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## Summer community structure of aerobic anoxygenic phototrophic bacteria in the western Arctic Ocean

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8 **Summer community structure of aerobic anoxygenic phototrophic bacteria in**  
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11 **the western Arctic Ocean**  
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49 **Running title:** AAP bacteria in the western Arctic Ocean  
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52 **Keywords:** photoheterotroph; aerobic anoxygenic phototrophic bacteria; *pufM* gene;  
53 bacteriochlorophyll; Arctic Ocean; Mackenzie River  
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## Abstract

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Aerobic anoxygenic phototrophic (AAP) bacteria are found in a range of aquatic and terrestrial environments, potentially playing unique roles in biogeochemical cycles. Although known to occur in the Arctic Ocean, their ecology and the factors that govern their community structure and distribution in this extreme environment are poorly understood. Here, we examined summer AAP abundance and diversity in the North East Pacific and the Arctic Ocean with emphasis on the southern Beaufort Sea. AAP bacteria comprised up to 10 and 14% of the prokaryotic community in the bottom nepheloid layer and surface waters of the Mackenzie plume, respectively. However, relative AAP abundances were low in offshore waters. Environmental *pufM* clone libraries revealed that AAP bacteria in the *Alphaproteobacteria* and *Betaproteobacteria* classes dominated in offshore and in river-influenced surface waters, respectively. The most frequent AAP group was a new uncultivated betaproteobacterial clade whose abundance decreased along the salinity gradient of the Mackenzie plume even though their photosynthetic genes were actively expressed in offshore waters. Our data indicate that AAP bacterial assemblages represented a mixture of freshwater and marine taxa mostly restricted to the Arctic Ocean and highlight the substantial influence of riverine inputs on their distribution in coastal environments.

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## 25 Introduction

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6 Aerobic anoxygenic phototrophic (AAP) bacteria are photoheterotrophs that require  
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8 oxygen for their growth and for bacteriochlorophyll *a* (Bchl *a*) synthesis. They inhabit a wide  
9  
10 variety of illuminated habitats in diverse terrestrial, freshwater, and marine environments  
11  
12 (Béjà *et al.*, 2002; Csotonyi *et al.*, 2010; Atamna-Ismaeel *et al.*, 2012). First discovered in  
13  
14 coastal marine waters (Shiba *et al.*, 1979), AAP bacteria have been intensively studied in the  
15  
16 marine environment (Cottrell *et al.*, 2006; Mašín *et al.*, 2006; Lehours *et al.*, 2010). Their  
17  
18 abundance and distribution vary greatly among oceanic regimes, suggesting that there is a  
19  
20 broad range of potential ecological niches for these microbes. AAP bacteria seem to be more  
21  
22 abundant in shelf and coastal areas than in the open ocean (Schwalbach & Fuhrman, 2005;  
23  
24 Sieracki *et al.*, 2006). Although their abundance can be high in some oligotrophic regions  
25  
26 (Lami *et al.*, 2007), AAP bacteria typically constitute a small percentage (2 to 4%) of total  
27  
28 prokaryotes in oceanic environments (Cottrell *et al.*, 2006; Jiao *et al.*, 2007). However, their  
29  
30 proportions can exceed 10% in eutrophic estuaries (Waidner & Kirchman, 2007). Despite the  
31  
32 lower abundances in most oligotrophic pelagic marine environments, AAP bacteria  
33  
34 constitute a very dynamic part of the bacterial community and potentially contribute  
35  
36 significantly to the cycling of organic carbon in the upper ocean (Koblížek *et al.*, 2007).  
37  
38 Culture-dependent and -independent studies have shown AAP bacteria to be genetically  
39  
40 diverse with members of the *Alpha*-, *Beta*-, and *Gammaproteobacteria* (Béjà *et al.*, 2002;  
41  
42 Yutin *et al.*, 2007; Jeanthon *et al.*, 2011). The environmental factors controlling the  
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44 population structure and diversity of these photoheterotrophic bacteria remain poorly  
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46 understood.  
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55 Arctic marine ecosystems are characterized by strong seasonality in incident light, ice  
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57 cover, temperature, and river discharge which affect salinity and other biogeochemical  
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3 properties. In the Beaufort Sea, the Mackenzie River delivers considerable freshwater,  
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5 50 nutrients and organic matter which exert strong controls on the productivity, salinity and  
6  
7 water circulation (Aagaard & Carmack, 1989; Opsahl *et al.*, 1999). Despite a short growing  
8  
9 season and extensive sea ice coverage, the most river-influenced of the world's oceans  
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11 supports a productive aquatic ecosystem. Recent studies over the Mackenzie Shelf in the  
12  
13 Beaufort Sea have revealed diverse and active communities of Bacteria and Archaea that  
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17 55 vary across salinity gradients (Galand *et al.*, 2006; Garneau *et al.*, 2006; Galand *et al.*, 2008).

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19  
20 Our current knowledge of the abundance, distribution, and activity of AAP bacteria in  
21  
22 perennially cold marine waters is still limited. In the only study in the Arctic, AAP bacteria  
23  
24 contributed 5 to 8% of the total prokaryotic community in spatially close coastal sites of the  
25  
26 western Beaufort and eastern Chukchi Seas (Cottrell & Kirchman, 2009). Seasonal shifts in  
27  
28  
29 60 the abundance of AAP bacteria between summer and winter paralleled changes in the  
30  
31 abundance of the total prokaryotic community. There were no substantial shifts in the  
32  
33 community structure of AAP bacteria between the two seasons. Although *pufM* genes in  
34  
35 Arctic surface waters could be assigned to taxonomic groups previously identified in  
36  
37 metagenomic shotgun sequences from the GOS expedition (Yutin *et al.*, 2007), their  
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39  
40 65 sequences were distinct from those retrieved in lower-latitude waters (Cottrell & Kirchman,  
41  
42 2009).

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45 In this study, we collected samples across a several-thousand kilometers transect  
46  
47 above the 60°N latitude that yielded an unprecedented resolution of the Beaufort Sea during  
48  
49 summer and explored transition zones between offshore and large Arctic rivers that have a  
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52 70 major influence on coastal ocean biogeochemistry. To examine the AAP bacterial community  
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54 structure and factors that govern it, we combined the use of infrared epifluorescence  
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56 microscopy and culture-dependent and independent approaches. The Beaufort Shelf surface  
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3 circulation is strongly influenced by ice, winds and freshwater input resulting from river  
4 discharge and sea-ice melt. We hypothesized that AAP bacterial abundance and composition  
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8 75 would change substantially along this transect and that riverine inputs would have a  
9  
10 substantial influence on the AAP bacterial populations.  
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## 14 **Materials and methods**

### 15 16 17 18 19 20 80 **Study area, sampling and oceanographic parameters**

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22 The MALINA cruise took place onboard the Canadian research icebreaker *CCGS Amundsen*  
23 during summer 2009 from Victoria (BC, Canada) to the Beaufort Sea (Leg 1b) and then  
24 throughout the Beaufort Sea (Leg 2b) (Fig.1). Most of the stations sampled on the west–east  
25 transect in the Beaufort Sea (leg2) were ice-free. However, surface waters of eastward  
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31 85 offshore waters were still ice-covered. Surface seawater samples were collected with an acid  
32 cleaned bucket during Leg 1b and in the Mackenzie plume (stations 395, 398, 694, and 697)  
33 during Leg 2b. In the Beaufort Sea, seawater was collected from six depths using Niskin  
34 bottles mounted on a CTD (conductivity temperature depth probe) rosette. Ancillary data of  
35 temperature, salinity, pH, dissolved oxygen, colored dissolved organic matter, inorganic and  
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42  
43 90 organic nutrients, and chlorophyll *a* are given in Table S1.  
44

45  
46 Bacterioplankton biomass for DNA and total RNA extraction were collected onboard.  
47  
48 Seawater samples were prefiltered through 47 mm diameter and 3  $\mu\text{m}$  pore size  
49 polycarbonate filters (Cyclopore, Whatman, USA) before the final collection of  
50  
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55 95 extraction or onto 27 mm diameter and 0.22  $\mu\text{m}$ -pore-size Durapore filters (Millipore, USA)  
56 for RNA extraction. Sterivex cartridges were filled with 1.6 ml of lysis buffer (0.75 M sucrose,  
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3 50 mM Tris-HCl, pH 8), immediately frozen in liquid nitrogen, and stored at -80°C until  
4  
5 analysis. To limit the degradation of mRNA, small volumes of seawater (~ 1 liter) were  
6  
7 filtered as rapidly as possible, immediately upon retrieval of the CTD. RNA filters were  
8  
9 transferred in collection tubes containing 0.8 ml of RLT buffer (Qiagen, Hamburg, Germany)  
10 100 with 1 % of  $\beta$ -mercaptoethanol, frozen in liquid nitrogen, and stored at -80°C until analysis.  
11  
12  
13  
14 The time from the start of filtration to storage was 15-20 min.  
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### 20 **Microscopic enumeration of AAP bacteria and total prokaryotes**

21  
22 105 Prokaryotes were enumerated by epifluorescence microscopy of paraformaldehyde-fixed  
23  
24 samples that were filtered onto 0.2- $\mu$ m pore-size black polycarbonate filters (Cottrell *et al.*,  
25  
26 2006). Filters were stored at -80°C for up to 4 months prior to analysis. Total prokaryotes  
27  
28 were enumerated after staining with 4',6-diamidino-2-phenylindole (DAPI), 1  $\mu$ g.ml<sup>-1</sup> final  
29  
30 concentration in 1 $\times$  phosphate-buffered saline (PBS) for 10 min. The AAP bacteria were  
31  
32 enumerated using an Intensified Retiga charge-couple device camera (Qimaging, Surry, BC  
33  
34 110 Canada) and image analysis routines to enumerate cells that fluoresce blue when stained  
35  
36 with DAPI and have infrared fluorescence (BChl *a* signal), but not red or orange fluorescence  
37  
38 (Chl *a* and phycoerythrin signals, respectively) (Cottrell *et al.*, 2006). AAP bacterial  
39  
40 abundance data were imported into Ocean Data View version 4 (Schlitzer, 2010) and  
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44  
45 115 interpolated using the DIVA gridding tool.  
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### 52 **Isolation and identification of AAP bacterial strains**

53  
54 Seawater samples (50-100  $\mu$ l) collected at the surface and at the deep chlorophyll maximum  
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57 120 (DCM) were spread onto low nutrient heterotrophic medium (Cho & Giovannoni, 2004)  
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3 prepared using seawater collected at station 860 at the surface and the DCM, respectively.  
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5 Dimethylsulfoniopropionate (DMSP) was added to the medium at a final concentration of  
6  
7 100 nM. Culture plates were incubated for up to 2 months at 4°C into thermostatic cabinets  
8  
9 illuminated by luminescent tubes at irradiance levels of 120  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  (for surface  
10  
11 samples) and 25  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  (for DCM samples). BChl *a*-containing colonies were  
125  
13 detected as previously described (Jeanthon *et al.*, 2011).  
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16  
17 Bacterial DNA was extracted using the QuickExtract™ Bacterial DNA Extraction Kit  
18  
19 (Epicentre Biotechnologies, Madison, WI, USA). Small-subunit (16S) rRNA genes were  
20  
21 amplified by polymerase chain reaction (PCR) using universal reverse primer 1492R and  
22  
23 *Bacteria*-specific forward primer 27F (Table 1), whose 5' ends were labeled with 6-  
24  
25 hexachlorofluorescein (6-HEX) and with 6-carboxyfluorescein (6-FAM), respectively. Reaction  
26  
27 mixtures (25  $\mu\text{L}$ ) contained 5 X buffer (5  $\mu\text{L}$ ), 2mM  $\text{MgCl}_2$ , 10 pmoles of each  
28  
29 deoxyribonucleotide triphosphate (dATP, dCTP, dGTP, dTTP; Eurogentec, Seraing, Belgium),  
30  
31 10 pmoles of each oligonucleotide primer, 1.25 U of GoTaq Flexi DNA polymerase (Promega,  
32  
33 Madison, WI, USA) and 1  $\mu\text{L}$  of cell lysate. The initial denaturation step consisted of heating  
34  
35 135 the reaction mixture at 95 °C for 5 min, and the thermal profile then consisted of 30 cycles of  
36  
37 denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 1.5 min.  
38  
39 A final extension step was carried out at 72 °C for 10 min. The PCR products were digested  
40  
41 with the restriction endonucleases *Hinf*I and *Msp*I (New England BioLabs, Ipswich, MA, USA)  
42  
43 140 for 4 h at 37°C. After enzymatic inactivation at 80°C for 20 min, T-RFLP digests were diluted  
44  
45 in Hi-Di™ formamide (Applied Biosystems, Carlsbad CA, USA) and terminal-restriction  
46  
47 fragments (T-RFs) were separated in a 3130 *xl* Genetic Analyzer (Applied Biosystems). Data  
48  
49 were analyzed using the PeakScanner™ Software (Applied Biosystems).  
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3 16S rRNA gene sequences of at least two randomly chosen strains from each T-RFLP  
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5 145 group were amplified by PCR using 8F-1492R primers (Table S2) following the same  
6  
7 conditions as described above. PCR products were purified by ExoSAP-IT PCR purification kit  
8  
9 (GE Healthcare) and sequenced using the latter primers with an ABI 3130 POP7 sequencer  
10  
11 (Applied Biosystems).  
12  
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14  
15 16S rRNA gene sequences were compared to sequences in public databases with  
16  
17 150 BLASTn (Altschul et al 1997). The new sequences were aligned using the Q-INS-I algorithm of  
18  
19 the MAFFT sequence alignment program version 6.5 (Kato et al 2009). A set of 16S rRNA  
20  
21 sequences with high similarity to those determined in this study were retrieved and added  
22  
23 to the alignment. Phylogenetic trees were computed using neighbor-joining, maximum  
24  
25 parsimony and maximum likelihood inference approaches using MEGA 5 (Tamura et al  
26  
27 2011). Bayesian analyses (MrBayes; (Ronquist and Huelsenbeck 2003) were also performed  
28  
29 155 to provide additional statistical support for the nodes.  
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33  
34 The presence of *pufM* was determined by using PCR amplification with specific  
35  
36 primers (Lehours *et al.*, 2010). When necessary, bands of the targeted size were isolated  
37  
38 from the gel using a DNA gel extraction kit (Millipore, Billerica, MA, USA). Gel-purified  
39  
40 160 products were concentrated with a Microcon YM50 centrifugal filter devices (Millipore) and  
41  
42 sequenced using *pufMF* and *pufM-WAW* primers (Table S2).  
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#### 48 **Diversity analysis of *pufM* gene**

49  
50 A total of 42 DNA samples were extracted as previously described (Marie *et al.*, 2006). To  
51  
52 165 select samples for *pufM* diversity analysis, amplification and fingerprinting of *pufM*  
53  
54 communities using temporal temperature gradient electrophoresis (TTGE) were performed  
55  
56 as described by Lehours et al. (2010). For the construction of partial *pufM* DNA clone  
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3 libraries, fresh PCR products were cloned directly or after gel purification using the TOPO-TA  
4  
5 cloning kit (Invitrogen) according to the manufacturer's instructions. The sequences were  
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7  
8 170 trimmed to remove any vector and primer sequences. DNA sequences were translated into  
9  
10 amino acid sequences and aligned using the ClustalW program in MEGA 5 (Tamura *et al.*,  
11  
12 2011). The resulting protein alignment was back-translated to nucleotide acid sequences  
13  
14 that were realigned manually. A conservative value of 94% nucleic acid sequence similarity  
15  
16 was chosen for clustering sequences into Operational Taxonomic Units (OTUs) using  
17  
18  
19 175 MOTHUR (Schloss *et al.*, 2009). Representative sequences (defined as the sequence with the  
20  
21 minimum distance to all other sequences in the OTU) were obtained using MOTHUR. The  
22  
23 coverage value (Mullins *et al.*, 1995), the Shannon index,  $H_{Shannon}$  (Shannon *et al.*, 1949) and  
24  
25 the bias-corrected richness-estimator,  $S_{Chao1}$  (Chao *et al.*, 2005) were calculated for each  
26  
27  
28 library.  
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30  
31 180 A *pufM* database containing 35 aligned sequences (720 total nucleotide positions) of  
32  
33 cultured species and environmental clones retrieved from GenBank and the GOS scaffold  
34  
35 nucleotide sequences was constructed using MEGA 5 (Tamura *et al.*, 2011) according to  
36  
37 (Yutin *et al.*, 2007). A phylogenetic tree was constructed by Bayesian inference using  
38  
39 MrBayes with substitution rates varying according to codon positions. Four parallel MCMC  
40  
41  
42 185 chains of 3.5 million generations were run; trees were sampled every 100 generations. A  
43  
44 consensus tree was constructed after the exclusion of 10.000 'burnin' trees. The *pufM*  
45  
46 database and the consensus tree were imported into ARB software (Ludwig *et al.*, 2004).  
47  
48 Representative sequences of each OTU (245 pb) and short *pufM* environmental reference  
49  
50 sequences were aligned as above and added to the consensus Bayesian tree using the  
51  
52  
53 190 ADD\_BY\_PARSIMONY algorithm implemented in ARB. Non-informative taxa were removed  
54  
55  
56 from the final tree.  
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3 For cluster analysis, the dataset was randomly resampled before analysis. A distance  
4 matrix was computed from the presence of OTUs in clone libraries at a 94% identity  
5 threshold using Dice coefficient (Dice, 1945) and a dendrogram was inferred with the  
6 unweighted pair-group average algorithm (UPGMA) as implemented in XLSTAT (Addinsoft,  
7 New York, USA).  
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### 18 ***In situ* abundance of OTU 6 members**

19 OTU 6, the most abundant group retrieved in the *pufM* libraries, was targeted by qPCR.  
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21  
22 200 Primers targeting specifically *pufM* genes from OTU 6 (Table S2) were designed using ARB.  
23  
24 Primer specificity was confirmed by constructing a clone library as described above and  
25 sequencing 31 clones. All sequences were identical to those of OTU 6 members. QPCR  
26 conditions were as in Table S2. Two plasmid clones from our *pufM* and 16S rRNA libraries  
27 were used as positive controls and for standard curves (10-fold dilution series) in the qPCR  
28 assays. Plasmid DNA was linearized with *HindIII* and quantified using the Picogreen assay  
29 (Invitrogen).  
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38 QPCR was performed in triplicate, with 1  $\mu\text{l}$  of environmental sample DNA normalized  
39 to 100  $\text{pg}\cdot\mu\text{l}^{-1}$  or plasmid DNA solution in a total reaction volume of 10  $\mu\text{l}$  using the SYBR  
40 Green/ROX qPCR *Master Mix* kit (Applied Biosystems). QPCR assay were performed using a  
41  
42  
43  
44  
45 210 Chromo4 Real-Time PCR Detection System and the Opticon Monitor software package (Bio-  
46 Rad) with the following thermal cycling conditions: 95°C for 15 min, 45 cycles of  
47 denaturation at 95°C for 45 s, primer annealing at the primer-specific annealing temperature  
48 (Table S2) for 45 s with fluorescence measurements after this step, and polymerase  
49 extension at 72°C for 15 s. Melting curves were generated after each assay to check the  
50 specificity of the amplification by heating from 65 to 95°C at a rate of  $0.1^\circ\text{C}\cdot\text{s}^{-1}$  and taking  
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3 fluorescence measurements every 0.5°C. Only single peaks were observed in the dissociation  
4  
5 curves for both the standards and samples, indicating specific amplification with each set of  
6  
7 primers. Average amplification efficiencies were as follows: 16S rDNA = 99.2% and OTU 6-  
8  
9 *pufM*= 97%.

10  
11  
12 220 No inhibition was detected when a known quantity of standard was added to DNA  
13  
14 extracts from surface waters of stations 697, 680, 620 and 398 (data not shown). The  
15  
16 estimates of OTU 6-*pufM* abundance were normalized to 16S rRNA gene abundance in order  
17  
18 to estimate the fraction of the bacterial community bearing OTU 6-*pufM* type (Campbell *et*  
19  
20 *al.*, 2008).  
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24 225

#### 25 26 **Activity of OTU 6 members**

27  
28 Reverse-transcription PCR was used to produce cDNA from mRNA transcripts to determine  
29  
30 whether *pufM* was being transcribed by OTU 6 members at the time of sampling. RNA  
31  
32 samples were extracted using the RNeasy Mini kit (Qiagen) following the manufacturer's  
33  
34 instructions with several modifications. Glass beads (50 µl) were added to the samples after  
35  
36 230 thawing on ice and the tubes were mixed vigorously for 10 min. Genomic DNA was removed  
37  
38 using a Turbo DNA-free kit (Ambion, Life Technologies, Paisley, UK). DNA removal in RNA  
39  
40 samples was confirmed by control PCR amplifications without the reverse transcription step.  
41  
42 No amplification was detected in these controls. ThermoScript RT-PCR system (Invitrogen,  
43  
44 Carlsbad, USA) was used for the reverse transcription of *pufM* mRNA from total RNA  
45  
46 235 samples. All reverse transcription reactions were performed in a total reaction volume of 10  
47  
48 µl. cDNA synthesis was performed at 55 °C using the reverse primer as gene-specific primer.  
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55 PCR products obtained as above were cloned directly or after gel extraction and sequenced.  
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3 *pufM* genes amplified using cDNA from station 620 were cloned and sequenced as described  
4  
5 240 above. Of 31 clones that were analyzed, all contained the expected *pufM* gene sequences.  
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9

### 10 **Nucleotide sequence accession numbers**

11  
12 Unique environmental *pufM* gene sequences obtained in this study are available in GenBank  
13  
14 database under the accession numbers JN248465 to JN248539. The 16S rRNA and *pufM*  
15  
16 245 gene sequences of the isolates are available in GenBank database under the accession  
17  
18 numbers JN248461 to JN248464 and JN378828 to JN378831, respectively.  
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## 24 **Results**

### 250 **Oceanographic context**

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29  
30 Using salinity and temperature profiles, several water masses were delineated in the  
31  
32 Beaufort Sea/Mackenzie system (Fig. S1; Table S1). Surface waters in the Mackenzie plume  
33  
34 stations and under the influence of the Firth river (station 680) displayed the highest  
35  
36 temperatures and lowest salinities. The influence of the Mackenzie plume decreased  
37  
38 eastward in the shelf where colder and denser surface waters were recorded, which was  
39  
40 255 likely due to the addition of ice melt from offshore waters. Below 40-50m depth, cold, high  
41  
42 salinity deep ocean waters consistent with Pacific Ocean intrusion were observed. Nitrate  
43  
44 concentrations in surface waters were low. Surface-water phosphate increased with salinity,  
45  
46 whereas silicate and nitrate decreased, as expected in this river-influenced region (Carmack  
47  
48 *et al.*, 2004). Similar to hydrographic features, total Chl *a* levels decreased greatly from the  
49  
50 260 river plume region towards the more oligotrophic marine shelf and offshore waters.  
51  
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### Abundance of total prokaryotes and AAP bacteria

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5 Total prokaryotic abundances ranged from  $9.1 \times 10^4$  cells  $\text{ml}^{-1}$  to  $3.4 \times 10^5$  cells  $\text{ml}^{-1}$  in  
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7  
8 265 surface waters sampled from the North Pacific Ocean to the Chukchi Sea. Along this transect,  
9  
10 AAP bacterial abundances as measured by infrared epifluorescence microscopy were low  
11  
12 (about 0.1% to 0.5%) except in the Chukchi Sea (ARC1) where their contribution reached  
13  
14 14.8% of the total prokaryotic community. In the Beaufort Sea, abundances of total  
15  
16 prokaryotes decreased slightly from the river plume to offshore surface waters (Table S1;  
17  
18 Fig. 2a to 2d). Similar to total Chl *a*, AAP abundances dropped 10-fold between the plume  
19  
20 270 and the shelf surface waters and by another 10-fold between the shelf and offshore surface  
21  
22 waters (Fig. 2e; Table S1). AAP bacterial abundances were highest in surface waters of the  
23  
24 Mackenzie plume (mean abundance of 6.3 %), reaching 14% of total prokaryotes at station  
25  
26 398 (Fig. 2f and 2g). High contributions (up to 10%) were also found in the bottom nepheloid  
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30  
31 275 layer (BNL) of transects 200 and 300 (Fig. 2g and 2h).

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33  
34 For the overall dataset, total prokaryotes and AAP bacterial abundances were  
35  
36 positively correlated with temperature and negatively with salinity (Table S3). AAP  
37  
38 proportions correlated significantly with ammonium, silicate, total Chl *a* and the different  
39  
40 forms of organic carbon, nitrogen, and phosphorus. In contrast to relative AAP bacterial  
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42  
43 280 abundance, total prokaryote abundance did not covary significantly with most of the  
44  
45 parameters. High AAP bacterial abundances in the BNL were related to dissolved inorganic  
46  
47 nitrogen and phosphorus, suggesting the presence of a diffusive flux of nutrients from the  
48  
49 organic-rich sediments, the BNL, or both (Sundby *et al.*, 1992).  
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### 285 Diversity and distribution of environmental *pufM* sequences

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3 Based on their TTGE band patterns and their location, 13 samples were selected for further  
4  
5 cloning and sequencing. Of 365 clones analyzed, 43 distinct OTUs were identified after  
6  
7 grouping the sequences at 94% nucleic acid sequence similarity. The diversity in the river-  
8  
9 influenced stations was high as indicated by the  $S_{\text{Chao1}}$  values (estimated numbers of  
10  
11 expected OTUs) and diversity indices (Table S4) while the coverage index was low. However,  
12 290 coverage estimates (> 83%) and rarefaction curves (data not shown) indicated that most of  
13  
14 the diversity was detected in the libraries from marine stations. The diversity of AAP bacteria  
15  
16 was generally lower in surface than in deeper waters. Cluster analysis of the AAP bacterial  
17  
18 community composition separated the Beaufort Sea samples in three clusters according to  
19  
20 water masses (Fig. S2). The two main clusters separated surface samples (upper polar mixed  
21  
22 layer) and waters below 50m (Pacific summer water). North Pacific Ocean and Bering Sea  
23  
24 295 samples grouped together.  
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31 A phylogenetic tree was constructed for all 43 OTUs from this study and from  
32  
33 previous studies of *pufM* sequences (Fig. 3). The phylogenetic analysis revealed that 41 OTUs  
34  
35 were tentatively assigned to *Alpha-* (22 OTUs, ~ 49 % of the sequences) or  
36 300 *Betaproteobacteria* (17 OTUs; ~ 48% of the sequences). Two OTUs belonged to  
37  
38 *Gammaproteobacteria*. Two singletons could not be confidently assigned.  
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43 The betaproteobacterial OTU 6 was the most frequent OTU (38 % of the sequences)  
44  
45 and had *pufM* sequences identical (153 bp out of 245 bp) to that of environmental clones  
46  
47 305 from the Monterey Bay (Béjà *et al.*, 2002). OTU 6 belonged to a large cluster of 13 OTUs that  
48  
49 also contained two sequences obtained by single-cell sequencing (Martinez-Garcia *et al.*,  
50  
51 2012) and a mixture of arctic sequences (Cottrell & Kirchman, 2009), but it did not have any  
52  
53 close relationship to known AAP bacteria. Two singleton OTUs belonged to  
54  
55 betaproteobacterial phylogroup I defined by Yutin *et al.* (2007) that formed a sister lineage  
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3 310 containing sequences of fosmid clones from the Delaware river (Waidner & Kirchman, 2005)  
4  
5 and *Rhodospirillum rubrum* (Page *et al.*, 2004). Betaproteobacterial sequences dominated in  
6  
7 clone libraries from river-influenced waters (Fig. 4). OTU 6, the most abundant OTU, was  
8  
9 recovered in all Beaufort Sea stations and in the Bering Sea but was absent in the North  
10  
11 Pacific Ocean and Chukchi Sea.

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13  
14 315 Alphaproteobacterial OTU 1 and OTU 5, the second and third most abundant OTU  
16  
17 (18.6% and 6.6% of the sequences, respectively) together with OTU 4, -12 and -29 were  
18  
19 closely related to environmental clones recovered from the Chukchi and Beaufort Seas  
20  
21 (Cottrell & Kirchman, 2009) (Fig. 3). These sequences dominated libraries from offshore  
22  
23 waters of the Beaufort and Chukchi Seas. Among them, OTUs 4 and 5 were only detected in  
24  
25 the Beaufort Sea (Fig. 4). In contrast, OTU 1 was retrieved from the North Pacific Ocean to  
26  
27 320 the Chukchi Sea with relative proportions increasing northwardly. It also occurred in most  
28  
29 Beaufort Sea samples.  
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32  
33 Other *Alphaproteobacteria* grouped mainly with cultivated members of the  
34  
35 *Bradyrhizobiaceae* and of genera *Citromicrobium*, *Methylobacterium* and *Sphingomonas* (Fig.  
36  
37 3). *Bradyrhizobiaceae*- and *Citrobacterium*-like sequences showed similar distribution  
38  
39 325 patterns. They co-occurred in surface waters of the North Pacific Ocean and in shelf and  
40  
41 offshore DCM layers of the Beaufort Sea. Sequences related to *Sphingomonas* and  
42  
43 *Methylobacterium* were only found at the surface in the Mackenzie mouth and at the DCM  
44  
45 level along the plume (Fig. 4). Gammaproteobacterial OTUs belonged to the phylogroup K  
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47  
48 330 defined by Yutin *et al.* (2007). They prevailed in surface water libraries of the North Pacific  
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51 Ocean but were not detected in higher latitude samples.  
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### Diversity of AAP isolates

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5 335 To gain more insights into the origin of the *pufM* genes obtained in this study, AAP bacteria  
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7 were isolated that grew on low nutrient heterotrophic medium. BChl *a*-containing colonies  
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9 were detected only from shelf and offshore surface waters of the Beaufort Sea. Based on T-  
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11 RFLP analyses of 16S rRNA gene, the 92 *pufM*-positive strains were distributed in four groups  
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13 (Table 1). In accordance with their position in the *pufM* phylogenetic tree (Fig. 3),  
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17 340 representative strains of these groups had 16S rRNA gene sequences closely related to  
18  
19 *Sulfitobacter* and *Loktanella* (Fig. 5). Interestingly, the *pufM* partial sequences of most  
20  
21 isolates were identical or nearly identical to environmental sequences obtained in this study.  
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23 About half of the isolates were affiliated with *S. marinus* (Fig. 5) and had *pufM* sequences  
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25 that matched with OTU 5, the third most abundant OTU in the *pufM* libraries (Fig. 3). The  
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29 345 16S rRNA gene sequences of both *Sulfitobacter* representatives RCC 2406 and RCC 2401  
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31 were most closely related with that of isolates from Antarctic Sea ice and Arctic marine  
32  
33 sediments, respectively (Table 1). The two other T-RFLP groups were affiliated to the genus  
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35 *Loktanella*. Both *Loktanella* groups had *pufM* sequences almost identical to that of OTU 29.  
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37 On the basis of their 16S rRNA gene sequences, both groups clustered with strains isolated  
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41 350 from Antarctic lakes (Van Trappen *et al.*, 2004), the major one having a sequence similar to  
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43 that of *Loktanella* sp. WM1 isolated from the Baltic Sea (Salka *et al.*, 2008). No gamma- or  
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45 betaproteobacterial AAP strains were isolated.  
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### Abundance and activity of OTU 6 members

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52 355 Since *pufM* in the OTU 6 clade clustered with betaproteobacterial sequences, we  
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54 hypothesized that OTU 6 members would have a freshwater origin. The relative abundance  
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56 of OTU 6 was investigated using quantitative PCR along the two salinity gradients of the  
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3 Mackenzie River (transects 300 and 600). Proportions of OTU 6-like *pufM* genes decreased  
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5 gradually from higher-temperature and lower-salinity to lower-temperature and higher-  
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8 360 salinity waters in both transects (Fig. 6). However, we noticed a high proportion of OTU 6 at  
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10 station 680 associated with increasing temperature and decreasing salinity. This contribution  
11  
12 was higher than the expected based on simple dilution. It could be related to a local input of  
13  
14 OTU 6 members released from the Firth River. A subsequent PCR survey of our samples  
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16 using specific primers detected OTU 6 members in all Beaufort Sea surface samples except at  
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19 365 the three northern offshore stations (110, 220, and 320).  
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22 To investigate if members of this clade actively express photosynthetic genes in cold  
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24 marine waters, we extracted mRNA from surface samples of transect 600 and amplified OTU  
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26 6-specific genes. We confirmed by sequencing that all *pufM* cDNA clones were OTU 6  
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28 members. Active OTU 6 members were detected in all samples including the most offshore  
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31 370 station 620, although their contribution to total abundance was low according to qPCR  
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33 assays (Fig. 6).  
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## 38 Discussion

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40 Little is known about AAP bacteria in polar environments. The main objective of this study  
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43 375 was to explore their abundance, diversity, and distribution patterns in a significant and  
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45 typical arctic region and to evaluate their ecology and biogeographical trends. Our results  
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47 indicate that the distribution patterns generally observed in oligotrophic open ocean and  
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49 eutrophic estuarine environments of temperate and tropical regions (Jiao *et al.*, 2007; Lamy  
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51 *et al.*, 2011; Waidner & Kirchman, 2008) also occur in the Beaufort Sea.  
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### Links between AAP population abundance and environmental variables

Our results indicate that particulate and dissolved organic matter and Chl *a* concentrations positively influenced the relative abundance of AAP bacteria but did not contribute to the success of the prokaryote community as a whole. This suggests that AAP bacteria may respond to organic supply differently and have higher mineralization capacities than the bacterial community. In addition to AAP bacteria associated with the thick surface turbidity layer, proportions of AAP bacteria were high in the thinner but more turbid bottom nepheloid layer. Interestingly, AAP bacteria in surface and bottom layers showed contrasting relationships to several environmental variables, suggesting that the two particle-rich environments represent substantially different habitats. The two habitats could differ because of different types of particles in the water column, phytoplankton, and mineral-rich fine particles in the surface layer and coarse aggregates of suspended sediments in the bottom layer (Doxaran *et al.*, 2012). These particle-rich waters might be important not only in terms of sediment transport but also as sites of microbially mediated organic transformations. The latter possibility is consistent with observations of increased microbial production and activity in particle-rich surface and bottom waters of the Mackenzie plume (Vallières *et al.*, 2008; Garneau *et al.*, 2009). Although we did not quantify the proportion of particle-associated AAP bacteria, earlier studies in other nutrient-rich environments (Waidner & Kirchman, 2007) suggest that they may represent a significant part of the AAP bacterial community in the Mackenzie plume. Being associated with particles seems to enhance the synthesis of BChl *a* in estuaries (Cottrell *et al.*, 2010). It is likely that the energy gained by AAP bacteria via phototrophy in these environments is suitable for cost-intensive

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3 405 metabolic processes such as degradation of humic-rich DOM from riverine and terrestrial  
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5 run-off.  
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### 10 **AAP bacterial diversity and distribution**

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12 Most OTUs recovered were closely related (>94% similarity) to existing Arctic sequences or  
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14 410 belonged to novel OTUs. Only 8 OTUs matched sequences from elsewhere. The arctic *pufM*  
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16 genes were also different from those in Antarctic sea-ice and coastal seawater (Koh *et al.*,  
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18 2011). Consistent with previous findings (Cottrell & Kirchman, 2009), this suggests that most  
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20 OTUs found in this study might be restricted to the Beaufort Sea, which seems to constitute  
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22 a microbial province favoring endemism (Lovejoy *et al.*, 2007).  
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26 415 The most striking observation from our data set is the widespread distribution of a  
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28 AAP betaproteobacterial clade (OTU 6) in the entire shelf. *Betaproteobacteria* are usually  
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30 low in abundance in the open ocean and the few betaproteobacterial sequences that have  
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32 been retrieved from the marine environment are from coastal environments (Rappé *et al.*,  
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34 2000; Riemann *et al.*, 2008). In contrast, they represent a consistently large fraction of the  
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36 bacterioplankton in freshwater lakes and diverse river types (Glöckner *et al.*, 2000; Zwart *et*  
37  
38 420 *al.*, 2002) including the Mackenzie River where they are abundant (Garneau *et al.*, 2006;  
39  
40 Galand *et al.*, 2008). Since the Arctic Ocean receives about 10% of global riverine discharge  
41  
42 (Aagaard & Carmack, 1989), the stratified surface waters of the Arctic Ocean share many  
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44 characteristics of an estuary where *Betaproteobacteria* form a minor fraction of the total  
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46 prokaryotic community (Galand *et al.*, 2009; Kirchman *et al.*, 2010). Most  
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48 425 betaproteobacterial OTUs recovered in this study were new (< 94% similarity to existing  
49  
50 sequences) and do not group with the *Rhodoferrax*, *Roseateles*, and *Rubrivivax pufM* clusters  
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52 often identified in other estuarine and freshwater systems (Waidner & Kirchman, 2008;  
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3 Salka *et al.*, 2011). They formed several clusters divergent from the phylogroup I (Yutin *et al.*,  
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5 430 2007) that include arctic sequences (Cottrell & Kirchman, 2009) and single amplified genome  
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7 (SAG) sequences from temperate freshwater lakes (Martinez-Garcia *et al.*, 2012).  
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9 Interestingly, the 16S rRNA sequences from the latter *pufM*-containing SAGs were primarily  
10  
11 related to the betaproteobacterial *Polynucleobacter* cluster that are common in the  
12  
13 Mackenzie river (Galand *et al.*, 2008). Since horizontal gene transfer of the photosynthetic  
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15 gene cluster is possible (Igarashi *et al.*, 2001), phylogenetic data need to be interpreted with  
16  
17 435 caution especially in groups with only a few species with known *pufM* and 16S sequences.  
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22 Our data clearly indicate that betaproteobacterial AAP bacteria exhibited a strong  
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24 river to ocean gradient, suggesting that these bacteria grew in the Mackenzie River and then  
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26 were mixed with Beaufort coastal waters. The distribution of OTU 6 in the Beaufort Sea was  
27  
28 440 consistent with satellite observations of the offshore extension of Mackenzie turbid waters  
29  
30 over the continental shelf (Doxaran *et al.*, 2012). However, the presence and activity of OTU  
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32 6 in Beaufort coastal waters contrasted with that of other betaproteobacterial OTUs found  
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34 only near the mouth of the Mackenzie River. Since mortality of freshwater bacteria can be  
35  
36 an important process in estuaries (Painchaud *et al.*, 1995), one explanation is that OTU 6  
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38 445 members have a broader salinity tolerance than other AAP betaproteobacteria. The  
39  
40 presence of partial *pufM* sequences identical to that of OTU 6 in marine waters of Monterey  
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42 Bay (Béjà *et al.*, 2002) supports this hypothesis. Further studies are required to estimate  
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44 their contribution in other marine Arctic regions and to identify parameters that control  
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46 their distribution.  
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52 450 Previous studies showed that some *Betaproteobacteria* are still active in marine  
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54 waters (Cottrell & Kirchman, 2004), including in the Arctic (Alonso-Sáez *et al.*, 2008). Here,  
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56 we showed that the OTU 6 *pufM* gene was actively transcribed in saline waters during the  
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3 arctic summer, which is surprising because BChl *a* synthesis is partly or totally inhibited by  
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5 light in AAP bacteria currently in culture (Yurkov & van Gemerden, 1993; Tomasch *et al.*,  
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7  
8 455 2011). Our data suggest that BChl *a* synthesis in OTU 6 members is not fully repressed by  
9  
10 light, like in *Dinoroseobacter shibae* and *Roseateles depolymerans* (Suyama *et al.*, 2002;  
11  
12 Tomasch *et al.*, 2011). In most AAP bacteria isolated so far, photoheterotrophy generates  
13  
14 additional metabolic energy enhancing AAP bacterial growth under light (Koblížek *et al.*,  
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16 2003; Cooney *et al.*, 2006). Considering the variety of sharp environmental gradients  
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19 460 encountered by OTU 6 members in the transition zone between freshwater and marine  
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21 waters, it is likely that, when light is sufficient, photoheterotrophy may have different  
22  
23 physiological roles in warm, organic matter-rich freshwater and in cold, saline oligotrophic  
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25 waters. The nature of their physiological adaptation and metabolic versatility needs to be  
26  
27 elucidated to understand the selective advantage that photoheterotrophy provides them.  
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31 465 To our knowledge, AAP strains have not previously been isolated from the Arctic  
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33 Ocean. In this study, the isolates provided a valuable set of data that allowed the taxonomic  
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35 identification of two predominant *pufM* phylotypes. We successfully isolated *pufM*-  
36  
37 containing bacteria, but only from surface waters, consistent with the phototrophic  
38  
39 character of AAP bacteria. Our arctic isolates were restricted to different species of the  
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41  
42 470 genera *Sulfitobacter* and *Loktanella*. Although these species were cultivated from temperate  
43  
44 to polar regions (Labrenz *et al.*, 2000; Van Trappen *et al.*, 2004; Salka *et al.*, 2008), the strains  
45  
46 isolated in this study were most similar to microorganisms residing in polar waters. Among  
47  
48 them, the *Sulfitobacter* strains clustering into OTU 5 were absent outside the Beaufort Sea,  
49  
50 suggesting that they potentially represent typical arctic members. *Sulfitobacter* and  
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53 475 *Loktanella* species are often found in surface waters and numerous interactions with  
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55 phytoplankton have been reported (Moran *et al.*, 2007). These metabolically versatile  
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3 bacteria can satisfy a significant part of their carbon and sulfur demands by assimilating  
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5 DMSP released during the decay of phytoplankton blooms (González *et al.*, 1999; Mou *et al.*,  
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7 2005). The Beaufort Sea waters sampled during this study exhibited post-bloom  
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10 480 characteristics, with low levels of Chl *a* in the surface layer. Since surface waters of the  
11  
12 Beaufort Sea are oligotrophic in summer, the capacity to derive energy from light and  
13  
14 reduced sulfur compounds would give a physiological advantage for competing in this  
15  
16 extreme environment. The influence of light and starvation on BChl *a* and biomass formation  
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18 has been studied in AAP bacteria such as *Sulfitobacter* and *Loktanella* that contain low  
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20  
21 485 amounts of BChl *a* (Biebl & Wagner-Döbler, 2006). Since this type of AAP bacteria seems to  
22  
23 use phototrophy genes only under simultaneous illumination and extreme shortage of  
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25 organic nutrients, their distribution in arctic waters may be linked to their  
26  
27 photoheterotrophic mode of metabolism.  
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31 AAP diversity was highest at the shelf and offshore DCM layers of the Beaufort Sea  
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33 490 where Pacific Summer Water mixes with the BNL. *Methylobacterium*- and *Sphingomonas*-  
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35 like bacteria, mostly absent in surface waters, were common at these depths along the  
36  
37 Mackenzie plume. Although a few species of both genera have been isolated from seawater  
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39 (Vancanneyt *et al.*, 2001; Wang *et al.*, 2007), these bacteria have a widespread distribution  
40  
41 in diverse terrestrial habitats including soil, freshwater, and lake sediments (White *et al.*,  
42  
43 495 1996; Green, 2006). Our data suggest that *Methylobacterium*- and *Sphingomonas*-like  
44  
45 bacteria share the same habitat, more likely associated with soluble humic material in river  
46  
47 water (Kirk, 2011). In line with this hypothesis, these bacteria were found to actively  
48  
49 participate in humic matter degradation (Balkwill *et al.*, 2006; Hutalle-Schmelzer *et al.*,  
50  
51 2010). Two groups of *Bradyrhizobiaceae*-related sequences were detected mostly in DCM  
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57 500 samples of the Beaufort Sea shelf. One group was loosely associated to the metabolically  
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3 versatile photosynthetic bacterium *Rhodopseudomonas palustris* that is widely distributed in  
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5 nature including coastal marine sediments (Oda *et al.*, 2003). The other grouped with strictly  
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7 aerobic anoxygenic phototrophic *Bradyrhizobium* strains that form nitrogen-fixing nodules  
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9 on stems and/or roots of many legumes (Giraud & Fleischman, 2004). Like  
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11  
12 505 *Methylobacterium* and *Sphingomonas* clusters, these clades were mostly recovered in the  
13  
14 deep layers of the Mackenzie plume, suggesting that they were also entrained by the river  
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16 sediment load. However, their distribution was similar to that of *Citromicrobium*-like clades  
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18 that are typical marine bacteria (Yurkov *et al.*, 1999; Jeanthon *et al.*, 2011). Furthermore, we  
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20 also retrieved them from North Pacific Ocean surface waters, supporting earlier reports of  
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24 510 *Bradyrhizobiaceae* in the North Pacific Ocean gyre and the Bering Sea where terrestrial  
25  
26 inputs are unlikely (Hu *et al.*, 2006). It is therefore tempting to speculate that arctic  
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28 *Bradyrhizobiaceae*-related *pufM* sequences are marine photosynthetic bacteria possibly  
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30 transported in Pacific waters.  
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## 36 515 **Conclusion**

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38 Our transect analysis in the coastal arctic ecosystem of the Mackenzie River and Beaufort  
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40 Sea shelf revealed strong spatial gradients in environmental properties and in the  
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42 abundance and composition of AAP bacterial communities. We showed that AAP bacteria  
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44 represent a significant fraction of the total prokaryotic community in the arctic coastal  
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48 520 environments impacted by riverine inputs. Our results highlight that OTU 6 members are an  
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50 important and widely distributed but largely unknown component of AAP bacterial  
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52 communities. However, we do not know the impact of phototrophic light harvesting on  
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54 energy requirements and carbon metabolism of these bacteria. Future studies are needed to  
55  
56 explore their role in biogeochemical cycling in coastal ecosystems.  
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For Peer Review



## Legends to figures

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8 **Fig. 1.** Locations of stations sampled during legs 1b and 2b of MALINA cruise. Leg 1b stations  
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10 760 (black squares) were sampled only at the surface; leg 2b stations were sampled at several  
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12 depths (black dots) or only at the surface (black triangles). Circles indicate stations where  
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14 *pufM* clone libraries were constructed. Grey shades correspond to bottom depths.  
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20 **Fig. 2.** Spatial distribution of total prokaryotes (cells ml<sup>-1</sup>) and AAP bacteria (%) in surface  
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22 765 waters of the Beaufort Sea (a, e) and along the transects 600 (b, f), 300 (c, g) and 200 (d, h)  
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24 as shown on the map (e).  
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30 **Fig. 3.** Phylogenetic tree showing the relationships between *pufM* gene sequences from  
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32 MALINA isolates (in bold) and environmental samples (in blue) and relatives retrieved in  
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34 770 GenBank (in black). Asterisks indicate the three most abundant OTUs in clone libraries. The  
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36 tree is based on a bayesian tree to which short sequences were added by ARB\_PARSIMONY.  
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38 Initial bayesian tree is shown in bold lines. The numbers on nodes represent branch  
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40 confidence values. Vertical bars indicate taxonomic groups defined by Yutin et al. (2007). The  
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42 scale bar represents dissimilarity between nucleotide positions.  
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48 775 **Fig. 4.** Relative abundance of phylogenetic clusters in samples from the North Pacific waters  
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50 (leg 1b) to the Beaufort Sea (leg 2b). Numbers of analyzed clones in each library are  
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52 indicated.  
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3 **Fig. 5.** Phylogenetic analysis based on the 16S rRNA sequences indicating the position of  
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5 780 MALINA isolates. Numbers show nodal supports from bayesian posterior probabilities, and  
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7 maximum likelihood, maximum parsimony and neighbor-joining analyses, respectively. The  
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9 scale bar represents dissimilarity percentage between nucleotide positions.  
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15 **Fig. 6.** Surface relative abundance of OTU 6 members (qPCR) and AAP bacteria (IR  
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17 785 microscopy) along transects 300 and 600 in relation with temperature and salinity.  
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**Table 1.** Main features of T-RFLP groups determined among the 92 AAP isolates and closest relatives based on the 16S rRNA and *pufM* gene sequences.

T-RF size (bp)		Nb of strains	Representative strains	16S rRNA gene closest relative			<i>pufM</i> gene closest relative		
FAM <sup>a</sup>	HEX <sup>b</sup>			Name	Accession number	% similarity	Name	Accession number	% similarity
300	121; 123	47	RCC 2406 (RCC 2405; RCC 2400)	<i>Sulfitobacter</i> sp. ZS5-11	FJ196046	99	Unc.bacterium MALINA OTU5	JN248469	100
298	123; 125	2	RCC 2401	<i>Sulfitobacter</i> sp. ZS2020	FJ889550	99.6	Unc.bacterium MALINA OTU4	JN248468	92
299	123; 125	41	RCC 2403 (RCC 2402; RCC 2404)	<i>Loktanella</i> sp. WM1	EF421433	100	Unc.bacterium MALINA OTU29	JN248513	99
298	123; 124;125	2	RCC 2642	<i>Loktanella</i> <i>salsilacus</i> LMG 22002	AJ582229	99.5	Unc.bacterium MALINA OTU29	JN248513	99

<sup>a</sup>FAM, carboxyfluorescein; <sup>b</sup>HEX, hexachlorofluorescein; T-RF, terminal restriction fragment;

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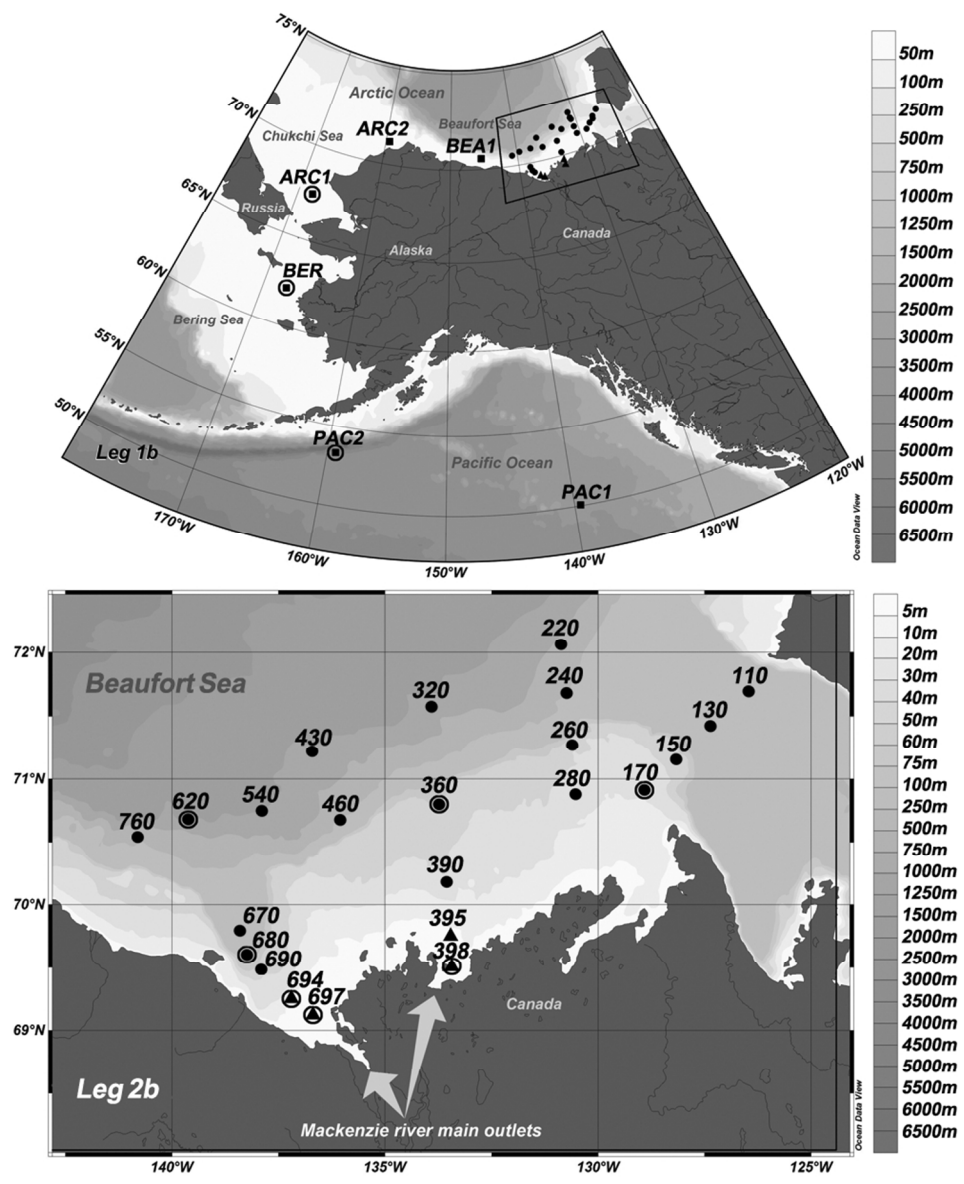


Fig. 1. Locations of stations sampled during legs 1b and 2b of MALINA cruise. Leg 1b stations (black squares) were sampled only at the surface; leg 2b stations were sampled at several depths (black dots) or only at the surface (black triangles). Circles indicate stations where pufM clone libraries were constructed. Grey shades correspond to bottom depths. 178x209mm (150 x 150 DPI)

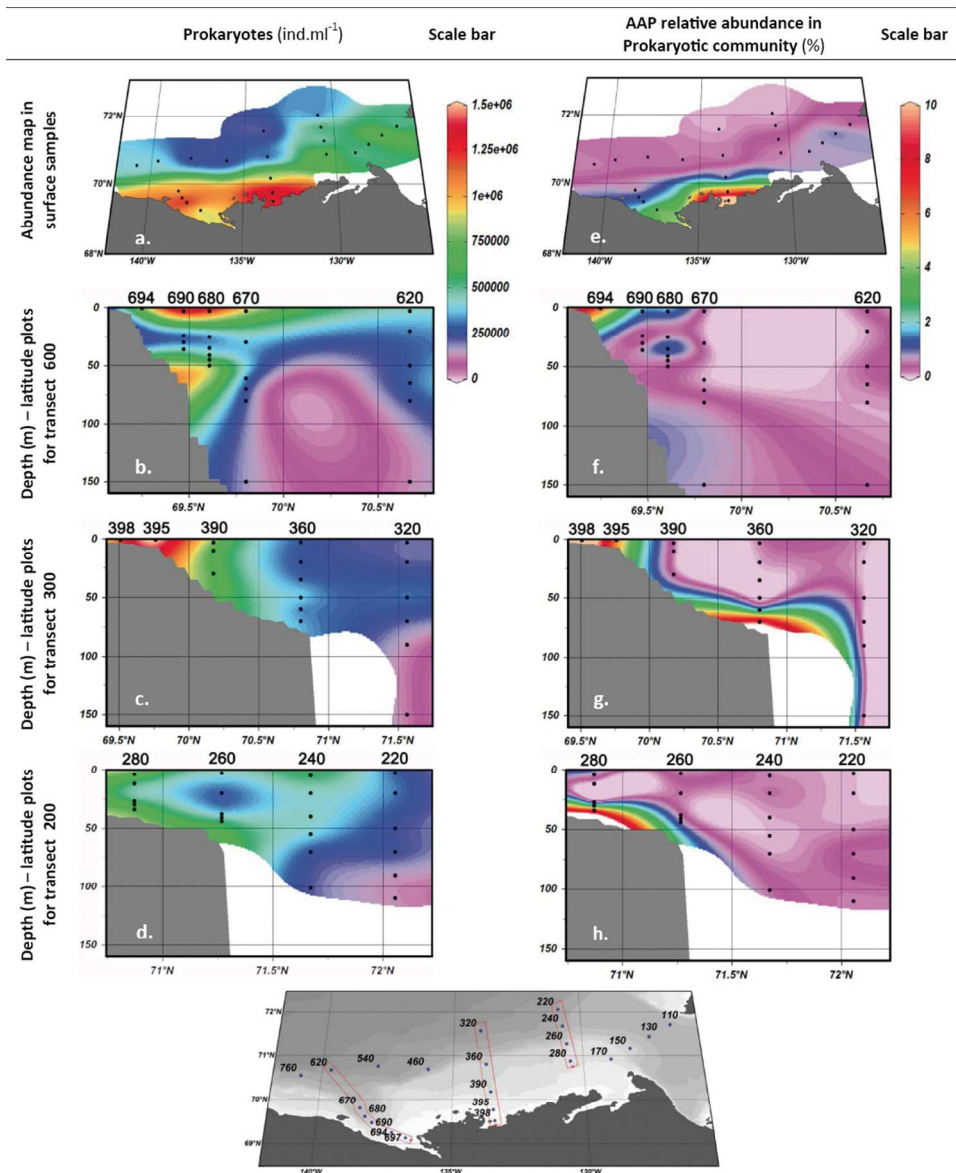


Fig. 2. Spatial distribution of total prokaryotes (cells.ml<sup>-1</sup>) and AAP bacteria (%) in surface waters of the Beaufort Sea (a, e) and along the transects 600 (b, f), 300 (c, g) and 200 (d, h) as shown on the map (e).  
187x236mm (150 x 150 DPI)

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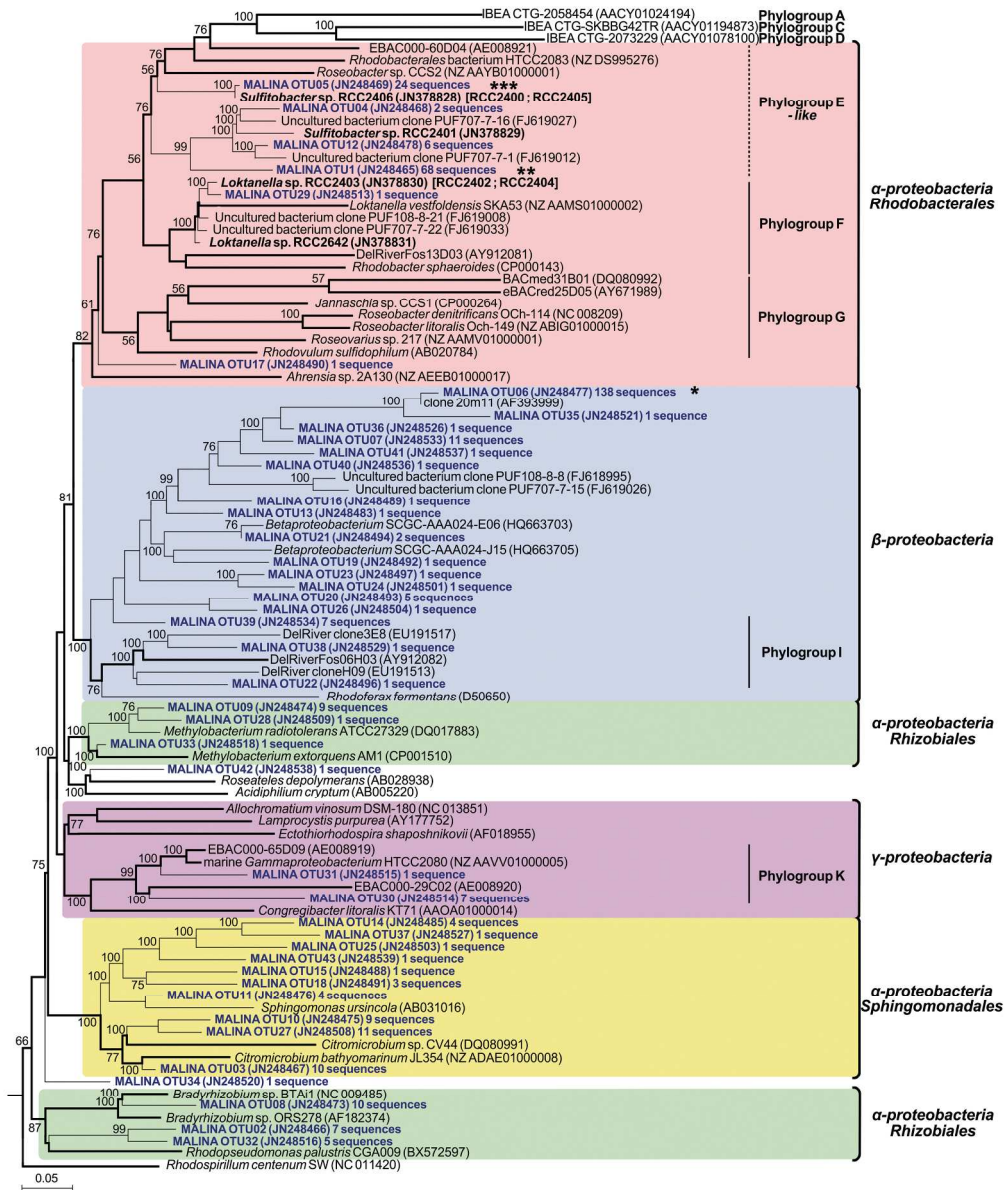


Fig. 3. Phylogenetic tree showing the relationships between *pufM* gene sequences from MALINA isolates (in bold) and environmental samples (in blue) and relatives retrieved in GenBank (in black). Asterisks indicate the 3 most abundant OTUs in clone libraries. The tree is based on a bayesian tree to which short sequences were added by ARB\_PARSIMONY. Initial bayesian tree is shown in bold lines. The numbers on nodes represent branch confidence values. Vertical bars indicate phylogroups defined by Yutin et al. (2007). The scale bar represents dissimilarity between nucleotide positions.

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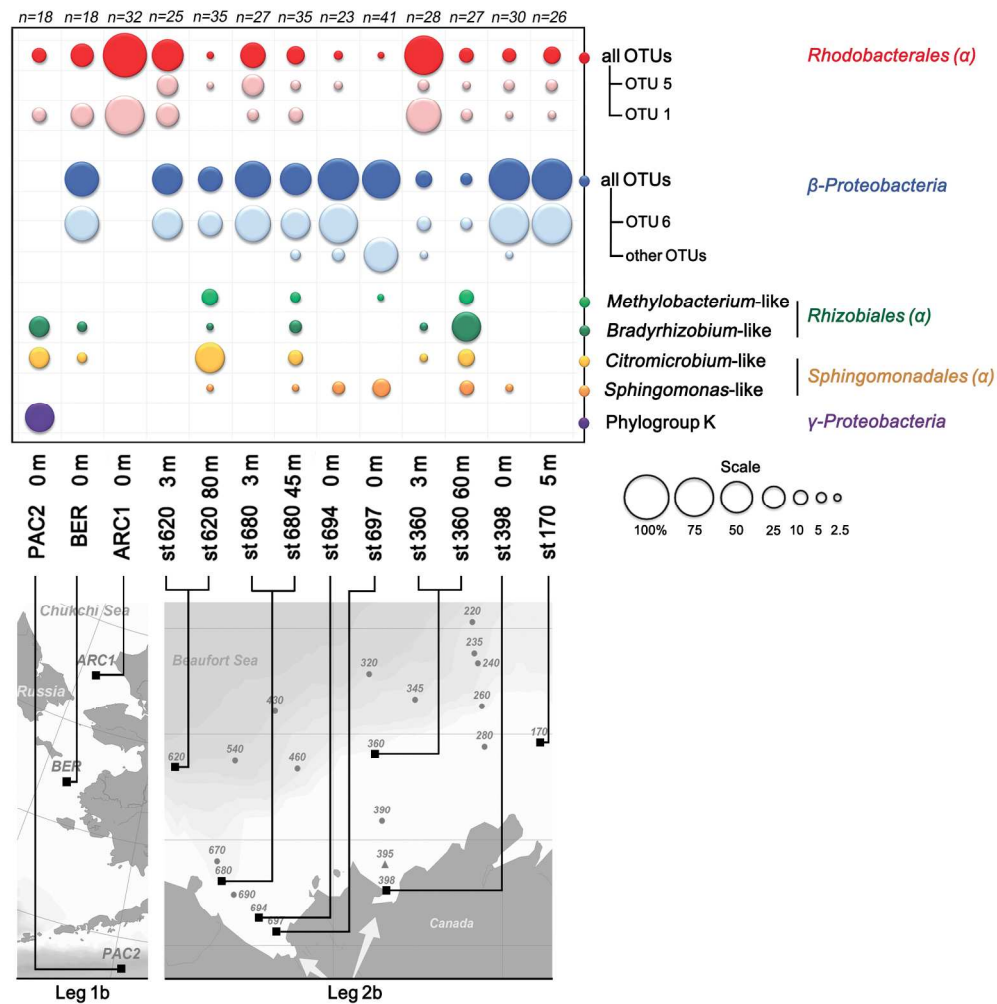


Fig. 4. Relative abundance of phylogenetic clusters in samples from the North Pacific waters (leg 1b) to the Beaufort Sea (leg 2b). Numbers of analyzed clones in each library are indicated.  
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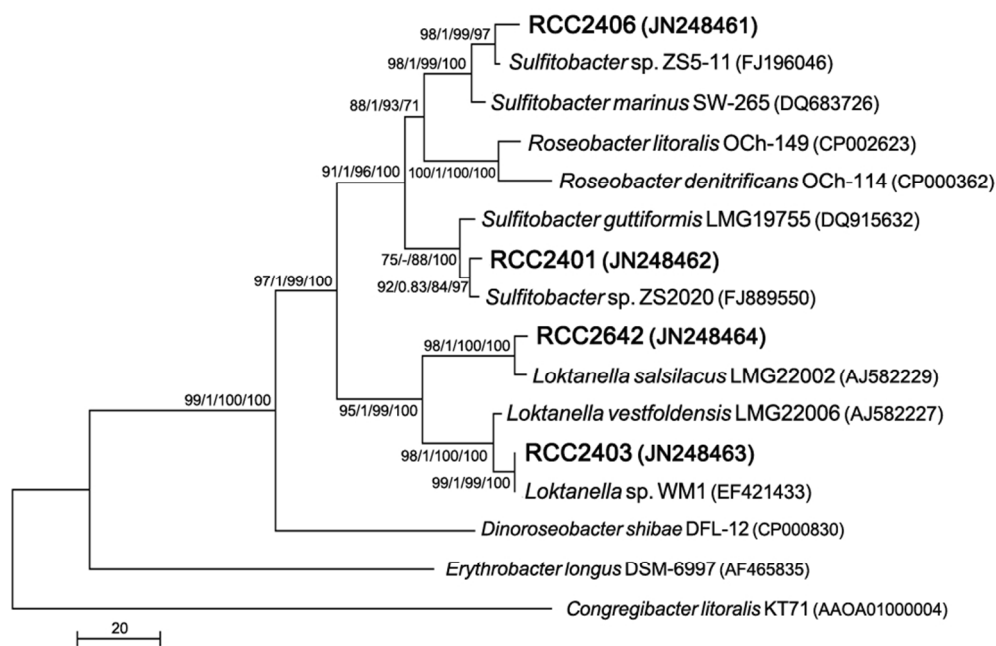


Fig. 5. Phylogenetic analysis based on the 16S rRNA sequences indicating the position of MALINA isolates. Numbers show nodal supports from bayesian posterior probabilities, and maximum likelihood, maximum parsimony and neighbor-joining analyses, respectively. The scale bar represents dissimilarity percentage between nucleotide positions.  
173x115mm (150 x 150 DPI)



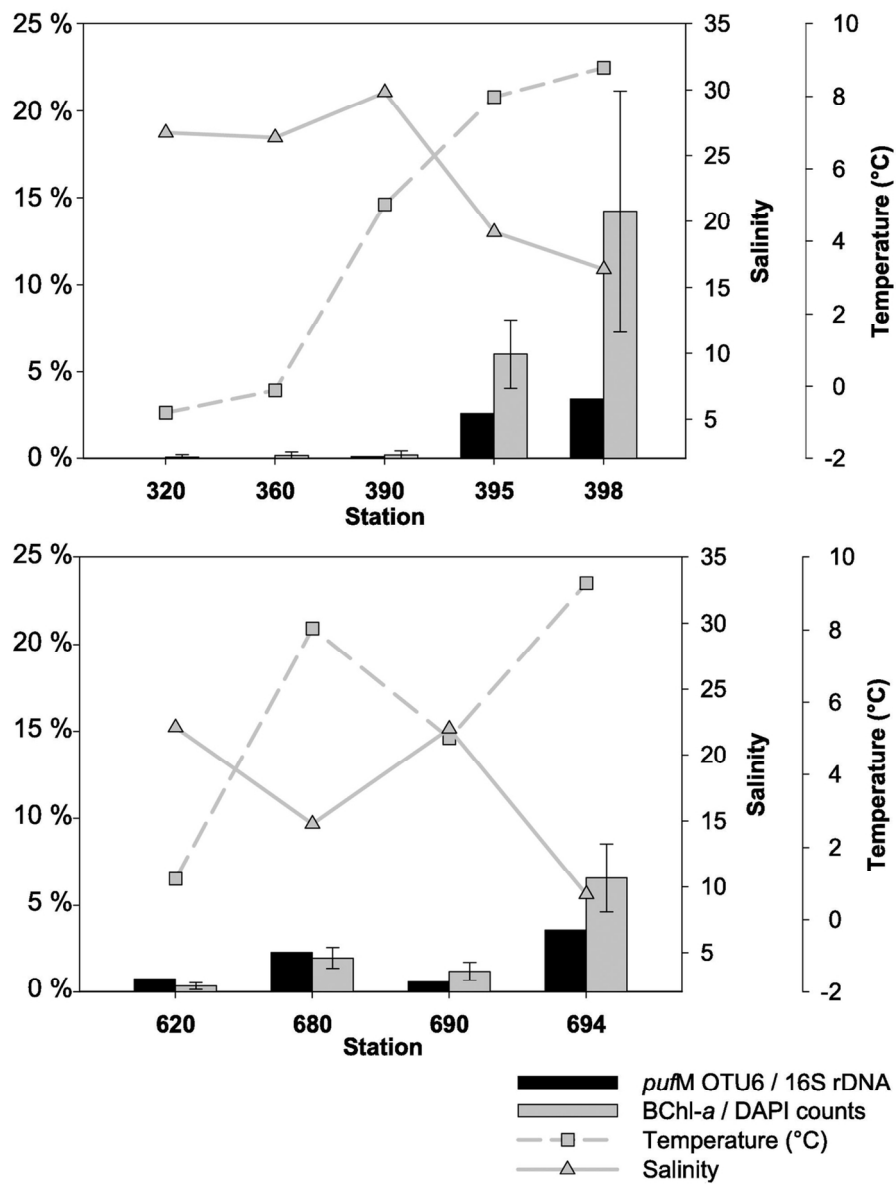


Fig. 6. Surface relative abundance of OTU 6 members (qPCR) and AAP bacteria (IR microscopy) along transects 300 and 600 in relation with temperature and salinity.  
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### Supporting information

The supporting information is composed of 6 files and contains 4 tables (Tables S1 to S4) and 2 figures (Fig. S1 and S2).

Below are the legends of the supplementary tables and figures:

**Table S1.** Summary of environmental parameters of water masses delineated during the MALINA cruise. For each, the median and range are reported.

**Table S2.** Summary of primers used in this study.

**Table S3.** Correlation of total and AAP bacterial abundances ( $p < 0.05$ ) over the entire data set and along Mackenzie-influenced transects.

**Table S4.** Clone library comparison of seawater samples collected during the MALINA cruise in summer 2009.

**Fig. S1.** Temperature-salinity diagram of the water masses observed in the Beaufort Sea during the MALINA cruise. Black dots correspond to water samples; numbers indicate the station and depth sampled. The inset gives an enlarged view of the diagram below.

**Fig. S2.** Dendrogram representing the similarity between the phylogenetic composition of *pufM* clone libraries. Clustering is based on a distance matrix computed with Dice similarity from the abundance of OTUs in clone libraries defined by a 94% identity threshold between sequences. The dendrogram was inferred with the UPGMA algorithm.

**Table S1.** Summary of environmental parameters of water masses delineated during the MALINA cruise. For each, the median and range are reported.

Parameters <sup>a</sup>	Water samples categories			
	River plume surface	Shelf surface	Offshore surface	Other depths
n =	5	29	17	60
Max depth sampled (m)	surface	50	50	151
Temperature (°C)	<b>8.8</b> (7.99 ; 10.32)	<b>1.03</b> (-0.87 ; 5.02)	<b>-0.58</b> (-1.04 ; 1.13)	<b>-1.21</b> (-1.43 ; -0.93)
Salinity (psu)	<b>14.77</b> (0.15 ; 19.17)	<b>30.17</b> (21.98 ; 32.2)	<b>28.02</b> (22.09 ; 30.2)	<b>31.92</b> (30.3 ; 33.65)
O <sub>2</sub> (mg.l <sup>-1</sup> )	<b>8.07</b> (7.34 ; 10.48)	<b>8.74</b> (6.94 ; 10.21)	<b>8.89</b> (7.98 ; 9.22)	<b>7.68</b> (6.18 ; 9.35)
pH	<b>8.04</b> (8.04 ; 8.32)	<b>8.1</b> (7.91 ; 8.34)	<b>8.11</b> (8.03 ; 8.15)	<b>8.02</b> (7.84 ; 8.17)
SiOH <sub>4</sub> (μM)	<b>30.27</b> (3.52 ; 64.53)	<b>3.92</b> (3.12 ; 27.9)	<b>3.27</b> (2.89 ; 7.82)	<b>17.95</b> (3.38 ; 37.19)
cDOM (V.m <sup>-1</sup> )	<b>0.12</b> (0.09 ; 0.15)	<b>0.09</b> (0.08 ; 0.14)	<b>0.08</b> (0.07 ; 0.13)	<b>0.1</b> (0.08 ; 0.12)
NO <sub>3</sub> (μM) <sup>b</sup>	<b>0.7</b> (0 ; 3.62)	<b>0.02</b> (0 ; 6.49)	<b>0.01</b> (0 ; 0.02)	<b>6.15</b> (0 ; 15.65)
NO <sub>2</sub> (μM) <sup>b</sup>	<b>0.01</b> (0 ; 0.07)	<b>0.01</b> (0 ; 0.12)	<b>0</b> (0 ; 0.03)	<b>0.05</b> (0 ; 0.15)
NH <sub>4</sub> (μM) <sup>b</sup>	<b>0.2</b> (0.07 ; 0.64)	<b>0.02</b> (0 ; 0.43)	<b>0</b> (0 ; 0.08)	<b>0.02</b> (0 ; 0.43)
PO <sub>4</sub> (μM)	<b>0.06</b> (0 ; 0.21)	<b>0.6</b> (0.31 ; 1.36)	<b>0.58</b> (0.33 ; 0.72)	<b>1.24</b> (0.62 ; 2.97)
TOC (μM)	<b>313.7</b> (199 ; 462.5)	<b>79.01</b> (66.15 ; 135)	<b>65.45</b> (60.62 ; 112)	<b>64.09</b> (52.55 ; 101)
POC (μM)	<b>16.15</b> (12.49 ; 52)	<b>7.91</b> (0 ; 41.16)	<b>2.18</b> (0.92 ; 5.61)	<b>3.15</b> (0.39 ; 43.2)
DOC (μM)	<b>295.8</b> (183 ; 454.6)	<b>68.65</b> (58.34 ; 107)	<b>62.34</b> (57.8 ; 108.4)	<b>60</b> (43.19 ; 83.2)
TON (μM)	<b>17.36</b> (12.65 ; 29.7)	<b>6.75</b> (5.11 ; 13.87)	<b>5.85</b> (4.52 ; 16.27)	<b>7.28</b> (3.21 ; 15.02)
PON (μM)	<b>3.42</b> (1.71 ; 15.84)	<b>0.83</b> (0 ; 3.14)	<b>0.28</b> (0.17 ; 0.77)	<b>0.36</b> (0.14 ; 2.65)
DON (μM)	<b>13.69</b> (10.94 ; 26.7)	<b>5.91</b> (4.43 ; 11.22)	<b>5.57</b> (4.34 ; 15.83)	<b>6.76</b> (3.02 ; 14.68)
TOP (μM)	<b>0.88</b> (0.23 ; 2.82)	<b>0.24</b> (0.11 ; 0.66)	<b>0.21</b> (0.12 ; 0.59)	<b>0.2</b> (0.02 ; 0.78)
POP (μM)	<b>0.56</b> (0.13 ; 4.96)	<b>0.06</b> (0 ; 0.24)	<b>0.02</b> (0.01 ; 0.06)	<b>0.02</b> (0.01 ; 0.24)
DOP (μM)	<b>0.22</b> (0.1 ; 1.99)	<b>0.17</b> (0.09 ; 0.53)	<b>0.19</b> (0.11 ; 0.56)	<b>0.17</b> (0 ; 0.76)
Total Chl <i>a</i> (μg.l <sup>-1</sup> )	<b>2.3</b> (0.18 ; 4.53)	<b>0.16</b> (0.02 ; 3.23)	<b>0.05</b> (0.01 ; 0.1)	<b>0.17</b> (0.01 ; 6.43)
Prokaryotes (cells.ml <sup>-1</sup> )	<b>2.56 10<sup>5</sup></b> (8.7 10 <sup>4</sup> ; 1.2 10 <sup>6</sup> )	<b>2.85 10<sup>5</sup></b> (6.3 10 <sup>4</sup> ; 1.0 10 <sup>6</sup> )	<b>2.78 10<sup>5</sup></b> (1.4 10 <sup>5</sup> ; 6.5 10 <sup>5</sup> )	<b>1.99 10<sup>5</sup></b> (3.7 10 <sup>4</sup> ; 9.7 10 <sup>5</sup> )
AAP bacteria (cells.ml <sup>-1</sup> )	<b>1.60 10<sup>4</sup></b> (1.2 10 <sup>4</sup> ; 2.3 10 <sup>4</sup> )	<b>1.02 10<sup>3</sup></b> (0 ; 8.6 10 <sup>3</sup> )	<b>1.51 10<sup>2</sup></b> (0 ; 2.2 10 <sup>3</sup> )	<b>2.33 10<sup>2</sup></b> (0 ; 3.6 10 <sup>4</sup> )
AAP bacteria (%)	<b>6.31</b> (1.96 ; 14.23)	<b>0.23</b> (0 ; 1.53)	<b>0.07</b> (0 ; 0.52)	<b>0.16</b> (0 ; 9.71)

<sup>a</sup> Ancillary data of temperature, salinity, pH, dissolved oxygen, colored dissolved organic matter, inorganic and organic nutrients, and chlorophyll *a* were provide by D. Doxaran, P. Raimbault, N. Garcia and J. Ras.

<sup>b</sup> The detection limit is 3 nM for nitrate and nitrite and 5 nM for ammonium.

**Table S2.** Summary of primers used in this study

Experiment	Gene	Group	Primer name	Primer sequence (5'-3')	Annealing temp (°C)	Amplicon length (bp)	Reference
t-RFLP	16S rDNA	Bacteria	27F	AGAGTTTGATCMTGGCTCAG	55	1465	(Lane <i>et al.</i> , 1991)
			1492R	CGGTTACCTTGTACGACTT			(Lane <i>et al.</i> , 1991)
PCR	16S rDNA	Bacteria	8F	AGAGTTTGATCCTGGCTCAG	55	1465	(Lane <i>et al.</i> , 1991)
			1492R	CGGTTACCTTGTACGACTT			(Lane <i>et al.</i> , 1991)
PCR / TTGE	<i>pufM</i>	AAPB	PufMF	TACGGSAACCTGTWCTAC	58	245	(Beja <i>et al.</i> , 2002)
			Puf-WAW	AYNGCRAACCACCANGCCCA			(Yutin <i>et al.</i> , 2005)
qPCR	16S rDNA	Bacteria	BACT1369F	CGGTGAATACGTTTCYCGG	56	142	(Suzuki <i>et al.</i> , 2000)
			PROK1492R	GGWTACCTTGTACGACTT			(Suzuki <i>et al.</i> , 2000)
qPCR	<i>pufM</i>	AAPB-	OTU6-f23	CCACGCACTATCCATTGT	60	146	This study
		OTU6	OTU6-r34	GGGCGGCGGCTCGGAAG			This study

**Table S3.** Correlation of total and AAP bacterial abundances ( $p < 0.05$ ) over the entire data set and along Mackenzie-influenced transects.

Variables	All samples ( $n=110$ )		All depths of transects 600 & 300 ( $n=40$ )	
	Prokaryotic cells	AAP proportions	Prokaryotic cells	AAP proportions
Temperature	0.24	0.46**	<i>ns</i> <sup>a</sup>	0.59**
Salinity	– 0.23	– 0.45**	<i>ns</i>	– 0.55**
O <sub>2</sub>	<i>ns</i>	– 0.20	<i>ns</i>	<i>ns</i>
cDOM	<i>ns</i>	0.28*	<i>ns</i>	0.32
PAR	0.2	<i>ns</i>	<i>ns</i>	<i>ns</i>
TIC	– 0.24	<i>ns</i>	– 0.32	<i>ns</i>
HCO <sub>3</sub>	– 0.24	<i>ns</i>	<i>ns</i>	<i>ns</i>
NO <sub>3</sub>	– 0.20	<i>ns</i>	– 0.31	<i>ns</i>
NO <sub>2</sub>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
NH <sub>4</sub>	<i>ns</i>	0.48**	<i>ns</i>	0.47*
PO <sub>4</sub>	– 0.23	<i>ns</i>	– 0.35	<i>ns</i>
SiOH <sub>4</sub>	<i>ns</i>	0.35**	– 0.34	0.47*
POC	0.24	0.42**	<i>ns</i>	0.57**
PON	<i>ns</i>	0.74**	<i>ns</i>	0.80**
POP	<i>ns</i>	0.77**	<i>ns</i>	0.82**
DOC	<i>ns</i>	0.65**	<i>ns</i>	0.74**
DON	<i>ns</i>	0.59**	<i>ns</i>	0.74**
DOP	<i>ns</i>	0.62**	<i>ns</i>	0.68**
TOC	<i>ns</i>	0.66**	<i>ns</i>	0.73**
TON	0.22	0.63**	<i>ns</i>	0.74**
TOP	<i>ns</i>	0.70**	<i>ns</i>	0.76**
Total Chl <sub>a</sub>	<i>ns</i>	0.43**	<i>ns</i>	0.78**

<sup>a</sup>*ns*, not significant ; \*,  $p < 0.005$  ; \*\*,  $p < 0.001$

**Table S4.** Clone library comparison of seawater samples collected during the MALINA cruise in summer 2009.

Location	Station-depth	Nb. of clones	Nb. of OTUs <sup>a</sup>	Coverage (%)	S <sub>Chao1</sub> (LCI-UCI) <sup>b</sup>	H <sub>Shannon</sub> (LCI-UCI)
<b>leg1b</b>	PAC2 0m	18	7	83	8 (7-18)	1.67 (1.29-2.05)
	BER 0m	18	4	89	5 (4-17)	0.98 (0.6-1.35)
	ARC1 0m	32	2	100	2 (2-2)	0.48 (0.28-0.69)
<b>leg2b</b>	170 3m	26	4	92	4.5 (4-12)	0.59 (0.19-0.98)
	360 3m	28	7	89	8 (7-18)	1.26 (0.82-1.69)
	360 60m	27	8	96	8	1.94 (1.71-2.17)
<b>Beaufort</b>	620 3m	25	3	100	3 (3-3)	1.05 (0.91-1.2)
<b>Sea</b>	620 80m	35	10	83	25 (13-78)	1.84 (1.53-2.14)
	680 3m	27	3	100	3 (3-3)	0.81 (0.56-1.07)
	680 45m	35	11	86	13.5 (11-28)	1.85 (1.48-2.23)
<b>leg2b</b>	694 0m	23	6	78	16 (8-58)	0.87 (0.37-1.38)
<b>Mackenzie</b>	697 0m	41	20	63	72.5 (34-213)	2.59 (2.27-2.91)
<b>plume</b>	398 0m	30	5	90	6.5 (5-20)	0.67 (0.27-1.08)

<sup>a</sup> Operational taxonomic units (OTUs) were defined with Mothur assuming a 94% sequence similarity level.

<sup>b</sup> LCI and UCI are the lower and upper bound of 95% confidence interval of the Chao1 and Shannon indexes, respectively.

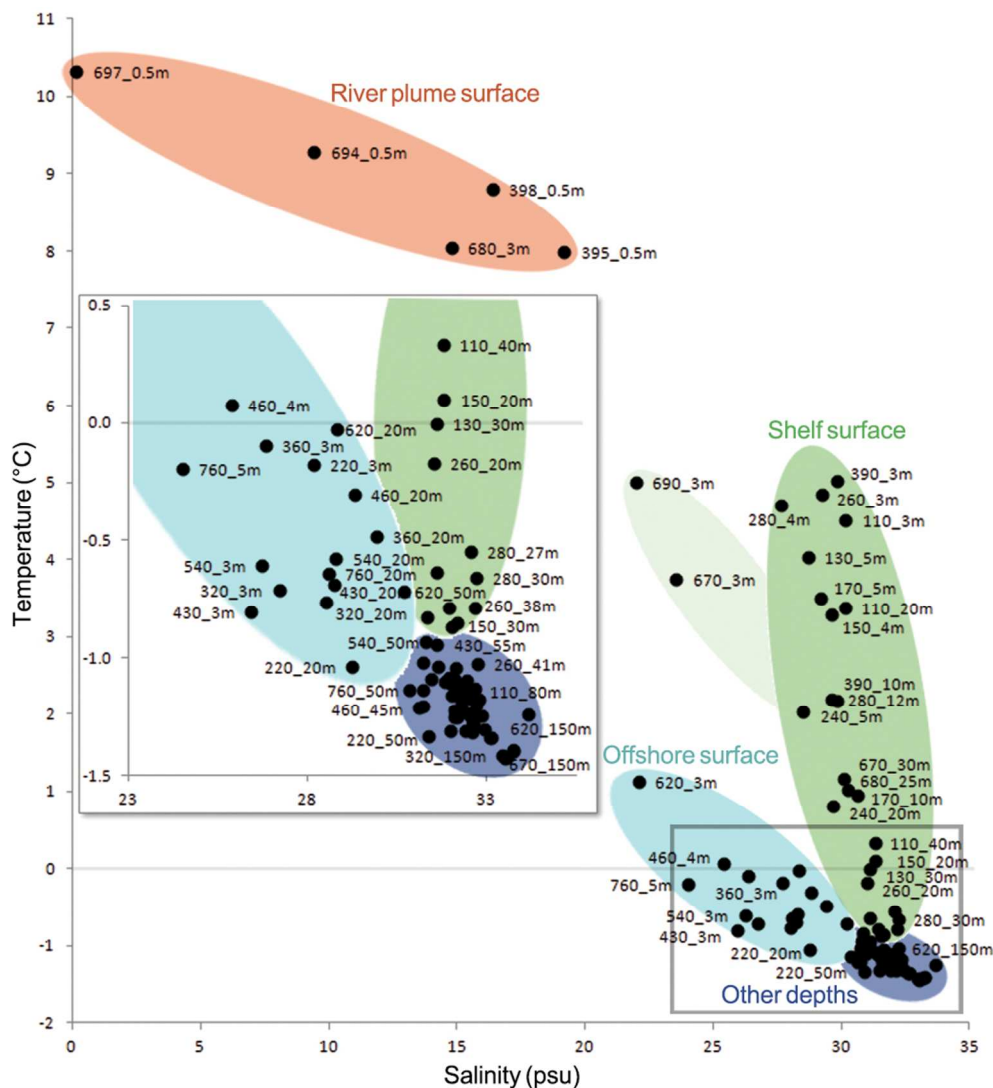


Fig. S1. Temperature-salinity diagram of the water masses observed in the Beaufort Sea during the MALINA cruise. Black dots correspond to water samples; numbers indicate the station and depth sampled. The inset gives an enlarged view of the diagram below.  
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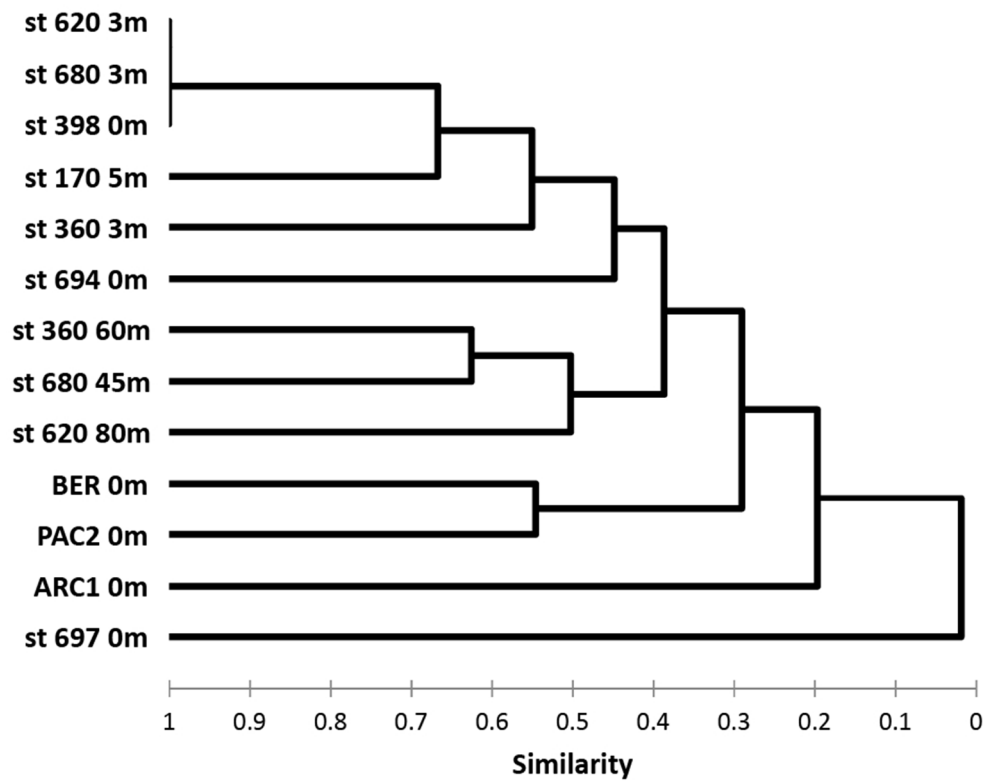


Fig. S2. Dendrogram representing the similarity between the phylogenetic composition of pufM clone libraries. Clustering is based on a distance matrix computed with Dice similarity from the abundance of OTUs in clone libraries defined by a 94% identity threshold between sequences. The dendrogram was inferred with the UPGMA algorithm.  
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