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Archaeal diversity and a gene for ammonia oxidation are coupled to oceanic circulation

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Summary

Evidence of microbial zonation in the open ocean is rapidly accumulating, but while the distribution of communities is often described according to depth, the other physical factors structuring microbial diversity and function remain poorly understood. Here we identify three different water masses in the North Water (eastern Canadian Arctic), defined by distinct temperature and salinity characteristics, and show that they contained distinct archaeal communities. Moreover, we found that one of the water masses contained an increased abundance of the archaeal alpha-subunit of the ammonia monooxygenase gene (*amoA*) and accounted for 70% of the *amoA* gene detected overall. This indicates likely differences in putative biogeochemical capacities among different water masses. The ensemble of our results strongly suggest that the widely accepted view of depth stratification did not explain microbial diversity, but rather that parent water masses provide the framework for predicting communities and potential microbial function in an Arctic marine system. Our results emphasize that microbial distributions are strongly influenced by oceanic circulation, implying that shifting currents and water mass boundaries resulting from climate change may well impact patterns of microbial diversity by displacing whole biomes from their historic distributions. This relocation could have the potential to establish a substantially different geography of microbial-driven biogeochemical processes and associated oceanic production.

Received 30 January, 2008; accepted 20 October, 2008. *For correspondence. E-mail pgaland@ceab.csic.es; Tel. (+34) 972336 101; Fax (+34) 972337 806. †Our colleague and mentor Grant Ingram died suddenly on 13 June 2007.

Introduction

Microbes are abundant and ubiquitous component of oceans, and are a fundamental element of the marine biotic engine (Karl, 2007). They drive fluxes of matter and energy, and through their role in biogeochemical cycling, they influence the earth's oceanic and atmospheric composition (Cotner and Biddanda, 2002). Traditionally, the distributions of the major marine prokaryotic groups have been described according to depth (Giovannoni *et al*., 1996; Massana *et al*., 1997; Murray *et al*., 1998; DeLong *et al*., 2006). *Bacteria* and *Euryarchaeota* are thought to be more abundant in the upper part of the water column, while *Crenarchaeota* are thought to represent a larger fraction of the prokaryotic community at greater depths (Massana *et al*., 2000; Karner *et al*., 2001; Church *et al*., 2003). Recent reports, however, indicate that the distribution of marine microorganisms may not be structured by depth *per se*, and water masses with distinct temperature (θ) and salinity (S) signatures possibly carry their own microbial assemblages (Lovejoy *et al*., 2002; Hamilton *et al*., 2008; Varela *et al*., 2008).

The productivity of the global ocean is affected by high northern latitude water masses. The upper 200 m waters from the North Pacific, which are depleted in nitrate and enriched in phosphate, flow through the Arctic and eventually into the North Atlantic (Yamamoto-Kawai *et al*., 2006). The quantity of excess phosphate to nitrate reaching more southern seas will have a profound influence on the net production and species composition of primary producers in those waters; therefore it is imperative to understand the details of nitrogen cycling in northern regions.

The goal of this study was to test whether distinct water masses have specific microbial communities, a crucial step to evaluate if future changes in ocean circulation will affect patterns of microbial biogeochemical cycling. We evaluated the molecular diversity of *Archaea* in the so-called North Water between Ellesmere Island and Greenland. This area is one of the most productive in the Arctic and was historically an important whaling region (Sterling, 1980). Here, outward flowing Arctic waters,

derived from a mix of waters with Pacific and Atlantic origins, enter from Nares Straight and converge with seasonally locally produced Baffin Bay waters. At the southern limit of the North Water, additional Arctic waters arrive after passing through the Canadian Archipelago, and there are further intrusions from the North Atlantic (Bacle *et al*., 2002). The end result is the presence of distinct interleaved water masses within a restricted geographical area. We targeted depths from the mid-water column, above the Arctic halocline and below the photic zone, to avoid the effect of deep Atlantic waters and of light on microbial zonation. We identified the water masses according to their θ–S and nutrient characteristics. Using quantitative PCR (qPCR) we further tracked the distribution of the alpha-subunit of the ammonia monooxygenase (*amoA*) gene and the most common archaeal taxonomic groups in the North Water, relative to parent water masses. The *amoA* gene, which mediates a key step in nitrification, is here a model biogeochemical cycling functional gene.

Results and discussion

Water masses and archaeal communities

Four sites were sampled in the North Water between 16 and 22 August 2005 (Fig. 1) and three different water masses were identified according to previous hydrographic definitions (Melling *et al*., 2001; Bacle *et al*., 2002): wintertime convection water (WCW) derived from brine rejection during ice formation the previous winter had high salinity ($S \geq 33.3$) and low temperature ($\theta \leq -0.9$°C); upper Arctic water (UAW) originating from the Arctic basin and entering the North Water via Smith Sound had cold but less saline water ($S \leq 33.0$ and $\theta \leq -0.9$°C); and Arctic Basin halocline (ABH) water intrusions were identified as temperature excursions with mid-range salinities ($\theta \geq -0.9$°C or $33 \leq S \leq 33.3$) (Fig. 2). Arctic Basin halocline waters most likely formed as a mixture of upper (Pacific derived) and lower (Atlantic derived) halocline waters (Jones and Anderson, 1986) stirred along a linear mixing line with Arctic thermocline waters (e.g. the upper domain of Atlantic waters) by tidal-forced mixing [mechanism similar to that described by Melling *et al*. (1984) as the water passes through Nares Strait].

Representative samples from the selected water masses were analysed for their archaeal communities and described in detail by their physical, chemical and biological characteristics (Table 1). In four cases, euryarchaeotal cluster IIa2 copy number was higher than total archaeal copy number (Table 1) suggesting that the general archaeal primer underestimated total archaeal

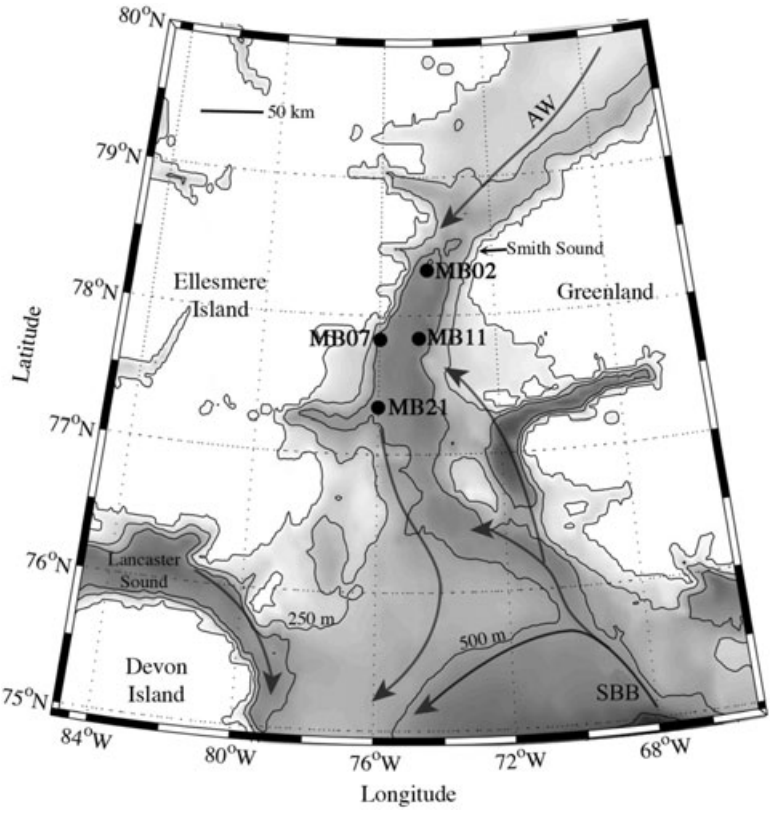

Fig. 1. Map of the North Water showing the locations of sampling stations MB02, MB07, MB11 and MB21. AW, Arctic water; SBB, southern Baffin Bay water.

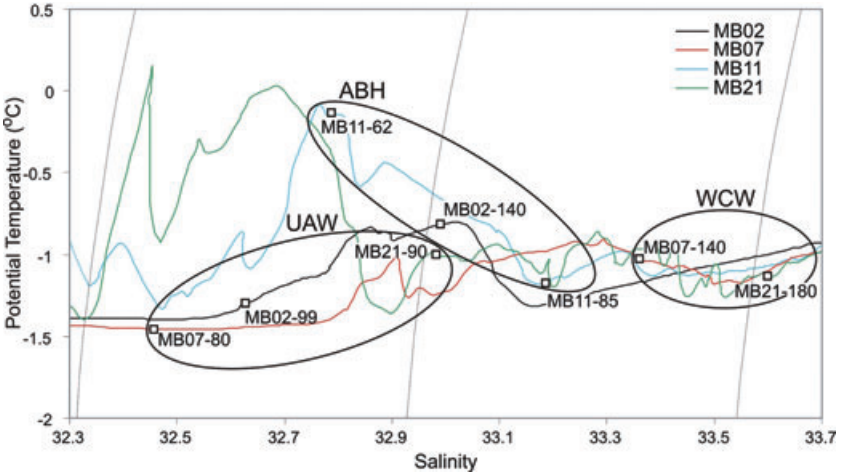

Fig. 2. θ–S diagram of the water masses at the sampling stations MB02, MB07, MB11 and MB21. Each coloured line represents the CTD cast at one station. Locations of water samples used to extract DNA are marked with an open square and the clone library name is indicated. Circles indicate water masses defined as described in *Experimental procedures*: upper Arctic water (UAW), Arctic Basin halocline intrusions (ABH) and wintertime convection water (WCW).

copy numbers. Since the aim of this study was to compare the relative composition of the archaeal communities in different water masses (Fig. 3) rather than the absolute numbers, we did not investigate this question further.

Each water mass was associated with specific archaeal taxa (Fig. 3) and the phylogenetic analysis separated the archaeal communities into three main clusters (Fig. 4) corresponding to their parent water masses [analysis of similarity (ANOSIM), $P = 0.01$] rather than to depth (Mantel test, $P = 0.05$). We found three major clades dominated the communities in clone libraries (Fig. 5). The most distinct archaeal community was from the ABH intrusions where quantification with specific qPCR primers showed that among three clades, Marine Group I.1a (MGI) *Crenarchaeota* were the most abundant (Fig. 3, Table 1). Sequence analysis of clone libraries revealed that all *Crenarchaeota* recovered from the ABH waters were affiliated with the MGI (Fig. 5), thought to be the dominant group of *Crenarchaeota* in the marine environment (DeLong *et al.*, 1998). In contrast, UAW and WCW were dominated by

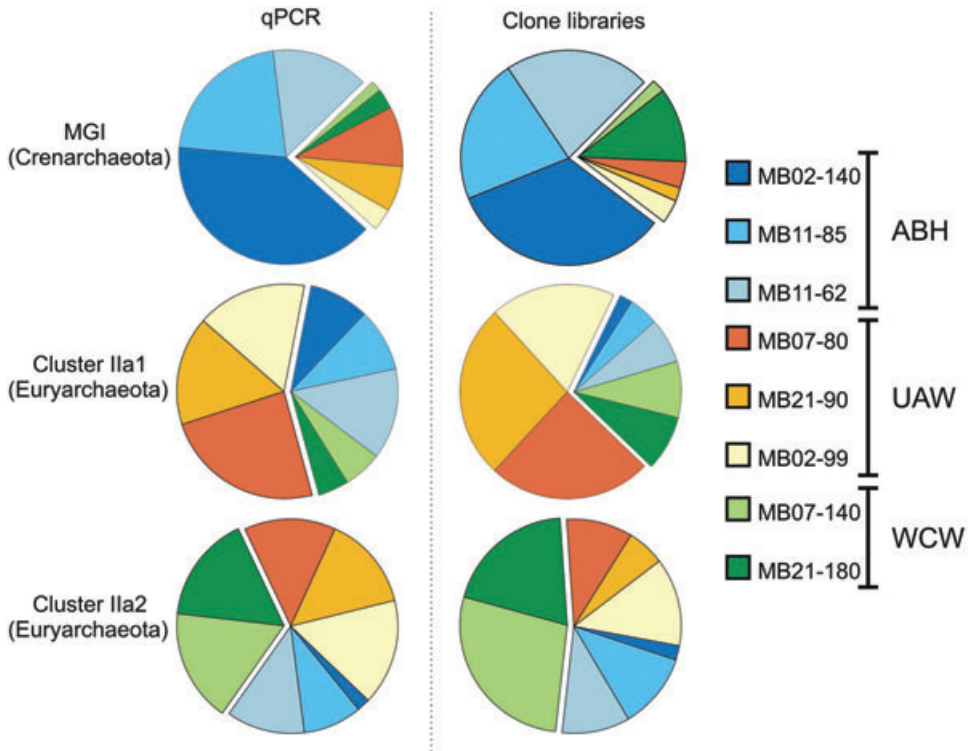

Fig. 3. Relative abundance of the three most common archaeal taxonomic groups (MGI, cluster IIa1, cluster IIa2) detected in the Arctic Basin halocline intrusions (ABH), upper Arctic water (UAW) and wintertime convection water (WCW), and quantified by qPCR (left) and abundance of sequences in clone libraries (right).

Table 1. Physical, chemical and biological characteristics of the water samples used for molecular analysis of archaeal communities in the Arctic Basin halocline intrusions (ABH), upper Arctic water (UAW) and wintertime convection water (WCW).

Water mass	Sample	Depth (m)	Temperature (°C)	Salinity	Nox (μM)	Silicate (μM)	Phosphate (μM)	Chlorophyll *a* (μg l^{-1})	*Archaea* 16S rRNA (SD)[a] (10^4 copies ml^{-1})	MGI 16S rRNA (SD) (10^4 copies ml^{-1})	*amoA* gene (SD) (10^4 copies ml^{-1})	Cluster IIa1 (SD) (10^4 copies ml^{-1})	Cluster IIa2 (SD) (10^4 copies ml^{-1})
ABH	MB11-62	62	–0.13	32.79	7.58	12.71	1.11	0.13	12.65 (0.43)	3.18 (0.27)	25.68 (1.17)	0.18 (0.02)	6.42 (1.26)
	MB02-140	140	–0.88	32.93	6.30	11.60	1.09	0.16	30.74 (3.69)	10.07 (1.37)	90.98 (5.60)	0.14 (0.01)	1.24 (0.30)
	MB11-85	85	–1.18	33.19	10.58	13.58	1.07	0.09	10.47 (0.80)	3.18 (0.07)	29.60 (0.93)	0.09 (0.00)	3.48 (0.45)
UAW	MB07-80	80	–1.45	32.40	7.67	10.93	0.99	0.20	4.78 (1.08)	1.11 (0.15)	11.99 (2.73)	0.14 (0.02)	4.52 (1.32)
	MB02-99	99	–1.34	32.61	6.38	11.49	1.04	0.21	2.26 (0.09)	0.45 (0.07)	7.54 (1.69)	0.13 (0.03)	6.39 (2.82)
	MB21-90	90	–1.15	32.93	5.82	8.66	1.02	0.09	3.55 (0.41)	1.48 (0.07)	10.66 (1.01)	0.17 (0.02)	9.80 (5.26)
WCW	MB07-140	140	–0.97	33.34	5.36	8.42	1.19	0.04	3.90 (1.15)	0.83 (0.21)	12.87 (1.83)	0.20 (0.02)	24.63 (0.27)
	MB21-180	180	–1.12	33.58	10.01	11.74	1.18	0.01	5.21 (0.78)	0.91 (0.02)	15.52 (2.15)	0.08 (0.00)	11.71 (1.52)

a. SD calculated from the triplicate measures of copy numbers by qPCR.
Nox, nitrate plus nitrite; MGI, Marine Group I *Crenarchaeota*.

Fig. 4. Dendrogram representing the similarity between the phylogenetic composition of eight archaeal 16S rRNA clone libraries from stations MB02, MB07, MB11 and MB21 of the North Water. The number following the station name indicates the depth from which the sample was retrieved. Parent water masses of the clone libraries are indicated: Arctic Basin halocline intrusions (ABH), upper Arctic water (UAW) and wintertime convection water (WCW). Clustering is based on a distance matrix computed with Bray-Curtis similarity from the abundance of OTUs in clone libraries defined by a 97% identity threshold between sequences. The dendrogram was inferred with the unweighted pair-group average algorithm (UPGMA).

Euryarchaeota cluster IIa1 and IIa2 respectively (Fig. 3) in both the qPCR and clone libraries analysis.

Archaeal diversity

Sequences from the eight archaeal libraries grouped within the two phyla *Crenarchaeota* and *Euryarchaeota*. *Euryarchaeota* grouped in seven different operational taxonomical units (OTUs) at the 97% threshold but most of the sequences belonged to two main clusters (IIa1 and IIa2, Fig. 5) classified as Group II.a *Euryarchaeota*. All libraries contained sequences from Group II.a, but the majority of Group II.a sequences recovered in this study were from libraries MB02-99, MB07-80, MB07-140, MB21-90 and MB21-180. At a finer level, sequences falling within cluster IIa1 were mostly sequences from library MB02-99, MB07-80 and MB21-90 while a majority of sequences from libraries MB07-140 and MB21-180 fell within cluster IIa2 (Fig. 3). Representatives from clusters IIa1 and IIa2 had a maximum sequence similarity of 95% between them and were affiliated to clones previously retrieved from the coastal Beaufort Sea (Galand *et al*., 2006). The only other euryarchaeotal clusters retrieved included few representatives from the Group II.b (four

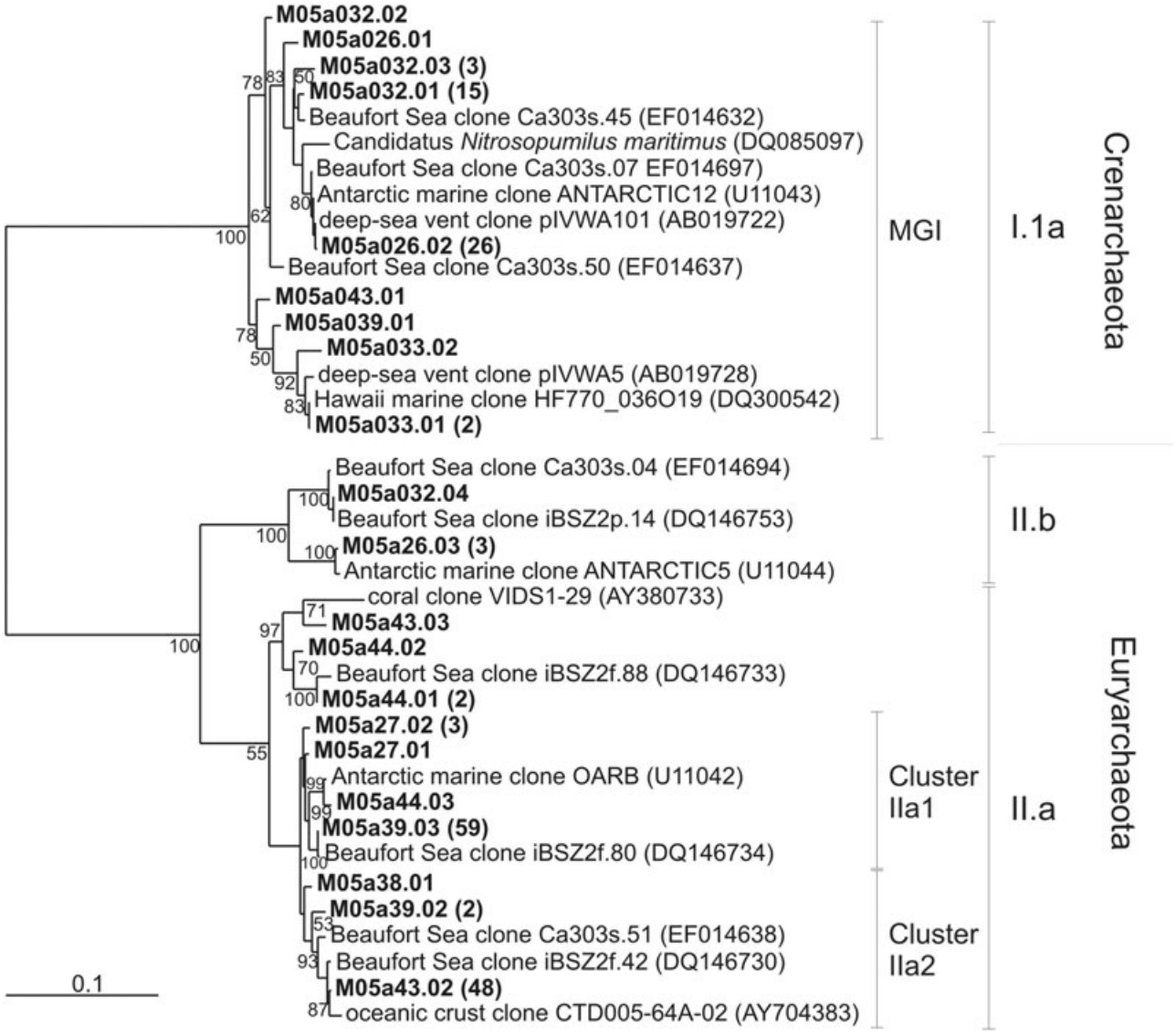

Fig. 5. Distance tree representing the position of *Archaea*. Sequences from this study are in boldface type. The number in parentheses indicates how many clones were represented by the sequence in the libraries. Bootstrap values >50 are shown expressed as a percentage of 1000 replicates. Analyses are inferred from 800-bp-long 16S rRNA sequences using FITCH distance matrix analysis (from the program PHYLIP). Scale bar represents 10% sequence divergence.

sequences) that were related to Arctic and Antarctic sequences (Fig. 5).

Crenarchaeotal sequences grouped within the MGI and were related to sequences from the Beaufort Sea and the Southern Ocean. Most of the MGI sequences were from libraries MB11-85, MB11-62 and MB02-140 (Fig. 3). At a 97% threshold for sequence separation, crenarchaeotal sequences were divided into 4 OTUs.

All eight archaeal libraries had a similarly low diversity with 2.5–5 phylotypes at a 97% threshold (Table 2) and an estimated total number of phylotypes not exceeding 7 (Chao1, Table 2). The percentage of coverage was in all but one case greater than 90% (Table 2) and rarefaction curves reached an asymptote (figure not shown) indicating that the sequencing effort was sufficient to cover the natural populations.

Abundance of amoA *genes*

The ABH had much higher abundance of *amoA* genes (> 70% of the *amoA* gene copies detected overall, Table 1) than the UAW and WCW, which may reflect that the distinct water masses harboured distinct microbial biogeochemical functions, here represented by the gene responsible for archaeal ammonia oxidation. There is mounting evidence that marine *Crenarchaeota* oxidize

Table 2. Diversity estimators calculated for eight archaeal 16S rRNA clone libraries from stations MB02, MB11, MB07 and MB21 of the North Water.

Water mass	Clone library	No. of clones	No. of phylotypes	Shannon H′	Chao1	Coverage (%)
ABH	MB11-62	21	4.5	1.24	4.5	95
	MB02-140	20	4	0.70	4.5	90
	MB11-85	23	5	1.17	7	87
UAW	MB07-80	22	3.5	0.56	4.6	91
	MB02-99	23	2.5	0.58	2.5	100
	MB21-90	22	3.5	0.68	3.5	95
WCW	MB07-140	21	3	0.80	3	95
	MB21-180	22	4.5	1.09	5.5	91

Enumeration of phylotypes and calculation of diversity estimators were completed at a 97% identity threshold between sequences. ABH, Basin halocline intrusions; UAW, upper Arctic water; WCW, wintertime convection water.

ammonia (NH_3) to nitrite (NO_2^-) as an energy source for growth (Konneke *et al*., 2005) and the recent detection of the *amoA* gene in various marine systems (Francis *et al*., 2005; Hallam *et al*., 2006; Wuchter *et al*., 2006; Mincer *et al*., 2007) implies a major role for *Crenarchaeota* in the global oceanic nitrogen cycle. The significant correlation between *amoA*, *Crenarchaeota* and *Archaea* (Fig. 6) indicates that crenarchaeotal mediated ammonia oxidation may be a major process occurring at mid-depths of the North Water. Activity measures however would be needed to assess whether the higher abundances of *amoA* genes yield higher rates of archaeal ammonia oxidation. This is the first report of the *amoA* genes in the Arctic, although earlier reports have found *Crenarchaeota* to be relatively abundant in other Arctic regions (Bano *et al*., 2004; Kirchman *et al*., 2007), including under the multiyear ice. The ubiquity of *Crenarchaeota* in these cold regions suggests that the production of nitrate through ammonia oxidation by *Archaea* could be an important biogeochemical process taking place in the nitrate- relative to phosphate-depleted waters that flow eastwards through the Arctic Ocean (Yamamoto-Kawai *et al*., 2006).

Our results indicated an *amoA* to 16S rRNA gene ratio close to 8, which is higher than previously reported in other oceanic regions. In samples from the Pacific (Mincer *et al*., 2007) and in an enrichment from the North Sea (Wuchter *et al*., 2006) the ratio was close to 1; in the Black Sea (Lam *et al*., 2007) it varied from 0.01 to 2.8 and in the Atlantic Ocean (Wuchter *et al*., 2006) from 0.18 to 5.22. Even though the ratios reported earlier varied greatly between and within sampling areas, none was as high as the one reported here. The most likely explanation for the elevated ratio would be related to methodological constraints; for example, MGI quantities may be underestimated due to lower qPCR efficiency of the 16S gene. Efficiency of the MGI primers (Takai *et al*., 2004) has not been reported earlier (Wuchter *et al*., 2006; Coolen *et al*., 2007) and could not be compared with ours. Alternatively, while the MGI primers have proven to be relatively robust there is always the possibility that undetected archaea

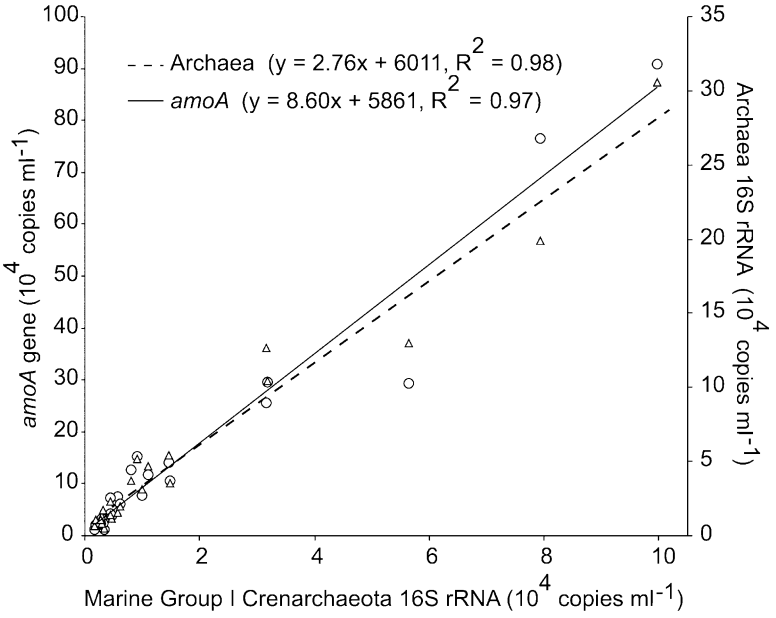

Fig. 6. Bivariance plot of Marine Group I *Crenarchaeota* (MGI) 16S rRNA versus *Archaea* 16S rRNA copies (triangle) and MGI 16S rRNA versus *amoA* gene copies (circles) quantified by qPCR. The figure includes samples from the three water masses (ABH, UAW and WCW) plus additional samples from undefined water masses of the North Water. Correlations between *amoA* and MGI and between MGI and *Archaea* were both significant ($P < 0.01$).

may carry the *amoA* gene. A less probable explanation would be that some species possess very high copy numbers of the *amoA* gene; if so, the Arctic species were substantially different from the ones found in other marine systems. Given the latitude and low annual temperatures this possibility cannot be dismissed. Irrespective of the absolute ratio, the comparison between samples clearly showed differences in the relative abundance of the different genes in the different water masses. This conclusion was well supported by the data from our eight clone libraries, and the qPCR quantification with different primers was consistent with that view.

In summary, we demonstrate that *Archaea* communities are closely tied to their parent water masses. Previously, protistan community structure and abundance have been linked to water masses in the Canadian Arctic (Lovejoy *et al*., 2002; Hamilton *et al*., 2008) and prokaryotes have been associated with marine regions and oceanic currents (Pinhassi *et al*., 2003; Teira *et al*., 2006; Varela *et al*., 2008). Those reports and our present results indicate that shifting currents will profoundly influence the distribution of microbial communities; such displacements have the potential of disrupting established geographic patterns of microbial-driven biogeochemical processes. For example, the ratio of excess phosphate to nitrate determines the balance between nitrogen fixers and other plankton in the North Atlantic (Yamamoto-Kawai *et al*., 2006). If changing currents disrupted the N:P stoichiometry of waters entering the region this would have repercussions for food web structure far beyond the Arctic. Anthropogenic forced changes in ocean circulation patterns are already being observed (Sarmiento *et al*., 2004) and to predict the effects of change on the global ecosystem it is essential to further identify microbial processes associated with water masses.

Experimental procedures

Study area and sample collection

The North Water is bounded by Ellesmere Island and Greenland between latitudes 76°N and 78.5°N (Fig. 1). Historically it is thought to be one of the most biologically productive regions in the high Arctic (Dunbar and Dunbar, 1972). The North Water was sampled between 16 and 22 August 2005 as part of a microbial diversity study on board the icebreaker CGGS *Amundsen*. Sample depths at each station were chosen by viewing the sensor profiles on the CTD (conductivity, temperature, depth) downcast and were determined to target temperature excursions indicating differences in water masses. We specifically targeted depths below the photic zone to avoid light masking other structuring parameters. Water samples were collected on the upcast with a Sea Bird SBE-911 CTD rosette sampler holding 24 12-L PVC bottles (Ocean Test Equipment). Microbial biomass was collected by sequentially filtering 6 l of seawater through a 53 μm nylon mesh, a 3 μm polycarbonate filter and then a 0.2 μm Sterivex filter unit. Filters were stored in buffer (50 mM tris, 40 mM EDTA and 0.75 M sucrose) and frozen at –80°C until the nucleic acid was extracted as in Lovejoy and colleagues (2006).

Water masses were identified based on their potential temperature (θ) and salinity (S) characteristics. Salinity was calibrated with water samples analysed by a Guideline Auto-Sal salinometer. Values of potential temperature (θ) and potential density were computed using algorithms from Fofonoff and Millard (1983). Oxygen concentrations were measured using a Seabird SBE43 oxygen sensor mounted on the CTD frame.

Nutrients and chlorophyll a

Concentrations of nitrite (NO_2^-), nitrate (NO_3^-), silicate (SiO_4^-) and phosphate (PO_4^{3-}) were determined immediately after sampling using a Bran and Luebbe Autoanalyser III with routine colorimetric methods (Grasshoff, 1976) and a detection limit of 0.05 μM.

For chlorophyll *a*, samples were filtered onto Whatman GF/F filters and stored at –80°C until analysis. Pigments were extracted from the filters in 95% ethanol at 70°C for 5 min (Nusch, 1980) and concentrations were determined by spectroflourometry (Cary Eclipse) before and after acidification (Strickland and Parsons, 1972).

PCR amplification and cloning

Archaeal 16S rRNA genes were amplified with primers 109F and 934R (Table 3) as described previously (Galand *et al*., 2006). PCR products were analysed by gel electrophoresis, purified with Qiaquick PCR Purification Kit (Qiagen) and cloned with TA cloning kit (Invitrogen). In total we constructed eight archaeal clone libraries. For each library, positive clones were picked and inoculated into LB media in 96-well plates. Clones containing inserts were chosen randomly and 24 clones from each library were sequenced directly using the vectors' T7p universal primer. Suspected chimeras were checked by using BLAST with sequence segments separately and then using the Chimera check program at Ribosomal Data Project II (Michigan State University; http://rdp8.cme.msu.edu/html/). Between one and four chimeras or poor-quality sequences were detected and excluded from each library. The 16S rRNA sequence data have been archived in the GenBank database under Accession Nos EF486586 to EF486606.

Phylogenetic analysis, diversity calculations and cluster analysis

Sequences were compared with those in the GenBank database using the BLAST server at the National Center for Biotechnology Information (NCBI). The approximately 800-base-pair sequences were aligned using the CLUSTAL W package (Thompson *et al*., 1994) and checked manually. Phylogenetic analyses were completed with the program PHYLIP (Felsenstein, 2004). DNADIST was used to calculate genetic distances with Kimura-2 model with 1000 data sets obtained by bootstrapping and the distance tree was estimated with FITCH.

Table 3. Characteristics of the PCR primers used in this study.

Application	Primers	Primer sequence	Targeted group	References
Cloning	109f	5′-ACK GCT CAG TAA CAC GT-3′	*Archaea*	Grosskopf *et al.* (1998)
	915r	5′-GTG CTC CCC CGC CAA TTC CT-3		Stahl and Amann (1991)
qPCR	Parch519f	5′-CAG CMG CCG CGG TAA-3	*Archaea*	Ovreas *et al.* (1997)
	Arch915r	5′-GTG CTC CCC CGC CAA TTC CT-3		Stahl and Amann (1991)
	AOA-amoA-f	5′-CTG AYT GGG CYT GGA CAT C-3′	Archaeal *amoA* gene	Wuchter *et al.* (2006)
	AOA-amoA-r	5′-TTC TTC TTT GTT GCC CAG TA-3′		Wuchter *et al.* (2006)
	C-IIa1f	5′-GGT TGT GAG AGC AAT AGC-3′	*Euryarchaeota* cluster IIa1	This study
	C-IIa1r	5′-GCT ACA CAA ATG TGT AGT C-3′		This study
	C-IIa2f	5′-TGT TGG GAG ACA TAG C-3′	*Euryarchaeota* cluster IIa2	This study
	C-IIa2r	5′-CAC TAT GTG CAT CCA C-3′		This study
	MCGI-391f	5′-AAG GTT ART CCG AGT GRT TTC-3′	Marine Group I	Takai *et al.* (2004)
	MCGI-554r	5′-TGA CCA CTT GAG GTG CTG-3′		Takai *et al.* (2004)

Sequences were grouped in OTUs at a 97% identity threshold with the program DOTUR (Schloss and Handelsman, 2005) through a Jukes–Cantor corrected distance matrix obtained using the DNADIST program from PHYLIP. The Shannon–Wiener diversity index and the Chao1 non-parametric richness estimator were also calculated at a 97% identity threshold with the program DOTUR. The coverage value (*C*) was calculated as $C = 1 - (n1/N)$ where $n1$ is the number of clones which occurred only once in a library of *N* clones (Good, 1953).

For cluster analysis, a distance matrix was computed from the abundance of OTUs in clone libraries at a 97% identity threshold using Bray–Curtis similarity and a dendrogram inferred with the unweighted pair-group average algorithm (UPGMA) as implemented in the program PAST (v 1.63, http://folk.uio.no/ohammer/past/).

Quantification of DNA

DNA concentrations were estimated using a fluorescence technique, 0.5 μl of DNA diluted in 49.5 μl of TE buffer was added to 50 μl of a 200-fold dilution of PicoGreen (Invitrogen) and incubated for 5 min at 22°C in the dark followed by detection of fluorescence intensity in a TBS-380 fluorometer (Turner Biosystems). A standard curve was generated using known amounts of Lambda DNA (Invitrogen). All measurements were performed in duplicate.

Quantitative PCR (qPCR)

Real-time PCR was performed on a Dyad Disciple thermal cycler with Chromo 4 Real-Time Detector (Bio-Rad, Hercules, CA) using primers and conditions listed in Table 4. All reactions were performed in 96-well white qPCR plates with adhesive seals (Bio-Rad). Reaction mixtures (20 μl) contained 10 μl of iQ SYBR Green Supermix (Bio-Rad, 100 mmol l^{-1} KCl, 40 mmol l^{-1} Tris-HCl, 0.4 mmol l^{-1} of each dNTP, iTaq DNA polymerase 50 units ml^{-1}, 6 mmol l^{-1} $MgCl_2$, SYBR Green I, 20 nmol l^{-1} fluoresein), 5 μl of template (5 ng), 0.2 mmol l^{-1} of primers and ultra pure sterile water. The reactions had an initial denaturing step for 5 min at 95°C, followed by 45 cycles including 30 s denaturing at 94°C, 40 s of primer annealing and 40 s of primer extension at 72°C. Annealing temperatures for the different primer sets are given in Table 4. The fluorescent signal was read in each cycle at 78°C for 25 s to ensure stringent product quantification. Reactions were run in triplicate with 5 ng of template DNA from each sample. Quantification standards consisting of 10-fold dilutions ranging from 10 to 10^6 copies of DNA fragment purified with Qiaquick PCR Purification Kit (Qiagen) were subjected to qPCR along with the samples. Overall, average efficiencies for all quantification reactions ranged from 57% for MGI *Crenarchaeota* 16S rRNA gene to 76% for *amoA* gene with R^2 values > 0.98. Standard DNA was obtained by PCR amplification of clones (Table 4). Control reactions included three reactions without DNA as control for

Table 4. PCR conditions for the primers used in this study.

Application	Primer pair	Annealing temperature	Control for standard curve	Amplicon length (bp)	Targeted group
Cloning	109f/915r	52°C	–	806	*Archaea*
qPCR	Parch519f/Arch915r	63°C	16S rDNA from clone M05a026.02	420	*Archaea*
	AOA-amoA-f/AOA-amoA-r	58.5°C	*amoA* DNA from clone generated with Arch-amoAF/Arch-amoAR[a]	256	*Archaea amoA* gene
	C-IIa1f/C-IIa1r	51°C	16S rDNA from clone M05a39.03	118	*Euryarchaeota* cluster IIa1
	C-IIa2f/C-IIa2r	53°C	16S rDNA from clone M05a43.02	114	*Euryarchaeota* cluster IIa2
	MCGI-391f/MCGI-554r	61°C	16S rDNA from clone M05a026.02	122	Marine Group I

a. From Francis and colleagues (2005).
–, not relevant.

contamination and three reactions containing 5 ng of non-target DNA as a control for the specificity of the primers. Primer specificity was also confirmed by running amplicons on agarose gel electrophoresis and by melting curve analyses.

Primer design

Quantitative PCR primers for the quantification of euryarchaeotal cluster IIa1 and IIa2 (Tables 3 and 4) were designed based on the alignment of 64 16S rRNA sequences belonging to cluster IIa1 and 51 sequences from cluster IIa2. During qPCR, known copy numbers of PCR-amplified clones from the non-target two euryarchaeotal clusters and from MGI *Crenarchaeota* were used as negative controls. The optimal annealing temperature was determined empirically to ensure maximum specificity of the PCR reaction.

Statistical analysis

Analysis of similarity (ANOSIM) statistics were used to verify the significance of the dendrogram clustering by testing the hypothesis that archaeal communities from a same cluster were more similar in composition with each other than with communities in different clusters. A Bray–Curtis similarity matrix computed from the abundance of OTUs in clone libraries at a 97% identity threshold was used to generate ANOSIM statistics with 1000 permutations (Clarke and Green, 1988). Analyses were conducted with the program PAST (v 1.63, http://folk.uio.no/ohammer/past/). Correlations between archaeal community structure and depth or water mass were tested with Mantel tests (999 permutations) on Euclidean distance matrices derived from the abundance of archaeal OTUs, depth and water mass categories. Analyses were conducted with the R package (Casgrain and Legendre, 2001).

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