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Structure and diversity of the bacterial community of an Arctic estuarine system (Kandalaksha Bay) subject to intense tidal currents.

Susanna Gorrasi[a], Chiara Pesciaroli[a], Paolo Barghini[a], Marcella Pasqualetti[a], Massimiliano Fenice[a,b*]

[a]Dipartimento di Ecologia e Biologia, University of Tuscia, Viterbo, 01100, Italy;

[b]Laboratorio di Microbiologia Marina Applicata, CONISMA, University of Tuscia, Viterbo, 01100, Italy

*Corresponding author

E-mail: fenice@unitus.it

Abstract

Kandalaksha Bay is a peculiar estuarine system located at the Arctic Circle within the White Sea closed basin (Russia). This sub-extreme marine environment combines features of temperate and Arctic seas. Its unusual hydrodynamic is due to seasonal high runoff of freshwater (caused by inputs from rivers and abundant precipitations), and tidal currents generating intense mixing mainly of surface waters. In order to get a survey of the bacterial community of this zone, seawater was sampled, at various distances from the shore and depths, in areas selected in relation to the tidal current pattern. Bacterial assemblages were characterised by 454-pyrosequencing to obtain detailed information on their diversity and structure. The phylum *Proteobacteria* was the most predominant in all samples (abundance ranged from 67% to 96%) being the class *γ-Proteobacteria*

its main fraction. *Cyanobacteria* was the second most abundant phylum (11-15%) in surface samples collected offshore in the main tidal stream while it was rather scarce in the coastal samples and very low at the maximum depth. Unexpectedly, no presence of *Synechococcus* was detected; in addition, the diffused occurrence of *Prochlorococcus,* generally very low or absent in polar waters, could be considered another effect of global change. *Bacteroidetes* showed abundance ≥ 5% in surface and shallow waters (15 m). At the genus level, biodiversity clearly decreased in the offshore samples, in particular at the highest depth, were high prevalence of *Halomonas* was recorded (> 65%).

The Redundancy Analysis revealed that water temperature and salinity represented the environmental factors determining the bacterial community structure. Moreover, the nMDS analysis evidenced high similarity only among samples taken in surface layers and involved in the water mixing by tidal currents, being the deepest sample (-70 meters) the most dissimilar.

Keywords

Kandalaksha Bay; Arctic estuarine system; Tidal currents; Biodiversity; Bacterial communities; 454 pyrosequencing

1. Introduction

Microorganisms represent the last boundary for life in regions that could not be colonized by other life forms. Those from extreme and sub-extreme cold environments, such as the highest mountains, the Arctic and Antarctic regions, provide optimal subjects for studying peculiar adaptation mechanisms and response to global change (Selbmann et al., 2012; Reboleiro-Rivas et al., 2013; Andrade et al., 2014). Obtaining information regarding the various groups within the microbial community of a certain environment is important for understanding their relative influence in the biogeochemical cycles and has become a fundamental ecological question (Cottrell

and Kirchman, 2000; Zeng et al., 2013). In this context, further acquisition of environmental awareness requires detailed investigation of the microbial communities' composition, structure and functionality. This allows understanding the various and composite dynamics to which microorganisms are subject and evidencing possible strategies established to counteract repeated and intense environmental stress (Reboleiro-Rivas et al., 2012; Pesciaroli et al., 2015a).

Kandalaksha Bay (KB) is one of the three southern bays of the White Sea (WS), which is a rather small semi-enclosed Arctic sea (NW of Russia) and communicates with the Arctic Ocean through the Barents Sea. The water exchanges between the Barents Sea and the WS are limited, but establish a transport system for particulate material, microorganisms and biogeochemical species (Howland et al., 1999; Berger and Naumov, 2000; Pantyulin, 2003). KB is a complex insular estuarine system having a special hydrological regime with rather large sea level differences during tides (shallow, asymmetrical and semi-diurnal), causing great cyclic variations in depths and horizontal displacements of the coastline and intense mixing of waters within 30 m from the surface (Melnikov et al., 2003; Savvichev et al., 2003). In some areas (i.e. the "Velikaja Salma" strait), both stratified and mixed regions are present. Actually, the topographic features of this area lead to an increase in tide height (up to 2.5 m), but especially in the tidal currents speed that can reach 80–120 cm/s (Pantyulin, 2003).

The pattern of currents is extremely complex and main tidal streams are often split in secondary flows by the various island; moreover, in some areas, peculiar mixing situations are generated also (Tzetlin et al., 1997; Berger and Naumov, 2000; Savvichev et al., 2003). The unique hydrodynamics of the bay is also affected by seasonal intense runoffs of freshwater, due to various rivers, streams and strong precipitations (Howland et al., 1999; Dolotov et al., 2005), which contribute to enrich the sea water with nutrients and microorganisms from the surrounding soils, forests and peatlands. On the other hand, these inputs, carrying particulate and humic substances, cause intense water darkening with consequent strong reduction of the photic zone, which is

confined in the first 10 m on average, and reaches 15-20 m in days with the highest solar radiation (Bobrov *et al*., 1995; Kravchishina *et al*., 2013).

KB is subject to wide fluctuations of environmental parameters (Savvichev et al., 2003). Annual temperature variability is very high; although global change begins to mitigate it, the winter season is long and quite severe (the sea surface is sheltered by ice for 5–6 months) and the meteorological conditions tend to be very unpredictable. Air temperature may fall to -40 °C, but occasionally rise to few degrees above 0 °C, due to warm Atlantic air streams; water temperature is about -1 °C to -2 °C. In the rather short summer, air temperature can rise up to 30 °C (15-20 °C, on average), while seawater temperature can reach 15 °C on surface layers, but is subject to a fast drop and remains steadily around 0 °C after a few dozen meters of depth (Berger and Gorbushin, 2001; Pantyulin, 2003; Shaporenko et al., 2005; Vershinin et al., 2006)

In this region, organisms must adapt to variable environment conditions in which factors (such temperature and salinity) change frequently (Savvichev et al., 2004; Kravchishina et al., 2008), and communities must be functionally organized to cope with the effects of sudden stressing conditions (Pesciaroli et al., 2015b).

Microbial communities in the White Sea and, particularly in the KB area, have been scarcely investigated and very little is known about their structure, organization and functionality. In addition, the majority of the studies characterised only the cultivable fraction of the communities (Savvichev et al., 2003, 2004; Kravchishina et al., 2008; Pesciaroli et al., 2015a). The sole attempt to depict KB total bacterial community composition and organization was the preliminary work of Pesciaroli and co-workers (Pesciaroli et al., 2015b), carried out by the PCR-TGGE fingerprinting technique. Although somehow useful for a basic screening, this low-throughput method could only allow for partial characterisation of the community diversity, resulting in incorrect information in relation to its structure. To the best of our knowledge, no study by new generation sequencing techniques has been carried out yet.

This work was aimed at achieving a first high-resolution information on the bacterial diversity in the Kandalaksha Bay peculiar environment: the community composition and structure of sea-water samples, collected at various depths (0 – 70 m) in different sites selected in relation to tidal currents, were analysed by the 454 pyrosequencing.

2. Materials and methods

2.1 Sample collection

Seawater samples (S1-S6) were collected in September 2008, at various depths and distances from the shore, in the "Velikaja Salma" strait (between Veliky Island and Cape Kindo peninsula), on the south-western coast of KB (Fig. 1). Samples were processed at the nearby "Nikolai Pertsov" White Sea Biological Station (WSBS, Moscow State University, "Lomonosov").

Sampling sites were selected according to known flows of tidal currents that represent a branch of the main KB current, which enters the inlet and is further split in secondary flows by several small islands located between the Veliky Island and the shore. An area where tidal stream presents a circular pattern is located in front of the WSBS at ca. 100 m from the Station pier (Tzetlin et al., 1997). S1 and S2 were manually collected, using sterile containers, at the lowest intertidal zone (intertidal pool) and at the nearby coastal line on water surface, ca. 30 and 35 m from the shore, respectively. S3 and S4 were collected using sterile containers by scuba divers in proximity of the mentioned circular pattern of the flow, on water surface and -15 m (ca. 1 m from the sea bottom to possibly avoid cross-contamination with sediments), respectively. S5 and S6 were collected using sanitised Niskin bottles in the inlet tidal current before it is split by the small islands, ca. 600 m from the shore on water surface and -70 m (close to the sea bottom, but distant enough to avoid cross-contamination with sediments), respectively. Table 1 reports the GPS coordinates for each sampling site and the environmental parameters recorded during sampling.

All samples were obtained pooling 3 different sub-samples. After sampling, 250 mL of seawater were vacuum-filtered on sterile membranes (0.22 µm, Millipore, USA) that were also

washed twice with sterile saline solution to remove possible nutrients from the filters. In addition, in order to avoid the bacterial growth on the filters before the DNA extraction (carried out within minutes from filtration), membranes were maintained at 4 °C in sterile tubes with sterile silica-gel.

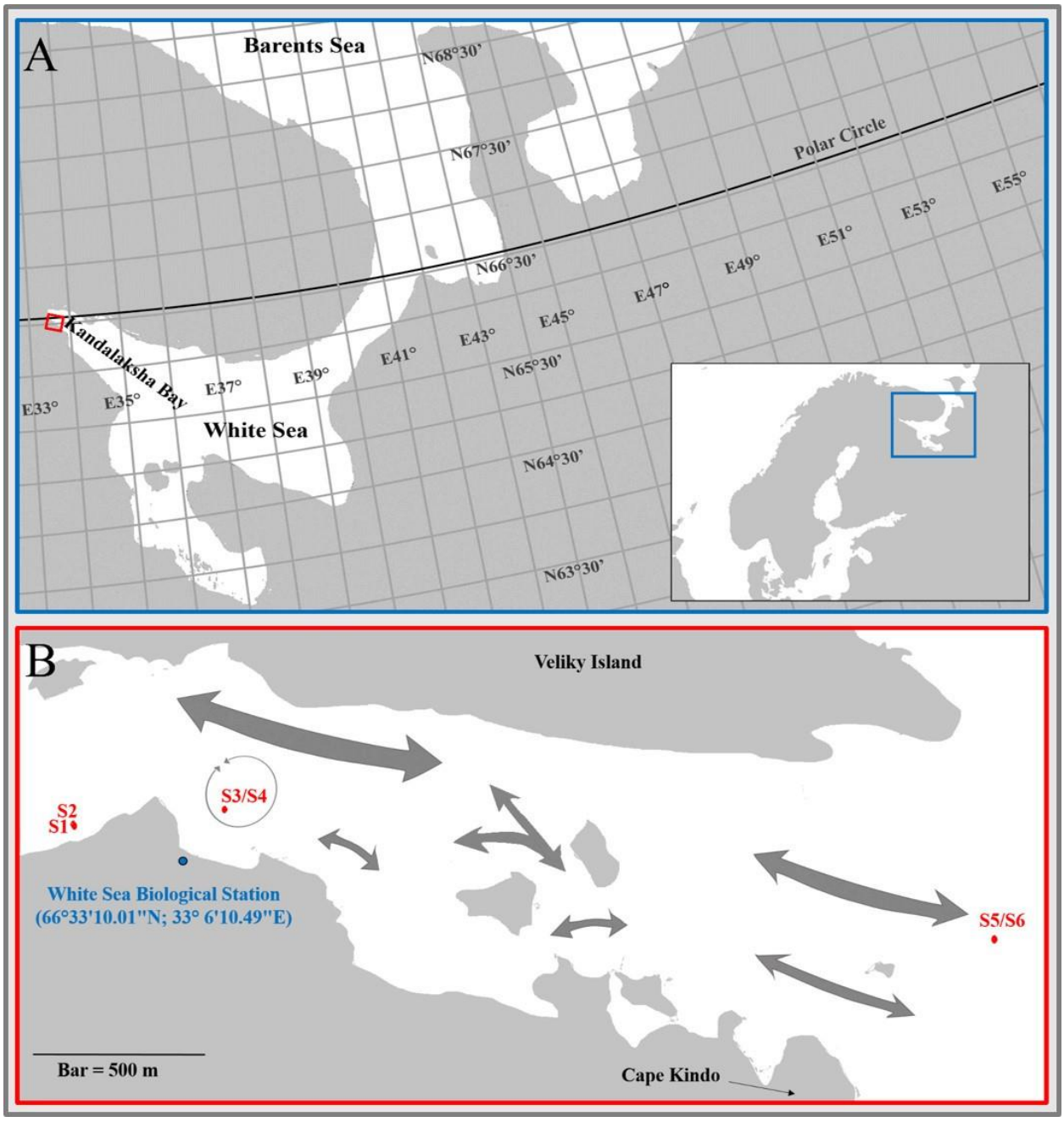

Fig. 1. Location of the sampling sites in the Velikaja Salma strait on the south-western shore of Kandalaksha Bay. (A) Overview of the White Sea region where the sampling area is located (red square). (B) Details of the sampling area with indication of the sampling sites (S1-S6) and tidal

currents (arrows). Arrow thickness approximately indicates current intensity and the round-shaped thin arrow represents the circular pattern of the current in front of the WSBS. Position and direction of tidal current flows have been superimposed on the map according to Tzetlin et al. (1997). Maps were generated using Google Earth Pro version 7.3.1. and graphically edited.

Table 1. GPS coordinates of the sampling sites in the Velikaja Salma strait and environmental parameters recorded during sampling.

Sample	Sampling site coordinates (Latitude - Longitude)	Water Temperature (°C)	Salinity (‰)	Depth (m)
S1	66°33'13.82"N - 33° 5'42.92"E	8.7	24.9	0.5
S2	66°33'13.82"N - 33° 5'42.92"E	8.4	24.4	0.5
S3	66°33'15.40"N - 33° 6'25.05"E	8.5	23.5	2.5
S4	66°33'15.40"N - 33° 6'25.05"E	6.6	24.2	15
S5	66°33'16.28"N - 33° 8'31.41"E	8.0	24.0	0.5
S6	66°33'16.28"N - 33° 8'31.41"E	0	25.4	70

2.2 DNA extraction, Amplicon Library Preparation and Sequencing

Membranes were processed for total DNA extraction as follows. Each membrane was transferred to a 15 ml sterile tube, containing 1,5 mL of sterile distilled water, and finely ground. Tubes were vortexed, in order to allow cell re-suspension. Suspensions were transferred to sterile microcentrifuge tubes, and cells pelleted by centrifugation (7,500 x g, 20 min.); supernatants were removed, leaving ca. 25 µl of liquid. The cell pellets were re-suspended by Vortex and processed using the MasterPureTM purification Kit (Epicentre® Biotechnologies, USA) accordingly to the manufacturer's instructions.

The PCR amplification of target region and the subsequent pyrosequencing, carried out as reported by De Filippis et al. (2013), were performed at the Department of Agricultural Sciences,

University of Naples Federico II, 80055 Portici, Italy). Amplification of bacterial 16S rRNA V1-V3 hypervariable regions was carried out using the universal primers Gray28F 59-TTTGATCNTGGCTCAG and Gray519r 59-GTNTTACNGCGGCKGCTG (obtaining 520 bp-fragments) (Ercolini et al., 2011). To allow sample multiplexing, the 454-adaptors were included in the forward primer followed by a 10 bp sample-specific Multiplex Identifier (MID). PCR reactions were performed in 50 µl of final volume containing 50 ng of template DNA, 0.4 µM of each primer, 0.50 mmol/L of each deoxynucleoside triphosphate, 2.5 mmol/L $MgCl_2$, 5 ml of 10 PCR buffer and 2.5 U of Taq polymerase (Invitrogen, Milano, Italy). PCR conditions were: 94 °C for 2 min, 35 cycles of 95 °C for 20 s, 56 °C for 45 s and 72 °C for 5 min, and a final extension at 72 °C for 7 min. Amplicons were purified twice by the Agencourt AMPure kit (Beckman Coulter, Milano, Italy) and quantified using the QuantiFluorTM (Promega, Milano, Italy). The equimolar pool of PCR products was sequenced on a GS Junior platform (454 Life Sciences, Roche Diagnostics, Italy) according to the manufacturer's instructions by using a Titanium chemistry.

2.3 Sequence processing and Data Analysis

Raw reads were preliminary filtered according to the 454 processing pipeline and subsequently processed using QIIME version 1.6.0 (Caporaso et al., 2010). After demultiplexing, in order to increase the accuracy of OTUs detection, sequences were submitted to a quality filtering step: reads shorter than 300 bp, with an average quality score lower than 25 and ambiguous base calls, were removed. The filtered sequences were denoised (Reeder and Knight, 2010) and singletons were excluded. Taxonomic annotation was carried out as follows: reads were clustered into OTUs, defined by a 97% of similarity, using the uclust method (Edgar, 2010); the representative sequences were submitted to the RDPII classifier (Wang et al., 2007) using the Greengenes 16S rRNA gene database (McDonald et al., 2012), obtaining the taxonomical assignment and the relative abundance of each OTU. Alpha diversity analyses were performed

using QIIME to calculate Observed OTUs, Good's coverage, Chao1 richness (Chao et al., 2002), and Shannon diversity indices (Shannon and Weaver, 1949), and to generate rarefaction curves.

Shared phylotypes among the samples were visualised by a six-way Venn diagram, generated using the JVenn tool (http://bioinfo.genotoul.fr/jvenn) (Bardou et al., 2014).

β-diversity was analysed by clustering analysis and non-metric multidimensional scaling (nMDS) performed using the software package PRIMER-E v.6.1.18 (Plymouth, UK) (Clarke and Gorley, 2005). The Bray-Curtis dissimilarity matrix was calculated from square root-transformed data of taxa relative abundance, and used for nMDS analysis (25 restarts; minimum Kruskal stress = 0.01). Clustering analysis (group average) was carried out to identify similarities among samples and used to generate the similarity contours superimposed on the nMDS ordination plot.

Finally, environmental drivers of community structure were analysed by Redundancy Analysis (RDA) using the software Canoco v. 5.0 (Microcomputer Power, Ithaca, NY, USA) (Ter Braak and Šmilauer, 2012), considering the following parameters: salinity, water temperature, and depth. Collinear variables were removed before the RDA analysis; parameters were tested by SYSTAT v. 8.0 (SPSS Inc., Chicago, IL, USA) for potential collinearity. OTU relative abundances were log-transformed for RDA analysis. The Monte Carlo permutation test was used to assess the statistical significance of the canonical axes.

3. Results

3.1 Composition and diversity of the bacterial communities

A total of 53870 validated reads were obtained, with an averaged value of 8978 reads/sample. Alpha diversity was estimated by Observed OTUs, Chao1, Shannon, and Good's coverage indices (Table 2). The Estimated Sample Coverage (ESC) was satisfactory (≥ 98%) for all samples. The number of observed OTUs and the estimated OTU richness (Chao 1), ranged from 296 to 610 and from 411.02 to 669.40, respectively; the Shannon diversity index ranged from 2.82

to 6.65. The intertidal pool sample (S1) showed the highest values for all indices, while S6 (-70 m) showed the lowest ones.

The taxonomical analysis allowed assigning reads to 20 phyla, accounted for ≥ 94% of the sequences across all samples; reads not classified ("Unassigned") at the phylum level were detected in all samples ranging from 0.8 to 6% (Fig. 2). *Proteobacteria* was the predominant phylum (67-96%); *Bacteroidetes* were also rather abundant (5-12%) in all samples except S6 (-70 m), while *Cyanobacteria* were mainly present on surface waters collected offshore (11 and 15 % for S3 and S5, respectively).

The class *γ-Proteobacteria* represented the main fraction (46-94%) in all samples, while *α-Proteobacteria* were rather abundant (11-31%) in S1-S5, which showed also fair presence of *Flavobacteria* (5-11%). The sub-class of *Synechococcophycideae* was relatively abundant in S3 and S5 (11 and 15%, respectively).

Overall, at the genus level, reads were assigned to 217 taxa, ranging from 93 to 159 across samples. Fig. 3 shows the pattern of genera in the various samples, evidencing those having percentage relative abundance (%Ra) ≥ 1 at least in one sample; minor genera (%Ra < 1) were gathered in "Others". Major genera (%Ra ≥ 1) were only few in each sample, but they accounted for the main fraction of the whole bacterial communities. In S1-S5 a total of 12-15 major genera were recorded, representing the 75-82% of the community. S1, S2, S4 and S5 communities were not characterised by the marked dominance of a single genus, but several majority taxa were present with similar abundance. In S3, predominance of *Psychrobacter* (37%) was quite evident. In S6 only 4 major genera were present, accounting for ca. 92% of the community, but the sole genus *Halomonas* constituted the 65% of the community. The other abundant genera were *Pseudoalteromonas* (18%), *Vibrio* (6%) and *Marinomonas* (3%). However, in all samples, *Pseudoalteromonas* represented one of the most abundant taxon (5-23%).

The number of shared phylotypes among the samples (Venn diagram) is reported in Fig. 4. Overall, 63 phylotypes (ca. 27% of the annotated phylotypes) were in common among all samples representing an important moiety of the communities (77-97%). Various phylotypes were found only in one community.

Table 2. Alpha-diversity indices of the six amplicon libraries.

Sample	Reads	Observed OTUs	Chao1	Shannon	ESC
S1	8448	610	669.40	6.65	99%
S2	9067	539	623.24	6.56	98%
S3	7135	568	640.57	5.80	98%
S4	8899	521	627.56	5.97	98%
S5	10664	437	613.55	5.97	98%
S6	9657	296	411.02	2.82	99%

OTU = operational taxonomic unit; ESC = estimated sample coverage.

Chao1, Shannon and ESC were calculated with Qiime at the 3% distance level.

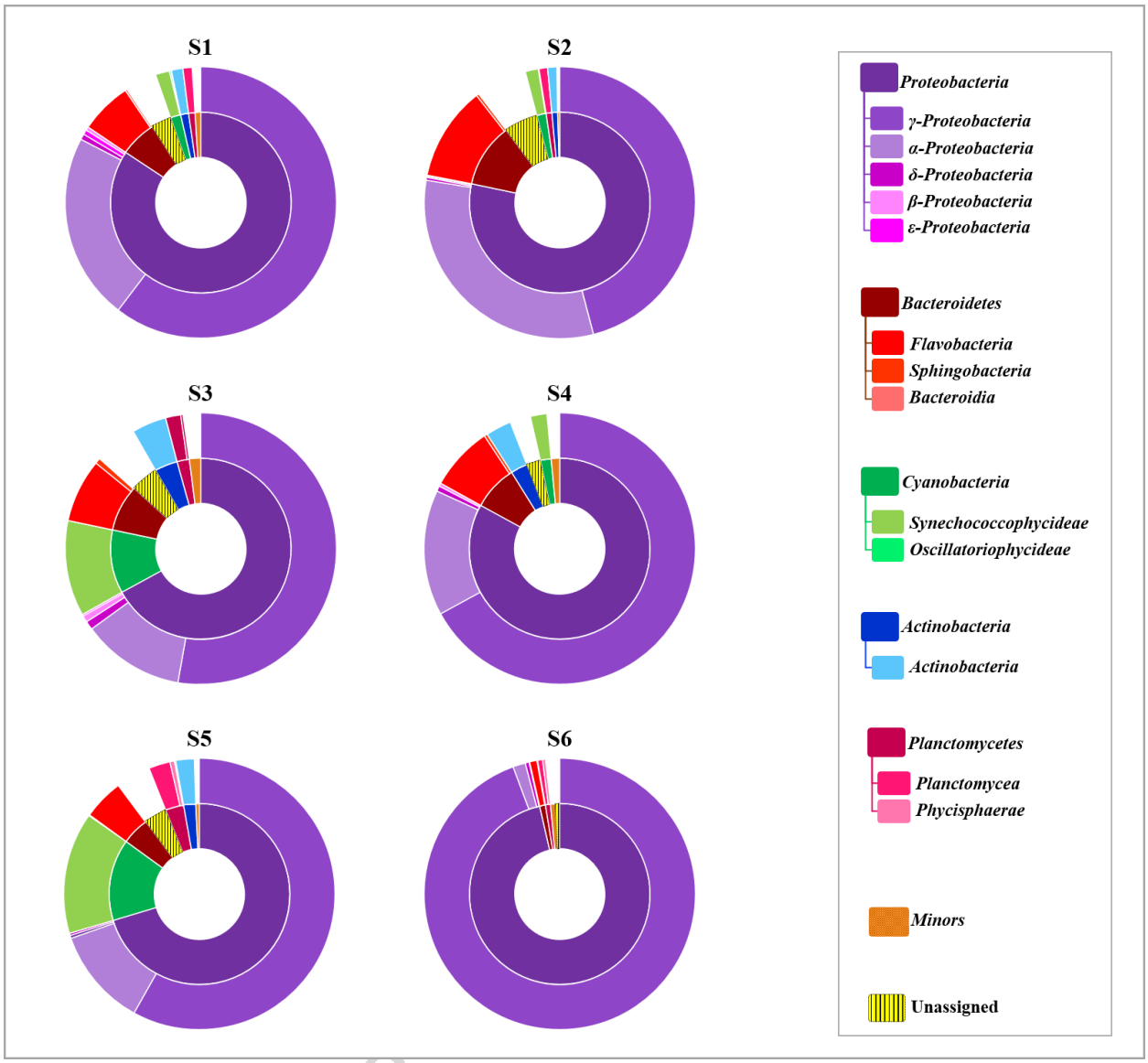

Fig. 2. Distribution of Phyla and relative Classes among the various KB samples. Phyla (inner rings) with Ra < 1% were gathered in "Minors".

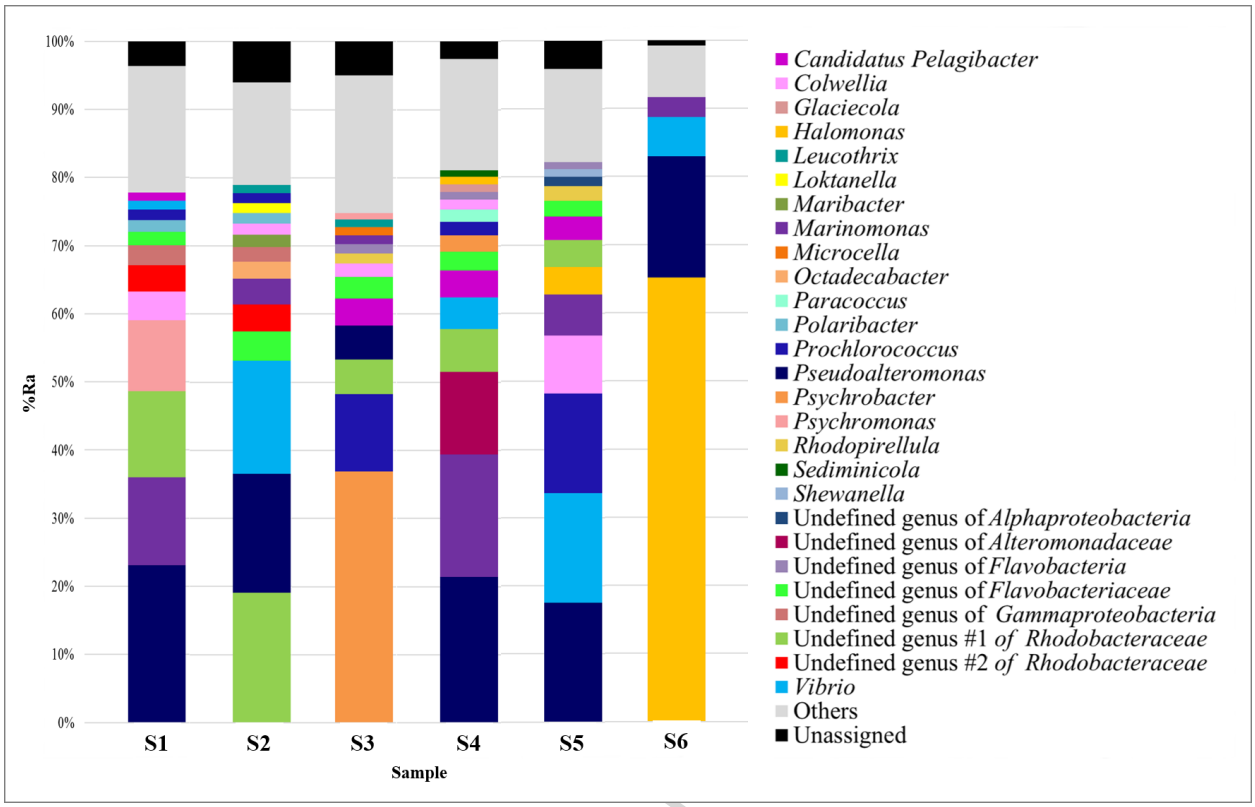

Fig. 3. Genus-level bacterial composition of the KB samples. Average distribution of the most abundant genera. Genera with Ra < 1% were gathered in "Others".

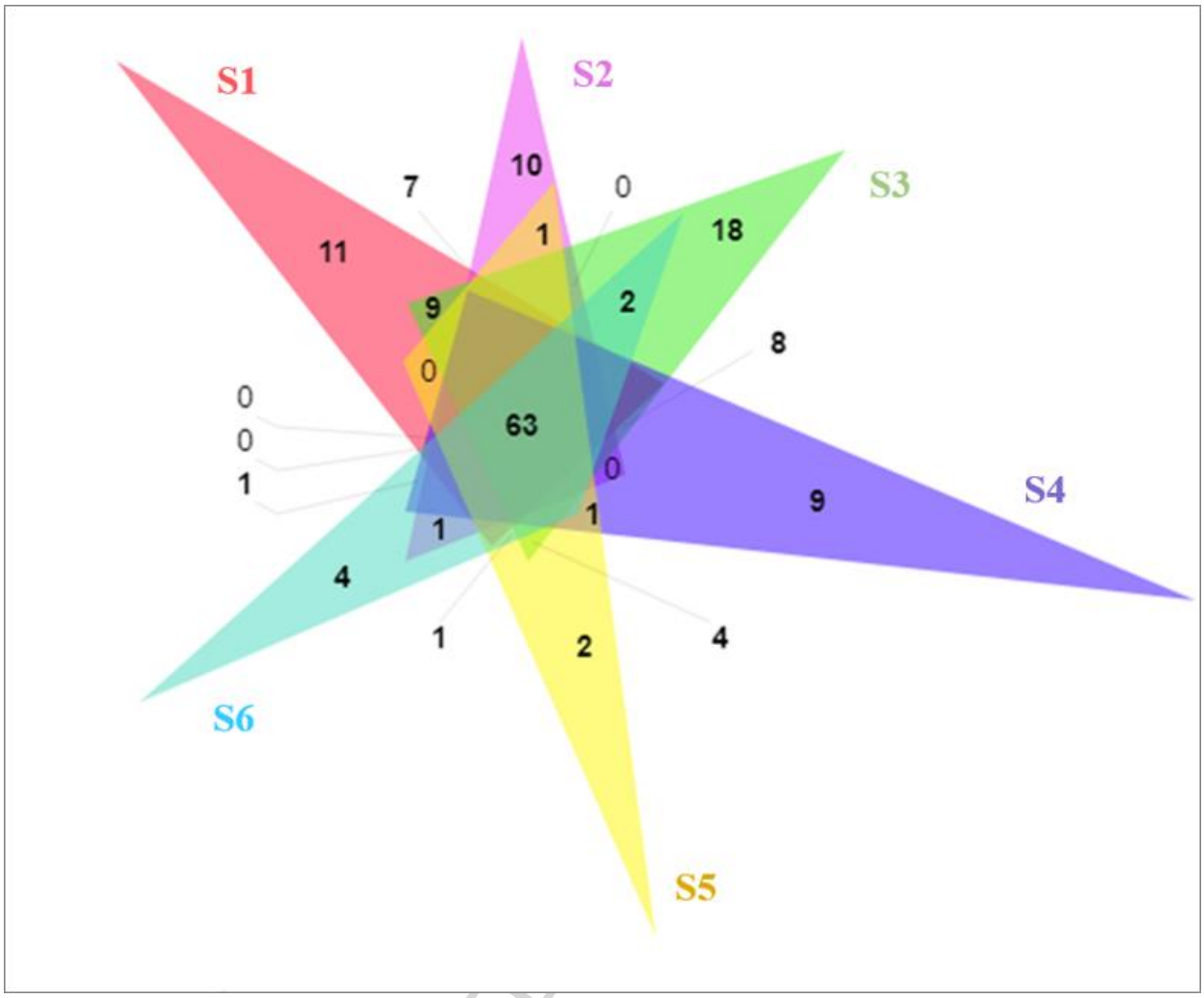

Fig. 4. Six-way Venn diagram depicting the number of exclusive and shared phylotypes among the KB samples.

3.2 Similarity among KB communities (nMDS) and environmental drivers of their structure (RDA)

The nMDS ordering, with superimposed similarity contours reflecting the cluster analysis, was used to display patterns of similarity among communities (Fig.5). The group comprising samples S1 and S2, showed the most similar communities (71% similarity); S3, S4 and S5, grouped together (65% similarity), while S6 revealed the most different community (47% similarity with other samples).

The environmental parameters included in the RDA analysis were water temperature and salinity; depth was excluded due to its high collinearity (-0.996) with water temperature. The RDA (Fig.6) explained 58.5% of the total variability (F=2.1; P=0.01) found in the relationship between environmental parameters (factors) and samples (variables). The two ordination axes accounted for 31.7% and 26.8% of the explained variability, respectively. The first axis was mainly correlated to water temperature, which describes 31.7% of total variance. As for the association between factors and variables, it is noteworthy that S6 showed the strongest relations with low water temperature and S3 with low salinity.

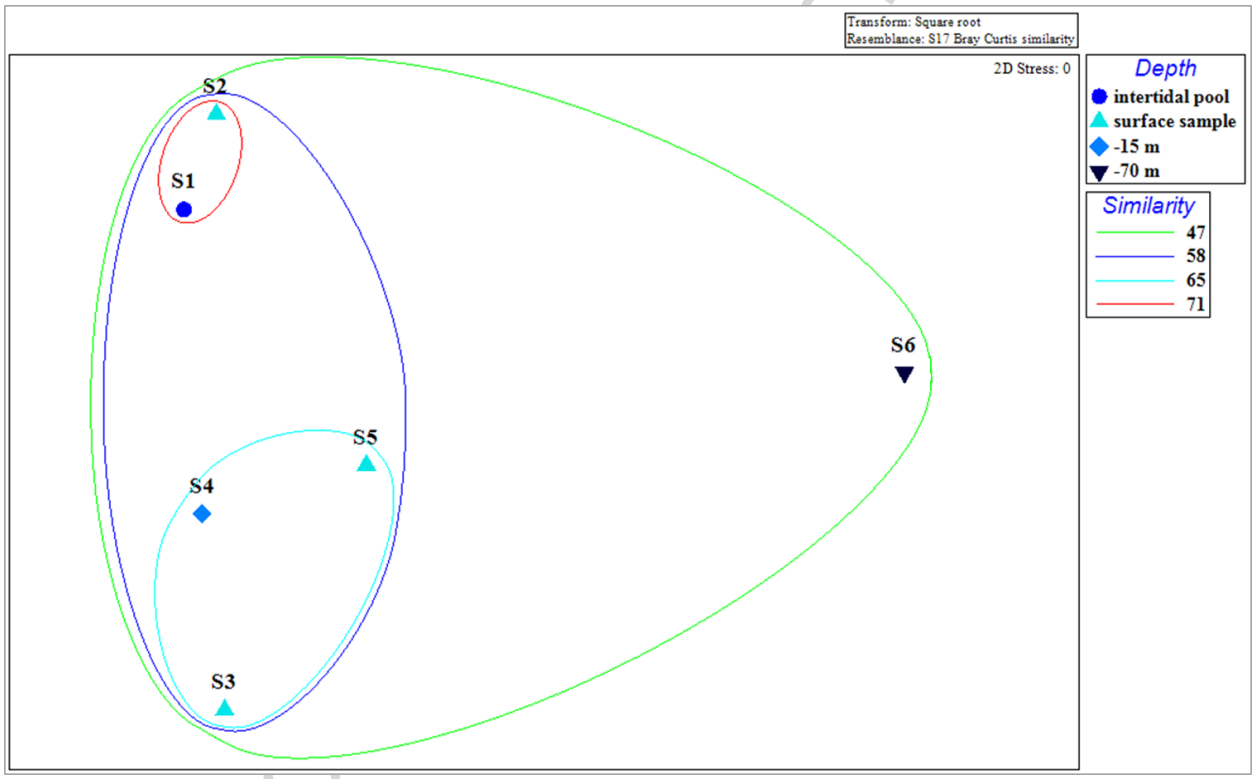

Fig. 5. Bray-Curtis based non-metric multidimensional scaling (nMDS) ordination of KB samples. The similarity contours superimposed on the nMDS plot derived from the Cluster Analysis calculated on Bray-Curtis distance matrix.

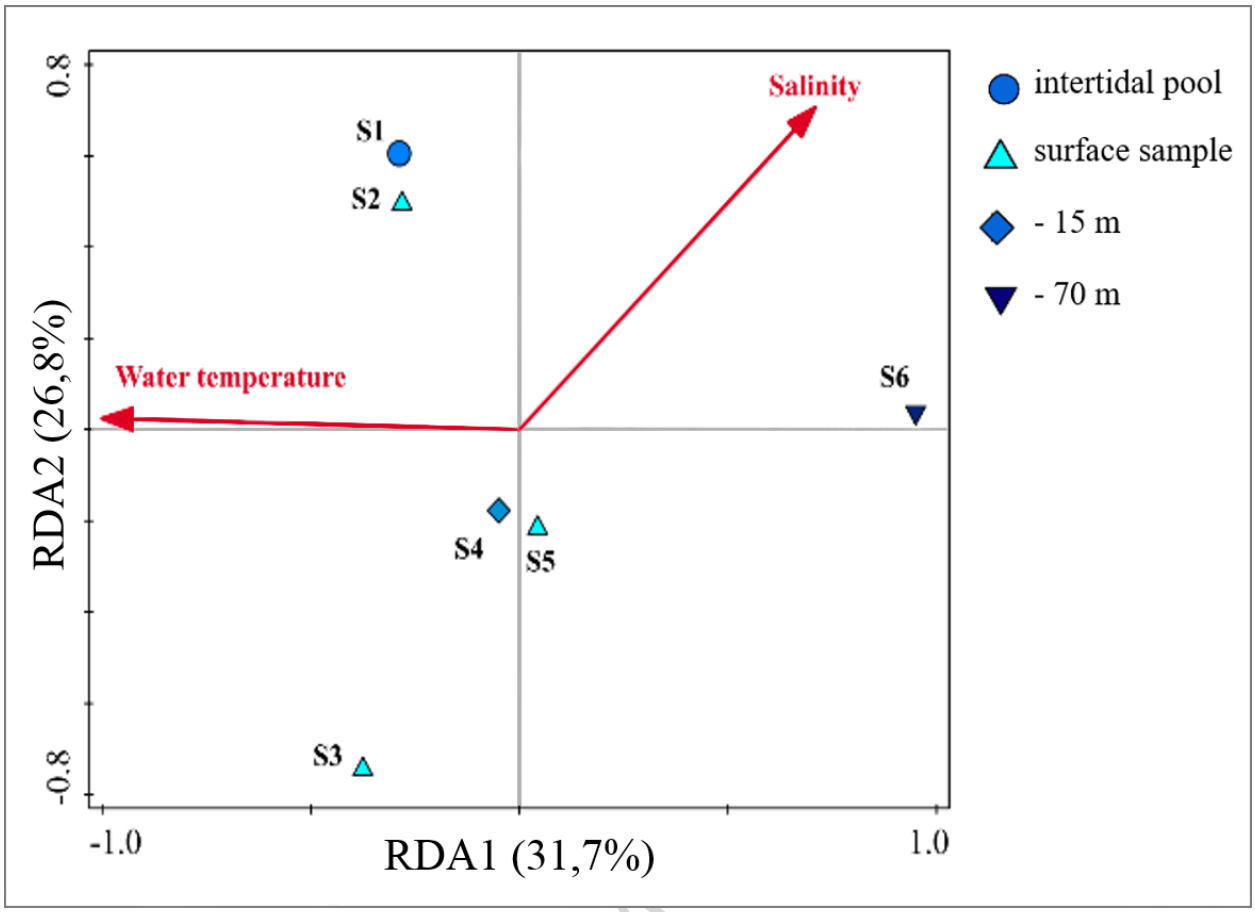

Fig. 6. Redundancy analysis (RDA) ordination diagram (biplot) showing samples and environmental parameters. Arrows indicate significant ($p < 0.05$) environmental parameters.

4. Discussion

The present study focused on producing a first high-resolution characterisation of the bacterial communities of some KB areas selected according to known flows of tidal currents. Although sampling did not involve the entire KB gulf and an extensive campaign, this work provides the first in-depth description of the bacterial communities in this region. Actually, the whole area has been overlooked from the microbiological point of view, and only a limited number of studies regarding the bacterial survey in KB gulf are available. Among them, some works dealt with phototrophs, others with invertebrate-associated bacteria (Gorlenko et al., 1985; Dul'tseva et al., 1996; Rabold et al., 2006; Gorelova et al., 2009) and the majority merely investigated the total

number of bacterial cells in a given area (Savvichev et al., 2003, 2004, 2008; Kravchishina et al., 2008). However, no study on whole bacterial communities is available.

In 2008 Pesciaroli and co-workers started a deeper investigation of the bacterial microbiota of KB seawater, carried-out both using culture-dependent and culture-independent methods. A number of isolates were studied for their ability to grow at different temperatures and investigated under the taxonomic/phylogenetic and nutritional point of view (Pesciaroli et al., 2012, 2015a; Leyva-Díaz et al., 2017; Timperio et al., 2017). In addition, the total KB bacterial community was preliminary analysed by the PCR-TGGE fingerprinting method (Pesciaroli et al., 2015b), supplying only a partial information regarding the bacterial diversity. The NGS approach of the present study allowed a deep survey and detailed profiling of the bacterial communities in this area, connoted by very interesting environmental features.

Since this work represents the first NGS investigation on the KB bacterial communities, the comparison of our results with those of others is rather difficult. In addition, no analogous data are available for the White Sea and the Barents Sea, which are closely in contact with our area of study. Comparable information is available only for other arctic or sub-arctic marine environments such as the Baltic Sea, Beaufort Sea, Chukchi Sea, and others. However, these Arctic areas are located quite far from KB and often show different environmental features (i.e. salinity, hydrodynamic regime and connection with the Arctic Ocean). In all these cold waters, evident preponderance of *Proteobacteria* (followed by *Bacteroidetes*) was detected (Kirchman et al., 2010; Koskinen et al., 2010; Comeau et al., 2011; Herlemann et al., 2011; Bowman et al., 2012; Ortega-Retuerta et al., 2013; Connelly et al., 2014; Dupont et al., 2014; Han et al., 2015; Pedrós-Alió et al., 2015; Rieck et al., 2015; Hu et al., 2016; Li et al., 2016; Wilson et al., 2017; Yergeau et al., 2017).

According to most of the cited works, both α- and *γ-Proteobacteria* are predominant, with particular relevance of the first class. Even if at the phylum level the composition of KB communities was coherent with the outcomes of the majority of the cited papers, at the class level

an opposite ratio Alpha/Gamma *Proteobacteria* was recorded since *γ-Proteobacteria* accounted for the main fraction of KB *Proteobacteria*. However, similar results were obtained in other Arctic zones, located much more north than KB, by Kirchman et al. (2010) and Wilson et al. (2017) for the bacterial communities of coastal and epipelagic Arctic zones. It is difficult to identify a general trend regarding the distribution of the various *Proteobacteria* classes within the Arctic seas**,** since they comprise heterogeneous environments. Nevertheless, dominance of *γ-Proteobacteria* in surface coastal waters and preponderance of *α-Proteobacteria* in offshore areas have been often demonstrated (Kirchman et al., 2010; Ortega-Retuerta et al., 2013; Connelly et al., 2014; Han et al., 2015; Li et al., 2016; Wilson et al., 2017).

In KB communities, among *Bacteroidetes*, the class *Flavobacteria* clearly represented the greatest proportion. In polar waters, *Bacteroidetes* (in particular Flavobacteria) were often found in correlation with the phytoplankton levels. *Flavobacteria* are abundant when light availability permits high phytoplankton growth (i.e. in epipelagic waters in summer) (Wilson et al., 2017). Our results showed a greater presence of this class in surface samples compared to the deepest sample, but it is worth noting that similar amounts of *Flavobacteria* were found in samples S3 (surface water) and S4 (-15 m, where light penetration is very low or null). This was probably due to the mixing of waters from different layers caused by the aforementioned circular pattern of tidal currents. *Cyanobacteria* were rather abundant in all samples except the deepest one (S6). Abundance of this phylum was particularly high in the surface samples (S3 and S5) collected in the main current stream. In S4 (-15 m), where light is almost absent (Bobrov et al., 1995, Kravchishina et al., 2013), the presence of this group with %Ra of ca. 2 could be due to a certain mixing with surface waters caused by the circular pattern of tidal currents.

The picocyanobacteria composition of KB community appeared quite unusual for an Arctic region. Incidence of these photosynthetic prokaryotes, affected mainly by light, nutrients and temperature, generally decreases with latitude increase (Boeuf et al., 2013; Celepli et al., 2017).

Prochlorococcus, less ubiquitous than *Synechococcus* for its greater sensitivity to low temperatures, was usually found to be abundant at relatively low latitudes (between 40° S and 45° N) and to sharply decline northward (Lee et al., 2014; Hess et al., 2016). Due to its temperature sensitivity, it is generally zoned in waters with temperatures above 15 °C; however, it has been found at 61°N in the north Atlantic (Buck et al., 1996) and it has also been recently detected in the Chukchi Sea (Lee et al., 2014) and the Gulf of Riga (Baltic Sea) (Tiirik et al., 2014). Nevertheless, it is often considered nearly absent in the polar oceans (Boeuf et al., 2013; Flombaum et al., 2013; Cadier et al., 2017). *Synechococcus* appears to be more widespread, showing a wider geographical distribution, ranging from tropical to polar waters (Hess et al., 2016; Cadier et al., 2017) including those from sub-Arctic and Artic regions (Michelou et al., 2007; Waleron et al., 2007; Zwirglmaier et al., 2008; Cottrell and Kirchman, 2009; Tremblay et al., 2009; Huang et al., 2012).

Although evidently in relation with depth and consequent light availability, presence of *Prochlorococcus* was observed in all KB samples; whereas *Synechococcus* was not detected at all. This feature of KB waters, that has to be confirmed using other molecular targets (i.e. ITS) for better taxonomic resolution of the two genera, seems to be quite unusual for an estuarine Arctic system, since *Synechococcus* was often found in arctic rivers and lakes and freshwater runoff is thought to represent a source of these bacteria to the Arctic Ocean (Fuhrman et al., 2015).

The results of the present study did not reveal whether the distribution of *Cyanobacteria* in the KB waters can be related to the specific environment peculiarities or, as suggested for other Arctic environments (Lee et al., 2014), to possible global change effects that favoured the establishment of *Prochlorococcus* populations further north, increasing its biogeographic distribution. In any case, this study supplies additional information about the presence of these taxa in cold waters and provides the bases for further investigations.

The taxonomic analysis at the genus level revealed that all samples appeared strongly dominated by few genera, accounting for ca. 50% of the community or more. Samples S3 and S6,

were strongly dominated by a single genus constituting a great proportion of the community (37% for *Psychrobacter* in S3) or even its main fraction (65% for *Halomonas* in S6). These outcomes are consistent with the α-diversity data; in fact, Shannon indices were low for all samples (and lowest for S6), indicating that KB bacterial communities were characterised by scarce diversity and equitability.

Another interesting issue evidenced by the present study is that tidal currents can somehow influence both distribution and composition of the KB bacterial communities. Overall, the results obtained from the taxonomical and α-diversity analyses together with those of the nMDS and RDA analyses seem to be explained by the strong water mixing caused by tidal currents, which affect in particular the surface and sub-surface layers (Pantyulin, 2003).

The nMDS and clustering analyses (Fig. 5) indicated that all surface samples, even those relatively far apart, showed rather similar communities. Such similarities could be explained by the strong mixing caused by the main flow of tide currents, while the differences recorded might depend on the position of the various sampling points in relation to the main current stream. S1 and S2 showed the highest similarity; these samples were the closest to the shore and, being located slightly apart from the main current and somehow shielded by a small promontory, were probably less affected by the tidal mixing. In addition, being in close relation with the intertidal zone**,** they might be more affected by inputs of microorganisms from this area.

Also S3, S4 and S5 were rather similar; the first two samples were collected from the same place but at different depths (surface and 15 m, respectively) and their similarity was probably due to the mixing caused by the circular pattern of the current recorded in that area (Fig.1). The similarity with S5, taken quite far away from S3 and S4 (ca. 2.5 km), could be attributed to the collection of the three samples within the main current stream (Fig. 5). In addition, the diversity of S3 appeared to be influenced by the low salinity as shown by the RDA analysis (Fig. 6). Actually, the lowest salinity value (23.5 ‰) was recorded in S3. However, the differences in salinity among the various samples are too small (min 23.5 ‰ in S3, max 25.4 ‰ in S6) to justify possible

differences in the communities. In general, deviations from the optimal conditions of few g per litres of salinity do not cause any significant physiological stress. Thus, no substantial reduction of the microbial growth, and consequent replacement of some microorganisms with more adapted ones, would occur. It is known that environmental bacteria can cope with much wider variations of salinity, as also demonstrated for those isolated in the Velikaja Salma strait (Barghini et al., 2018). The variations of salinity recorded in the surface layers of the sampling area could be mainly ascribed to freshwater inputs, which are rather well mixed with seawater by the tidal currents. The circular pattern of the current in S3, attenuating the mixing action of the main tidal flow in this area, probably allows an increased persistence of freshwater and its microbial communities. Hence, all this could explain from an ecological point of view the RDA results obtained for S3.

S6 (-70 m) definitely showed the most dissimilar community (nMDS analysis), which was characterised by the lowest equitability (Shannon index), and strongly influenced by water temperature (RDA analysis), and consequently by depth (being water temperature highly correlated with depth). The deepest cold layers represent a stable environment with strong dominance of adapted microorganisms, with scarce contacts with the surface.

It is worth noting that the temperature differences recorded between the surface and deepest layers are sufficient to select microorganisms having different adaptation abilities with regards to temperature. Sampling at KB was carried out at the end of the Arctic summer. In this period water temperature started to drop from the average summer values (ca. 15 °C) that could easily support mesophilic-psychrotolerant and/or psychrotolerant bacteria (Pesciaroli et al., 2012). On the bottom layers, temperature is constantly around 0°C (Pantyulin, 2003), representing the optimal conditions for psychrophilic bacteria, requiring stable low temperatures with very limited variations (Helmke and Weyland 2004; Pesciaroli et al., 2012).

The effects of depth are related to various factors. First of all, increasing depth, variation of water temperature and salinity occurs. Moreover, with increasing depth increasing hydrostatic pressure and light diminution are recorded. However, the limited differences of depth recorded

among our samples (just 70 m, 7 bar) would be insufficient to select communities with diverse adaptation to pressure (for example from piezosensitive to piezotolerant bacteria). It is a matter of general knowledge that the shift in communities with regards to pressure needs hundreds of bars. By contrast, intense light variations, that in this region occur within a few meters from the surface (Bobrov *et al*., 1995; Kravchishina *et al*., 2013) heavily affect the community composition due to the different presence of primary producers and consequent availability of nutrients.

5. Conclusions

Kandalaksha Bay is an extremely dynamic marine transition area, where bacterial communities must be well adapted to repeated variations of environmental conditions and factors such as nutrient availability, temperature and salinity change frequently. Although it represents an interesting study area, it has been overlooked from the microbiological point of view. This work was the first attempt to outline the KB bacterial communities with a high-throughput method (454 pyrosequencing) and resulted in a snapshot of their structure with a deep resolution. Our results showed how KB community assemblages was clearly influenced by environmental parameters (salinity and temperature) which are affected by tidal currents that strongly contributed to the peculiar patterns of water mixing.

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Highlights

- First NGS survey of bacterial communities of Kandalaksha Bay Arctic estuarine system
- Proteobacteria dominated in all samples with γ-Proteobacteria as its main fraction
- Diffused occurrence of *Prochlorococcus* (low or absent in polar waters) was recorded
- Community driving factors were water temperature and salinity (RDA analysis)
- Water mixing by currents influenced distribution and composition of the communities