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Vertical structure of archaeal communities and the distribution of ammonia monooxygenase A gene variants in two meromictic High Arctic lakes

Jérémie Pouliot,[1] Pierre E. Galand,[1,2] Connie Lovejoy[1] and Warwick F. Vincent[3]*
[1]*Département de Biologie and Québec-Océan, Université Laval, Québec, QC G1V 0A6, Canada.*
[2]*Limnology Unit, Department of Continental Ecology, Centre d'Estudis Avançats de Blanes – CSIC, 17300 Blanes, Spain.*
[3]*Département de Biologie and Centre d'Études Nordiques, Université Laval, Québec, QC G1V 0A6, Canada.*

Summary

The distribution of archaeal *amoA* and 16S rRNA genes was evaluated in two marine-derived, meromictic lakes in the Canadian High Arctic: Lake A and Lake C1 on the northern coast of Ellesmere Island. The *amoA* gene was recorded in both lakes, with highest copy numbers in the oxycline. Sequence analysis showed that *amoA* from the two lakes shared 94% similarity, indicating at least two phylogenetically distinct clusters. Clone libraries of archaeal 16S rRNA genes from Lake A revealed strong vertical differences in archaeal community diversity and composition down the water column. The oxic layer was dominated by one group of Euryarchaeota affiliated to the Lake Dagow Sediment (LDS) cluster. This group was absent from the oxycline, which had an extremely low archaeal diversity of two phylotypes. Both belonged to the Crenarchaeota Marine Group I (MGI), the marine group that has been linked to archaeal *amoA*; however, there was a low ratio of *amoA* to MGI copy numbers, suggesting that many MGI Archaea did not carry the *amoA* gene. The anoxic zone contained representatives of the RC-V (Rice Cluster-V) and LDS clusters of Euryarchaeota. These results show the strong vertical differentiation of archaeal communities in polar meromictic lakes, and they suggest archaeal nitrification within the oxycline of these highly stratified waters.

Received 15 June, 2009; accepted 22 November, 2009. *For correspondence. E-mail warwick.vincent@bio.ulaval.ca; Tel. (+1) 418 656 5644; Fax (+1) 418 656 2043.

Introduction

Ammonia oxidation is a key process in the biogeochemical nitrogen cycle and until recently was attributed to Bacteria, specifically members of *Betaproteobacteria* and *Gammaproteobacteria*. Past studies in the ocean have drawn attention to the often sparse concentrations of nitrifying Bacteria (0.1% of bacterial assemblages; Bothe *et al*., 2000), despite the evident oxidation of ammonia to nitrate. This apparent anomaly has been resolved with the discovery of widespread ammonia-oxidizing Archaea that contain the archaeal ammonium monooxygenase enzyme involved in the first step of nitrification (Könneke *et al*., 2005; Wuchter *et al*., 2006; Coolen *et al*., 2007). Archaeal ammonia oxidizers are believed to belong to the Marine Group I (MGI) Crenarchaeota (Könneke *et al*., 2005). The widespread distribution of Crenarchaeota (Karner *et al*., 2001) and the detection of the archaeal ammonia monooxygenase subunit A gene *amoA* in various marine systems (Francis *et al*., 2005; Hallam *et al*., 2006; Wuchter *et al*., 2006; Mincer *et al*., 2007) suggest that Crenarchaeota play a major role in the marine nitrogen cycle. Despite the newly discovered global relevance of archaeal ammonia oxidizers, they have received little attention to date in lakes, and few studies have considered Crenarchaeota in lake ecosystems (Auguet and Casamayor, 2008).

Meromictic (permanently stratified) lakes are found in both the north and south polar regions, and are characterized by vertical gradients in their physical and chemical properties (Priscu, 1997; Van Hove *et al*., 2006), providing diverse potential habitats for microbial growth and niche differentiation. There is evidence of nitrous oxide maxima in Antarctic meromictic Lake Vanda (Vincent *et al*., 1981) and in other lakes of the McMurdo Dry Valleys (Priscu, 1997). The presence of ammonia-oxidizing bacteria has already been described in these lakes (Voytek and Ward, 1995), consistent with the accumulation of nitrous oxide as a nitrification product in their saline waters. To date however, the archaeal *amoA* gene and nitrifying Archaea have not been investigated. Analogous meromictic lakes occur along the northern coastline of Ellesmere Island in the Canadian High Arctic, but comparatively little is known about their aquatic nitrogen cycle.

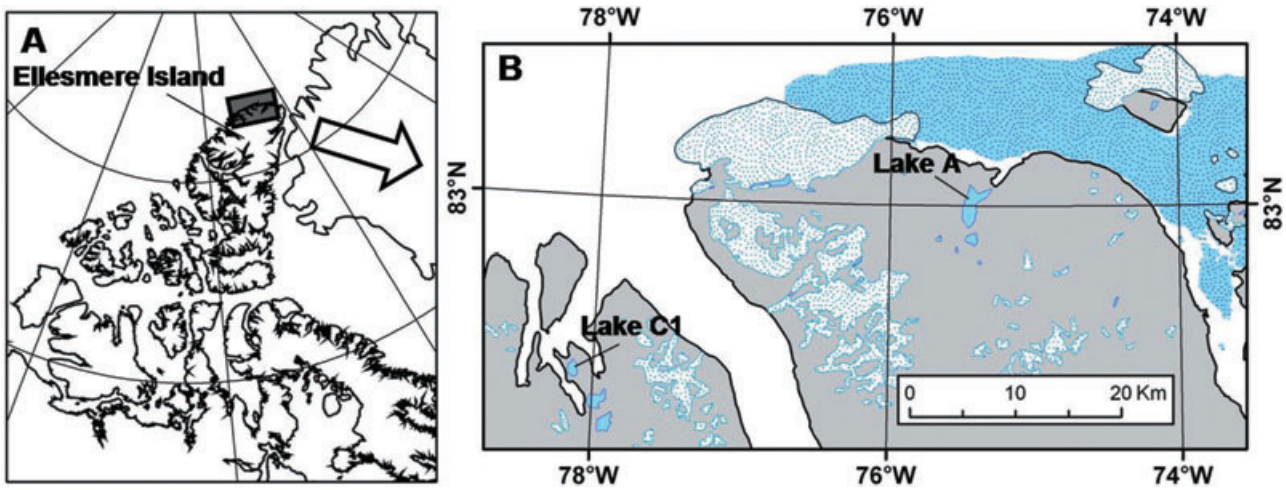

Fig. 1. Map of the sampling sites.
A. Canadian High Arctic and northern coast of Ellesmere Island.
B. Location of Lake A and Lake C1.

The first objective of this study was to determine whether the archaeal *amoA* gene could be detected in saline lakes, given the importance of archaeal nitrifiers in marine waters. First, we used *amoA*-specific primers to test whether the gene was present in DNA collected from two meromictic lakes that were cut off from the Arctic Ocean several thousand years ago (Jeffries *et al.*, 1984). We then quantified the abundance of this gene within the different density-stratified layers of the water column in both lakes using quantitative PCR (qPCR). We assessed the diversity of the *amoA* gene by constructing *amoA* gene clone libraries and sequencing the resulting clones. We then addressed the local biogeography of *amoA*-containing organisms by comparing two libraries from these lakes, which lie 40 km apart and have separate catchments. Finally, we constructed archaeal 16S rDNA gene libraries to estimate archaeal diversity in these high latitude lakes and to test for ecological or niche separation by comparing communities from different depths. These highly stratified meromictic lakes with distinct physical and chemical structure are attractive systems to test whether diverse habitats allow the development of niche-specialized microbial communities.

Results

Physico-chemical characteristics of the lakes

Lake A and Lake C1, situated along the northern coast of Arctic Canada (Fig. 1), had similar conductivity profiles with freshwater in the upper water column, and increasing salinity with depth reaching sea water values below the oxic zone (Fig. 2A). Temperature (Fig. 2B) and pH (Fig. 2D) profiles were also similar in both lakes with lowest pH and highest temperature recorded in the oxycline (Table 1). Total phosphorus (TP) and dissolved

Table 1. Environmental characteristics of Lake A and Lake C1.

Depth (m)	Temp (°C)	DO (mg l^{-1})	pH	Cond (mS cm^{-1})	TP (μg P l^{-1})	DOC (mg C l^{-1})	Prokaryotes (10^6 cells ml^{-1})	Chl *a* (μg l^{-1})
Lake A								
2	1.11	18.46	8.15	0.39	< 0.5	0.83	1.69	0.23
10	5.72	14.70	7.92	0.91	< 0.5	0.71	0.65	0.12
12	7.01	5.38	7.34	5.07	2.6	0.81	1.51	0.18
29	6.40	0.56	7.93	29.08	15.1	3.20	2.39	0.16
32	5.89	0.57	7.91	29.87	63.8	5.70	2.52	0.23
Lake C1								
2	2.44	20.42	8.43	0.40	< 0.5	1.21	2.57	0.18
9	8.97	> 25.00	7.67	0.62	< 0.5	1.37	1.80	0.09
18	12.64	1.62	7.27	19.69	2.6	1.48	1.00	0.03
32	7.49	0.58	7.79	29.99	3.2	3.00	1.68	0.16

Temp, temperature; DO, dissolved oxygen; Cond, conductivity; TP, total phosphorus; DOC, dissolved organic carbon; Chl *a*, chlorophyll *a*, < or >, data under/over detection limits.

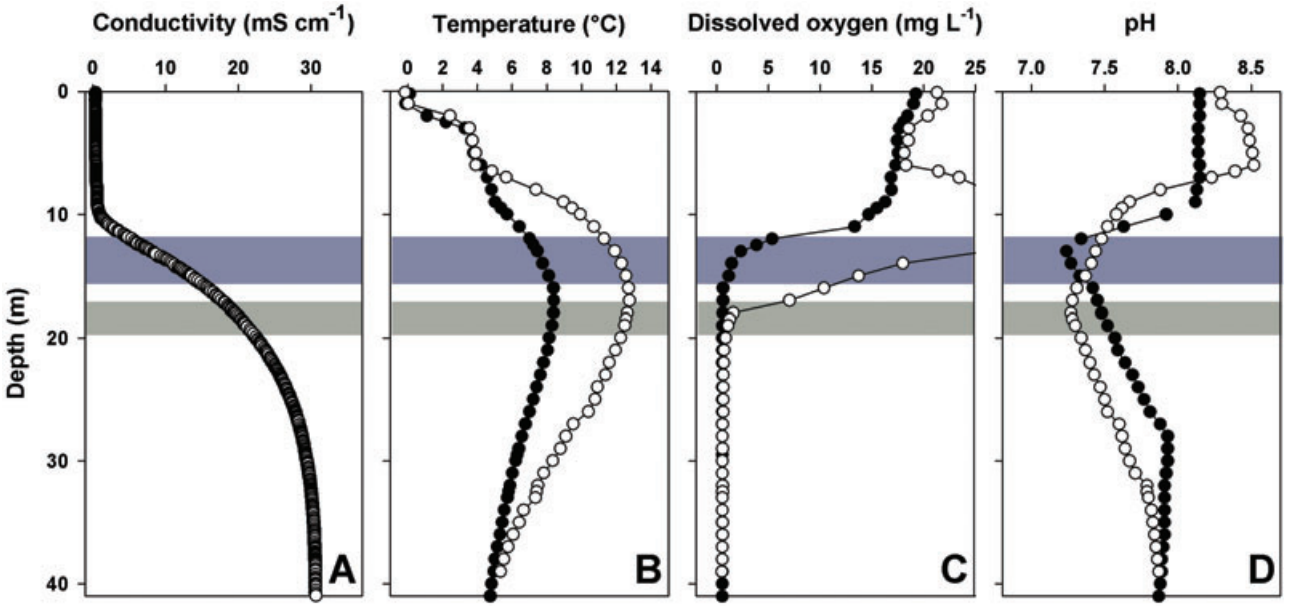

Fig. 2. Depth profiles for conductivity (A), temperature (B), dissolved oxygen (C) and pH (D) for Lake A (filled circles) and Lake C1 (open circles). The dark and light shadings indicate the oxycline of lakes A and C1 respectively. Note that the conductivity profiles were almost identical in both lakes.

organic carbon (DOC) concentrations increased with depth in both lakes. Chlorophyll *a* profiles were also similar in the two lakes, with high concentrations beneath the ice, lower concentrations at mid-depths and higher concentrations in the deep waters (Table 1).

Despite the similarities of many features, there were also pronounced differences between the two lakes. Temperature rose to a higher maximum in Lake C1 (Fig. 2B) and there were pronounced differences in oxygen profiles. The oxycline (0.5–5.5 mg O_2 l^{-1}) extended over 5 m, from 11 to 16 m, in Lake A, but over only 3 m, from 17 to 20 m, in Lake C1. Unlike Lake A, Lake C1 had a sharp peak in oxygen concentrations that exceeded the range of the oxygen profiler (Fig. 2C, > 20 mg l^{-1}). The concentration of TP was an order of magnitude higher in the anoxic waters of Lake A compared with C1 (Table 1). The vertical distribution of prokaryotes [4,6-diamidino-2-phenylindole (DAPI)-stained cells] differed between the two lakes, but both had lowest cell concentrations in the oxycline (Table 1).

Quantification of archaeal amoA *and 16S rDNA genes*

Following initial tests using standard PCR we applied qPCR analysis with specific primers (see below). We found archaeal *amoA* genes in both Lake A and Lake C1. The quantitative distribution of *amoA* genes varied with depth for both lakes, with highest gene copy numbers in the oxycline (12 and 18 m for Lake A and Lake C1 respectively) (Fig. 3). The gene was less abundant or absent in the surface and anoxic layers of the lakes (Fig. 3). Depths with highest copy number of *amoA* genes corresponded to highest 16S rDNA copies of MGI Crenarchaeota (Fig. 3). Archaeal 16S rDNA genes were present throughout the water column of Lake A (Fig. 3A) and Lake C1 (Fig. 3B) with highest gene copy numbers in the oxic layers of the lakes. The abundance of archaeal, MGI and *amoA* genes differed between duplicate samples taken 500 m away from the main sampling site; however, the duplicate values at specific depths were generally of the same order of magnitude and the duplicate profiles gave the same pattern with depth (Fig. S1).

Diversity of the amoA *gene*

The composition of the *amoA* gene libraries differed between Lake A and Lake C1. The Lake A library was dominated by the phylotype LA_amo.14 (> 77% of the sequences) while Lake C1 was dominated by LC1amo.16 (> 85% of the sequences) (Fig. 4). The Lake A phylotype was only distantly related to previously published *amoA* sequences with 94% identity to sequences from the Black Sea and 92% identity to the *amoA* gene of the marine isolate *Nitrosopumilus maritimus* (Fig. 4). In Lake C1, the dominant *amoA* cluster was related to sequences from marine sediment (98% identity) and had only 88% identity to *N. maritimus* (Fig. 4). We tentatively named those two oxyclinic clusters the LA and LC1 clusters (standing for Lake A and Lake C1 clusters respectively) (Fig. 4), and defined them as clusters containing meromictic lake *amoA* genes. There was 94% sequence similarity

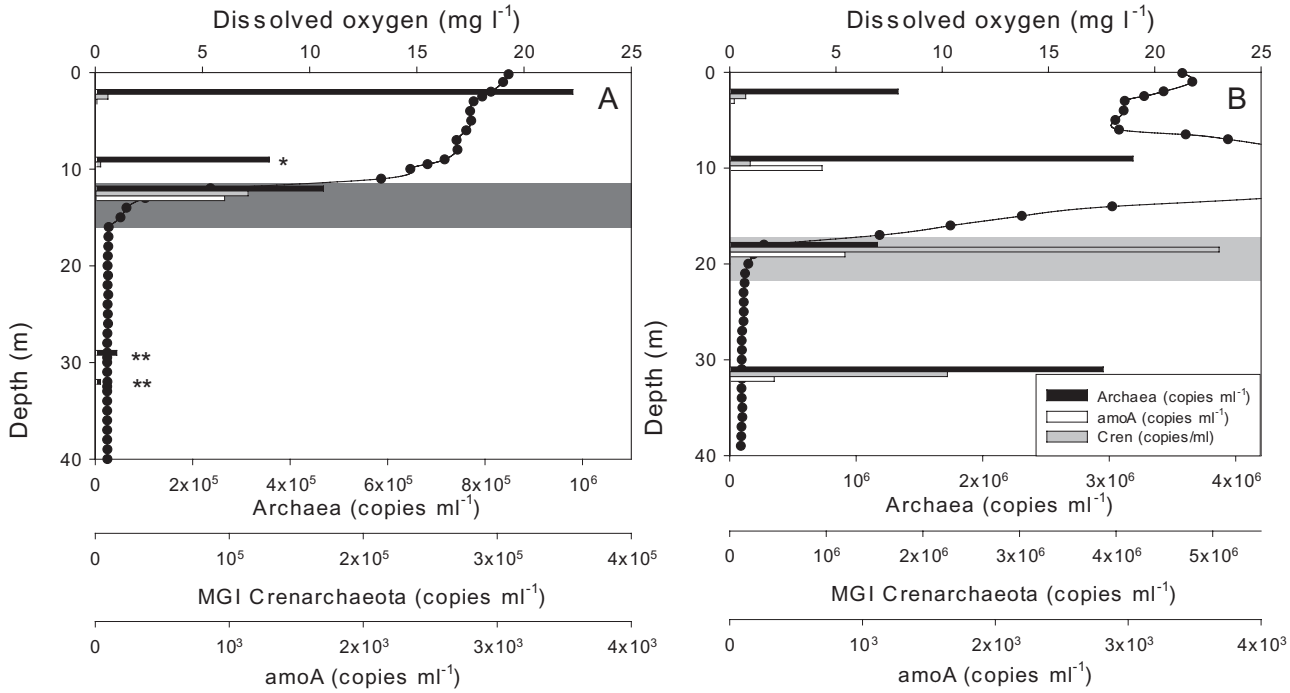

Fig. 3. Abundance of 16S rDNA and *amoA* genes in the water column of Lake A (A) and Lake C1 (B) in relation to dissolved oxygen profile (filled circles). The shading indicates the oxycline. *: < 2 *amoA* copies ml^{-1}; **: < 0.4 *amoA* and MGI Crenarchaeota copies ml^{-1}.

between LA and LC1 clusters. In both Lake A and Lake C1, the *amoA* gene diversity was very low with only two or three different phylotypes detected. The coverage percentage was higher than 95% (Table 2) indicating that Lake A and Lake C1 libraries covered well the natural diversity of *amoA* genes.

Diversity of archaeal communities in Lake A

There were pronounced differences in archaeal community composition between depths in Lake A. The oxic layer (2 and 10 m) contained 10–20 phylotypes (Table 2) and was dominated by one group of sequences belonging

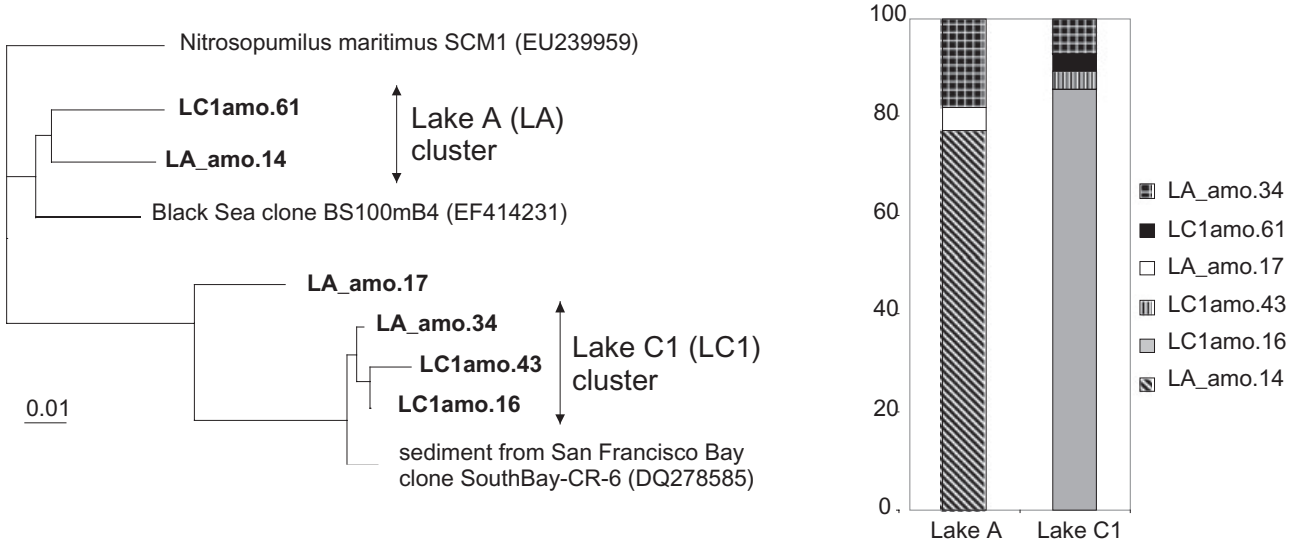

Fig. 4. Community composition of *amoA* clone libraries from the oxyclines of Lake A and Lake C1. The name of the different groups is given in the distance tree representing the phylogenetic position of *amoA* OTUs. Only representative OTUs (< 99% sequence identity) are shown in the tree (in bold).

Table 2. Diversity of clone libraries for the *amoA* gene in the oxycline of Lake A and Lake C1 and archaeal 16S rDNA in Lake A.

Depth (m)	No. of clones	No. of phylotypes	Shannon index	Chao index	Coverage of libraries (%)	Evenness index
amoA gene						
Lake A	22	3	0.61	2.67	95	1.00
Lake C1	28	2	0.14	1.90	96	1.27
Archaeal 16S rDNA						
2	49	20	2.69	23.60	84	1.00
10	41	10	1.99	11.33	93	0.84
12	63	2	0.03	1.40	98	1.29
29	43	20	2.66	27.40	77	0.88
32	35	17	2.54	28.25	71	0.71

Enumeration of phylotypes and calculation of diversity estimators (Shannon and Chao) were done at a 3% distance level between sequences.

to operational taxonomic unit (OTU) 6 (Fig. 5). OTU 6 sequences belonged to the Euryarchaeota and were affiliated to the LDS cluster (Lake Dagow Sediment) (Glissmann *et al*., 2004) (Fig. 6B). They were absent from oxycline and rare (one sequence) in the anoxic waters. The LDS sequences were related to clones previously retrieved from the Mackenzie River, Canadian Arctic (Fig. 6B). The 2 m and 10 m samples each contained a site-specific group of euryarchaeotal sequences (OTU 4 and OTU 2 respectively, Fig. 5) affiliated to the RC-V cluster (Rice Cluster-V) (Grosskopf *et al*., 1998b) and related to clone sequences from Arctic peat and river samples (Fig. 6C). Some Crenarchaeota were also detected in the oxic layer (OTU 11); they belonged to the MGI (DeLong, 1998) and were related to clones from salt marsh sediments (Fig. 6A).

The oxycline at 12 m had a strikingly low archaeal diversity with only two phylotypes, both belonging to OTU 10 (Fig. 5, Table 2); of these, one phylotype dominated and accounted for 62 (> 98%) of the 63 sequenced clones from the sample. OTU 10 belonged to crenarchaeotal MGI and clustered separately from the upper oxic layer Crenarchaeota. The oxycline sequences had a 96% similarity to *N. maritimus,* the only marine mesophilic Crenarchaeota isolated to date, but were most similar to clones from the marine-influenced arctic Lake Mackenzie and to deep groundwater (Fig. 6A).

In the anoxic layers, from samples taken at 29 and 32 m, we recovered 20 and 17 different phylotypes respectively (Table 2) and two predominant groups of sequences (OTUs 1 and 7, Fig. 5) that fell within the RC-V and LDS clusters of Euryarchaeota respectively. Representatives of OTUs 1 and 7 were also found in the oxic zone but the sequences were distinct from the anoxic layer sequences (Fig. 6B and C). RC-V and LDS sequences were related to clones from diverse environments such as the coastal Beaufort Sea and hydrothermal vents. Two less abundant clusters were detected exclusively in the anoxic layers: OTU 3, which belonged to

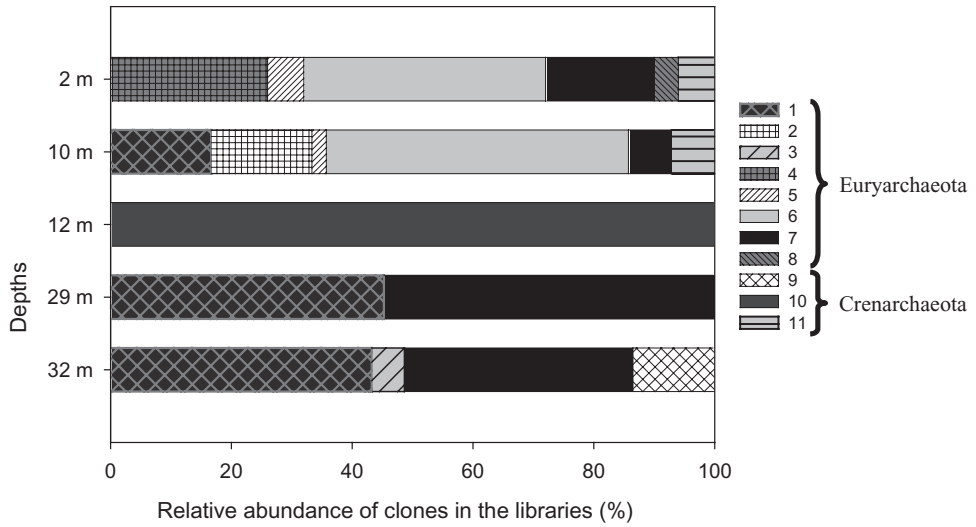

Fig. 5. Composition of archaeal 16S rRNA gene clone libraries at five depths of Lake A. Communities are represented by relative abundances of clones belonging to the different OTUs defined in Fig. 6.

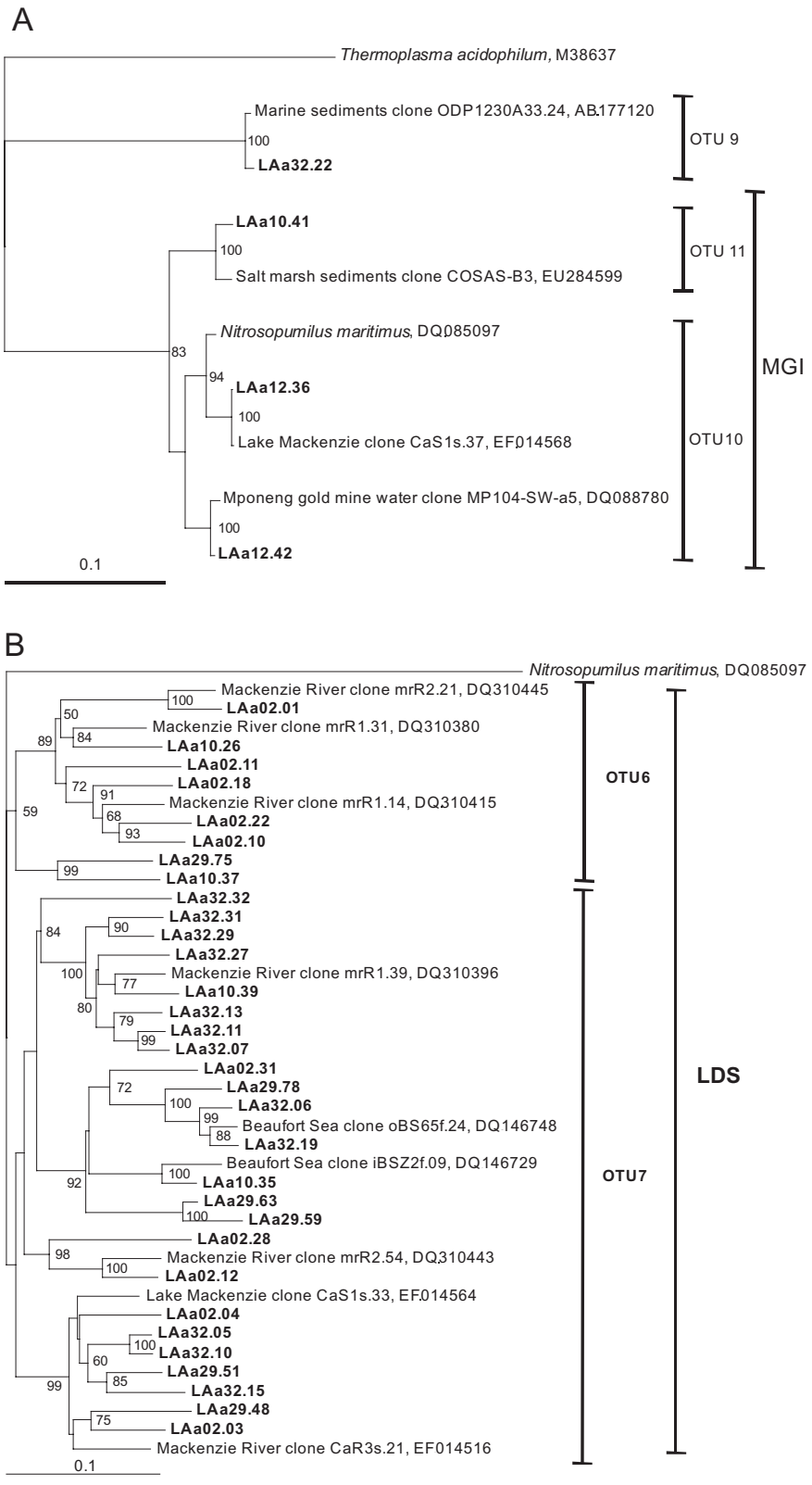

Fig. 6. Distance tree representing the position of Crenarchaeota (A) and Euryarchaeota LDS (B) and RC-V clusters (C) from Lake A. Analyses are inferred from 800-base-pair-long 16S rRNA gene sequences using FITCH distance matrix analysis (from the program PHYLIP). Sequences from this study are shown in bold, with depth of origin indicated (LAa[depth].[clone]). Bootstrap values > 50 are shown. Scale bar: 10% sequence divergence.

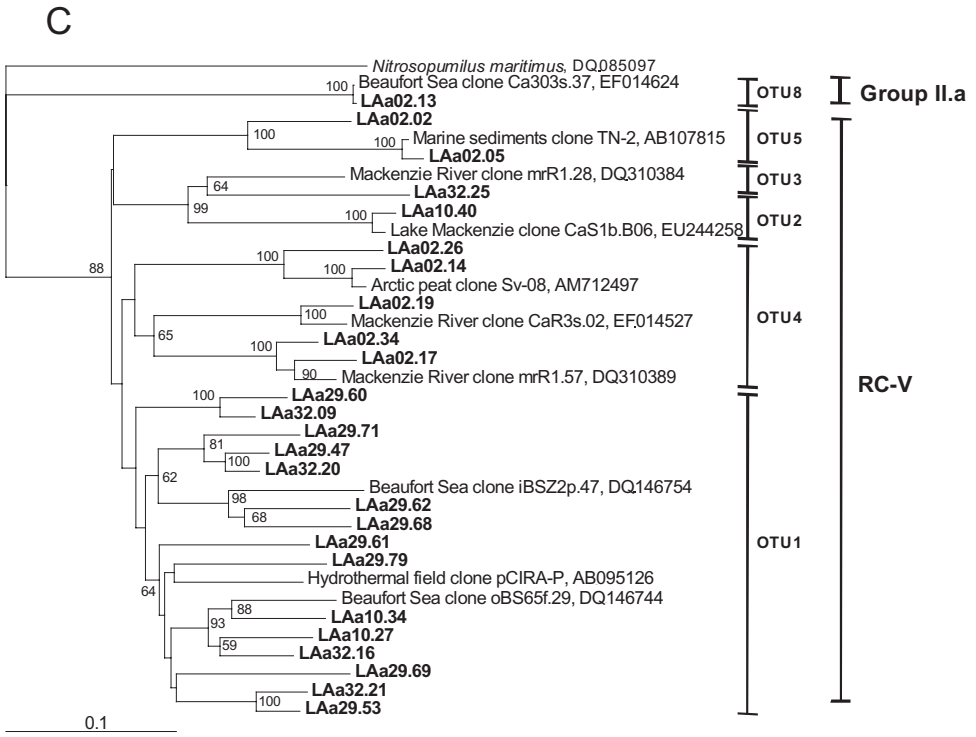

Fig. 6. *cont.*

Euryarchaeota, and OTU 9 related to crenarchaeotal sequences from marine sediments (Fig. 6A and C).

Diversity was highest in samples from the upper oxic layer (2 m) and from the anoxic layer (29 and 32 m), as shown by the estimated number of phylotypes (Chao1, Table 2) and diversity indices (Shannon, Table 2). At 10 m, the diversity was lower than at 2, 29 and 32 m, but much higher than in the oxycline (12 m).

Discussion

Our analyses revealed the presence of the archaeal *amoA* gene in both Lake A and Lake C1, and thereby extend the records of putative archaeal ammonia oxidation to arctic saline lakes. The presence of the *amoA* gene has been recorded in a number of marine waters, and both marine and freshwater sediments indicating a major role for Archaea in the biogeochemical cycle of nitrogen (Francis *et al*., 2007). This first evidence of planktonic archaeal *amoA* genes in these unusual lakes further highlights the likely importance of archaeal mediated nitrification in diverse environments. In both lakes, the highest *amoA* copy numbers were detected in the microaerophilic layers (oxycline). Similar results have been reported for the Black Sea where quantification of the *amoA* gene revealed highest abundance in the suboxic zone (Coolen *et al*., 2007). The Black Sea and our High Arctic meromictic lakes have similar physico-chemical stratification, with an upper oxic layer separated from a deeper anoxic layer by a marked chemocline, consistent with the similar distribution of the *amoA* gene in both systems. The presence of the *amoA* genes in the two Arctic lakes suggests that, as in the Black Sea, Archaea are likely involved in ammonia oxidation at the oxycline where the combined supply of ammonia from below and oxygen from above provide the optimal conditions for nitrification. Evidence to date strongly supports the notion that archaeal ammonia oxidation is carried out by Crenarchaeota belonging to the MGI (Könneke *et al*., 2005; Wuchter *et al*., 2006). The depths where the *amoA* gene was most abundant in lakes A and C1 corresponded to the depths of highest copy number of crenarchaeotal MGI 16S rDNA genes, suggesting that at least some of the MGI Crenarchaeota contain the *amoA* gene, as reported from marine systems.

The ratio *amoA* to 16S rRNA was always very low in our analyses (ranging from 0.0001 to 0.045), and much lower than the ratio of 1 reported elsewhere (Wuchter *et al*., 2006; Mincer *et al*., 2007). This conspicuously low ratio may be because the *amoA* primers were not able to amplify all *amoA* genes present in the lakes or because not all MGI Archaea have the *amoA* gene. Several earlier studies have reported *amoA*:16S rRNA ratios varying from 0.01 to 2.8 in the Black Sea (Lam *et al*., 2007) and from 0.18 to 5.22 in the Atlantic Ocean (Wuchter *et al*., 2006). The primers we used were initially designed from marine sequences (Coolen *et al*., 2007) and thus may

contain mismatches to more brackish or freshwater *amoA* genes. There are few reports of *amoA* genes in these latter systems, which may be a result of undersampling, or poor recovery by the marine primers. The low ratio we observed could also imply that not all of the Crenarchaeota quantified by qPCR contained the *amoA* gene, and that Crenarchaeota encompass a broad range of trophic characteristics apart from ammonia oxidation. We recovered only two phylotypes in the Lake A 12 m clone library, with one of the phylotypes representing < 2% of all clones. It could be speculated that only the less abundant phylotype contained the *amoA* gene, making the ratio very close to two *amoA* copies per cell. Regardless of such a relationship, the maximum numbers of copies for *amoA* and MGI Crenarchaeota were from the oxycline.

Archaea were found at all depths in the two lakes, and the lake communities included both Crenarchaeota and Euryarchaeota. However, our phylogenetic analysis of 16S rDNA sequences revealed that the Archaea present in the oxycline were exclusively restricted to the MGI Crenarchaeota. The limited diversity of Crenarchaeota in this layer provides little ambiguity for the conclusion that the archaeal *amoA* genes belonged to MGI Crenarchaeota. We found that *amoA* genes were present at only low concentrations in the anoxic zone of Lake C1 and were non-detectable in the anoxic zone of Lake A. In these deeper layers, the aerobic archaeal nitrifiers would likely be replaced by anaerobic ammonium oxidation (anammox) bacteria, such as those detected in deep anaerobic waters of the Black Sea (Kuypers *et al*., 2003). Further molecular analysis targeting nitrifying, denitrifying and anammox bacteria are needed to resolve the community of nitrogen cycling microorganisms in these and other lakes.

The phylogenetic analysis of *amoA* genes revealed differences between the Lake A and Lake C1 clone libraries. Each lake was characterized by specific sequence clusters (LA and LC1) distantly related to each other (94% similarity), indicating a clear divergence between archaeal *amoA* containing communities of the two lakes that were only a few tens of km apart and that shared many limnological features. The extent of divergence may indicate niche separation between two phylotypes of ammonia oxidizers where physical or chemical factors were sufficiently different between lakes to select for the different phylotypes. Alternatively, it may reflect differences in founder populations. Our sequences in the LA and LC1 clusters were only distantly related to *amoA* sequences retrieved earlier from marine environments and the two novel clusters may be uniquely lacustrine microorganisms. Additional sampling over different seasons and times and in similar lakes would be valuable in determining the role of ecology versus evolution on this genetic variability.

The results from Lake A 16S rRNA gene analysis provide compelling evidence of vertical structure in archaeal community composition. There were large differences among strata of the lake, with disparate phylogenetic groups dominating the different depths. Depth stratification of archaeal communities has also been found in meromictic Lake Pavin (Lehours *et al*., 2005; 2007) and perennially ice-covered Lake Fryxell in Antarctica (Karr *et al*., 2006). In those two studies, as well as the archaeal community of the anoxic layers of Mono Lake (Scholten *et al*., 2005), many of the Archaea detected belonged to methanogenic Euryarchaeota. In contrast, we did not recover methanogenic sequences, which suggests that methane-related biogeochemical pathways may be little used in these saline Arctic lakes. This observation is consistent with the absence of methane accumulation in many Antarctic saline lakes where sulfate-reducing bacteria likely outcompete methanogens for acetate and hydrogen (Vincent, 1988), and is consistent with the high H_2S concentrations in the anoxic zone of these lakes (up to 48 mmol m^{-3} in Lake A; Gibson *et al*., 2002). Rather than methanogens, we found that both the oxic and anoxic layers of Lake A were dominated by the euryarchaeotal LDS and RC-V clusters, whose functions remain unknown. Both LDS and RC-V are phylogenetically diverse and reported from a large variety of environments (Galand *et al*., 2006), suggesting diverse metabolic characteristics and physiologies. Sequences belonging to those clusters have previously been reported from freshwater systems such as an Arctic river (Galand *et al*., 2008) and a temperate lake (Glissmann *et al*., 2004). RC-V has also been reported from Mono Lake (Scholten *et al*., 2005), an alkaline lake that occasionally is meromictic. The presence of LDS sequences in High Arctic lakes extends the record of this group to saline and anoxic waters. RC-V and LDS sequences in the deep layers were different from those higher in the water column, suggesting that the deep Euryarchaeota were specifically adapted to saline, anoxic waters. Sinking phytoplankton may have provided a transport mechanism to introduce particle-attached Archaea from surface to bottom waters. However, high-performance liquid chromatography (HPLC) pigment analysis showed that most of the chlorophyllous pigments in the deep layers was associated with photosynthetic sulfur bacteria, which were confined to the anoxic zone (D. Antoniades, unpublished). The pigment data also indicated a highly stratified microbial community structure in these lakes.

The oxycline of Lake A was the most phylogenetically distinct, with a low diversity community of only a few crenarchaeotal sequences. These sequences belonged to a cluster related to the only cultivated Crenarchaeota capable of ammonia oxidation (Könneke *et al*., 2005). The combined presence of Crenarchaeota and *amoA* genes is a strong indication for putative ammonia-oxidation by Crenarchaeota in the microaerophilic (hypoxic) layer of

these far northern lakes. Nutrient profiles for these lakes show that the location of the *amoA* maximum was between the upper zone of high oxygen and the deep zone of high ammonia, which in Lake A rises from undetectable in the surface (subice) waters to 650 mmol m^{-3} at depth (Gibson *et al*., 2002). This intersection of gradients would provide optimal conditions for nitrification, as observed elsewhere (e.g. Lake Vanda, Antarctica; Vincent *et al*., 1981).

Lake A is a highly stratified ecosystem as a result of strong salinity gradients and perennial ice cover that prevents wind-induced mixing. This stratification results in a great variety of physico-chemical conditions within the water column, which in turn would favour diverse microbial niches. Community stratification of Bacteria was earlier reported during a period of meromixis in highly saline, alkaline Mono Lake, California (Humayoun *et al*., 2003), and for Archaea in Lac Pavin (Lehours *et al*., 2005), and was attributed to niche differentiation across REDOX gradients in these waters. In polar meromictic lakes, the perennial ice cover reduces exchange of gases such as oxygen with the atmosphere and also results in greenhouse conditions that heat the water column (Spigel and Priscu, 1998; Vincent *et al*., 2008). Annual loss of ice cover during summer as a result of climatic variability or global warming (ACIA, 2004) would radically alter the stratification regime of these lakes (Vincent *et al*., 2008) and would likely cause a massive disruption of microbial habitats. The localized and likely specialized archaeal communities in the oxycline would be especially vulnerable to this disruption.

Conclusions

The presence of archaeal *amoA* genes in Arctic meromictic lakes provides further support for the global role of Archaea in the nitrogen cycle. The concomitant presence of MGI crenarchaeotal and *amoA* genes indicates the potential for crenarchaeotal ammonia oxidation in these lakes; however, further activity and expression measurements are needed to confirm the biogeochemical significance of *amoA* in these lakes. The spatial concordance of highest copy number of *amoA* genes, unique crenarchaeotal sequences and conditions conducive to nitrification in the oxycline indicate a niche-specific occurrence of ammonia oxidizers. Our results also show the presence of distinct *amoA* gene clusters in different lakes, indicating *amoA* diversity in lacustrine and brackish systems. Although process measurements and more detailed molecular studies are required to understand the role of the different Archaea down the water column, these analyses provide evidence of vertical niche separation, as well as potential geographical separation of archaeal niches between lakes.

Experimental procedures

Sampling

We sampled two permanently stratified (meromictic) lakes of the northern coast of Ellesmere Island (latitude 83°N, Nunavut, Canada). These lakes result from isostatic rebound and trapping of seawater between 2500 and 4000 years ago (Lyons and Mielke, 1973; Jeffries and Krouse, 1985; Ludlam, 1996). Since that time, they have been completely closed off from exchange with the sea. Because of extreme cold temperatures in this region (average annual air temperature of −20°C; Vincent *et al*., 2008), they have an ice cover that persists through most or all of the year and preventing wind-induced mixing of the water. The waters are further stabilized by strong salinity gradients, from recent snow and ice melt water at the surface over relict seawater at the bottom.

We sampled Lake A and Lake C1 between 29 May and 3 June 2006. Both lakes are along the northern coastline of Ellesmere Island, Canadian High Arctic (Fig. 1). Lake A (83°00′ N, 75°30′ W) has a maximum depth of 128 m, a surface area of 5 km^2 and a catchment area of 37 km^2 (Van Hove *et al*., 2006). Background limnological information for Lake A is given in Hattersley-Smith and colleagues (1970), Jeffries and colleagues (1984), Ludlam (1996), Gibson and colleagues (2002) and Van Hove and colleagues (2006). Lake C1 is 40 km to the west of Lake A (82°51′ N, 78°12′ W), and has a surface area of 1.1 km^2, a catchment area of 3.3 km^2 and maximum depth of 65 m. Previous limnological studies on Lake C1 are reported in Ludlam (1996) and Van Hove and colleagues (2006).

At the time of sampling, both lakes were covered by a layer of ice (1.25 m for Lake A and 1.07 m for Lake C1). Profiling and sampling were conducted from a mid-lake site through a 22 cm diameter hole drilled with a battery-powered auger (Strikemaster). Profiles for temperature, specific conductivity, pH and dissolved oxygen were measured with a Surveyor 3 profiler (Hydrolab Corporation). Sampling depths were selected in relation to the dissolved oxygen gradient (Table 1) to target the upper oxic zone, the lower anoxic zone and the oxycline, the transition between the two first zones containing a gradient of dissolved oxygen concentrations over the range 0.5–5.5 mg l^{-1}. Water was sampled with a 6.6 l Kemmerer bottle and transferred to an acid-washed opaque 20 l plastic container and stored in the dark at 4°C until processing. Five depths were sampled in Lake A and four depths in Lake C1 (Table 1). Three additional depths were sampled from a second site in Lake A to evaluate the extent spatial heterogeneity across the lake. These duplicate samples were at a site 500 m away from the original station, from the oxic zone (2 m), the oxycline (12 m) and the anoxic zone (32 m).

Cell counts and chlorophyll analysis

Total bacteria and archaea were enumerated from DAPI-stained cells (Porter and Feig, 1980). Microscope slides were prepared in the field by filtering 10 ml of water that had been fixed with glutaraldehyde (1% final concentration) in the dark for 6 h, through a 0.2 μm black 25-mm-diameter polycarbonate filter (Poretics) and the filters were stained with DAPI (5 mg ml^{-1} final concentration, Sigma) for 5 min. Filters were

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

Application	Primers	Primer sequences	Annealing temperature	Targeted group	Reference
Cloning	Arch109f	5′-ACK GCT CAG TAA CAC GT-3′	52°C	Archaea	Grosskopf *et al.* (1998a)
	Arch915r	5′-GTG CTC CCC CGC CAA TTC CT-3′			
	Arch-*amoA*-f[a]	5′-ST AAT GGT CTG GCT TAG ACG-3′	53°C	Archaeal *amoA* gene	Francis *et al.* (2005)
	Arch-*amoA*-r	5′-GC GGC CAT CCA TCT GTA TGT-3′			
qPCR	Parch519f	5′-CAG CMG CCG CGG TAA-3′	63°C	Archaea	Coolen *et al.* (2007)
	Arch915r[b]	5′-GTG CTC CCC CGC CAA TTC CT-3′			
	AOA-*amo*Af	5′-CTG AYT GGG CYT GGA CAT C-3′	58.5°C	Archaeal *amoA* gene	Wuchter *et al.* (2006)
	AOA-*amo*Ar	5′-TTC TTC TTT GTT GCC CAG TA-3′			
	MCGI-391f	5′-AAG GTT ART CCG AGT GRT TTC-3′	61°C	Marine Group I	Takai *et al.* (2004)
	MCGI-554r	5′-TGA CCA CTT GAG GTG CTG-3′			

a. Amplify essentially the entire gene.
b. Also referred as 934r.

mounted onto slides with non-fluorescent mounting oil (Immersol 518 M). Slides were stored in the dark at 4°C and subsequently transferred to a –20°C freezer until epifluorescence microscopy counting at ×1000 magnification with an Olympus BX51 epifluorescence microscope. Samples for pigment analysis were filtered onto GF/F glass-fibre filters that were stored frozen until analysis. The chlorophylls were extracted and then analysed by HPLC, as described in Bonilla and colleagues (2005).

Chemical analyses

Samples for chemical analysis of DOC, TP and soluble reactive phosphorus were filtered through 0.2 μm membrane filters and stored in the dark at 4°C until analysis. The analyses were performed by the Institut National de la Recherche Scientifique (Quebec City, QC, Canada) according to standard protocols. Dissolved organic carbon was analysed by a Shimadzu TOC-5000 A carbon analyser calibrated with potassium biphthalate. Total phosphorus was determined after perchlorate digestion and manual spectrophotometric measurement as in Stainton and colleagues (1977).

DNA sampling and extraction

Microbial biomass was collected by filtering 3–6 l of water through 0.2 μm pore-size Sterivex filter unit (Millipore) after pre-filtration through a 47-mm-diameter, 3 μm pore-size polycarbonate filter. Filters were stored in buffer (50 mmol l^{-1} Tris, 40 mmol l^{-1} EDTA, and 0.75 mol l^{-1} sucrose) and frozen immediately. These filters were subsequently transferred to a –80°C freezer until nucleic acid was extracted as described in Lovejoy and colleagues (2006).

PCR and cloning

Part of the 16S rDNA and *amoA* genes were amplified by PCR with the archaeal specific primers and conditions described in Table 3. After validation by gel electrophoresis, PCR products were purified with Quiaquick PCR Purification Kit (Quiagen) and cloned using TOPO cloning kits (Invitrogen) following the manufacturer's instructions. A total of five 16S rDNA gene clone libraries were constructed from five depths of Lake A (2, 10, 12, 29 and 32 m) and two *amoA* gene clone libraries were constructed from the oxycline of Lake A and Lake C1 (12 and 18 m respectively). For 16S rDNA libraries, positive colonies were screened by restriction fragment length polymorphism (RFLP) following digestion by Taq I restriction enzyme (Invitrogen). Clones with the same RFLP pattern were grouped and considered as members of the same phylotype (Díez *et al.*, 2001), and representatives were sequenced using the vectors' T7p universal primers. Several representatives of the most numerous RFLP groups from each sample were sequenced to verify that the single patterns were closely related sequences. The similarity between sequences from the same pattern was always > 99%. For the *amoA* gene, between 25 and 30 clones were picked randomly from each library and sequenced directly without an RFLP step. Sequencing was done by the Sequencing Center of Laval University Hospital Center (CHUL; Quebec City, QC, Canada). The 16S rRNA and *amoA* gene sequence data obtained in this study have been archived in the GenBank database under accession numbers EU750834 to EU750894 and EU781996 to EU782024 (16S rRNA) and EU667426 to EU667431 (*amoA*).

Diversity and phylogenetic analysis

A total of 231 16S rRNA and 50 *amoA* clones were used for diversity and phylogenetic analysis (Table 2). The coverage of the clone libraries was calculated as $(1 - (n/N)) \times 100$, where n is the number of unique clones detected in the library of size N (Good, 1953; Mullins *et al.*, 1995). Diversity calculations (Shannon and Chao) were made with the DOTUR package (Schloss and Handelsman, 2005) based on 3% difference between sequences. Sequence and phylogenetic analysis was done as described in Galand and colleagues (2006). In brief, the approximately 800 bp sequences were aligned using the CLUSTAL W package (Thompson *et al.*, 1994) and checked manually. Sequences were compared with those in the GenBank database using BLAST at the National Center for Biotechnology Information. Phylogenetic distances were compiled with the Kimura-2 model and trees built with Fitch from the Phylip software (Felsenstein, 2004).

Quantitative PCR analysis

Quantitative 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 3. 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 (2 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. The fluorescence signal was read in each cycle at 78°C for 25 s to ensure stringent product quantification. Reactions were run in triplicate with 2 ng of template DNA from each sample. Quantification standards consisting of 10-fold dilutions ranging from 10^2 to 10^8 copies of DNA of a PCR amplicon of a known clone of the gene of interest. All PCR products were purified with a Qiaquick PCR Purification Kit (Qiagen) and were then analysed by qPCR along with standards. Overall, average efficiencies for all quantification reactions ranged from 44% for MGI Crenarchaeota 16S rRNA gene to 65% for Archaea. R^2 values were always > 0.99. Control reactions included three reactions without DNA as control for contamination and three reactions containing 2 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.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Replicability of the community quantification. qPCR results for Lake A samples from 2, 12 and 32 m taken at the

main site and at a duplicate site 500 m away. (A) Archaeal (B) MGI crenarchaeotal and (C) *amoA* genes expressed as number of copies of genes ml^{-1}. **: less than 0.4 copies ml^{-1} for main and duplicate site.

