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Archaeal *amoA* and *ureC* genes and their transcriptional activity in the Arctic OceanSUBJECT AREAS:
ARCHAEA EVOLUTION
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23 December 2013Accepted
26 March 2014Published
11 April 2014

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Thaumarchaeota and the gene encoding for a subunit of ammonia monooxygenase (*amoA*) are ubiquitous in Polar Seas, and some *Thaumarchaeota* also have a gene coding for *ureC*, diagnostic for urease. Using quantitative PCR we investigated the occurrence of genes and transcripts of *ureC* and *amoA* in Arctic samples from winter, spring and summer. *AmoA* genes, *ureC* genes and *amoA* transcripts were always present, but *ureC* transcripts were rarely detected. Over a 48 h light manipulation experiment *amoA* transcripts persisted under light and dark conditions, but not *ureC* transcripts. In addition, maxima for *amoA* transcript were nearer the surface compared to *amoA* genes. Clone libraries using DNA template recovered shallow and deep *amoA* clades but only the shallow clade was recovered from cDNA (from RNA). These results imply environmental control of *amoA* expression with direct or indirect light effects, and rare *ureC* expression despite its widespread occurrence in the Arctic Ocean.

The global nitrogen cycle depends on the remineralization of organic nitrogen to ammonium and nitrate, with microbially mediated ammonia oxidation by ammonia monooxygenase being a key intermediate step. This process is commonly inferred by the presence of the ammonia monooxygenase α -subunit (*amoA*). The *amoA* gene, found in some Bacteria and some Archaea, is part of a larger gene family of copper-containing membrane-bound monooxygenases¹. Evidence to date indicates that oceanic archaeal *amoA* is involved in converting ammonia to nitrite, a rate limiting step in aerobic nitrification². The discovery of non-extremophile marine group I (MGI) Archaea that could grow chemolithoautotrophically by aerobically oxidizing ammonia to nitrite³, and the occurrence of the *amoA* gene in other uncultivated, non-extremophile MGI^{4,5}, provided an added perspective on the nitrogen cycle. The widespread detection of both MGI and archaeal *amoA* in the ocean combined with the rarity of bacterial nitrifiers has led to the new paradigm that Archaea are largely responsible for marine nitrification^{6–8} and in particular may contribute substantially to nitrification in the upper few hundred meters of the ocean⁹. Interestingly, nitrification by bacteria is inhibited by light, but ammonia oxidizing Archaea (AOA) are often retrieved from the euphotic zone, and the effect of light on *amoA* is not resolved^{10,11}.

MGI are sufficiently distinct that they are considered a phylum of Archaea, *Thaumarchaeota*¹², but the diversity and metabolic capacity of these largely uncultivated microorganisms remains to be fully elucidated. In addition to potentially oxidizing ammonia, genomic surveys indicate that some MGI may use urea, broken down to ammonium (NH₄⁺) and inorganic carbon (C), as an energy and carbon source. The genes that code for urease have been reported in *Cenarchaeum symbiosum*⁴ and “*Candidatus Nitrosopumilus sediminis*” AR2 from Svalbard¹³. Recently *ureC*, the gene that codes for a subunit of *urease* has been amplified from the deep Mediterranean Sea¹⁴, and detected in metagenomes from a deep hydrothermal plume¹⁵ and the deep Pacific Ocean¹⁶. The *ureC* gene has also been reported from surface waters of the Gulf of Maine¹⁷ and in the winter Arctic Ocean¹⁸. Alonso-Saez and colleagues¹⁸ amplified the *ureC* gene from deep Antarctic waters, but they failed to detect it in summer Antarctic surface waters. In that same study the Archaeal *amoA* gene was amplified from the same surface waters, suggesting that the potential for MGI Archaea to use urea is not universal. In fact, *ureC* and the *urease* are not found in cultivated *N. maritimus*¹⁹.

Over much of the Arctic, nitrogen availability limits photosynthetic productivity, with nitrate rapidly depleted in surface waters during the short summer growing season. However, nitrate concentrations are substantial in and below the halocline²⁰. Interestingly, Northern Baffin Bay stands out from much of the Arctic, with high



photosynthetic rates continuing throughout the Arctic summer, despite low standing stocks of inorganic nitrogen in the euphotic zone^{21,22}. Galand and colleagues²³ found that MGI and archaeal *amoA* genes were abundant in Northern Baffin Bay, particularly in the Arctic halocline, leading to the hypothesis that the archaeal community could influence photosynthetic community structure by rapidly recycling inorganic nitrogen in the euphotic zone. Nitrate concentrations in the surface waters of Baffin Bay are often below the detection limits (ca. 0.03 $\mu\text{mol N L}^{-1}$)^{24,25}, but ammonium concentrations can be substantial²⁶, suggesting bacterial degradation of fixed organic material and possible release of ammonium. The high affinity of MGI Archaea for ammonium²⁷ could enable MGI to successfully compete with phytoplankton for ammonium, and result in *amoA* expression nearer the surface. In contrast to NH_4 , there have been few measures of urea in the Arctic, but in general, concentrations are variable and sporadic^{28,29}, which leads to the hypothesis that expression of genes coding for urease would also be variable. The presence of archaeal *ureC* genes in Arctic MGI Archaea is intriguing¹⁸ but to date there is no evidence of *ureC* being expressed in polar waters.

The main objective of our study was to quantify the *amoA* and *ureC* transcripts in three regions of the Arctic Ocean (Amundsen Gulf, Lancaster Sound and Baffin Bay) and to gain an understanding of conditions associated with gene expression. To this end, we investigated *amoA* and *ureC* transcripts by qPCR during polar darkness and around the vernal equinox. We then experimentally investigated *amoA* and *ureC* expression in natural communities exposed to typical irradiances of the Arctic Ocean euphotic zone. Finally we investigated the depth distribution of genes and transcripts from MGI *Thaumarchaeota*, archaeal *amoA* and *ureC*, along a north to south gradient of the hydrographically complex center of the Northern Baffin Bay³⁰, targeting water masses with different bio-physical properties. Phylogenetic differences between the more likely active and more likely dormant *Thaumarchaeota* with *amoA* were also examined through cloning and sequencing the *amoA* gene from both DNA and cDNA (from total RNA).

Results

Effect of light on *ureC* and *amoA* transcripts. In the Amundsen Gulf region (Western Canadian Arctic), *amoA*, transcripts, *ureC* transcripts and MGI 16S rRNA were quantified on 3 dates as ambient light conditions increased over time (January, March and the beginning of April). In surface waters, *amoA* and MGI copies mL^{-1} were greatest in January. At the halocline, more transcripts were recorded in April. There were always more transcripts at the halocline than at surface. *UreC* transcripts were only detected in January (Figure 1).

In the initial samples from Lancaster Sound (Eastern Canadian Arctic) used for the light-manipulation experiment, *amoA* transcripts were detected at T_0 from both 1% (80 m deep) and 25% (25 m shallow) surface irradiance (Figure 2), with an order of magnitude (ca. 10^5 versus 10^4) fewer transcripts near the surface. *UreC* transcripts were successfully quantified from the freshly collected deeper sample (T_0), with 10 copies per mL of seawater, but not in the sample from 25% irradiance. There were no significant changes in MGI 16S rRNA copies mL^{-1} over the 48 hours, and no effect of light (3 way ANOVA) on MGI 16S rRNA (Figure 2). There were, however, significantly more copies of MGI 16S rRNA in the deeper sample compared to the 25 m water over the 48 h incubation ($P < 0.001$).

The *amoA* transcripts were significantly greater in the dark than in the light overall, but the effect of collection depth was not significant (see below). The multiple pairwise comparisons revealed several interesting trends. After 24 hours, *amoA* transcripts were always greater in the dark (ANOVA- pairwise multiple comparison, $P = 0.008$) compared to bottles exposed to light. After 48 h, the *amoA* transcripts from the two depths diverged, with a significant increase under both light and dark conditions in the 25 m origin treatments ($P < 0.001$), conversely in the 80 m origin treatments, *amoA* transcripts decreased significantly ($P < 0.001$) (Figure 2). Transcripts of *ureC* were not detected in the experimental treatments.

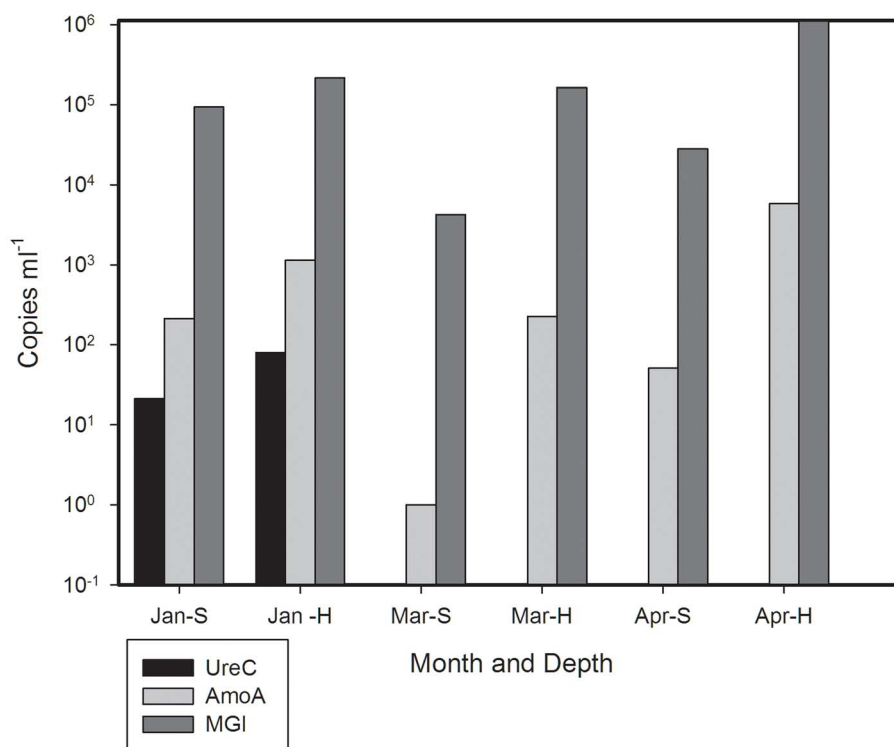


Figure 1 | Number of MGI rRNA, *ureC* and *amoA* transcripts from winter Amundsen Gulf waters. Standard errors of triplicates were smaller than the size of the symbols. The abbreviation for the month is followed by relative depth of collection: surface (S) or halocline (H).

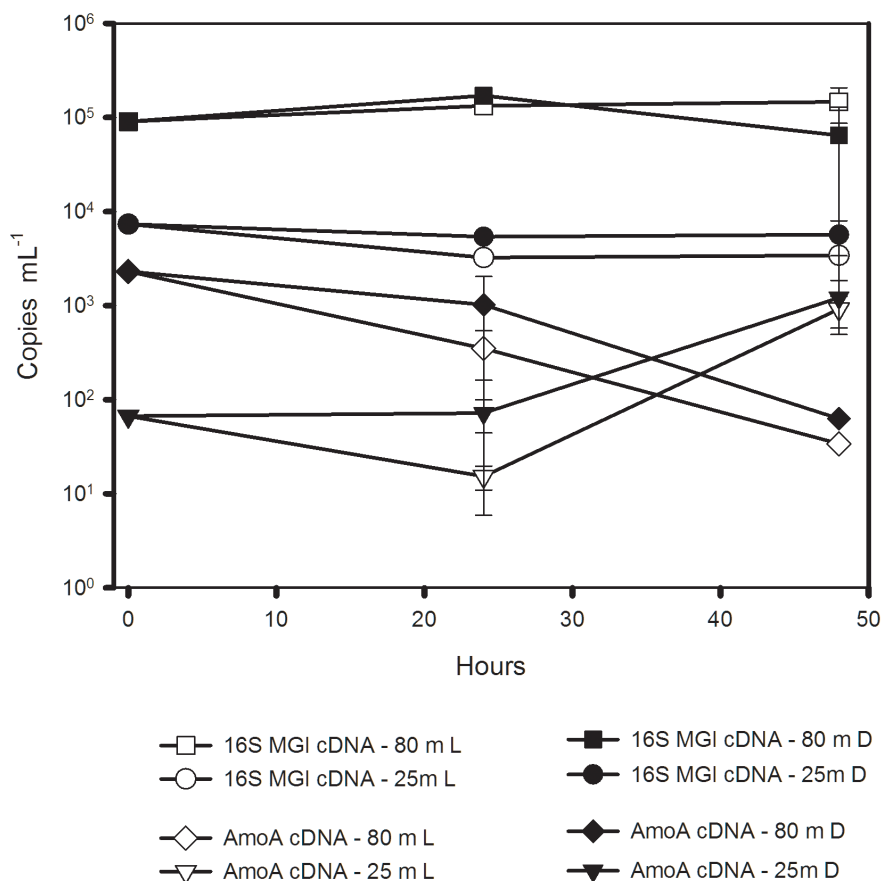


Figure 2 | Number of MG1 rRNA and *amoA* transcripts in the light manipulation experiment from Lancaster Sound samples, which were collected at 25 m (25% surface irradiance) and 80 m (1% surface irradiance). Light treatment (L, open symbols), dark treatment (D, closed symbols).

Environmental and biological conditions in Baffin Bay. The Baffin Bay water columns (Stns. 129, 123 and 109) were density stratified with a subsurface chlorophyll maximum (SCM) just above a pycnocline (from the maximum rate of change in sigma theta) at 25–30 m (Figure 3). Secondary chlorophyll fluorescence peaks were also detected at ca. 70–100 m. The highest extracted Chl *a* values were 1.4 $\mu\text{g L}^{-1}$ at Stn 129–22 m and 1.3 $\mu\text{g L}^{-1}$ at Stn 109–25 m. Most other values were below 1 $\mu\text{g L}^{-1}$ (Supplementary Table S1). Dissolved oxygen concentrations ranged from 279 to 356 $\mu\text{mol kg}^{-1}$ (Figure 3). At Stn123, there was a low oxygen intrusion between 200 and 300 m (Figure 3) coinciding with slight increases in salinity and temperature (not shown). Bacteria concentrations were ca. 10^5 cells mL^{-1} and tended to decrease with depth, with slightly higher concentrations above the pycnocline (Supplementary Table S1). Nitrite concentrations ranged from 0.05 to 0.17 $\mu\text{mol kg}^{-1}$, nitrate concentrations ranged from below the detection to 15.34 $\mu\text{mol kg}^{-1}$ (Supplementary Table S1). The nitrite maxima were above nitrate maxima and between 100–200 m at the three stations (Fig. 4). Ammonium concentrations were variable with higher values in the upper waters (Supplementary Table 1).

In September 2006, the sun was above the horizon for ca. 15 h on Sept. 11 to ca. 12 h on Sept. 20. Surface values of photosynthetically active radiation (PAR) were lowest at station 129 (1.74 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), which was sampled at 05:17 UTC, during twilight prior to apparent sunrise at the latitude of sampling. The other three stations were sampled with the sun higher above the horizon and surface PAR values were much greater, especially at Station 109, sampled on a clear day ca. 4 h after apparent sunrise (Table 1). Subsurface PAR values for samples collected at depth were consequently greater at Stn 109 compared to the other stations.

Transcript and gene quantification in the water column. MG1 16S rRNA (from cDNA) qPCR values ranged from ca. 175 to 4.5×10^5 copies mL^{-1} of seawater collected, and *amoA* transcripts from 18 to 1829 copies mL^{-1} (Supplementary Fig. S1). In contrast to *amoA*, where transcripts were detected in all samples tested, *ureC* transcripts were never detected from the Baffin Bay samples.

Baffin Bay *amoA* transcripts varied down the water column and between stations. The station (Stn) 129 maximum for *amoA* transcripts was at 35 m, which was above the nitrite maximum (Figure 4; note the log scale for depth). For Stn 123, the maximum *amoA* transcripts were just below the nitrite maximum. Finally for Stn 109, the *amoA* transcript maximum was at 90 m, which was above the nitrite maximum (Fig. 4).

In Baffin Bay, gene abundance (from DNA) also varied with depth and station. Stn 129 maxima for *amoA*, *ureC* and MG1 16S rRNA genes were detected in the 120 m sample (Figure 5a). At Stn. 123, the maxima for MG1, *amoA* and *ureC* genes were at 280 m and coincided with the nitrate maximum at the base of a low oxygen intrusion (Figs. 3 and 5a). At station 109, gene copies of MG1, *amoA* and *ureC* were also all greatest nearer the nitrate maximum at 195 m (Fig. 5a).

Baffin Bay qPCR values from DNA template ranged from 16 to 3756 copies of MG1 (16S rRNA) genes mL^{-1} , 24 to 5270 *amoA* gene copies mL^{-1} , and 45 to 2780 *ureC* gene copies mL^{-1} . Overall *amoA* gene copy numbers were significantly correlated with MG1 rRNA genes (Tau, $T = 0.74$, $p < 0.001$) as were *ureC* gene copies to MG1 rRNA genes ($T = 0.66$, $p < 0.001$) (Fig. 5b). *UreC* gene copy numbers also correlated with *amoA* gene copies ($T = 0.79$, $p < 0.001$), and genes followed depth related variables, such as silica, phosphate and nitrate (Supplementary Fig. S2). The ratio of *amoA* genes to MG1 16S

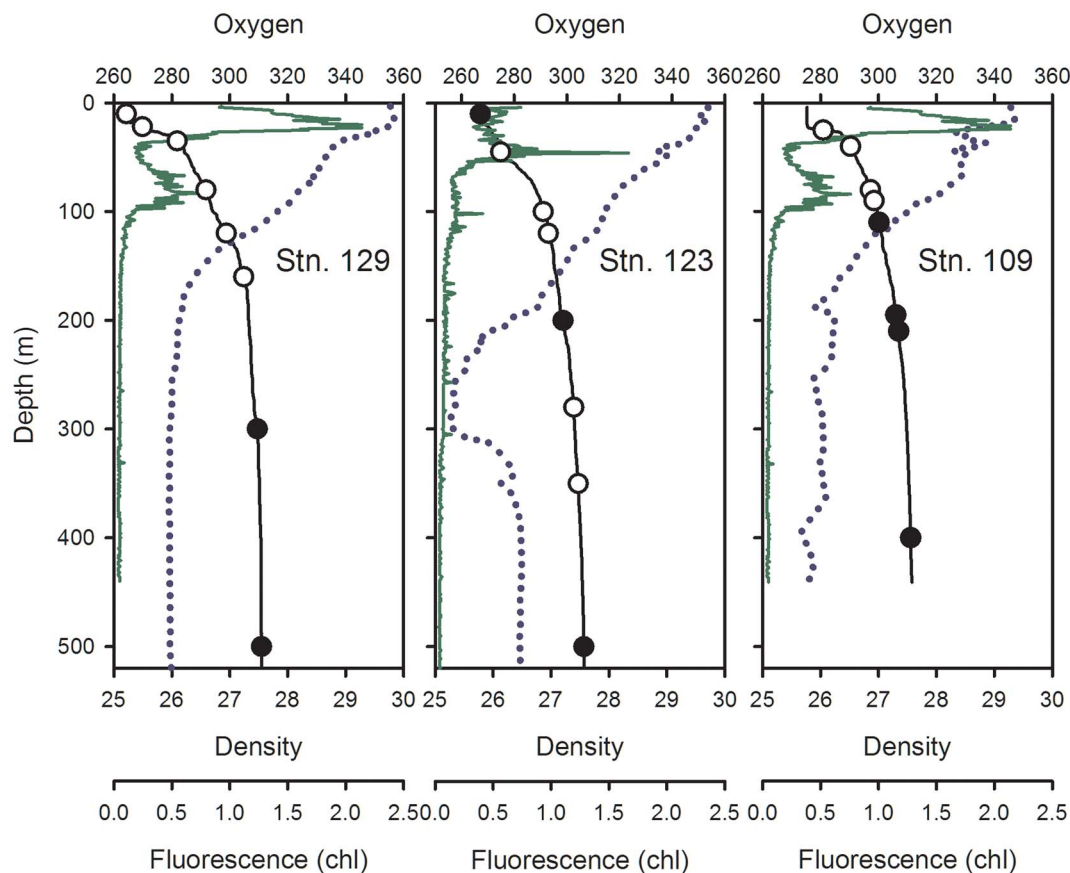


Figure 3 | Depth profiles of Northern Baffin Bay Stations 129, 123 and 109. Density (σ_t) is indicated by the solid black line, circles embedded in these lines show sample depths for DNA and RNA (open circles) and for DNA only (filled circles). In situ chlorophyll fluorescence (relative units) is shown by the green line and oxygen concentrations ($\mu\text{mol kg}^{-1}$) by the dotted line.

rRNA genes was on average 1.8 ranging from 0.2 to 5.5. There were fewer *ureC* gene copies than *amoA* copies with an average ratio of 0.4 *ureC* genes to MGI 16S rRNA genes (Figure 5b).

The maximum abundance of *amoA* transcripts did not correspond to the same depths as the maximum gene copies in any of the water column profiles. Furthermore there was no significant correlation between archaeal *amoA* transcripts and gene copy numbers estimated from the DNA template, nor between MGI 16S rRNA genes and 16S rRNA copies from cDNA.

Across stations and depths from both sides of the Canadian Arctic we found a significant positive relationship between MGI 16S rRNA and *amoA* transcripts (analysis on log transformed data with $r^2 = 0.80$ and $p < 0.001$) (Supplementary Fig. S1). The PCA indicated that nutrients, including silica and phosphate, which were associated with the deepest samples, best explained gene concentrations along the X axis. Chl *a* and ammonium were more associated with *amoA* transcripts and MGI 16S rRNA (Supplementary Fig. S2).

Diversity and phylogeny of *amoA* in Baffin Bay. A total of 423 cloned *amoA* gene sequences with the correct target size (635 nt) from 8 libraries passed chimera and other quality tests. The two libraries constructed using cDNA as a template were asymptotic. There were five OTUs at the 97% similarity level out of 32 clones at Stn 123–200 m and a single OTU from 46 clones at station 109–110 m (Table 2). The DNA libraries were also asymptotic. At 97% similarity, there were 4 OTUs out of 19 clones from Station 109–110 m. Only 6 clones were recovered from the shallowest library (station 129–35 m) and each clone was unique. At the two stations and depths where both DNA and cDNA were used as templates, the DNA derived communities were more diverse

(Table 2). All closest matches were to uncultured environmental archaeal *amoA* genes (Fig. 6).

Maximum likelihood (not shown) and Neighbor joining phylogenies (Figure 6) placed all of the *amoA* sequences into previously defined marine groups, corresponding to clades A and B defined by Francis *et al.*³¹, with no sequences within their group C. All *amoA* sequences from RNA source libraries grouped within clade A, which is thought to represent shallow ecotypes³². The most common cDNA sourced *amoA* sequences grouped together in a sub-clade that we annotated A.A, which corresponds to the group A of Kalanetra *et al.*³³ (Figure 6). Our sub-clade A.A sequences were > 99% similar to other polar sequences (not shown). At Stn 123–200 m, *amoA* clones from two clusters (indicated as A.B. and A.A.a in Figure 6) were most often from cDNA (representing > 50% of cDNA clones). At Stn. 109–110 m, only A.A.a was retrieved from cDNA. A third Arctic sub-cluster in the A clade (A.C. in Figure 6) had no cDNA representatives. DNA sequences fell into both A and B clades³². Overall 40% of the sequences from the DNA libraries fell into the B clade.

There was some *amoA* clone clustering according to depth. The shallow A.A.a group was, for example, was absent from the 400 m sample and one deep B sub-clade was overrepresented in the deepest samples (Figure 6).

Discussion

Taken together, the lines of evidence suggest that *amoA* expression was influenced by light but *amoA* containing MGI persisted in the upper waters of the Arctic Ocean, and were possibly more active when irradiance (PAR) was lower. Our qPCR results indicated that *amoA* transcripts were more abundant in surface waters during

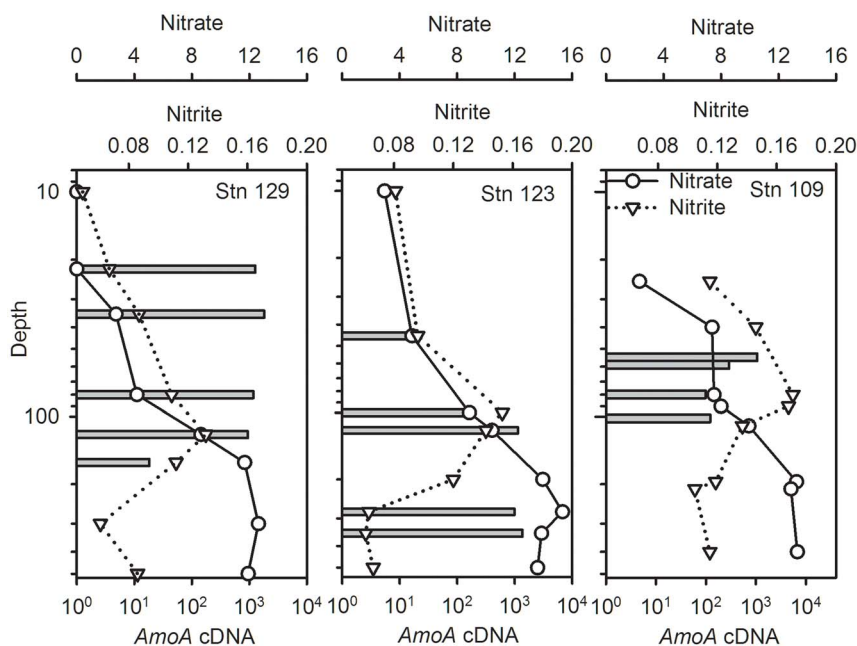


Figure 4 | Nitrate (solid lines with open circles) and nitrite (dotted line with open triangles) concentrations down the water column ($\mu\text{mol L}^{-1}$), note log scale for depth. Bars indicate copies mL^{-1} of *amoA* from cDNA.

January, the darkest winter month, and later more abundant in the halocline where light levels were lower. In the light manipulation experiment *amoA* transcript levels were significantly higher in the dark. We found that actual irradiance levels at the time of sample collection likely influenced transcript abundance, with more transcripts detected in samples from the euphotic zone (defined as $>1\%$ of surface PAR), when surface irradiance was lower. Light may inhibit *amoA* activity directly, promote more rapid degradation of transcripts, or have secondary influence on substrate availability, for example phytoplankton may be better able to compete with ammonium oxidizing Archaea for ammonium when light levels are greater. Similarly, in the Pacific, Church *et al.*³⁴ reported peak *amoA* transcript abundance at the bottom of the euphotic zone.

Our results indicated that *amoA* transcriptional activity probably occurs throughout winter. Compared to other oceanic regions, Arctic and Antarctic MGI 16S rRNA genes are more abundant nearer the surface, especially in winter and early spring³⁵, with values lower in summer³⁶. Collins *et al.*³⁷ also reported MGI 16S rRNA genes in newly formed sea ice in the Beaufort Sea in October when light levels are rapidly decreasing. The lower expression that we observed nearer the surface compared to the halocline in March and April could be due to direct light inhibition or lack of substrate. The net effect was a decline in *amoA* near surface activity in spring when phytoplankton activity begins^{38,39}.

In Baffin Bay, distribution profiles of the *amoA* transcripts down the water column were closely correlated with the MGI 16S rRNA indicating that the MGI were in a state consistent with being metabolically active and with ammonium oxidation having occurred around the time of sampling. This was notable since the transcript distribution differed from the gene distribution (from DNA) (Figure 3); suggesting that environmental gene distribution may not accurately reflect activity. In the Pacific, Church *et al.*³⁴ reported similar results with relatively more *amoA* transcripts nearer the surface, despite higher gene numbers at depth. The relationship between the euphotic zone and nitrification activity was reported in a North-South transect of the Atlantic Ocean, ammonia oxidizing activity was greatest at the base of the euphotic zone, and decreased with depth⁴⁰. But this depth dependent distribution, did not occur in the Black Sea and the highest expression of *amoA* is within a nitrification zone coinciding with low oxygen levels⁴¹. At Stn 123, where we sampled a lower oxygen layer, *amoA* transcripts were high compared to other stations sampled at the same depth, but higher still at the base of the euphotic zone. While we did not directly measure nitrification, we speculate that in the Arctic *amoA* gene maximum activity differed from DNA gene distribution due to substrate availability over relevant time spans, with *amoA* transcripts associated with Chl *a* and ammonium concentrations in the PCA analysis (Supplementary Fig. S2). In Baffin Bay, dissolved organic matter (DOM) is released by

Table 1 | Sampling dates and time in Universal Coordinated Time (UTC), latitudes and longitudes, and surface values for photosynthetically available radiation (Surface PAR; $\mu\text{mol photons m}^{-2} \text{sec}^{-1}$) at the time of sampling. UTC apparent sunrise and sunset calculated using the NOAA ESRL calculator. <http://www.srrb.noaa.gov/highlights/sunrise/sunrise.html>. Data not available (n/a)

Station	UTC Date-Time	Latitude	Longitude	UTC sunrise	UTC sunset	Surface PAR
Northern Baffin Bay						
129	11/09/06 05:17	78° 19.75	74° 00.84	09:07	00:36	1.74
123	13/09/06 12:55	77° 20.55	74° 38.44	09:30	0:14	74.71
109	17/09/06 14:19	76° 15.28	74° 10.39	10:00	23:38	369.50
Lancaster Sound						
301	20/09/06 23:03	74° 7.44	83° 20.69	11:00	23:51	46.92
Amundsen Gulf						
D14	08/01/08 14:05	71° 31.58	125° 35.76	never	n/a	0
D29	10/03/08 17:26	71° 2.31	123° 54.65	20:30	01:58	0
D36	09/04/08 17:31	71° 18.43	124° 34.32	12:32	04:11	n/a

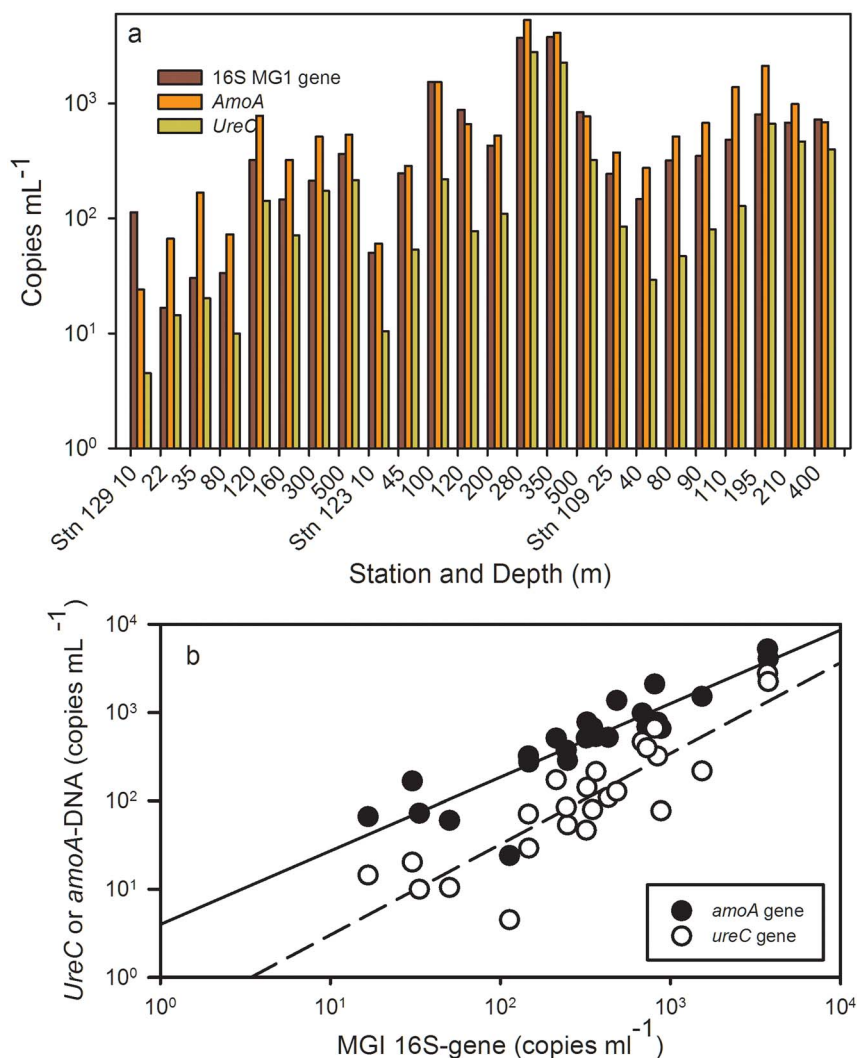


Figure 5 | Panel (a) Northern Baffin Bay, qPCR copies mL⁻¹ of MGI, *amoA* and *ureC* genes (DNA template). Station number and depth of sampling are indicated along the X axis. Panel (b) Log-log plots of gene copies of *ureC* (open circles) and *amoA* (closed circles) compared the 16S rRNA genes of MGI. Lines indicate significant linear regressions of the log transformed data; the dashed line is *ureC* versus MGI ($r^2 = 0.76$, $p < 0.001$), the solid line is *amoA* versus MGI ($r^2 = 0.78$, $p < 0.001$). Standard errors of triplicates and standards were smaller than the size of the symbols.

phytoplankton leakage²¹, and zooplankton and protist grazing⁴². This DOM is quickly broken down by bacterial activity⁴³. The higher probable microbial activity at the base of the euphotic zone stands out as the overriding factor associated *amoA* transcript abundance. In contrast, the surface MGI populations, are most likely maintained by

diurnal activity during periods of low incoming solar radiation each day.

In early September, the greatest concentrations of MGI 16S rRNA and *amoA* genes in Baffin Bay were at the nitrate maximum, similarly in the Pacific, the same two genes tracked nitrate concentrations down the water column¹¹. However, our PCA showed that phosphorus and silica, with higher concentrations in deeper waters, had a stronger relationship with gene copy numbers in Northern Baffin Bay, consistent with the oceanography of the region²¹. The abundance of genes, but not transcripts, below the subsurface chlorophyll maximum layer may be linked to loss rates. For example, protists that graze on prokaryotes are also more abundant above the nitracline⁴⁴. Alternatively, the lower numbers of *amoA* transcripts per MGI 16S RNA gene deeper in the water column could also be due to inherent biological properties of dominant clades in deeper waters⁴⁵.

For *ureC*, we detected the gene at all depths and stations, and in a parallel study, the gene was reported in Amundsen Gulf in March and February¹⁸, indicating that MGI with the *ureC* gene are widely distributed in the Arctic, irrespective of season or depth. In contrast to *amoA* transcripts, which were detected in all samples, we only recorded *ureC* transcripts in samples from January, when photosyn-

Table 2 | Summary of clone libraries constructed targeting archaeal *amoA* using DNA and cDNA as templates. Number of clones from each library, and number of OTUs defined at a similarity level of $\geq 97\%$. Depth of initial water sample collection in meters (m)

Station	Depth	Template	Clones	Library	OTUs
129	35	DNA	6	a.129.35.d	6
129	120	DNA	91	a.129.120.d	12
123	200	DNA	51	a.123.200.d	8
123	200	cDNA	32	a.123.200.r	5
123	280	DNA	86	a.123.280.d	11
109	110	DNA	19	a.109.110.d	4
109	110	cDNA	46	a.109.110.r	1
109	400	DNA	92	a.109.400.d	13

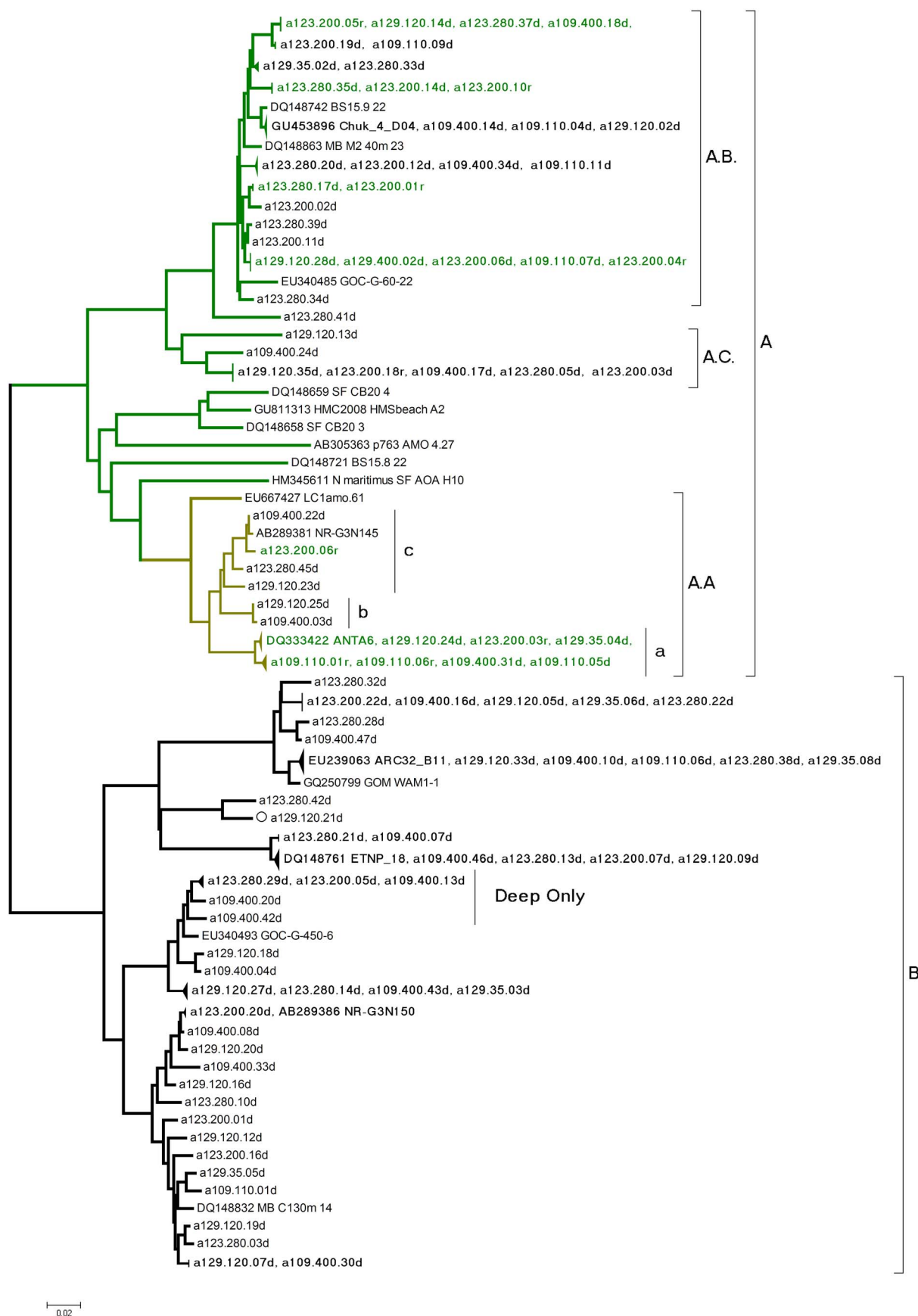


Figure 6 | Distance tree of the *amoA* gene from the Northern Baffin Bay. Major clades A and B from (Francis *et al.* 2005) are indicated; A.a coincides with A in Kalanetra *et al.* (2009). All reference sequences except *N. maritimus* were from open water (Francis *et al.* 2005). Sequence names in this study consist of station number and sample depth followed by d for DNA or r for RNA (from cDNA).



thetic primary production was not possible because there is no light, and from a sample, collected from the bottom of the euphotic zone in Lancaster Sound, which is a region of high biological activity²⁵.

The phylogeny of the *amoA* gene implies the presence of at least two distinct MGI ecotypes. All *amoA* transcripts grouped within the clade A³¹, which is classed as a shallow group³². More specifically, in our clone libraries the most abundant cDNA *amoA* clones were closely related to each other and within a single sub-clade (A.A). This sub-clade was originally identified by Kalanetra *et al.*³³ from the central Arctic Ocean, with representatives from the Southern Ocean, and may be typical for cold waters. In contrast, *amoA* sequences from cluster B, which is thought to be more representative of the deep ocean³², were never recovered from cDNA clone libraries suggesting this clade was dormant in Baffin Bay at the time of sampling. DNA based and cDNA based clone libraries from the central California Current recovered both A and B clusters and the A cluster dominated in all but the deepest samples⁹. Recently Sintez *et al.*⁴⁵ explored *amoA* gene diversity using *amoA* primers designed to detect two distinct clades of the AOA; one associated mostly with less deep waters and high ammonium concentrations (HAC) and the second clade, occurring in deeper waters and with low ammonium concentrations (LAC). Both primer pairs detected high concentrations of *amoA* HAC and LAC in an Arctic coastal site with co-occurrence down the water column.

The co-occurrence of the two AOA clusters in the Arctic Ocean, even near the surface, is intriguing. Inspecting gene ratios from our qPCR results we note the potential of 2 copies of the *amoA* gene within ca. 60% of the MGI (with a 1.8 to 1 ratio of *amoA* genes to 16S rRNA genes), but fewer MGI containing the *ureC* gene (with a ratio of 0.4). In effect, the persistence of a dormant B clade at similar occurrence rates (40% of our DNA template clones) and the observation of a *ureC* gene to MGI 16S rRNA gene ratio of 0.4 may be linked.

Apart from its initial discovery in the coral reef endosymbiont⁴⁶, the archaeal *ureC* gene has been most often reported from deeper waters or winter surface waters where concentrations of fresh organic material would seem to be low⁴⁷. The sporadic amplification of *ureC* transcripts over a range of samples from the Arctic, is telling. As mentioned earlier, there appeared to be a light effect, but the rarity and weak concentrations of *ureC* transcripts may also be a result of the ephemeral occurrence of urea itself in the Arctic environment²⁹. Zooplankton are the main source of urea in the Arctic, with some copepod species persisting and feeding near the surface and at mid-depths throughout the Arctic winter⁴⁸. Interestingly, one copepod species, *Acartia tonsa*, releases different forms of nitrogen depending on diet; with a mixed diet of heterotrophic protists and diatoms resulting in greater release of urea compared to either dietary source alone⁴⁹. Copepod species in the Arctic are mostly opportunists and able to switch to the dominant food source⁵⁰ and thus the taxonomy of the protist plankton prey could also result in the variable urea concentrations. The episodic but poorly understood input of urea into the surrounding waters could be sufficient for maintaining *Thaumarchaeota* with the capacity to scavenge and use urea as a carbon and energy source. While the archaeal *ureC* gene has been reported in the Arctic and elsewhere¹⁸, transcriptional activity to date has only been reported in very deep waters¹⁴ and a coral symbiont⁴. The similarity between the two types of environments is not obvious, except possibly the availability of animal generated urea.

Overall we found evidence of two MGI populations, one active nearer the surface, and a second, deeper more dormant population, which may be reliant on sporadic inputs of urea as a source of energy and carbon. Finally, while *amoA* transcript abundance was greater in the dark, light inhibition was not complete and transcripts persisted under low irradiance. The distribution of AOA in the Arctic suggests multiple links between biological and physical properties in this region.

Methods

Sampling. Samples were collected from Amundsen Gulf during the Circumpolar Flaw Lead System Study (CFL)⁵¹ in winter 2008–2009, the North Water region of Northern Baffin Bay between Ellesmere Island and Greenland from 11–17 September 2006 and from near Lancaster Sound on 20 Sept 2006 (Table 1). Samples for both missions were collected from the research icebreaker CCGS Amundsen. Water from the Amundsen Gulf was sampled from 2 depths: surface (ca. 10 m) and at the halocline that is indicative of Pacific origin waters³⁹ (ca. 50 m) in January, March and April. The three Northern Baffin Bay stations, 109, 123 and 129, were sampled along a south–north transect. Since the cruise was multidisciplinary, with constrained logistics, samples were taken opportunistically over the 24 h period (Table 1). All seawater samples were collected with 12-L Niskin type bottles (Ocean Test Equipment) mounted on carousel rosette system equipped with a SBE-911 plus conductivity–temperature–depth (CTD) profiler with a dissolved oxygen sensor. Other instruments on the rosette system recorded fluorescence (Seapoint), transmissivity (WetLabs C-Star Transmissometer), photosynthetically active radiation (PAR; Biospherical Instruments), and relative nitrate concentrations from an in situ ultraviolet spectrometer (ISUS) probe (Satlantic). Water sample depths were selected on the downward cast⁵². In particular, we followed the nitrate profile and targeted peak and minimum concentrations indicated from the ISUS trace. Eight depths at each station were selected for DNA and four to six for RNA analysis. DNA and RNA samples were filtered separately, both using a peristaltic pump system. Both filtrations included an in-line 3 µm pre-filtration through a 47 mm polycarbonate filter. The entire system used to filter RNA was treated with RNaseZap[®] prior to filtration. One to seven L were filtered for RNA at 4 °C in the dark, with a maximum filtration time of 3 hours. Samples for RNA were collected on 47 mm diameter 0.2 µm pore size polycarbonate (PC) filters, which were placed in 1.8 mL cryovials with RLT buffer (QIAGEN RNeasy) mixed with 1% beta-mercaptoethanol. The vials were immediately flash frozen in liquid nitrogen and stored at –80 °C. For DNA, six L of water were filtered through 0.2 µm Sterivex filter units (Millipore) and stored in buffer at –80 °C²³.

Nutrient concentrations: nitrite (NO₂⁻), nitrate (NO₃⁻), silicic acid (Si(OH)₄), and soluble reactive phosphate (SRP), were determined on board with a Bran and Luebbe Autoanalyzer III using standard protocols⁵³, and detection limit of 0.03 µmol L⁻¹ for the three nutrients. Ammonium was determined manually with the sensitive fluorometric method of Holmes *et al.*⁵⁴ and a detection limit of 0.01 µmol L⁻¹. Oxygen values from the CTD were calibrated at the Institut National Recherche Scientifique in Québec, Canada, using samples taken at discrete depths. Samples for total chlorophyll *a* (Chl *a*) were collected onto Whatman GF/F filters. These were stored at –80 °C until extraction in 95% ethanol⁵⁵ and concentrations determined by spectrofluorometry. Flow cytometry (FCM) samples were fixed with paraformaldehyde, flash frozen in liquid nitrogen and stored at –80 °C^{56,57}. This method does not distinguish Bacteria and Archaea and we use the term bacteria for convenience.

Light manipulation experiment. Samples from two photic depths, 25% and 1% of surface PAR, were collected from near Lancaster Sound, where Arctic water enters Northern Baffin Bay (Stn 301, Table 1). The water from 25 m (25% PAR) and 80 m (1% PAR) was collected into 18 1–L PC bottles, and incubated at 4 °C, at either in the dark or at 0.60 µmol photons m⁻² s⁻¹ in bottles shaded with blue filters, which is typical for September at the 25% photic depth (25% of surface PAR) in the study region. One bottle from each depth was immediately filtered for RNA, as above. After incubations of 24 and 48 h, two replicate bottles for each treatment were filtered as above.

Molecular biological techniques. DNA was extracted using a salt extraction method⁵⁸ as modified by Harding *et al.*⁵⁹ and RNA extracted as in Church *et al.*⁶⁰. RNA extracts were reverse transcribed and PCR amplified (RT-PCR) by using a high capacity cDNA archive kit (Applied Biosystems) as specified by the manufacturer.

Quantitative PCR was performed on a Dyad Disciple thermal cycler with Chromo 4 Real-Time Detector (Bio-Rad) in 96 well opaque qPCR plates with adhesive seals (Bio-Rad). Primers and qPCR conditions, as well as *amoA*, *ureC* and MGI positive controls and standard curves were the same as in Alonso-Saez *et al.*¹⁸. Primer specificity was verified by running PCR products on gels, and sequencing the resulting single bands from the individual primer sets. The triplicate standard curves and negative controls (without DNA added) were subjected to qPCR along with the samples. Copy number per mL of seawater was calculated as described in Zhu *et al.*⁶¹.

Eight clone libraries targeting the archaeal *amoA* gene and transcripts were constructed using Arch-*amoA*F and Arch-*amoA*R primers³¹. Three separate PCR reactions containing 2, 3 or 4 µL of template were amplified to counter primer competition and increase the probability of recovering common gene variants^{62,63}. The three PCR products were pooled, and then purified with Qiaquick PCR Purification Kit (Qiagen), and cloned using the TA cloning kit (Invitrogen) according to the manufacturer's instructions. Inserts were amplified with the vectors' M13 primers and resulting amplicons were analyzed by restriction fragment length polymorphism (RFLP) following digestion with *RsaI* (New England Biolab). Up to 10 clones per pattern were sequenced to cover potential RFLP variants. Sequencing (single read) used the vector's T7p universal primer and was performed at Centre Hospitalier de l'Université Laval (CHUL), Québec, with an ABI 3730xl system (Applied Biosystems).

The sequences were compared to the NCBI GenBank nr/nt database using NCBI BLAST⁶⁴. Non-redundant nucleotide and translated amino acid sequences are



deposited under accession numbers HQ713534-HQ713542 for the cDNA clones and JF272615-JF272702 for the DNA clones.

Phylogenetic and statistical analysis. In addition to the 202 archaeal *amoA* gene sequences from this study, 148 reference sequences were selected from Francis *et al.*³¹ and the resulting 350 sequences were aligned using Clustal X v.1.83, then manually checked and trimmed using GeneDoc, v. 2.6.0.2. After trimming, additional sequences including nearest matches to our sequences, were added for phylogenetic tree construction using the DNAML program from PHYLIP (available at <http://evolution.genetics.washington.edu/phylip.html>). The topology of dendrograms using maximum likelihood (ML) and neighbor-joining (NJ) were similar. This larger tree placed all of our sequences fell into Marine Group I (MGI), clades A and B defined in Francis *et al.*³¹. Non-marine and redundant sequences from our study were then removed from the tree leaving only representative A and B sequences in the final tree where evolutionary history was inferred by NJ, with evolutionary distances computed using the Maximum Composite Likelihood method. Phylogenetic analyses were conducted in MEGA4⁶⁵.

To estimate library coverage using Chao1, sequences were clustered at a similarity level $\geq 97\%$ using DOTUR⁶⁶. For the light experiment, significance of the 3 way (depth of collection, light level and time of incubation) ANOVA was tested on log transformed qPCR data following a general linear model after testing for main effects. Pairwise multiple comparisons using the Holm-Sidak method were used to test the significance of interactions.

Kendall's correlation analysis was used to test correlations among all quantitative variables from Northern Baffin Bay. A principal components analysis (PCA) was conducted using environmental and qPCR data, and plotted in a coordinate system, given by the two main components, to explore possible relationships between variables. Missing data were handled by pairwise deletion. PCA was done with PAST⁶⁷. ANOVA, Kendall's correlation and regression analysis on log transformed qPCR data were carried using SigmaPlot version 11 (Systat Software Inc).

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Acknowledgments

This work is an ArcticNet contribution, with additional funds from the Natural Science and Engineering Research Council (NSERC) of Canada, including a Discovery and other grants to C.L. Financial support from Fonds de recherche du Qu  bec - nature et technologies (FRQNT) to Qu  bec Oc  an is gratefully acknowledged. We thank W.K.W. Li for flow cytometry data and Mary Thaler for cell counts, advice on statistics and comments on the manuscript. We would also like to thank and the Canadian Coast Guard crew of the CCGS Amundsen and scientists who helped with sampling over the numerous missions.

Author contributions

C.L. and P.E.G. wrote the main manuscript text and E.P., M.P., J.E.T. contributed technical sections and reviewed the manuscript. E.P. carried out the light experiment, Baffin Bay sampling and clone library construction, M.P. optimized and carried out qPCR protocols and J.E.T. contributed nutrient data.

Additional information

Accession codes: Non-redundant nucleotide and translated amino acid sequences are deposited under accession numbers HQ713534-HQ713542 for the cDNA clones and JF272615-JF272702 for the DNA clones.

Supplementary information accompanies this paper at <http://www.nature.com/scientificreports>

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Pedneault, E., Galand, P.E., Potvin, M., Tremblay, J.-   & Lovejoy, C. Archaeal *amoA* and *ureC* genes and their transcriptional activity in the Arctic Ocean. *Sci. Rep.* **4**, 4661; DOI:10.1038/srep04661 (2014).



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