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Spatial variability of particle-attached and free-living bacterial diversity in surface waters from the Mackenzie River to the Beaufort Sea (Canadian Arctic)

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Abstract

We explored the patterns of total and active bacterial community structure in a gradient covering surface waters from the Mackenzie River to the coastal Beaufort Sea, Canadian Arctic Ocean, with a particular focus on free-living vs. particle-attached communities. Capillary electrophoresis-single strand conformation polymorphism (CE-SSCP) showed significant differences when comparing river, coast and open sea bacterial community structures. In contrast to the river and coastal waters, total (16S rDNA-based) and active (16S rRNA-based) communities in the open sea samples were not significantly different, suggesting that most present bacterial groups were equally active in this area. Additionally, we observed significant differences between particle-attached (PA) and free-living (FL) bacterial communities in the open sea, but similar structure in the two fractions for coastal and river samples. Direct multivariate statistical analyses showed that total community structure was mainly driven by salinity (proxy of DOC and CDOM), suspended particles, amino acids and chlorophyll *a*. 16S rRNA genes pyrosequencing of selected samples confirmed these significant differences from river to sea and also between PA and FL fractions only in open sea samples, and PA samples generally showed higher diversity (Shannon, Simpson and Chao indices) than FL samples. At the class level, *Opitutae* was most abundant in the PA fraction of the sea sample, followed by *Flavobacteria* and *Gammaproteobacteria*, while the FL sea sample was dominated by *Alphaproteobacteria*. Finally, the coast and river samples, both PA and FL fractions, were dominated by *Betaproteobacteria*, *Alphaproteobacteria* and *Actinobacteria*. These results highlight the coexistence of particle specialists and generalists and the role of particle quality in structuring bacterial communities in the area. These results may also serve as a basis to predict further changes in bacterial communities should climate change lead to further increases in river discharge and related particles load.

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1 Introduction

The Arctic Ocean is subject to profound changes due to global warming (ACIA, 2005). On one hand, increases in temperature, a reduction in the extension of the ice pack and prolonging of ice-free periods (Perovich, 2011) and an increase in ultraviolet radiation will change the abundance, activity and distribution of primary producers, as well as the duration and timing of phytoplankton blooms (Arrigo and van Dijken, 2004; Lavoie et al., 2010). On the other hand, river runoff has increased by 7 % in the last sixty years due to higher precipitation and glacier melting (Peterson et al., 2002). This last fact will increase the importance of riverine nutrient inputs, to sustain primary and secondary production, in an area that already receives 10 % of the global river discharge (Dittmar and Kattner, 2003). This is especially important in the Eastern Beaufort shelf (coastal Arctic Ocean). This area receives approximately 128 tons of sediment particles per year from the Mackenzie River, the main source of particles and of 95 % of the shelf sediment supply (Macdonald et al., 1998). Additionally, Arctic warming contributes to the increase of soil organic carbon in the Arctic Rivers due to permafrost melting (Guo and Macdonald, 2006). Autochthonous particulate organic matter (POM) inputs are also high in the area due to the occurrence of algal blooms associated with the retreat of sea ice (Forest et al., 2008). These contrasting sources lead to a composite of inorganic and organic suspended materials of diverse origin and physico-chemical properties, which may be subject to substantial alteration in size and chemical composition.

Marine particles have been identified as “hotspots” of high microbial abundance and activity (Smith et al., 1992; Azam et al., 1994). Particle-attached (PA) bacteria can show higher specific metabolic activity (Mével et al., 2008) and higher enzymatic activity rates (Murrell et al., 1999) than their free-living (FL) counterparts. In addition, elevated enzymatic activity on particles may release nutrients to the surrounding water, creating hot spots of high microbial activity around them (Azam and Malfatti, 2007). The contribution of the PA fraction to the total bacterial activity is highly variable, but can be as high as 90 % of the total bacterial production in estuarine habitats (Crump et al., 1998). This

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contribution depends mainly on the concentration of attached bacteria and on the size, quantity and quality of the suspended particles. For instance, a previous experimental study showed higher bacterial growth associated with organic than to mineral particles (Kernegger et al., 2009). In the Beaufort Sea, previous studies have highlighted the importance of bacteria degrading POM in aggregates (Kellogg et al., 2011; Forest et al., 2012) and reported high variability (from 0 up to 98 %) of the contribution of PA bacteria to total bacterial production (Garneau et al., 2009).

Whether particles can host a distinct microbial community (Acinas et al., 1997; Rath et al., 1998) is still unclear, as some studies have reported a high degree of specialization of PA bacteria (Acinas et al., 1999; Crump et al., 1999; Ghiglione et al., 2009), while others have proposed that the majority of bacterial groups are ubiquitous and exchange from FL to PA lifestyles (Hollibaugh et al., 2000; Ghiglione et al., 2007). Previous studies in the Arctic and subarctic have found different bacterial communities in PA fractions from those FL (Hodges et al., 2005; Garneau et al., 2009; Lapoussiere et al., 2011), although clear niche segregation between the two fractions has not been always observed (Hollibaugh et al., 2000). Owing to the different metabolic capacities (e.g. different enzyme sets) associated to different bacterial populations, exploring patterns of bacterial community structure in different particle fractions can give us hints about major metabolic pathways, and hence help understand nutrient fluxes and elemental cycles.

If a complex spatiotemporal variability of substrate facilitates the diversification of bacteria by different physiological adaptations to the environment, bacterial diversity is expected to be high in coastal environments influenced by river outflows, where abrupt changes in salinity, temperature and substrate quality, including particles, may lead to diverse habitats and niche differentiation (Crump et al., 1999). A change in river discharge and sediment load will also likely impact the dynamics of PA and FL bacterial communities in the area.

In the present study we describe summer bacterial diversity and community structure in surface waters from the Mackenzie River to the Beaufort Sea, Canadian Arctic.

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At this period surface waters had the maximum of bacterial activity (Ortega-Retuerta et al., 2012b). We evaluated spatial differences in community structure from the river to the open sea, separating into free-living vs. particle-attached bacterial communities. For our purposes, we combined the use of molecular fingerprinting and 454 tag-pyrosequencing based on 16S rRNA genes. As metabolically active bacteria contain more rRNA than resting or starved cells (Kemp et al., 1993), we compared 16S rDNA vs. 16S rRNA community structure in order to address which members of the bacterial community are metabolically active.

2 Material and methods

2.1 Sampling

The MALINA campaign was carried out in the Southern Beaufort Sea, Arctic Ocean, in late summer, from 30 July to 27 August 2009 aboard research icebreaker CCGS *Amundsen*. The study area presented high ice concentration when compared with previous years at the same period (Forest et al., 2012). Mackenzie River discharge presented its maxima at the end of spring (June–July), decreasing thereafter (Doxaran et al., 2012).

For this study, surface waters from 15 stations were selected covering two transects from the Mackenzie River mouth to the open sea (bottom depth 1538 m) (Fig. 1). Correspondence between station codes and numbers in the general MALINA sampling grid are detailed in supplemental Table 1. In the Mackenzie River mouth, water was collected manually from a zodiac (top-surface, 0 to 0.5 m deep) using clean 5 l carboys. For other stations, water was collected from the *Amundsen* using a SBE Rosette equipped with PVC bottles (12 and 24 l each, Ocean Test) equipped with a SBE911-plus CTD profiler.

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2.2 Chemical and biological analyses

Methods for chemical and biological analyses used to test environmental drivers of bacterial community structure are detailed elsewhere in this issue; i.e. (Doxaran et al., 2012; Le Fouest et al., 2012; Matsuoka et al., 2012; Shen et al., 2012). Bacterial production was measured by ^3H -leucine incorporation (Smith and Azam, 1992). Samples (1.5 ml in triplicate plus one killed control) were added to sterile microcentrifuge tubes, containing 10 to 20 nM [4,5- ^3H]-leucine (specific activity 139 Ci mmole^{-1}) (Ortega-Retuerta et al., 2012b). After filtering water aliquots by gravity through $3\text{ }\mu\text{m}$ polycarbonate filters, free-living bacterial production was measured in the filtrate. This was subtracted from BP determined in whole seawater to calculate the percentage of particle-attached BP (%).

2.3 Bacterial community structure by capillary electrophoresis single-strand conformation polymorphism (CE-SSCP) fingerprinting

Samples for bacterial community structure were sequentially filtered through $3\text{ }\mu\text{m}$ polycarbonate filters (diameter, 47 mm) (particle-attached fraction) and $0.2\text{ }\mu\text{m}$ Sterivex filters (free-living fraction) using a peristaltic pump under low pressure. The fraction larger than $3\text{ }\mu\text{m}$ was confirmed to contain only bacteria attached to particles by visual examination in DAPI stained samples using epifluorescence microscopy (data not shown). One to two liters of water were filtered depending on suspended solids concentration to avoid filter clogging. Samples were subsequently stored at -80°C until processing at the home laboratory. DNA and RNA co-extraction, PCR and reverse transcriptase (RT)-PCR were performed as previously described (Ghiglione et al., 2009). Both DNA and cDNA were used as a template for PCR amplification of the variable V3 region of the 16S rRNA gene with primers w49F fluorescently 5'-labeled with phosphoramidite (TET, Eurogentec) and w34R according to (Ghiglione et al., 2005). CE-SSCP and analysis of the electrophoregrams were performed using the 310 Genetic Analyzer and Genescan

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analysis software (Applied Biosystems), as described in a previous paper (Ghiglione et al., 2008).

2.4 Bacterial diversity by pyrosequencing

Bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) was performed as described previously using Gray28F (5' TTTGATCNTGGCTCAG) and Gray519r (5' GTNTTACNGCGGCKGCTG) (Dowd et al., 2008) amplifying 400 bp of the V6 region of the 16S rRNA gene. Initial generation of the sequencing library was accomplished by a one-step PCR with a total of 30 cycles using the HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA) and amplicons originating and extending from the 28F primer for bacterial diversity. Tag-encoded FLX amplicon pyrosequencing analyses utilized the Roche 454 FLX instrument with Titanium reagents and procedures performed at the Research and Testing Laboratory (Lubbock, TX) based upon RTL protocols.

Sequences were processed and analyzed using the Qiime software (Caporaso et al., 2010). Briefly, samples were denoised using the AmpliconNoise program and checked for chimeras using Perseus (Quince et al., 2011). The resulting clean sequences were clustered using Operational Taxonomic Units (OTUs) at a 97 % sequence identity level using the Uclust algorithm (Edgar, 2010). A representative sequence from each OTU was classified using the RDP classifier (Wang et al., 2007) using the Greengenes training set. Taxonomic identification of the sequence reads (tags) followed the approach by Sogin et al. (2006) and Huse et al. (2010). To normalize the number of tags sequenced between samples, tags were randomly re-sampled to the sample with the fewest tags (2826 tags) using DaisyChopper v. 0.6 (Gilbert et al., 2009). This step was performed on operational taxonomic unit (OTU) files clustered at a distance of 0.03 (Ghiglione and Murray, 2012).

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2.5 Diversity estimators and cluster analysis

All OTU and diversity analyses were performed on the randomly re-sampled data sets. We used SPADE (Species Prediction and Diversity Estimation; <http://chao.stat.nthu.edu.tw/>) to calculate non-parametric species richness estimators ACE and Chao1. PAST was used (PAleontological STatistics v 1.19; <http://folk.uio.no/ohammer/past/>) to generate rarefaction curves. Both Simpson and Shannon diversity indexes were calculated using PRIMER 6 software (PRIMER-E, UK). Ordination of Bray-Curtis similarities among normalized sample CE-SSCP profiles or OTU table file were used to build dendrograms by unweighted-pair group method with arithmetic averages (UPGMA). A similarity profile test (SIMPROF, PRIMER 6) was performed on a null hypothesis that a specific sub-cluster can be recreated by permuting the entry species and samples. The significant branch (SIMPROF, $p < 0.05$) was used as a prerequisite for defining bacterial clusters. One-way analysis of similarity (ANOSIM, PRIMER 6) was performed on the same distance matrix to test the null hypothesis that there was no difference between bacterial communities of different clusters.

2.6 Direct multivariate analysis

To investigate the relationships between bacterial community structure and environmental parameters, we used a direct-gradient approach, i.e. a canonical correspondence analysis (CCA) using the software package CANOCO, version 4.5 for Windows, as previously described (Berdjeb et al., 2011). Spearman rank pairwise correlations between the environmental variables (Table 1) helped to determine their significance. To statistically evaluate the significance of the canonical axes we used a Monte Carlo permutation full model test with 199 unrestricted permutations. Significant variables were chosen using a forward-selection procedure and 999 permutations, a nd explanatory variables were added until further addition of variables failed to contribute significantly ($p < 0.05$) to a substantial improvement to the model's explanatory power.

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3 Results

3.1 Study area characterization

According to physical and chemical water properties (Table 1), we defined three zones relative to the proximity of the river in both Eastern and Western Mackenzie River transects. Stations with salinity 0 were named “river” (R). These stations were characterized, aside from freshwater, by higher temperature (average 10.7 °C) and suspended particulate matter (SPM; average 111.8 mg l⁻¹). Coastal stations and open sea stations were separated according to physical and chemical features: stations considered “coastal” (C) were characterized by Mackenzie River water influence, showing salinity below 24, surface temperature higher than 4 °C and SPM concentrations higher than 1 mg l⁻¹. “Open sea” (S) stations had salinity higher than 26 ‰ except for station WS1, which was influenced by ice melt waters, temperatures lower than 4.5 °C and SPM below 1 mg l⁻¹. The western river transect showed a higher influence by river waters than the eastern transect (i.e. lower salinity and higher SPM concentration, data not shown).

Bacterial production was significantly higher in the Mackenzie River mouth and decreased offshore (Table 1). The proportion of bacterial production due to particle-attached (PA) bacteria was not different in marine and coastal stations (average 41 and 38 % respectively, Table 1). In contrast, most of the bacterial production was performed by PA bacteria in riverine stations (average 99 % of the total bacterial production).

3.2 Changes in bacterial community structure measured by CE-SSCP

Overall, we observed significant spatial differences in bacterial community structure in both western and eastern transects from the Mackenzie River to the Beaufort Sea (Table 2, Fig. 2). The highest differences were found between river and open sea samples (ANOSIM $R^2 = 0.63$, $p < 0.001$), whereas no clear difference was observed between samples from the eastern (Kugmallit Bay) and western (Shallow Bay) transects (Table 2). Coastal communities were more similar to open sea than river samples

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(ANOSIM $R^2 = 0.33$, $p < 0.001$ and $R^2 = 0.36$, $p < 0.001$, respectively). Hierarchical clustering based on Bray Curtis similarity between CE-SSCP profiles showed a clear organization according to the distance to the coast in each river, coastal and open sea clusters (Fig. 2). Consequently, samples from river, coast and open sea were treated separately for further detailed comparisons.

When comparing DNA- and RNA-based fingerprints, significant differences were obtained for riverine or coastal samples, but not for open sea samples (Table 2). The highest difference was found between DNA- and RNA-based profiles from river samples (ANOSIM $R^2 = 0.7$, $p < 0.05$), as depicted in Fig. 1 in the Supplement.

In open sea samples, we observed significant differences between PA and FL communities (Table 2) and, indeed, PA sea samples formed a single cluster with Bray Curtis similarities to the rest of samples lower than 60% in DNA-based profiles and lower than 50% in RNA-based profiles (Fig. 2). The differences were also significant for coastal samples ($p < 0.05$), although the percentage of explained variance was low ($R^2 = 0.12$). No significant differences were found between PA and FL communities in the river samples.

3.3 Environmental drivers of bacterial community structure

A strong Spearman's rank pairwise correlation was found between CDOM, DOC, Si and salinity (data not shown). Therefore, salinity was used as a proxy of these parameters for further direct multivariate statistical analysis. We performed canonical correspondence analysis (CCA) in order to explore which environmental factors drive the spatial distribution of PA and FL bacterial communities. As no significant differences were observed between DNA and RNA-based fingerprints (Table 2), we present only DNA-based profiles for CCA analyses. Similar results were found for the RNA fraction.

In both PA and FL DNA samples, the cumulative percentage of variance of the species-environment relationship indicates that the first and second canonical axis explained 47% and 22.5% of this variance, respectively, for PA and 44% and 30% of

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this variance respectively, for FL (Table 3). Consequent axes accounted for less than 17% of the variance each, and are not considered further here. In both PA and FL DNA-based CCA, the first canonical axis was highly positively correlated with salinity (and phosphate in the case of PA fraction, but not in the FL fraction) and negatively correlated with the concentration of chlorophyll *a*, amino acids and SPM (Fig. 3). The concomitant effect of these parameters explained 59% and 48% (ratio between the sum of all canonical eigenvalues and the sum of all eigenvalues) of the changes in bacterial community structure found in the PA and FL fractions, respectively (Table 3).

3.4 Bacterial diversity by tag-pyrosequencing

According to CE-SSCP results (Fig. 2), we selected three samples in the western transect representative of the river (R₂W), coast (C₄W) and open sea (S₁W) for pyrosequencing-based analysis of the PA and FL fractions. After removing low-quality tags, a total of 60 544 reads were obtained for the six samples analyzed, with a minimum of 2826 tags per sample. All the further analyses were based on the OTU table following re-sampling to the lowest abundant tag (2826) and clustering at a distance of 0.03. Out of the total 323 OTUs found in our samples, 195 OTUs were found in the river, 210 OTUs in the coast and 112 OTUs in the open sea (Fig. 5). All richness and diversity indexes showed higher values at the coastal and river stations than at the open sea station (Table 4, Fig. 2 in the Supplement).

We compared the presence vs. absence of OTUs merging both fractions in sea, coast and river samples (Fig. 5). 82 OTUs (24.3% of total OTUs) were exclusive to the river, 43 OTUs (12.8%) were exclusive to the coast and 33 OTUs were exclusive to the sea sample (9.8%). Only 24 OTUs (7.1%) were shared at all three zones, and there was a higher degree of OTUs sharing between coast and river (26.4%) than between sea and coast (17.2%) (Fig. 5).

In all the three samples, the diversity of PA was always higher than for FL bacteria (Table 4). Shared OTUs had a higher proportion of those found in both PA and FL fractions (60% for those shared between sea and coast, 78.6% for those shared

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between coast and river). Conversely, relatively more OTUs exclusive from either sea or river samples were also exclusive from one fraction, namely the PA fraction (43.3% of OTUs for the sea sample, 32.9% for the river sample, Fig. 5).

Hierarchical clustering of tag occurrence profiles showed similar clustering to CE-SSCP profiles, albeit with fewer samples (Fig. 4). Bacterial community structure was different among coast, river and sea samples. Looking at specific areas, PA bacteria were very different than FL bacteria in sea samples (Fig. 4), with only 15.7% of similarity whilst there were much less differences between PA and FL communities in the coast and river samples (78.8 and 67.5% similarity, respectively). Differences between PA and FL fractions in open sea were also visible upon closer inspection of their respective taxonomic compositions. At the class level, the PA open sea samples were dominated by the classes *Opitutae* (31% of all reads), *Flavobacteria* (29%) and *Gammaproteobacteria* (19%). The free-living fraction of the open sea waters was dominated by *Alphaproteobacteria* (54% of all reads), followed by *Actinobacteria* and *Flavobacteria* (12% and 12%, respectively). In the coast sample, in both PA and FL fractions, the most abundant class was *Alphaproteobacteria* (34%), followed by *Betaproteobacteria* (14% and 19% in PA and FL, respectively) and *Actinobacteria* (13% and 11% in PA and FL, respectively). Finally, the river sample was dominated by *Betaproteobacteria* (26% and 31% in PA and FL samples, respectively). *Actinobacteria* was more abundant in the PA fraction (24%) than in the FL one (11%), while *Alphaproteobacteria* relative abundance was similar in the two fractions (22% and 25%, Fig. 6). Some OTUs that were exclusive from the river included members of *Nostocales* (*Cyanobacteria*), *Caulobacteriales* (*Alphaproteobacteria*) or different *Betaproteobacteria* groups (e.g. *Burkholderiales*, *Methylophilales*), while some OTUs exclusive from the sea included OTUs belonging to *Flavobacteriales* (*Flavobacteria*) and *Oceanospirillales* (*Gammaproteobacteria*).

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4 Discussion

The MALINA Arctic campaign, that sampled the Mackenzie Delta-Beaufort shelf area in summer 2009, revealed an ecosystem characterized by its oligotrophy (i.e. low primary production (Ortega-Retuerta et al., 2012b), dissolved amino acids, and labile organic matter; Shen et al., 2012), partially sustained by Mackenzie River inputs that structured a complex gradient of suspended particles with mineral to organic content (Doxaran et al., 2012) as well as a shelf-basin gradient of sinking particles and sediment flux (Forest et al., 2012). Under these conditions, we found that almost half of the bacterial production in the open sea and 99% in the river was due to PA bacteria, which is consistent with previous measurements in the Mackenzie River and Beaufort Sea (Galand et al., 2008; Vallieres et al., 2008; Garneau et al., 2009). The investigation of bacterial community structure associated with those particles is of interest to gain knowledge about particle remineralization pathways and nutrient cycling in the system. This will serve as a basis to predict further changes in bacterial communities in the area, and hence on biogeochemical processes, if particle loads to the system increase via river discharge (Peterson et al., 2002) and primary production increases (Arrigo and van Dijken, 2011). To our knowledge, this is the first study using CE-SSCP and tag-sequencing to differentiate PA and FL bacteria in the Arctic Ocean. The combination of these techniques has proven robust for ecological studies on aquatic microbial diversity (Ghiglione and Murray, 2012).

4.1 Spatial variability of bacterial community structure

The principal factor determining bacterial community structure at the surface in our system was the sample location (river, coast, open sea). Previous studies have documented a transition from freshwater to marine communities (Riemann and Middelboe, 2002; Troussellier et al., 2002; Campbell and Kirchman, 2012). For instance, bacterial community composition in river and sea samples (if we merged PA and FL fractions together) was similar to the one reported by Galand et al. (2008), hence we report

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5 a transition from a community dominated by members frequently found to dominate in freshwaters, such as *Actinobacteria* (Holmfeldt et al., 2009) or *Betaproteobacteria* (Garneau et al., 2006), to a typical marine bacterial community dominated by *Alphaproteobacteria* (Fig. 6). The low degree of overlap between river and sea groups (Fig. 5) indicates the low dispersal potential, or low viability in marine waters of riverine bacteria. Even when the Mackenzie River plume extended more than 200 km offshore, studies performed during the same campaign reported that SPM was limited to close to the river mouth (Doxaran et al., 2012). CCA analyses confirmed a spatial structuring of bacterial community driven by salinity, chlorophyll *a*, amino acids and SPM. In this area, we showed previously that bacterial abundance and activity (production and respiration) was controlled by the availability of labile organic matter, with DOC, amino acids and primary production as proxies (Ortega-Retuerta et al., 2012b). This supports the significant role of these factors in structuring bacterial communities (salinity was included in the CCA analysis as a proxy of DOC and CDOM), given the importance of bacterial community structure on the bulk bacterioplankton activity (e.g. Obernosterer et al., 2010). As the station location was the main factor determining bacterial communities, both PA and FL seemed to be driven by similar environmental variables. The influence of river inputs and temperature on bacterial community structure was also shown in a previous study in the Arctic (Kellogg and Deming, 2009) and in a global comparison of bacterial diversity including Arctic samples (Ghiglione et al., 2012).

4.2 Role of particles on bacterial community structure

Contrary to expected, significant differences between PA and FL bacteria were only observed in open sea samples, neither in the river nor in the coastal samples, even when particle concentration was much higher in the river and coast (Table 1). This result contrasts to previous observations in the Mackenzie River–Beaufort Sea system (Garneau et al., 2009), but is similar to the work of Kellogg and Deming (2009), also performed in an Arctic shelf (Laptev Sea, influenced by the Lena River), where no differences were observed in PA (> 3µm) vs. FL (< 3µm) bacterial communities. We suggest that

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particle quality, rather than their quantity, would play a major role structuring bacterial communities. The relationship between particulate organic carbon (POC) to SPM can give us information about the nature of the particles, from mineral to organic (Wozniak et al., 2010). During the MALINA cruise, particles in the river and coast samples had POC : SPM of 2.2 % (i.e. mineral-rich particles) while particles in the open sea had POC : SPM ratios of 12.2 % (i.e. organic-rich) (Doxaran et al., 2012). Therefore, the organic substrates provided by particles were likely influencing the bacterial community growing on them.

These results were reinforced by tag-pyrosequencing of selected samples. The higher bacteria diversity in PA than in FL samples (Table 4) and the presence of more unique OTUs in the PA fraction than in the FL one (Fig. 5) indicates the existence of particle specialists. Indeed, within the shared OTUs (i.e. cosmopolitan OTUs) there was a higher proportion of OTUs found on both PA and FL fractions. This suggests that cosmopolitan OTUs were also generalists in their lifestyle. Conversely, within OTUs exclusive from river or marine locations, we could observe more specialists, mainly to particles (Fig. 5). A closer look into community composition in those samples suggests different underlying mechanisms: in the open sea sample, groups found to prevail at the PA fraction suggest adaptation to PA lifestyles. For instance, *Flavobacteria*, highly represented in our PA sea sample (Fig. 6), are specialized in adhesion to particles, as shown in a recent study using metaproteomics (Williams et al., 2012). Also, members of *Verrucomicrobia*, dominant in our PA open sea samples, are known to be active polysaccharide degraders (Martinez-Garcia et al., 2012). Conversely, some of the groups found in PA fraction from the river and the coast samples were bacteria previously observed in sediments or in land, such as the groups *Rhodocyclales* and *Methylophilales*, involved in denitrification processes (Ginige et al., 2004; Ishii et al., 2009). These groups will likely not spread far offshore, and may be partially the cause for the higher presence of non-active OTUs in river samples. This may explain the significant differences between DNA- and RNA-based fingerprints in river samples (Table 2, Supplement Fig. 1). Previous studies have also found higher diversity at the

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PA fraction, such as in deep samples of the Puerto Rico trench, using clone libraries (Eloe et al., 2011), in the upper euphotic layer of the Mediterranean Sea using 454 tag-pyrosequencing (Crespo et al, unpublished) or in suboxic zones of the Black Sea using 454 tag-pyrosequencing (Fuchsman et al., 2012). However, this is in contrast with other studies in marine and freshwaters (Ghiglione et al., 2007) including the Arctic (Kellog and Deming, 2009) where a higher diversity in the FL fraction has been observed. Additional work is needed to establish which conditions favor bacterial specialization, or where generalists will prevail.

4.3 Conclusions and implications

In this study we have applied for the first time a combination of fingerprinting and 454 tag-pyrosequencing to investigate patterns of PA and FL bacterial community structure in an Arctic system. Despite the outcome that location (river, coast, open sea) was the main structuring factor was expected, the reported spatial patterns of bacterial community structure can give us hints about potential biogeochemical processes. For instance, we noted the presence of *Nostocales* in the River sample. This group has been previously observed in the same location and recognized as potential N₂ fixers (Blais et al., 2012). N fixation has been observed in the Mackenzie shelf (Blais et al., 2012) and may play a crucial role in N cycling by partially alleviating strong N limitation in the area (Tremblay and Gagnon, 2009; Ortega-Retuerta et al., 2012a). Other groups of biogeochemical relevance are aerobic anoxygenic phototrophic bacteria (AAPs), which contribution is higher in the Mackenzie River delta and belong mainly to the phylum *Betaproteobacteria* (Boeuf et al. personal communication, 2012) which also dominated in our river sample.

The distinction between PA and FL communities allowed us to observe a high degree of specialization in Beaufort Sea communities, where particles are likely organic, but a high degree of overlap between PA and FL communities in coast and Mackenzie River samples, where particles have a higher mineral content. These contrasting patterns also suggest different metabolisms within particles, which will likely affect the fate of

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these particles in the water column (rem mineralization and sedimentation processes). Previous studies in the area have reported bacteria attenuate marine particle fluxes via enzymatic hydrolysis (Kellogg et al., 2011) and contribute to the remineralization of sinking particles (Forest et al., 2012) while others have reported a low degree of bacterial remineralization in phytoplankton-derived particulate matter (Rontani et al., 2012). Further work is needed to link particle composition, as a proxy for substrate quality, to bacterial metabolism and diversity.

Supplementary material related to this article is available online at:
[http://www.biogeosciences-discuss.net/9/17401/2012/
bgd-9-17401-2012-supplement.pdf](http://www.biogeosciences-discuss.net/9/17401/2012/bgd-9-17401-2012-supplement.pdf).

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Table 1. Average and range of primary physical, chemical and biological parameters in the different sections of the study area.

Parameter	River (<i>n</i> = 2)	Coast (<i>n</i> = 10)	Sea (<i>n</i> = 8)
Temp (°C)	10.2 (10.1–10.3)	8.0 (5.2–9.3)	2.6 (–0.8–4.5)
Sal	0.2 (0.2–0.2)	17.0 (7.0–23.6)	26.3 (22.5–27.9)
SPM (mg l ⁻¹)	111.8 (82.1–141.6)	7.0 (1.0–20.6)	0.4 (0.04–1.0)
NO ₃ (μM)	3.46 (3.31–3.62)	0.55 (0.00–2.60)	0.01 (0.00–0.02)
NH ₄ (μM)	0.21 (0.20–0.22)	0.34 (0.02–1.48)	0.02 (0.00–0.09)
PO ₄ (μM)	0.02 (0.02–0.02)	0.19 (0.06–0.36)	0.47 (0.33–0.60)
Si (μM)	64.3 (64.2–64.5)	25.8 (9.4–47.2)	4.6 (2.8–7.8)
DOC (μM)	487.9 (454.6–521.2)	238.6 (98.4–392.0)	86.7 (68.3–103.4)
DON (μM)	14.4 (13.7–15.2)	12.3 (6.9–26.7)	5.6 (4.4–7.9)
TDAA (nM)	532.7 (527.4–537.9)	456.2 (317.3–551.6)	266.4 (169.0–364.5)
DOP (μM)	0.22 (0.22–0.22)	0.52 (0.17–1.99)	0.17 (0.10–0.26)
POC (μM)	62.4 (52.0–72.8)	19.0 (6.9–26.7)	5.5 (2.5–7.1)
PON (μM)	10.4 (8.3–12.5)	4.2 (0.9–15.8)	0.8 (0.2–1.1)
POP (μM)	5.2 (5.0–5.5)	0.6 (0.1–1.9)	0.1 (0.0–0.1)
CDOM (a ₃₅₀ , m ⁻¹)	5.9 (5.6–6.3)	3.3 (1.1–5.6)	0.4 (0.1–0.8)
Chl <i>a</i> (mg m ⁻³)	3.1 (3.0–3.2)	1.2 (0.2–2.7)	0.7 (0.1–2.2)
BP (μg C m ⁻³ d ⁻¹)	136.7 (106.6–166.9)	115.9 (91.6–145.0)	44.0 (6.9–131.0)
%PA BP	99.4 (99–99.7)	38.2 (0–81.7)	41.1 (22.8–97.0)

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Table 2. Results of ANOSIM analyses to test significant differences in community structure between DNA (indicative of all present OTUs) and RNA (indicative of active OTUs), between transects (western and eastern) and between locations (river, coast and sea). From those groups showing significant differences in community structure, we then tested the different groups separately for differences between attached and free-living community structure and between total and active community structure. R^2 = explained variance. p level = level of significance. NS = non significant.

Sample chosen	Factor	R^2	p level
All	DNA-RNA	0.09	0.003
All	Transect	0.08	0.004
All	Location	0.39	0.001
Sea	Fraction	0.80	0.001
Coastal	"	0.12	0.005
River	"	NS	NS
Sea	DNA-RNA	0.01	NS
Coastal	"	0.20	0.001
River	"	0.71	0.029

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Table 3. Results of canonical correspondence analysis (CCA) of PA and FL bacterial community structure constrained by environmental parameters (shown in Fig. 3)

Axes	1	2	3	4	
DNA Attached					
Eigenvalues	0.178	0.085	0.049	0.035	
Species-environment correlations	0.937	0.896	0.829	0.858	
Cumulative percentage variance of species data	27.8	41.2	48.9	54.4	
Cumulative percentage variance of species-environment relation	47.0	69.5	82.5	91.8	
Sum of all eigenvalues					0.638
Sum of all canonical eigenvalues					0.378
DNA Free					
Eigenvalues	0.088	0.059	0.033	0.020	
Species-environment correlations	0.963	0.899	0.891	0.718	
Cumulative percentage variance of species data	21.1	35.3	43.2	48.0	
Cumulative percentage variance of species-environment relation	44.1	73.6	90.1	100.0	
Sum of all eigenvalues					0.414
Sum of all canonical eigenvalues					0.199

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Table 4. Number of OTUs at 0.03 level of clustering, and Chao1, Shannon and Simpson diversity indexes after 454 tag-pyrosequencing for the selected samples.

Sample	Fraction	<i>N</i> reads	<i>N</i> OTUs	Chao1	Shannon	Simpson
Sea	Attached	2826	105	137.5 ± 17.2	2.97	0.1859
	Free	"	80	103.0 ± 12.5	2.67	0.1798
Coast	Attached	"	179	232.1 ± 18.8	3.82	0.2559
	Free	"	162	240.1 ± 26.6	3.62	0.2302
River	Attached	"	166	192.5 ± 10.9	3.49	0.1975
	Free	"	144	179.9 ± 14.6	3.21	0.1718

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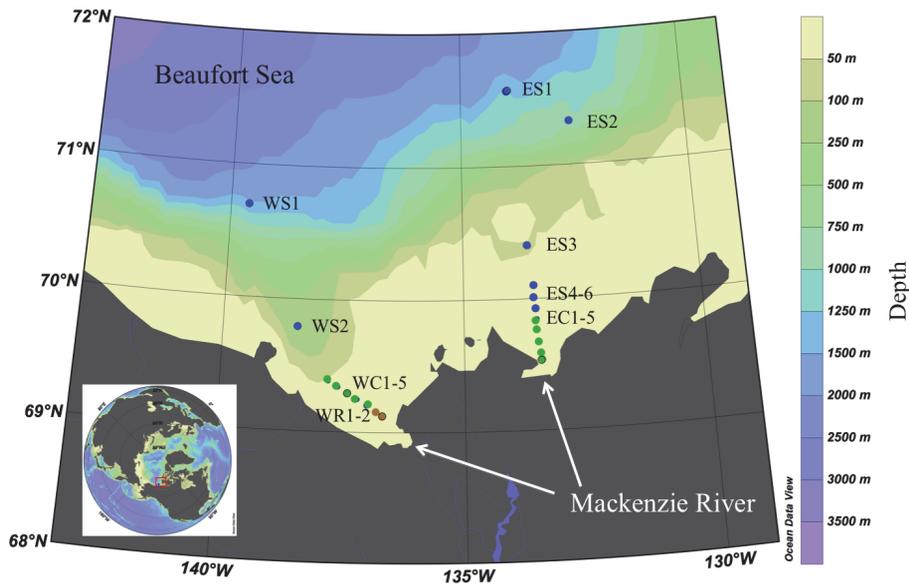


Fig. 1. Study area and station locations. Blue dots: stations considered as “open sea”. Green dots: stations considered as “coast”. Brown dots: stations considered as “river”.

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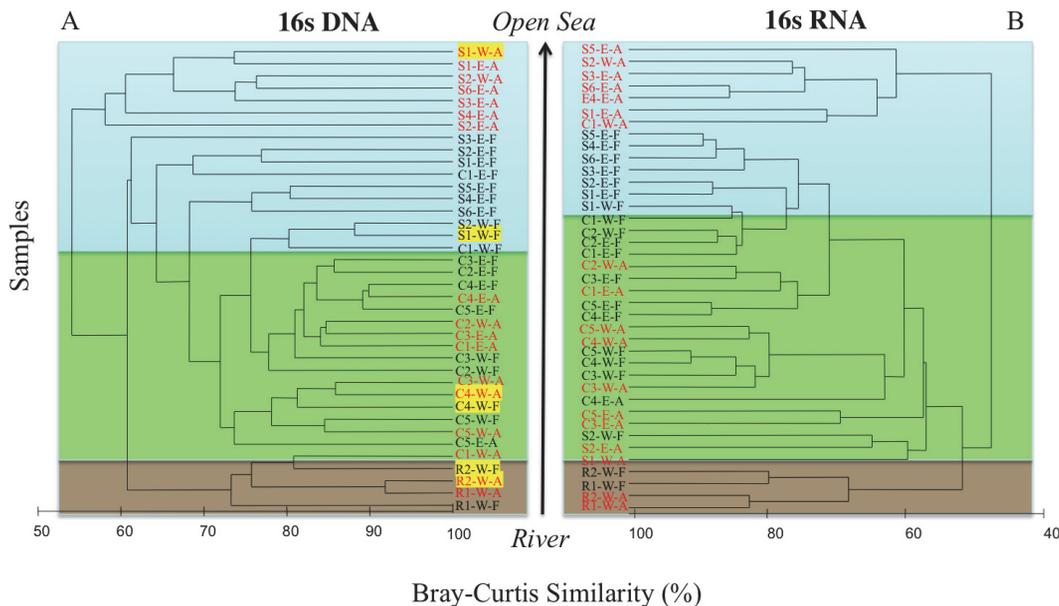


Fig. 2. Unweighted Pair Group Method with Arithmetic mean (UPGMA) dendrograms based on Bray-Curtis similarities of DNA-based (A) and RNA-based (B) CE-SSCP fingerprints of particle-attached (PA) and free-living (FL) bacteria. S: open sea C: coast R: river. W: western transect E: eastern transect. Samples selected for tag-pyrosequencing are highlighted in yellow.

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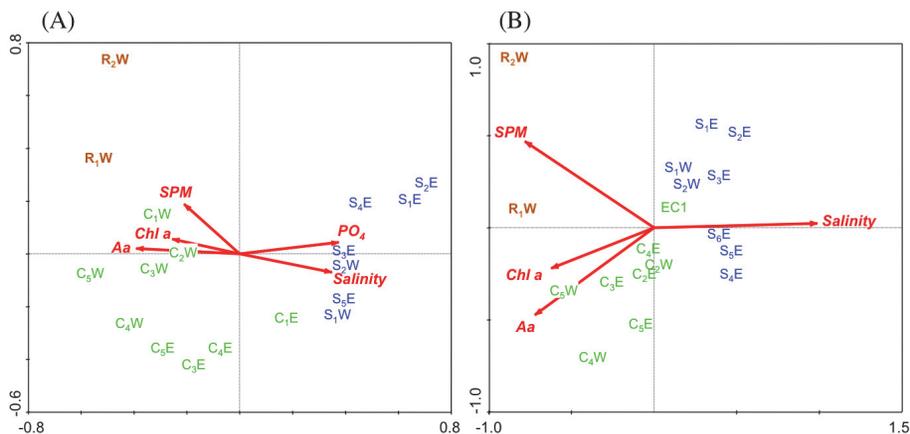


Fig. 3. Canonical correspondence analysis of bacterioplankton community structure from PA **(A)** and FL **(B)** samples using environmental variables. The length of the arrow represents degree of correlation with presented axis. SPM: Suspended particulate matter. Chl *a*: chlorophyll *a* concentration. Aa: amino acid concentration.

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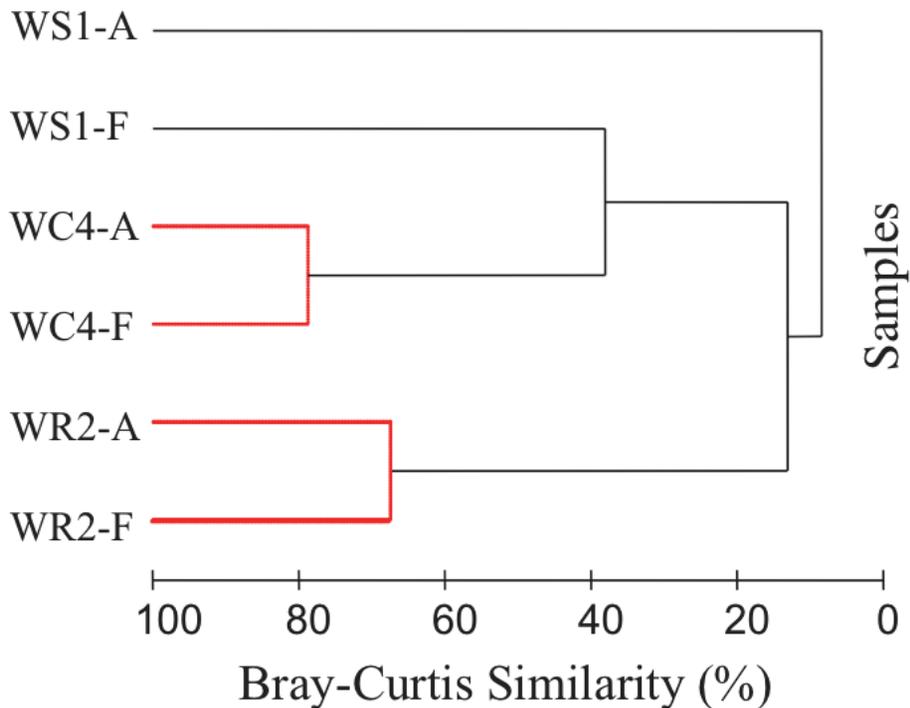


Fig. 4. UPGMA dendrograms based on Bray-Curtis similarities of DNA 454 tag-sequences. A: Particle-attached bacteria. F: free-living bacteria. S: sea C: coast R: river. Single clusters with non-significant differences between samples are marked in red.

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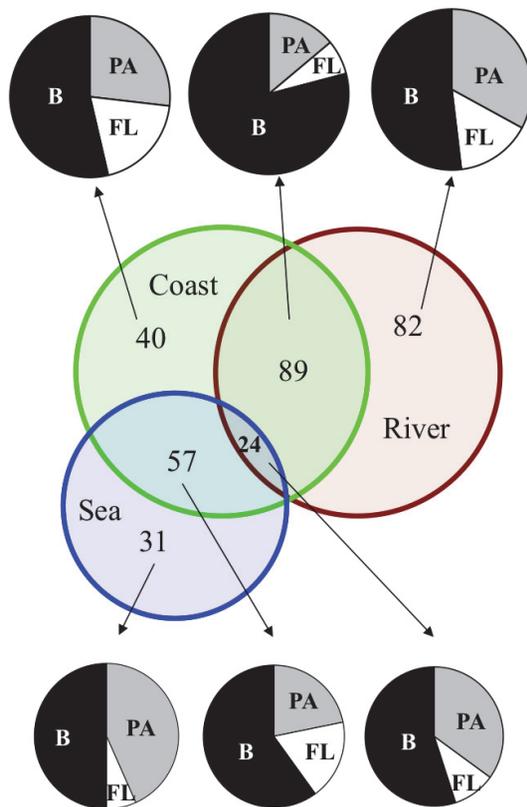


Fig. 5. OTUs distribution between open sea (blue circle), coast (green circle) and river (brown circle) (areas are proportional to the number of OTUs). Relative abundance of all exclusive and shared OTUs in the particle-attached fraction (PA), in the free-living fraction (FL) or in both fractions (B) is also shown.

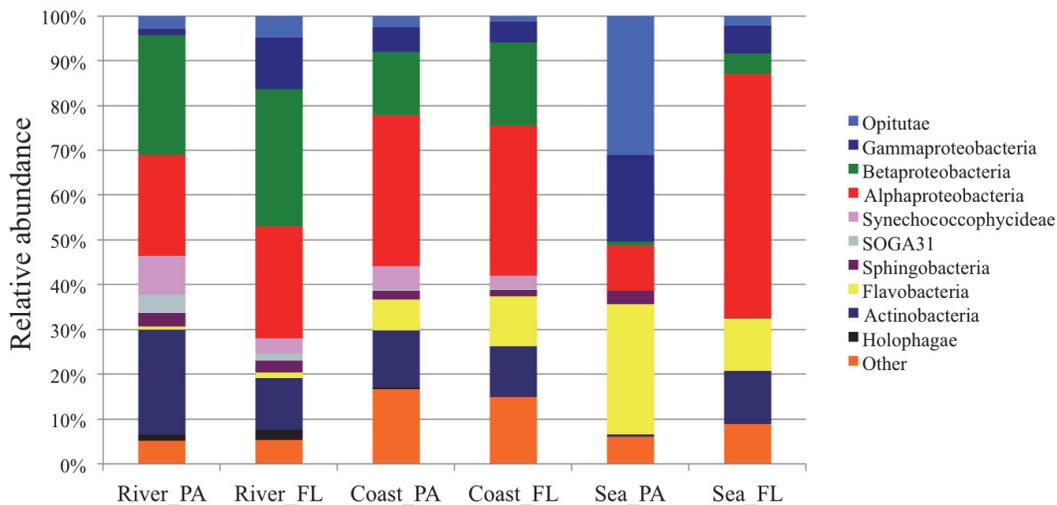


Fig. 6. Cumulative bar charts comparing the relative class abundances in PA and FL selected samples.

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