i> 	This Study
Illumina MiSeq sequencing	<ul style="list-style-type: none"> • <i>Proteobacteria</i>, <i>Bacteroidetes</i>, <i>Verrucomicrobia</i>, <i>Actinobacteria</i> and <i>Chloroflexi</i> as the dominant members of the bacterial group along the sediment sea floor (0–10 cm) of Arctic fjord. • Within the phylum <i>Proteobacteria</i>, most sequences were related with γ and δ-<i>Proteobacteria</i>. 	Fang et al. 2019
Metagenomic sequencing in Hiseq X Ten platform	<ul style="list-style-type: none"> • Metagenomic analysis of sediment from Hadal Biosphere at the Yap Trench as γ-<i>Proteobacteria</i> was the predominant group of bacteria and other major groups in sediment included δ, α-<i>Proteobacteria</i> and <i>Firmicutes</i>. 	Zhang et al. 2018
Metabarcoding of 16S rRNA genes	<ul style="list-style-type: none"> • Bacterial diversity related to the metalliferous deposits of hydrothermal vent fields. • <i>Proteobacteria</i>, <i>Epsilonbacteraeota</i>, <i>Bacteroidetes</i> and <i>Planctomycetes</i> were the major phyla reported. 	Storesund et al. 2018
454 pyrosequencing	<ul style="list-style-type: none"> • <i>Proteobacteria</i>, <i>Bacteroidetes</i>, <i>Chloroflexi</i>, <i>Acidobacteria</i>, <i>Planctomycetes</i>, <i>Actinobacteria</i> and <i>Gemmatimonadetes</i> were the most dominant phyla in Arctic Kongsfjorden and Sub Arctic Northern Bering sea sediments. 	Zeng et al. 2017
454 pyrosequencing	<ul style="list-style-type: none"> • <i>Proteobacteria</i>, <i>Bacteroidetes</i> and <i>Chloroflexi</i> constituted the largest proportions in Arctic sediment (0–10 cm). 	Li et al. 2015
Pyrosequencing	<ul style="list-style-type: none"> • γ-<i>Proteobacteria</i> and <i>Bacteroidetes</i> were the predominant members identified in Arctic water. 	Zeng et al. 2013
16S rDNA clone library analysis	<ul style="list-style-type: none"> • Reported the Actinobacterial diversity in the sediments of the Arctic. 	Zhang et al. 2014
16S rRNA gene clone library analysis	<ul style="list-style-type: none"> • γ-<i>Proteobacteria</i>, <i>Bacteroidetes</i> and <i>Actinobacteria</i> were the principal lineages of bacteria in surface (0–5 cm) sediments of Arctic. 	Xuezheng et al. 2014
16S rRNA gene clone library analysis	<ul style="list-style-type: none"> • δ-<i>Proteobacteria</i> was the dominant bacteria followed by γ-<i>Proteobacteria</i> and <i>Acidobacteria</i> in sediments of Northern Bering sea. • The dominance of α, ε-<i>Proteobacteria</i>, <i>Bacteroidetes</i>, <i>Actinobacteria</i>, <i>Firmicutes</i>, <i>Planctomycetes</i>, <i>Verrucomicrobia</i>, <i>Chloroflexi</i>, and <i>Spirochaetes</i> were also observed. 	Zeng et al. 2011
16S rRNA gene clone library analysis	<ul style="list-style-type: none"> • <i>Proteobacteria</i>, <i>Bacteroidetes</i>, <i>Planctomycetes</i>, <i>Acidobacteria</i> and <i>Chloroflexi</i> were observed in the sediments of Arctic fjord. • Within the phylum <i>Proteobacteria</i>, γ-<i>Proteobacteria</i> and α-<i>Proteobacteria</i> more abundant in surface sediments (0–2 cm) and δ-<i>Proteobacteria</i> more predominant in subsurface (3–9 cm) sediments. • <i>Chloroflexi</i> and <i>Planctomycetes</i> were also dominant in subsurface and <i>Bacteroidetes</i> were dominant in surface sediments. 	Teske et al. 2011
16S ribosomal DNA clone library analysis	<ul style="list-style-type: none"> • <i>Proteobacteria</i>, <i>Acidobacteria</i>, <i>Bacteroidetes</i>, <i>Chloroflexi</i>, <i>Actinobacteria</i>, <i>Firmicutes</i>, <i>Planctomycetes</i>, <i>Spirochaetes</i>, and <i>Verrucomicrobia</i> were the dominant phyla in surface sediments (0–5 cm) of the Pacific Arctic ocean. • Within the phylum <i>Proteobacteria</i>, γ-<i>Proteobacteria</i> were the most dominant bacterial family. 	Li et al. 2009
16S rRNA gene analysis	<ul style="list-style-type: none"> • Phylum <i>Proteobacteria</i> (α, β, γ and δ), <i>Bacteroidetes</i>, <i>Actinobacteria</i>, <i>Verrucomicrobia</i> were observed both in surface and bottom water at the central part of the Kongsfjorden. • <i>Planctomycetes</i> were observed only in bottom water and γ and α-<i>Proteobacteria</i> were also more detected in bottom water. 	Zeng et al. 2009
DNA clone library analysis	<ul style="list-style-type: none"> • Predominance of bacteria related to the sulfur cycle, δ and γ-<i>Proteobacteria</i> were dominated in the permanently cold marine sediments of Arctic. 	Ravenschlag et al. 1999

In our study, genus *Lutibacter* of family *Flavobacteriaceae* under the phylum *Bacteroidetes* was the more dominant OTU in surface sediments. Several studies have reported the phylum have been consistently enriched in phytoplankton blooms (Kirchman 2002; Abell and Bowman 2005; Teeling et al. 2012). *Bacteroidetes* are extensively distributed in marine environments, have the capacity to degrade particulate organic matter in the ocean and also play a significant role in ocean carbon cycling (Riemann et al. 2000; Kirchman 2002; Bauer et al. 2006). They are being adapted to the degradation of polymeric substances in ocean (González et al. 2008) mainly polysaccharides and proteins (Cottrell and Kirchman 2000). We have found that, genus *Sulfurovum* of family *Sulfurovaceae* under the phylum *Epsilonbacteraeota* was found to be more dominant OTU in subsurface sediments. Same OTU was seem to be second most dominant in surface sediments. In contrast, OTU of phylum *Epsilonbacteraeota* more dominant in subsurface than surface sediments. OTU of family Desulfobulbaceae belonging to δ -*Proteobacteria* was found to be the second most dominant in subsurface sediments. At the genus level, *Lutibacter* (6.43%) and *Marinifilum* (3.63%) of phylum *Bacteroidetes*, *Sulfurovum* (3.63%) and *Sulfurimonas* (2.10%) belonging to phylum *Epsilonbacteraeota*, *Psychromonas* (2.80%) and *Woeseia* (2.52%) of phylum γ -*Proteobacteria* were found to be dominant in surface sediments. Comparatively, genus *Sulfurovum* (24.51%) and *Sulfurimonas* (7.38%) were found to be more predominant in subsurface sediments than in surface sediments. *SEEP-SRB1* (3.25%) of phylum δ -*Proteobacteria* dominant in subsurface sediment. Genera *Sulfurimonas* and *Sulfurovum* in this study depicted a considerable portion of bacteria in the sediments of Kongsfjorden, which is agreeing with the study of Zeng et al. (2017). Regarding sulfur cycling, *Epsilon-Proteobacteria* play a significant role in marine environment (Campbell et al. 2006). Several studies reported that the bacterial members of δ , γ and ε -*Proteobacteria* mediate sulfide reduction and oxidation (Sahm et al. 1999; Nakagawa et al. 2004; Campbell et al. 2006; Muyzer and Stams 2008). Sulfate reducer phylotypes belonging to δ -*Proteobacteria* have been reported in Arctic sediment (Ravenschlag et al. 2001; Zeng et al. 2011). Moreover δ -*Proteobacteria* sulfate reducer, the γ -*Proteobacteria* sulfate oxidizer phylotypes were also discovered in Arctic sediments (Ravenschlag et al. 1999). Phylotypes belonging to ε -*Proteobacteria*, were affiliated with sulfur oxidizing genera of *Sulfurovum* and *Sulfurimonas* and have been reported earlier (Table 3).

Diverse of bacterial communities harbouring in surface and subsurface sediments in Kongsfjorden have a potential role in biogeochemical cycling. In this study, it was found that a significant fraction of retrieved sequences from both surface and subsurface sediments do not belong to any taxonomic division and they are categorized into unknown and uncultured bacteria. Similar findings have been reported in various studies (Polymenakou et al. 2009), the presence of uncultivated bacterial diversity in polar sediments was also reported by several molecular studies (Ravenschlag et al. 1999; Bowman and McCuaig 2003). Our studies revealed, only slight differences in bacterial community composition of surface and subsurface sediments. However, there were distinct differences in the predominance of bacterial communities between surface and subsurface sediments. When compared with previous reports, our results also indicated that *Proteobacteria* were dominant in the Arctic marine sediment and within this phylum, γ -*Proteobacteria* were dominant in surface and δ -*Proteobacteria* were seemed to be dominant in subsurface sediments of Kongsfjorden. The higher relative abundance of γ and α -*Proteobacteria*, *Bacteroidetes* and *Verrucomicrobia* in the surface sediments may be due to the higher quantity of phytoplankton derived organic matter in surficial sediments. They have a significant function in carbon cycle in the ocean. In marine sediments, bacterial abundance and community composition control by the abundance and quality of organic carbon present (Orcutt et al. 2011). Mostly, easily degradable organic matter is used by the microorganisms in surface sediments (Cowie and Hedges 1994; Wakeham et al. 1997). Microorganisms accumulated on the surface sediment from the water column are progressively buried into deeper layers as sedimentary particulate matter deposits on the surface of the sediment. Certain selection processes filter out the bacterial community during burial and producing subsurface and deeper layers below the subsurface, that are populated by a rare group of bacteria in the surface sediment (Jochum et al. 2017). Persisting populations such as *Chloroflexi* can adapt into the deep biosphere which was also present in the subsurface sediment below the bioturbation zone (Petro et al. 2019). Sulfate reduction is a significant bacterial process in marine sediment involved in nearly 50% remineralization of total organic carbon (Canfield et al. 1993). We have identified the predominance of sulfate reducing δ -*Proteobacteria*, sulfur oxidizing *Epsilonbacteraeota*, and γ -*Proteobacteria* in both surface and subsurface sediments. In this study, *Sulfurovum* and *Sulfurimonas* have important role in sulfur oxidation and genera *SEEP-SRB1* of class δ -*Proteobacteria* which are involved in sulfate reduction found to be predominant in subsurface sediment.

Our studies revealed that the phylum γ , α -*Proteobacteria* and *Bacteroidetes* were more predominant in surface sediments. Surface sediments contain higher proportions of labile algal derived organic matter which could support these bacterial groups. Several researchers reported their marked presence in marine waters and sediments for the degradation of algal derived organic matter (Teeling et al. 2012; Miyatake et al. 2014; Ruff et al. 2014; Landa et al. 2014). Studies of Cottrell and Kirchman (2000) reported that, rather than utilizing polymers γ -*Proteobacteria* found to be more adapted to monomers. PICRUSt analysis revealed that the genes in the bacterial community structure in surface and subsurface sediments were involved in carbohydrate and amino acid metabolism in higher abundance. Predicted gene clusters for xenobiotic degradation and metabolism, biosynthesis of secondary metabolites, and genes for membrane transport were also observed. In carbohydrate metabolism, genes responsible for degradation complex polysaccharide represented endoglucanase, β -

glucosidase, glycogen phosphorylase was observed and this indicates the important ecological function in carbon cycling by the sediment bacterial community of Kongsfjorden, Arctic.

5. Conclusion

High throughput sequencing analysis disclosed a highly diverse bacterial community composition in the surface and subsurface sediments of Kongsfjorden. *Proteobacteria* were found to be the predominant phylum in both surface and subsurface sediments. The γ , δ -*Proteobacteria*, and *Epsilonbacteraeota* which are having a potential role in the carbon remineralization have appeared in both surface and subsurface sediments with relatively higher abundance. Analysis of functional profiles indicated that the bacterial community have the potential to degrade complex organic matter such as glycans, glycosaminoglycans, polycyclic aromatic hydrocarbons etc. The present study thus demonstrates that the significant variability in the community composition of bacteria between the surface and sub surface sediments during the onset of winter in Kongsfjorden could be considerably influenced by the availability of total carbon and organic carbon in the sediments.

Declarations

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Conflict of interest

The authors declared that there is no conflict of interest.

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Figures

Figure 1

Map showing the sampling locations in the Kongsfjorden.

Figure 2

Microbial community composition at phylum level. Only top 10 enriched phyla are shown in the figure. Those sequences that could not be assigned to phylum level were marked as unknown.

Figure 3

Microbial community composition at (a) class (b) order (c) Family (d) genus levels. Only top 10 enriched categories are shown in the figure. Those sequences that could not be assigned to a particular level were marked as unknown.

Figure 4

Venn diagram showing shared and unique OTUs at 97% identity among surface and subsurface sediments of Kongsfjorden.

Figure 5

The relative abundance of predicted functional genes of microbial communities from surface and subsurface sediments based on KEGG database.

Supplementary Files

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