Metagenomic Analysis of The Effects of Salinity On Microbial Diversity and Functional Gene Diversity in Kongsfjorden Estuary

Lidong Lin
Qingdao University

Nengfei Wang ( wangnengfei@fio.org.cn )
First Institute of Oceanography Ministry of Natural Resources

Wenbing Han
First Institute of Oceanography

Botao Zhang
Qingdao University

Jiaye Zang
First Institute of Oceanography

Qinxin Li
Qingdao University

Yiling Qin
First Institute of Oceanography

Long Wang
Qingdao University of Science & Technology

Long Fang
Qingdao University

Jie Liu
Qingdao University of Science & Technology

Research Article

Keywords: Metagenome, Kongsfjorden, Marine microorganism, Bacterial diversity, Nitrogen cycle

Posted Date: November 22nd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1094012/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License
Metagenomic analysis of the effects of salinity on microbial diversity and functional gene diversity in Kongsfjorden estuary

Lidong Lin† · Nengfei Wang* · Wenbing Han · Botao Zhang · Jiaye Zang · Qinxin Li · Yiling Qin · Long Wang · Long Fang · Jie Liu

*Correspondence: N. Wang
†These authors have contributed equally to this work

L. Lin · B. Zhang · Q. Li · L. Fang
College of Chemistry and Chemical Engineering, Qingdao University, Qingdao 266071, China

N. Wang* · W. Han · J. Zang · Y. Qin
First Institute of Oceanography, Ministry of Natural Resources, Qingdao 266061, China
e-mail: wangnengfei@fio.org.cn

L. Wang · J. Liu
Department of Bioengineering, College of Marine Sciences and Biological Engineering, Qingdao University of Science & Technology, Qingdao 266042, China

Abstract

Due to the inflow of meltwater from the Midre Lovénbreen glacier upstream of Kongsfjorden, the salinity of Kongsfjorden increases from the estuary to the interior of the fjord. Our goal was to determine which bacterial taxa and metabolism-related gene abundance were affected by changes in salinity, and whether salinity is correlated with genes related to nitrogen and sulfur cycling in fjord ecosystem using metagenomic analysis. Our data indicate that changes in salinity may affect some bacterial taxa, such as the relative abundance of Alphaproteobacteria and Deltaproteobacteria is higher at high salinity sites, while the relative abundance of Gammaproteobacteria and Betaproteobacteria is more dominant at low salinity sites. In addition, the relative abundance of some bacteria at the high and low salinity sites was different at the family level. For example, Rhodobacteraceae, Pseudoalteromonadaceae, Flavobacteriaceae, Vibrionaceae at the high salinity site Colwelliaceae, Chromatiaceae and Alteromonadaceae at the low salinity site are affected by salinity. In terms of functional gene diversity, our study proved that salinity could affect the relative abundance of related genes by affecting the metabolic mechanism of microorganisms. In addition to salinity, functional attributes of microorganisms themselves were also important factors affecting the relative abundance of metabolism-related genes. In addition, salinity has a certain effect on the relative abundance of genes related to nitrogen and sulfur cycling.

Keywords
Metagenome · Kongsfjorden · Marine microorganism · Bacterial diversity · Nitrogen cycle

Introduction

Over the past few decades, the global climate has changed dramatically, with the effects becoming
more pronounced in the polar regions (Wang et al. 2019). This would lead to higher temperatures in the Arctic Ocean, including Spitsbergen, causing the glaciers to melt earlier in the annual cycle and then freeze, increase precipitation and reduce sea ice cover. In addition, glacial melt drains downstream, leading to changes of biogeochemical and nutrient salt in downstream ecosystems (Bhatia et al. 2013; Hawkings et al. 2015; Hood et al. 2015; Hood Berner 2009; Lawson et al. 2014; Schroth et al. 2014). Due to the strong changes in salinity and chemical properties of sea water, the structure and abundance of microbial community in downstream fjords and estuaries will change. Microorganisms in marine ecosystems are the most important drivers of biogeochemical cycling on a global scale (Azam et al. 1983), as they are responsible for the remineralization of organic matter and the transfer of nutrients and energy to higher levels in the ocean (Mason et al. 2009; Nealson 1997). However their taxonomic composition and metabolic function are influenced by "bottom-up" physical and chemical factors, such as salinity, nutrient concentration and availability (Lozupone Knight 2007; Yokokawa et al. 2004). Therefore, the natural processes that cause physiochemical dynamics changes greatly affect the bacterial community, resulting in rapid changes in community structure and function, and thus have an impact on the aquatic environment (Newton et al. 2018).

Kongsfjorden, located on the west coast of Svalbard at 79°N, is a glacier-open fjord in the Arctic. Since arctic glacial fjords are characterized by the discharge of fresh water and suspended matter from glaciers on top of fjords, the stable ocean at the entrance to the fjord becomes a very unstable brackish water in the inner basin of the fjord (Svivitski et al. 1987). As a passage connecting the Atlantic Ocean and the Arctic Ocean, Kongsfjorden has attracted extensive research attention in recent years. Previous studies have shown that changes in salinity and sediment load due to the inflow of glacial meltwater into Kongsfjorden are the main determinants of changes in microbial community composition and diversity in Kongsfjorden (Piquet et al. 2010), consistent with another research which indicated salinity is a selective pressure governing global bacterial distribution (Lozupone Knight 2007). However, the characteristics and metabolic potential of the fjord bacterial community have not been determined. Since bacteria are the basis of major biogeochemical cycles, the changes of high trophic levels and the whole ecosystem will determine the community characteristics and functions. Therefore, how salinity changes affect bacterial communities is important.

Metagenomics overcomes the constraints faced by culture-oriented microbiology institutions and can be used as a search tool for detailed screening of microbial community species present in ecosystems (Martinez-Porchas et al. 2017). Because DNA extracted from environmental samples is a microcosm of the entire microbial community of an ecosystem, metagenomic analysis can provide a more comprehensive assessment of the entire microbial community (Lagkouvardos et al. 2016). Thus advances in community analysis, using metagenomic methods, have made it possible to characterize bacterial taxonomic composition and functional metabolic potential. In this case, the aim of this study was to investigate whether salinity is an important factor affecting the bacterial community structure and relative abundance of metabolic genes in the fjord. To do this, we set up three sampling sites in Kongsfjorden: the intersection of glacial meltwater and the edge of the fjord (S5), the coastal waters (S6) and the interior of Kongsfjorden (S7). The salinity of the three sites varies significantly due to the input of glacial meltwater and their location in the fjords, our hypothesis is that salinity is an important factor affecting bacterial community structure and major metabolic genes in the fjord ecosystem, therefore, in this study, we focused on the effects of salinity changes on the bacterial community structure and the relative abundance of functional genes.
Materials and Methods

Study sites and sample collection

Kongsfjorden is a polar fjord located west of Svalbard located between 78°04'N-79°05'N and 11°03'E-13°03'E. The fjord is characterized by a low tidal difference (~2 m) and is strongly influenced by topography and the adjacent ocean. The western coastal waters of Svalbard are affected by the northernmost extension of the North Atlantic Current. The Midre Lovénbreen glacier is located in the Kongsfjorden region. Glacial meltwater flows into the Kongsfjorden through glacial runoff and provides the main fresh water resources. So there is an increasing trend in salinity from the glacial melt water into the estuary to the fjord interior (Piquet et al. 2010).

Three sampling points will be set up in the waters of Kongsfjorden, namely a place where the estuary of Kongsfjorden (S5, 12°01'50.9"E and 78°54'54.2"N), the coastal sea area (S6, 12°02'6.9"E and 78°54'55.7"N), and interior of the fjord (S7, 12°02'32.8"E and 78°55'2.7"N) (Fig. 1). The sampling points are 500 meters apart, water samples were directly collected into TWIRL'EM sterile sampling bags (Labplas Inc., Canada). The microbial samples were then collected by filtering 1000 ml of the water samples. The microbial biomass was successively trapped onto 47-mm-diameter, 0.2-μm-pore-size membrane filters (Pall Corporation, USA). Membrane filters were placed in centrifuge tubes at −20 °C in the Yellow River Station (China) and taken to the laboratory by plane. Filters were then frozen at −80 °C until nucleic acid extraction.

![Fig.1 Sampling sites in the Kongsfjorden.](image)

The chemical properties of sea water

Temperature and salinity were measured by a CTD (SBE 19 plus CTD, Sea-Bird Electronics, Bellevue, Washington, USA) at each sampling site. pH of seawater was measured by pH electrode (PHS-3C, Shanghai REX Instrument Factory, Shanghai, China). NO\textsubscript{3}-N, NO\textsubscript{2}-N, NH\textsubscript{4}+-N, SiO\textsubscript{4}–Si and PO\textsubscript{4}3–-P concentration of seawater samples were measured by nutrient automatic analyzer.
Metagenomic sequencing and analyses

The total DNA was extracted from the water sample using the PowerWater DNA Isolation Kit (MO BIO Laboratories, Inc., USA) according to the manufacturer's instructions. A total of 1.0 μg DNA per sample was used for library construction with a NEBNext Ultra DNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA, USA) following the manufacturer’s recommendations and corresponding index sequences. Briefly, DNA samples were fragmented by sonication to 350 bp, and fragments were end-polished, A-tailed, and ligated with the full-length adaptor for PCR amplification. PCR products were purified (AMPure XP system; Beckman Coulter, Brea, CA, USA), and libraries were analyzed for size distribution by an Agilent 2100 Bioanalyzer and quantified using real-time PCR. The clustering of index-coded samples was performed on a cBot Cluster Generation System according to the manufacturer’s instructions. After cluster generation, library preparations were sequenced on an Illumina HiSeq 2000 and paired-end reads were generated and deposited to NCBI SRA (accession number: SRP319744).

In the metagenome analysis, reads of low quality (containing 40 or more bases with scores <20) or with more than 10 unknown bases (Ns) were removed using SOAPaligner (Santamans et al. 2017). Clean reads from each sample were assembled using SOAPdenovo2 using default parameters (Gu et al. 2013). The reads not assembled into scaftigs (continuous sequences within scaffolds) in each sample were pooled together to form a mixed sample, which was reassembled. All scaftigs shorter than 500 bp were discarded.

Open reading frames in the scaftigs of each sample and of the mixed sample were predicted using MetaGeneMark (Luo et al. 2012). Redundant genes in each sample were removed using CD-HIT with 95% identity and 90% coverage (Zhu et al. 2010). The resulting genes were further filtered with the number of reads assembled, and those assembled with less than two reads were discarded to generate a gene catalog (unigenes). Unigenes were then filtered using DIAMOND (W. Li et al. 2012) against the reference genes in the NCBI-nr database at a cut-off e-value of 1E-10. In the functional analysis, DIAMOND was used to map unigenes against reference genes in KEGG (Buchfink et al. 2015).

Results and Discussion

Chemical properties analysis of seawater

Due to the damage of a parallel sample S7.2 from the sampling site S7 during transportation, there are only two parallel samples of S7 in the subsequent experiment. However, from the perspective of various indicators of chemical properties, S7 parallel sample has very good parallelism, so it does not affect the subsequent experimental results.

Salinity of the three sampling sites varies greatly (one-way ANOVA, P < 0.05; Table S1), showing an increasing trend from S5 to S7 (Table 1). In addition to salinity, other six chemical factors which are important parameters in marine research were determined at the three sampling sites: pH, NH$_4^+$-N, NO$_3^-$-N, NO$_2^-$-N, PO$_4^{3-}$-P and SiO$_4^{2-}$-Si. The results showed that the maximum values of SiO$_4^{2-}$-Si (92.48 μg/g) and NO$_3^-$-N (5.26 μg/g) were detected in S7 samples, the maximum values of NH$_4^+$-N (33.29 μg/g) and NO$_2^-$-N (3.24 μg/g) were detected at S6 sampling points. In general, salinity is the chemical property with the greatest difference among the three sampling sites, so we infer that salinity may have a certain influence on the driving bacterial community structure and gene diversity. Moreover,
our research focuses on the effects of salinity on bacterial community diversity and abundance of functional genes, so we rarely discuss the effects of other physical and chemical properties on bacterial diversity and gene diversity.

### Table 1: Chemical properties of three sampling sites in Kongsfjorden

| Sample | pH | Salinity (ppt) | NH$_4^+$-N (μg/g) | SiO$_4^{2-}$-Si (μg/g) | NO$_2^-$-N (μg/g) | PO$_4^{3-}$-P (μg/g) | NO$_3^-$-N (μg/g) |
|--------|----|----------------|-------------------|------------------------|------------------|------------------|------------------|
| S5.1   | 8.01 | 5.08           | 33.213            | 91.039                | 3.151            | 5.384            | 5.251            |
| S5.2   | 8.00 | 5.16           | 31.221            | 90.081                | 3.230            | 5.390            | 5.153            |
| S5.3   | 8.02 | 5.06           | 33.199            | 89.370                | 3.222            | 5.313            | 5.043            |
| **Average** | **8.01** | **5.10** | **32.544** | **90.163** | **3.201** | **5.362** | **5.149** |
| S6.1   | 8.06 | 15.21          | 32.570            | 92.410                | 3.240            | 5.357            | 5.247            |
| S6.2   | 8.02 | 15.09          | 32.641            | 91.550                | 3.205            | 5.370            | 5.248            |
| S6.3   | 8.04 | 15.06          | 33.290            | 91.510                | 3.250            | 5.221            | 5.246            |
| **Average** | **8.04** | **15.12** | **32.834** | **91.823** | **3.232** | **5.316** | **5.247** |
| S7.1   | 7.95 | 30.10          | 32.195            | 92.480                | 3.051            | 5.334            | 5.251            |
| S7.2   | 8.00 | 30.12          | 32.573            | 90.653                | 3.191            | 5.280            | 5.260            |
| S7.3   | 7.93 | 30.11          | 32.413            | 91.120                | 3.153            | 5.070            | 5.257            |
| **Average** | **7.96** | **30.11** | **32.394** | **91.418** | **3.132** | **5.228** | **5.256** |

Statistical significance was assessed by one-way ANOVA followed by Tukey’s HSD test, and significant differences were accepted when $p < 0.05$ between the two groups. The letters a, b, and c were used to show statistically significant differences.

### Metagenomic sequencing results and gene prediction

Illumina HiSeq sequencing platform was used to obtain the original data, and the following data were obtained after preprocessing and statistics (Table 2). A total of 111,529.18 Mbp sequences were obtained from all the samples, and the average sequencing number of each sample reached 13941.14 Mbp. After quality control, the remaining 111402.52 Mbp sequences averaged 13925.315 Mbp per sample, with an average effective rate of 99.89%. The average percentage of the number of bases with sequencing error rate less than 0.01 (mass value greater than 20) in CleanData was 97.03, indicating a low error rate in the sample. The average GC content of base in CleanData was 44.26%.

We conducted gene count statistics for each sampling site and drew a Venn diagram (Fig. 2), among which 1193970 genes were shared by the three sampling sites. The number of unique genes for S5, S6 and S7 was 293182, 79733 and 43216, respectively.

Unigenes was sequenced and annotated with NCBI NR database for bacteria, fungi, archaea and virus respectively. It was found that the microorganisms in the samples were mainly bacteria, accounting for 84% (S5), 77.1% (S6) and 82.3% (S7), Viruses for 3.8% (S5), 5% (S6) and 4% (S7), Eukarya for 0.3% (S5), 0.7% (S6) and 0.5% (S7) and archaea for 0.2% (S5), 0.2% (S6) and 0.2% (S7). In the metagenomic library of 3 sample sites, 11.7%, 17% and 13% of the genes were unannotated, respectively.

### Table 2: Data preprocessing statistics

| Sample | RawData (Mbp) | CleanData (Mbp) | Clean_Q20 (%) | Clean_Q30 (%) | Clean_GC (%) | Effective (%) |
|--------|---------------|----------------|---------------|---------------|--------------|---------------|
Effect of salinity on bacterial community

Since we wanted to explore the effect of salinity on bacterial community structure, we analyzed the correlation between salinity and bacterial taxa with large differences in relative abundance between sampling sites, and the bacteria taxa with high correlation coefficient with salinity were screened out (Table 3). Proteobacteria are abundant in all three sampling sites at 58.51%-62.98% (Fig. 3a). Alphaproteobacteria (25.79%) and Deltaproteobacteria (0.35%) are relatively abundant in low salinity sites, while the abundance of Gammaproteobacteria (39.67%) and Betaproteobacteria (1.5%) is higher in high salinity sites (Fig. 3b, one-way ANOVA, P < 0.05; Table S2). At the family level, the abundance of Rhodobacteraceae (12.23%), Pseudoalteromonadaceae (8.45%), Flavobacteriaceae (10.44%) and Vibrionaceae (0.69%) is relatively high in low salinity sites (Fig. 3c, one-way ANOVA, P < 0.05; Table S3). With the increase of salinity gradient, the abundance of Actinobacteria (0.38%-1.2%) increases gradually. Especially, the abundance of Acidimicrobiaceae (0.06%-0.26%) and Microbacteriaceae (0.08%-0.68%), which belong to Actinobacteria, is higher in S6 and S7 than in S5. At the family level, the abundance of Colwelliaceae (8.33%), Chromatiaceae (0.46%) and Alteromonadaceae (11.42%) is higher in high salinity sites than that in low salinity sites (one-way ANOVA, P < 0.05; Supplementary Table S4).

Dissimilarities between samples using PCoA were examined based on weighted UniFrac distances (Fig. 4). As expected, the three groups of samples are significantly separated, and the S5 sample is separated from the other two groups, distributing along the first principal component. The distribution pattern of S6 sample is distributed along the second principal component, which is opposite to S5.
Salinity can be an important factor in driving microbial diversity (Bouvier del Giorgio 2002; Lozupone Knight 2007; Tamames et al. 2010; Wu et al. 2006) and controls global microbial distribution (Lozupone Knight 2007). Our data confirm that certain bacterial groups are strongly correlated with salinity. The number of genes in low-salinity loci was higher than that in high-salinity loci, and Banda et al. (2020) had pointed out that species diversity would decrease in high-salinity environments. We first screened out bacterial taxa that were strongly correlated with salinity and discussed their relative abundance differences. As can be seen from the figure (Fig. 3b), Alphaproteobacteria and Deltaproteobacteria have high abundance in S5. These classes are ubiquitous in the marine environment and contain many marine species with high abundance (Biers et al. 2009; Capo et al. 2020). The high abundance of Rhodobacteraceae and Pseudoalteromonadaceae observed in S5 due to the rapid response of these families to the input of foreign nutrients caused by glacial melt water (Fig. 3c), their high abundance may be an indication of coastal surface water disturbance events (Allers et al. 2007; Nogales et al. 2011). In addition, high abundance of Flavobacteriaceae and Vibrionaceae are also detected in S5. The high abundance of the former due to the fact that this bacterium prefers to utilize complex organic matter by directly attaching to algal cells and algal derivative clastic particles (Y. Li et al. 2018), while the fresh water of S5 is more conducive to the growth of algae (Buchholz Wiencke 2016), so salinity indirectly affects the abundance of Flavobacteriaceae. The high abundance of Vibrionaceae can be ascribed to its association with soil microbiota (Reen et al. 2006), suggesting that Vibrionaceae may be terrigenous microorganism, which are imported into the fjords by glacial meltwater and are subjected to salinity stress, which is not so abundant in high-salinity areas.

Actinobacteria are common degrading bacteria in soil and ocean (Bull et al. 2005; Magarvey et al. 2004), which were initially thought to be transferred to marine environment through terrestrial runoff (Bull et al. 2005). However, in our figure, the abundance of Actinobacteria in S6 and S7 is higher than that of S5 (Fig. 3a), and the abundant of Acidimicrobiaceae and Microbacteriaceae in S6 and S7 confirm that Actinobacteria are the resident members of marine environment (Cottrell et al. 2005; Han et al. 2003; Rusch et al. 2007) and high-salt environment (Ghai et al. 2011) (Fig. 3c). The abundance of Gammaproteobacteria is higher in high salinity S7 (Fig. 3b), which is consistent with previous studies (Paver et al. 2018). Systematic evolution of the proteobacteria along salinity gradients shows the effect of salinity on the structure (Wu et al. 2006), suggests that salinity is an important factor controlling the composition of the microbial community in Kongsfjorden. The high abundance ratios of Colwelliaceae, Chromatiaceae and Alteromonadaceae observed in high salinity S7 may be attributed to their sensitivity to salinity (Fig. 3c), proving that they may belong to marine bacterium (Kwak et al. 2012; Methé et al. 2005; Pfennig Trüper 1981) and they may have "exclude salt" mechanisms such as osmotic balance and prevent dry (Banda et al. 2020). In this adaptation, the microorganism synthesizes the corresponding solute to help stabilize the cell membrane structure.

| Table 3 Pearson correlation between bacterial taxa and water chemistry |
|-----------------------|---------------|-------------|-------------|-------------|-------------|-------------|-------------|
|                     | pH    | salinity | NH₄⁺-N   | SiO₂⁻-Si  | NO₂⁻-N   | PO₄³⁻-P   | NO₃⁻-N   |
| Actinobacteria       | r    | -0.548   | **0.857** | -0.310    | 0.381     | -0.571     | 0.281     | 0.04       |
|                      | P    | 0.160    | 0.007    | 0.454     | 0.352     | 0.139      | 0.5       | 0.925      |
| Alphaproteobacteria  | r    | 0.338    | **-0.912** | 0.081    | **-0.714** | 0.19       | 0.103     | -0.04      |
|                      | P    | 0.412    | 0.002    | 0.85      | 0.047     | 0.651      | 0.809     | 0.925      |
| Deltaproteobacteria  | r    | 0.586    | **-0.946** | 0.029    | **-0.738** | 0.429      | 0.009     | -0.056     |
| Family                | r   | P   | r   | P   | r   | P   |
|----------------------|-----|-----|-----|-----|-----|-----|
| Gammaproteobacteria  | -0.416 | 0.940** | -0.057 | 0.714* | -0.286 | -0.249 | 0.012 |
| Betaproteobacteria   | -0.649 | 0.822* | 0.013 | 0.667 | -0.429 | 0.127 | 0.054 |
| Rhodobacteraceae     | 0.234 | -0.864** | 0.016 | -0.810* | 0.048 | 0.192 | -0.033 |
| Pseudoalteromonadaceae | -0.040 | -0.857** | 0.083 | -0.69 | 0.119 | 0.056 | -0.02 |
| Flavobacteriaceae    | 0.170 | -0.832* | -0.017 | -0.524 | -0.024 | 0.33 | 0.012 |
| Vibrionaceae         | 0.158 | -0.817* | -0.085 | -0.524 | 0.286 | 0.339 | -0.142 |
| Acidimicrobiaceae    | 0.043 | 0.931** | 0.047 | 0.571 | -0.357 | -0.325 | 0.07 |
| Microbacteriaceae    | -0.213 | 0.901** | -0.199 | 0.595 | -0.643 | 0.291 | 0.068 |
| Colwelliaceae        | -0.399 | 0.968** | -0.3 | 0.714* | -0.071 | -0.041 | -0.116 |
| Chromatiaceae        | -0.943* | 0.815* | -0.289 | -0.238 | -0.881** | 0.217 | 0.053 |
| Alteromonadaceae     | -0.268 | 0.949** | -0.413 | 0.476 | -0.048 | -0.002 | -0.179 |

*Correlation is significant at the 0.05 level.

**Correlation is significant at the 0.01 level.
Fig. 3 Microbial UPGMA clusters and relative abundance of species at the phyla level (a). A heatmap of the top 35 abundant class (b). A heatmap of the top 35 abundant family (c).
Starting from the results of Unigenes annotation, KEGG database was selected for annotation. A total of 780765 genes were annotated, and a total of 45 pathways were obtained. As shown in the figure (Fig. S1), the abscissa on the bar chart shows the number of Unigenes on the notes, and the ordinate with different colors represents the six metabolic pathways in KEGG database, including Cellular Processes, Environmental Information Processing, Genetic Information Processing, Human Diseases, Metabolism and Organismal Systems. Different columns represent different pathways of the six metabolic fluxes, and the corresponding explanations are shown in the legend on the left. In the samples, the main genes are distributed in Amino acid metabolism (96066 Unigenes), Carbohydrate metabolism (86517 Unigenes), Metabolism of cofactors and vitamins (60104 Unigenes) and Energy metabolism (56892 Unigenes).

By distributing the top 35 functional genes with the highest gene abundance (Fig. 5), it can be found that there are great differences in gene functional abundance among the three sampling points, especially S5 and S7. We focused on the abundance of metabolism related genes and found that most metabolism related genes are abundant in S5, especially in Energy metabolism (3.09%), Amino acid metabolism (5.05%), and Carbohydrate metabolism (4.31%), while Nucleotide metabolism (2.39%) and Lipid metabolism (1.33%) are abundant in S7.

The abundance of most metabolism related genes in S5 is high, suggesting that the Kongsfjorden estuary is a highly competitive environment where bacteria must exhibit the ability to take advantage of changing nutrient conditions by demonstrating multiple metabolic pathways (Newton et al. 2018). Most of the S5 metabolism related genes are concentrated in energy metabolism, amino acid metabolism and carbohydrate metabolism (Fig. 5). The influx of glacial meltwater in the upper reaches of Kongsfjorden promotes the accumulation of carbon-rich substances, and the conversion of inorganic carbon to organic matter through carbon sequestration is the main functional attribute of the ecosystem. Chemoautotrophs also use inorganic carbon to some extent (Feisthauer et al. 2008). Although heterotrophic prokaryotes utilize organic carbon, they can integrate dissolved inorganic carbon through extensive carboxylation reactions (Reddy et al. 2019). The high abundance of energy metabolism related genes and carbohydrate related genes at S5 site is due to the rich microbial community at the
low salinity site, which constitutes an effective carbon sequestration system in the Fjord estuary through autotrophic and heterotrophic mechanisms. Proteobacteria and actinomycetes have previously been reported to play a major role in carbohydrate metabolism and carbon fixation (Reddy et al. 2019). The enhancement of amino acid metabolism can enable microorganisms to successfully obtain nutrients in the context of intense nutrient competition. For example, increased nutrient acquisition by highly reactive microbial groups in S5 leads to the production of reactive oxygen species through nutrient oxidation, metabolism, and cellular respiration (Cabriscol Català et al. 2000) and causes overexpression of cellular responses to oxidative stress, of which glutathione is a major component (Klatt Lamas 2000).

In high salinity environment S7, halophilic and salt-tolerant microorganisms must maintain their cytoplasm at least isosmotic with their environment in order to withstand high salinity environment (Oren 2002). The strategy of some of these organisms is to remove as much salt from the cytoplasm as possible and to accumulate organic solutes to provide osmotic equilibrium. There are a variety of compounds that can be used for this purpose, from glycerol and other sugar alcohols to cytidine (Galinski 1995), leads to high abundance of genes related to fat metabolism and nucleotide metabolism in high-salt environments.

Effect of salinity on nitrogen cycle

In the KEGG annotated gene, a total of six nitrogen metabolism patterns and related genes were detected (Fig. 6a), including nitrification (amoC, nxrB and hao), denitrification (nirS, nirK, norC and norB), dissimilatory nitrate reductase (narG, narI, nirD, nirB and nrfA), assimilatory nitrate reductase (narH, narB and nirA), anammox (hdh) and nitrogen fixation (nifH). Two exogenous ammonia input related genes, glutamine synthetase (GS) and glutamate dehydrogenase (gdh) are widely distributed throughout the sampling site. Ammonia Monoxygenase Subunit C (amoC) is only detected in S5.
Denitrification related genes (nirK, nirS, norC, norB and nosZ) and Anammox related genes (hdh) are rarely distributed in S7. In addition, in terms of abundance, the abundance of most genes related to nitrogen cycle in S5 is higher than that in the other two sample sites. We analyzed the correlation between the abundance and chemical properties of nitrogen cycling related genes at each sampling site (Table 4). Some genes of dissimilated nitrate reductase and assimilative nitrate reductase were not highly correlated with salinity, while most of the genes related to nitrogen cycle were highly correlated with salinity.

In order to distinguish the microbial groups to which the genes related to nitrogen metabolism belong, the relative abundance map of the groups to which the genes belong was drawn (Fig. 6b). The results show that different nitrogen cycling related genes belong to different microbial groups. For example, the denitrification related gene nosZ related microbial taxa in S5 are mainly composed of Gammaproteobacteria, Alphaproteobacteria and Flavobacteria, while the denitrification related gene nosZ related microbiota in S6 and S7 are mainly composed of Gammaproteobacteria. However, in general, the microbial taxa belonging to nitrogen cycling related genes at low salinity sites are mainly composed of Gammaproteobacteria and Alphaproteobacteria, while those at high salinity sites are mainly composed of Gammaproteobacteria.

The abundance of nitrogen cycling related genes and the microbial community structure carrying nitrogen cycling related genes are studied under different salinity. We believe that salinity has an effect on the abundance of genes associated with the nitrogen cycle. When salinity increases from 5.10 to 30.11 ppt, the abundance of most genes related to nitrogen cycle decrease (Fig. 6a). The abundance of nitrification related genes (amoC, hao and nxrB) and denitrification-related genes (nirK, nirS, norB, norC and nosZ) is high at low salinity sites. Previous studies have found that estuarine nitrifiers grow best at 5-10 ppt and will be inhibited if the salinity exceeds 10 ppt (Zhou et al. 2017), which means that nitrification related genes are most abundant at sites with salinity less than 10 ppt. Denitrification, which returns nitrogen to the atmosphere as N₂O and N₂, shows a different relationship with salinity. In some estuaries, the abundance of denitrification related genes is negatively correlated with salinity in the range of 0-36 ppt (Giblin et al. 2010). The abundance and potential of denitrifying bacteria are associated with low salinity around 5 ppt (Franklin et al. 2017; Marton et al. 2012). Salinity can affect denitrification by altering the organic substrates necessary for heterotrophic bacteria (Franklin et al. 2017). Therefore, the high concentration of NO₃⁻-N at high salinity can be attributed to the low abundance of denitrifying bacteria at high salinity. In addition, the reduction of dissimilated nitrate to ammonium (DNRA) (nirB nirD and nrfA) can compete with denitrification for nitrate, we observed higher concentrations of NH₄⁺ at the lower salinity sites (Table 1), indicating less NH₄⁺ consumption, which may be due to increased DNRA activity, further counteract the NH₄⁺ consumption (Marchant et al. 2014). A recent study also showed that, although anammox may be inhibited at higher salinity, increases in salinity below 15 ppt can stimulate anammox (Jin et al. 2012). This may explain our observation that the abundance of anammox-associated genes (hdh) is also high when salinity ranges from 5.1 to 15.16 (Fig. 6a).

We suggest that salinity affects the abundance of genes associated with the nitrogen cycle by affecting gene-carrying microbial populations. Sahan and Muyzer (2008) showed that salinity was the main factor controlling the distribution of microorganisms associated with the nitrogen cycle. Gammaproteobacteria and Alphaproteobacteria are dominant in most of the nitrogen cycling related genes in the three sample sites, but Alphaproteobacteria has a slight limitation under high salinity (Fig. 6b), which is consistent with the low abundance of Alphaproteobacteria in high-salinity areas (Fig. 3b).
Fig. 6 Pie chart of relative abundance of nitrogen cycling-related genes (a). Microbial taxa of nitrogen cycling-related genes and their relative abundance in each sample (b).

| Gene | pH  | salinity | NH$_4^+$-N | SiO$_2^-$-Si | NO$_2^-$-N | PO$_4^3^-$-P | NO$_3^-$-N |
|------|-----|----------|------------|---------------|-------------|--------------|------------|
| amoC | r   | -0.002   | -0.749**   | 0.165         | -0.843**    | 0.281        | 0.331      |
|      | P   | 0.996    | 0.033      | 0.697         | 0.009       | 0.792        | 0.5        |
| narB | r   | -0.138   | -0.753*    | 0.014         | -0.703      | -0.047       | 0.461      |
|      | P   | 0.745    | 0.031      | 0.973         | 0.052       | 0.912        | 0.25       |
| narG | r   | -0.117   | -0.252     | -0.54         | 0.2         | -0.113       | 0.648      |
|      | P   | 0.783    | 0.547      | 0.167         | 0.636       | 0.789        | 0.082      |
| narH | r   | -0.169   | -0.194     | -0.148        | -0.081      | -0.02        | -0.087     |
|      | P   | 0.69     | 0.645      | 0.726         | 0.848       | 0.963        | 0.837      |
| narI | r   | 0.138    | -0.379     | -0.231        | 0.287       | 0.071        | 0.484      |
|      | P   | 0.745    | 0.355      | 0.582         | 0.491       | 0.867        | 0.225      |
| nirA | r   | 0.284    | 0.24       | 0.383         | 0.327       | 0.278        | -0.850**   |
|      | P   | 0.496    | 0.567      | 0.349         | 0.429       | 0.506        | 0.008      |
| nirB | r   | 0.23     | -0.806*    | 0.219         | -0.516      | 0.156        | 0.366      |
|      | P   | 0.584    | 0.016      | 0.602         | 0.19        | 0.713        | 0.373      |
| nirD | r   | -0.026   | -0.664     | -0.038        | -0.303      | -0.084       | 0.368      |
|      | P   | 0.95     | 0.072      | 0.929         | 0.465       | 0.843        | 0.369      |

Table 4 Pearson correlation between nitrogen cycling related genes and chemical properties
Effect of salinity on sulfur cycle

In our KEGG annotated gene, the sulfur cycle is mainly controlled by two metabolic processes, namely sulfur oxidation and sulfur reduction (Cao et al. 2014) (Fig. 7a). Our results indicate that sulfur cycling related genes account for the largest proportion in S5. The sulfur oxidation genes (SoxAX, SoxY, SoxZ and SoxB) are similar in composition of related microorganisms (Fig. 7b), mainly carried by Alphaproteobacteria, Betaproteobacteria and Gammaproteobacteria. The cysD and cysN genes show similar microbial groups distribution among the three sampling sites, which are mainly carried by Flavobacteria and Gammaproteobacteria. In addition, in S5, both dsrA and dsrB genes are carried only by the Gammaproteobacteria, while in both S6 and S7, dsrA is carried only by Betaproteobacteria. About half of the sulfur cycling-related genes in our statistics are highly correlated with salinity, all of these sulfur cycling-related genes were negatively correlated with salinity (Table 5).

Fjords are an important part of the global ecosystem and play an important role in the sulfur cycle. Sulfur oxidation (Sox) is usually driven by sulfur-oxidizing bacteria, which oxidize reduced sulfide compounds, including elemental sulfur, sulfides, and thiosulfates (Yang et al. 2013). The Sox enzyme system is widely present in known sulphur oxidizing bacteria. In this study, the Sox enzyme system was detected to contain four gene: SoxAX, SoxY, SoxZ and SoxB (Fig. 7a). Sox related genes are mainly composed of Gammaproteobacteria, Betaproteobacteria and Alphaproteobacteria (Fig. 7b). Yang et al. (2013) have found that the response of a given sulphur oxidizing bacteria population to increases of salinity consists of successive changes in community structure but not of gradual adaptation of the sulphur oxidizing bacteria population. Because sulphur oxidizing bacteria belong to various classes of Proteobacteria, the response pattern of sulphur oxidizing bacteria to increases of salinity is consistent with that of proteobacterial classes (Yang et al. 2013). Our study shows that Alphaproteobacteria is dominant at low salinity, while Gammaproteobacteria is dominant at high salinity (Fig. 3b). Consistently, in this study, Alphaproteobacterial sulphur oxidizing bacteria with high abundance is detected in low salinity sites, while Gammaproteobacterial sulphur oxidizing bacteria with high abundance is detected in high salinity sites (Fig. 7b). Bacterial sulfate reduction has important ecological and geochemical significance in marine high-salt sediments (Oren 1988). Notably, besides the carry of the genes related to sulfate reduction by Gammaproteobacteria and

|   | r  | -0.871** | -0.027 | -0.765* | 0.132 | 0.516 | 0.044 |
|---|----|----------|--------|---------|-------|-------|-------|
|   | P  | 0.991    | 0.005  | 0.949   | 0.027 | 0.755 | 0.191 |
| nirK |     | 0.944    | 0.042  | 0.832   | 0.521 | 0.832 | 0.016 |
|   | P  | 0.946    | 0.011  | 0.72    | 0.019 | 0.71  | 0.164 |
| norB |     | 0.329    | -0.893** | 0.143   | -0.666 | 0.447 | 0.34  |
|   | P  | 0.426    | 0.003  | 0.736   | 0.071 | 0.267 | 0.41  |
| norC |     | 0.252    | -0.935** | 0.003   | -0.635 | 0.329 | 0.566 |
|   | P  | 0.547    | 0.001  | 0.994   | 0.091 | 0.427 | 0.144 |

*Correlation is significant at the 0.05 level.

**Correlation is significant at the 0.01 level.
Alphaproteobacteria, Flavobacteria is dominant in the microbial populations belonging to sulfate reduction related genes cysD and cysN (Fig. 7b). In addition, Flavobacteria plays an important role in sulfate reduction, which is consistent with the research results of Y. Li et al. (2018).

Fig. 7 Pie chart of relative abundance of sulfur cycling-related genes (a). Microbial taxa of sulfur cycling related genes and their relative abundance in each sample (b).

Table 5 Pearson correlation analysis of sulfur cycling related genes and chemical properties

|        | pH   | salinity | NH₄⁺-N | SiO₄²⁻-Si | NO₃⁻-N | NO₂⁻-N | PO₄³⁻-P | NO₃⁻-N |
|--------|------|----------|--------|-----------|--------|--------|---------|--------|
| soxY   | r    | 0.017    | -0.436 | 0.245     | -0.194 | 0.011  | -0.232  | -0.148 |
|        | P    | 0.967    | 0.281  | 0.558     | 0.645  | 0.98   | 0.58    | 0.726  |
| soxB   | r    | 0.125    | -0.823*| 0.199     | -0.538 | 0.194  | 0.144   | -0.081 |
|        | P    | 0.767    | 0.012  | 0.636     | 0.169  | 0.645  | 0.734   | 0.848  |
| soxZ   | r    | 0.628    | -0.815*| 0.331     | -0.19  | 0.635  | -0.062  | -0.085 |
|        | P    | 0.095    | 0.014  | 0.423     | 0.653  | 0.09   | 0.884   | 0.841  |
| soxX   | r    | 0.183    | -0.465 | 0.47      | -0.14  | 0.158  | -0.321  | -0.032 |
|        | P    | 0.664    | 0.246  | 0.24      | 0.742  | 0.71   | 0.439   | 0.941  |
| soxA   | r    | 0.175    | -0.433 | 0.342     | -0.349 | 0.345  | -0.455  | -0.084 |
|        | P   | 0.678 | 0.284 | 0.407 | 0.396 | 0.403 | 0.257 | 0.843 |
|--------|-----|-------|-------|-------|-------|-------|-------|-------|
| cysD r  | 0.005 | -0.28 | -0.217 | -0.158 | -0.103 | **0.804** | 0.143 |
|        | P   | 0.99 | 0.501 | 0.605 | 0.708 | 0.809 | 0.016 | 0.735 |
| cysN r  | 0.484 | **-0.708** | 0.664 | -0.425 | 0.472 | -0.21 | 0.354 |
|        | P   | 0.224 | 0.049 | 0.073 | 0.294 | 0.237 | 0.618 | 0.389 |
| sat r   | 0.052 | -0.464 | 0.418 | -0.316 | 0.084 | -0.332 | 0.017 |
|        | P   | 0.902 | 0.247 | 0.303 | 0.445 | 0.844 | 0.422 | 0.968 |
| aprA r  | 0.275 | **-0.807** | 0.261 | -0.557 | 0.394 | -0.101 | 0.036 |
|        | P   | 0.51 | 0.016 | 0.532 | 0.152 | 0.334 | 0.811 | 0.932 |
| aprB r  | 0.305 | -0.376 | 0.36 | -0.492 | 0.328 | -0.169 | 0.696 |
|        | P   | 0.462 | 0.358 | 0.382 | 0.216 | 0.427 | 0.689 | 0.055 |
| dsrA r  | 0.5 | -0.524 | 0.628 | -0.407 | 0.542 | -0.522 | 0.492 |
|        | P   | 0.207 | 0.183 | 0.095 | 0.317 | 0.166 | 0.185 | 0.215 |
| dsrB r  | 0.47 | **-0.951** | 0.174 | -0.571 | 0.543 | 0.323 | 0.157 |
|        | P   | 0.24 | 0.001 | 0.681 | 0.139 | 0.165 | 0.435 | 0.71  |

*Correlation is significant at the 0.05 level.

**Correlation is significant at the 0.01 level.

**Conclusion**

We used metagenomic analysis to demonstrate that changes in salinity can affect the relative abundance of some bacterial taxa on the one hand, and also change the relative abundance of functional genes and genes related to the nitrogen and sulfur cycles on the other hand.

**Acknowledgements**

We thank the First Oceanographic Institute, Ministry of Natural Resources for supporting this study.

**Declarations**

**Funding**

This research was funded by the National Natural Science Foundation of China (No.41776198), the Natural Foundation of Shandong Province National (No.ZR2020KC036), the Key R&D Program of China (2018YFC1406700), and Basic Scientific Fund for National Public Research Institutes of China (Nos.GY0219Q10).

**Conflicts of interest**

The authors declare that they have no conflicts of interest.

**Availability of data and material**

Raw data have been deposited into the NCBI Sequence Read Archive (SRA) database (Accession Number: SRP319744).

**Code availability**

Not applicable.

**Authors’ contributions**

Conceptualization: NW, WH, BZ, JZ and JL; methodology: BZ; software: WH; validation: LL, YQ and LF; formal analysis: JZ; investigation: NW; resources: NW and JL; data curation: LL, QL and LW; writing—original draft preparation: LL; writing—review and editing: NW, WH and LL; visualization: LL, YQ and LW; supervision: WH, LF and QL; project administration: NW; funding acquisition: NW.
Ethics approval
Not applicable.

Consent to participate
Not applicable.

Consent for publication
All authors read and approved the final version of the manuscript.

References
Allers E, Gómez-Consarnau L, Pinhassi J, Gasol JM, Šimek K, Pernthaler J (2007) Response of Alteromonadaceae and Rhodobacteriaceae to glucose and phosphorus manipulation in marine mesocosms. Environmental microbiology 9(10): 2417-2429. [http://doi.org/doi:10.1111/j.1462-2920.2007.01360.x]

Azam F, Fenchel T, Field JG, Gray J, Meyer-Reil L, Thingstad F (1983) The ecological role of water-column microbes in the sea. Marine ecology progress series 10: 257-263. [http://doi.org/doi:10.3354/meps010257]

Banda JF, Lu Y, Hao C, Pei L, Du Z, Zhang Y, et al. (2020) The Effects of Salinity and pH on Microbial Community Diversity and Distribution Pattern in the Brines of Soda Lakes in Badain Jaran Desert, China. Geomicrobiology Journal 37(1): 1-12. [http://doi.org/doi:10.1080/01490451.2019.1654568]

Bhatia MP, Kujawinski EB, Das SB, Breier CF, Henderson PB, Charette MA (2013) Greenland meltwater as a significant and potentially bioavailable source of iron to the ocean. Nature Geoscience 6(4): 274-278. [http://doi.org/doi:10.1038/NGEO1746]

Biers EJ, Sun S, Howard EC (2009) Prokaryotic genomes and diversity in surface ocean waters: interrogating the global ocean sampling metagenome. Applied and environmental microbiology 75(7): 2221-2229. [http://doi.org/doi:10.1128/AEM.02118-08]

Bouvier TC, del Giorgio PA (2002) Compositional changes in free-living bacterial communities along a salinity gradient in two temperate estuaries. Limnology and oceanography 47(2): 453-470. [http://doi.org/doi:10.4319/lo.2002.47.2.0453]

Buchfink B, Xie C, Huson DH (2015) Fast and sensitive protein alignment using DIAMOND. Nature methods 12(1): 59-60. [http://doi.org/doi:10.1038/nmeth.3176]

Buchholz CM, Wiencke C (2016) Working on a baseline for the Kongsfjorden food web: production and properties of macroalgal particulate organic matter (POM). Polar Biology 39(11): 2053-2064. [http://doi.org/doi:10.1007/s00300-015-1828-3]

Bull AT, Stach JE, Ward AC, Goodfellow M (2005) Marine actinobacteria: perspectives, challenges, future directions. Antonie Van Leeuwenhoek 87(1): 65-79. [http://doi.org/doi:10.1007/s10482-004-6562-8]

Cabisco Català E, Tamarit Sumalla J, Ros Salvador J (2000) Oxidative stress in bacteria and protein damage by reactive oxygen species. International Microbiology, 2000, vol. 3, núm. 1, p. 3-8 3(1): 3-8. [http://doi.org/doi:10.0000/PMID10963327]

Cao H, Wang Y, Lee OO, Zeng X, Shao Z, Qian P-Y (2014) Microbial sulfur cycle in two hydrothermal chimneys on the Southwest Indian Ridge. MBio 5(1): e00980-00913. [http://doi.org/doi:10.1128/mBio.00980-13]

Capo E, Bravo AG, Soerensen AL, Bertilsson S, Pinhassi J, Feng C, et al. (2020) Deltaproteobacteria and Spirochaetes-like bacteria are abundant putative mercury methylators in oxygen-deficient
water and marine particles in the Baltic Sea. Frontiers in microbiology 11(574080): 1-11. 
http://doi.org/doi:10.3389/fmicb.2020.574080

Cottrell MT, Waidner LA, Yu L, Kirchman DL (2005) Bacterial diversity of metagenomic and PCR libraries from the Delaware River. Environmental Microbiology 7(12): 1883-1895. 
http://doi.org/doi:10.1111/j.1462-2920.2005.00762.x

Feisthauer S, Wick LY, Kästner M, Kaschabek SR, Schlömann M, Richnow HH (2008) Differences of heterotrophic 13CO2 assimilation by Pseudomonas knackmussii strain B13 and Rhodococcus opacus 1CP and potential impact on biomarker stable isotope probing. Environmental Microbiology 10(6): 1641-1651. http://doi.org/doi:10.1111/j.1462-2920.2008.01573.x

Franklin RB, Morrissey EM, Morina JC (2017) Changes in abundance and community structure of nitrate-reducing bacteria along a salinity gradient in tidal wetlands. Pedobiologia 60: 21-26. http://doi.org/doi:10.1016/j.pedobi.2016.12.002

Galinski EA (1995) Osmoadaptation in bacteria. Advances in microbial physiology 37: 273-328. 
http://doi.org/doi:10.1016/S0065-2911(08)60148-4

Ghai R, Pašić L, Fernández AB, Martín-Cuadrado A-B, Mizuno CM, McMahon KD, et al. (2011) New abundant microbial groups in aquatic hypersaline environments. Scientific reports 1(1): 1-10. http://doi.org/doi:10.1038/srep00135

Giblin AE, Weston NB, Banta GT, Tucker J, Hopkinson CS (2010) The effects of salinity on nitrogen losses from an oligohaline estuarine sediment. Estuaries and Coasts 33(5): 1054-1068. 
http://doi.org/doi:10.1007/s12237-010-9280-7

Gu S, Fang L, Xu X (2013) Using SOAPaligner for short reads alignment. Current protocols in bioinformatics 44(1): 11.11. 11-11.11. 17. http://doi.org/doi:10.1002/0471250953.bi111s44

Han SK, Nedashkovskaya OI, Mikhailov VV, Kim SB, Bae KS (2003) Salinibacterium amurskyense gen. nov., sp. nov., a novel genus of the family Microbacteriaceae from the marine environment. International journal of systematic and evolutionary microbiology 53(6): 2061-2066. http://doi.org/doi:10.1099/ijs.0.02627-0

Hawkins J, Wadham J, Tranter M, Lawson E, Sole A, Cowton T, et al. (2015) The effect of warming climate on nutrient and solute export from the Greenland Ice Sheet. Geochem. Perspect. Lett 1(1): 94-104. http://doi.org/doi:10.7185/geochemlet.1510

Hood E, Battin TJ, Fellman J, O'neel S, Spencer RG (2015) Storage and release of organic carbon from glaciers and ice sheets. Nature geoscience 8(2): 91-96. 
http://doi.org/doi:10.1038/NGEO2331

Hood E, Berner L (2009) Effects of changing glacial coverage on the physical and biogeochemical properties of coastal streams in southeastern Alaska. Journal of Geophysical Research: Biogeosciences 114(G3): 1-10. http://doi.org/doi:10.1029/2009JG000971

Jin R-C, Yang G-F, Yu J-J, Zheng P (2012) The inhibition of the Anammox process: a review. Chemical engineering journal 197: 67-79. http://doi.org/doi:10.1016/j.cej.2012.05.014

Klatt P, Lamas S (2000) Regulation of protein function by S-glutathiolation in response to oxidative and nitrosative stress. European journal of biochemistry 267(16): 4928-4944. http://doi.org/doi:10.1046/j.1432-1327.2000.01601.x

Kwak M-J, Song JY, Kim BK, Chi W-J, Kwon S-K, Choi S, et al. (2012). Genome sequence of the agar-degrading marine bacterium Alteromonadaceae sp. strain G7. In (Vol. 194, pp. 6961-6962): Am Soc Microbiol.

Lagkouvardos I, Joseph D, Kapfhammer M, Giritli S, Horn M, Haller D, et al. (2016) IMNGS: a
Lawson EC, Wadham JL, Tranter M, Stibal M, Lis GP, Butler CE, et al. (2014) Greenland Ice Sheet exports labile organic carbon to the Arctic oceans. Biogeosciences 11(14): 4015-4028. http://doi.org/doi:10.5194/bg-11-4015-2014

Li W, Fu L, Niu B, Wu S, Wooley J (2012) Ultrafast clustering algorithms for metagenomic sequence analysis. Briefings in bioinformatics 13(6): 656-668. http://doi.org/doi:10.1093/bib/bbs035

Li Y, Jing H, Xia X, Cheung S, Suzuki K, Liu H (2018) Metagenomic insights into the microbial community and nutrient cycling in the western subarctic Pacific Ocean. Frontiers in microbiology 9(623): 1-15. http://doi.org/doi:10.3389/fmicb.2018.00623

Lozupone CA, Knight R (2007) Global patterns in bacterial diversity. Proceedings of the National Academy of Sciences 104(27): 11436-11440. http://doi.org/doi:10.1073/pnas.0611525104

Luo R, Liu B, Xie Y, Li Z, Huang W, Yuan J, et al. (2012) SOAPdenovo2: an empirically improved memory-efficient short-read de novo assembler. Gigascience 1(1): 2047-217X-2041-2018. http://doi.org/doi:10.1186/2047-217X-1-18

Magarvey NA, Keller JM, Bernan V, Dworkin M, Sherman DH (2004) Isolation and characterization of novel marine-derived actinomycete taxa rich in bioactive metabolites. Applied and environmental microbiology 70(12): 7520-7529. http://doi.org/doi:10.1128/AEM.70.12.7520–7529

Marchant HK, Lavik G, Holtappels M, Kuypers MM (2014) The fate of nitrate in intertidal permeable sediments. PloS one 9(8): e104517. http://doi.org/doi:10.1371/journal.pone.0104517

Martinez-Porchas M, Villalpando-Canchola E, Suarez LEO, Vargas-Albores F (2017) How conserved are the conserved 16S-rRNA regions? PeerJ 5: e3036. http://doi.org/doi: 10.7717/peerj.3036

Marton JM, Herbert ER, Craft CB (2012) Effects of salinity on denitrification and greenhouse gas production from laboratory-incubated tidal forest soils. Wetlands 32(2): 347-357. http://doi.org/doi:10.1007/s10270-012-0270-3

Mason OU, Di Meo-Savoie CA, Van Nostrand JD, Zhou J, Fisk MR, Giovannoni SJ (2009) Prokaryotic diversity, distribution, and insights into their role in biogeochemical cycling in marine basalts. The ISME journal 3(2): 231-242. http://doi.org/doi:10.1038/ismej.2008.92

Methé BA, Nelson KE, Deming JW, Momen B, Melamud E, Zhang X, et al. (2005) The psychrophilic lifestyle as revealed by the genome sequence of Colwellia psychrerythraea 34H through genomic and proteomic analyses. Proceedings of the National Academy of Sciences 102(31): 10913-10918. http://doi.org/doi:10.1073/pnas.0504766102

Nealson KH (1997) Sediment bacteria: who's there, what are they doing, and what's new? Annual Review of Earth and Planetary Sciences 25(1): 403-434. http://doi.org/doi:10.1146/annurev.earth.25.1.403

Newton K, Jeffries TC, Smith RJ, Seymour JR, Seuront L, Mitchell JG (2018) Taxonomic and metabolic shifts in the Coorong bacterial metagenome driven by salinity and external inputs. Journal of Oceanology and Limnology 36(6): 2033-2049. http://doi.org/doi:10.1007/s00343-018-7387-z

Nogales B, Lanfranconi MP, Píña-Villalonga JM, Bosch R (2011) Anthropogenic perturbations in marine microbial communities. FEMS Microbiology reviews 35(2): 275-298. http://doi.org/doi:10.1111/j.1574-6976.2010.00248.x

Oren A (1988) Anaerobic degradation of organic compounds at high salt concentrations. Antonie van
Leeuwenhoek 54(3): 267-277. http://doi.org/doi:10.1007/BF00443585

Oren A (2002) Diversity of halophilic microorganisms: environments, phylogeny, physiology, and applications. Journal of Industrial Microbiology and Biotechnology 28(1): 56-63. http://doi.org/doi:10.1038/sj/jim/7000176

Paver SF, Muratore D, Newton RJ, Coleman ML (2018) Reevaluating the salty divide: phylogenetic specificity of transitions between marine and freshwater systems. Msystems 3(6): e00232-00218. http://doi.org/doi:10.1128/mSystems.00232-18

Pfennig N, Trüper HG. (1981). Isolation of members of the families Chromatiaceae and Chlorobiaceae. In The prokaryotes (pp. 279-289): Springer.

Piquet A-T, Scheepens J, Bolhuis H, Wiencke C, Buma A (2010) Variability of protistan and bacterial communities in two Arctic fjords (Spitsbergen). Polar biology 33(11): 1521-1536. http://doi.org/doi:10.1007/s00300-010-0841-9

Reddy B, Pandey J, Dubey SK (2019) Assessment of environmental gene tags linked with carbohydrate metabolism and chemolithotrophy associated microbial community in River Ganga. Gene 704: 31-41. http://doi.org/doi:10.1016/j.gene.2019.04.004

Reen FJ, Almagro-Moreno S, Ussery D, Boyd EF (2006) The genomic code: inferring Vibionaceae niche specialization. Nature Reviews Microbiology 4(9): 697-704. http://doi.org/doi:10.1038/nrmicro1476

Rusch DB, Halpern AL, Sutton G, Heidelberg KB, Williamson S, Yooseph S, et al. (2007) The Sorcerer II global ocean sampling expedition: northwest Atlantic through eastern tropical Pacific. PLoS Biol 5(3): e77. http://doi.org/doi:10.1371/journal.pbio.0050077

Sahan E, Muyzer G (2008) Diversity and spatio-temporal distribution of ammonia-oxidizing Archaea and Bacteria in sediments of the Westerschelde estuary. FEMS microbiology ecology 64(2): 175-186. http://doi.org/doi:10.1111/j.1574-6941.2008.00462.x

Santamans AC, Boluda R, Picazo A, Gil C, Ramos-Miras J, Tejedo P, et al. (2017) Soil features in rookeries of Antarctic penguins reveal sea to land biotransport of chemical pollutants. PLoS One 12(8): e0181901.

Schroth AW, Crusius J, Hoyer I, Campbell R (2014) Estuarine removal of glacial iron and implications for iron fluxes to the ocean. Geophysical Research Letters 41(11): 3951-3958. http://doi.org/doi:10.1002/2014GL060199

Syvitski JP, Burrell DC, Skei JM. (1987). Fjords: processes and products: Springer Science & Business Media.

Tamames J, Abellán JJ, Pignatelli M, Camacho A, Moya A (2010) Environmental distribution of prokaryotic taxa. BMC microbiology 10(1): 1-14. http://doi.org/doi:10.1186/1471-2180-10-85

Wang N, Guo Y, Li G, Xia Y, Ma M, Zang J, et al. (2019) Geochemical-compositional-functional changes in arctic soil microbiomes post land submergence revealed by metagenomics. Microbes and environments 34(2): 180-190. http://doi.org/doi:10.1264/jsme2.ME18091

Wu QL, Zwart G, Schauer M, Kamst-van Agterveld MP, Hahn MW (2006) Bacterioplankton community composition along a salinity gradient of sixteen high-mountain lakes located on the Tibetan Plateau, China. Applied and Environmental Microbiology 72(8): 5478-5485. http://doi.org/doi:10.1128/AEM.00767-06

Yang J, Jiang H, Dong H, Wu G, Hou W, Zhao W, et al. (2013) Abundance and diversity of sulfur-oxidizing bacteria along a salinity gradient in four Qinghai-Tibetan lakes, China.
Yokokawa T, Nagata T, Cottrell MT, Kirchman DL (2004) Growth rate of the major phylogenetic bacterial groups in the Delaware estuary. Limnology and Oceanography 49(5): 1620-1629. http://doi.org/doi:10.4319/lo.2004.49.5.1620

Zhou M, Butterbach-Bahl K, Vereecken H, Brüggemann N (2017) A meta-analysis of soil salinization effects on nitrogen pools, cycles and fluxes in coastal ecosystems. Global change biology 23(3): 1338-1352. http://doi.org/doi:10.1111/gcb.13430

Zhu W, Lomsadze A, Borodovsky M (2010) Ab initio gene identification in metagenomic sequences. Nucleic acids research 38(12): e132-e132. http://doi.org/doi:10.1093/nar/gkq275
Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- tableS1.xlsx
- tableS2.xlsx
- tables3.xlsx
- tableS4.xlsx
- S1.png