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Ammonia-oxidizing *Archaea* in the Arctic Ocean and Antarctic coastal waters

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Summary

We compared abundance, distributions and phylogenetic composition of *Crenarchaeota* and ammonia-oxidizing *Archaea* (AOA) in samples collected from coastal waters west of the Antarctic Peninsula during the summers of 2005 and 2006, with samples from the central Arctic Ocean collected during the summer of 1997. Ammonia-oxidizing *Archaea* and *Crenarchaeota* abundances were estimated from quantitative PCR measurements of *amoA* and 16S rRNA gene abundances. *Crenarchaeota* and AOA were approximately fivefold more abundant at comparable depths in the Antarctic versus the Arctic Ocean. *Crenarchaeota* and AOA were essentially absent from the Antarctic Summer Surface Water (SSW) water mass (0–45 m depth). The ratio of *Crenarchaeota* 16S rRNA to archaeal *amoA* gene abundance in the Winter Water (WW) water mass (45–105 m depth) of the Southern Ocean was much lower (0.15) than expected and in sharp contrast to the ratio (2.0) in the Circumpolar Deep Water (CDW) water mass (105–3500 m depth) immediately below it. We did not observe comparable segregation of this ratio by depth or water mass in Arctic Ocean samples. A ubiquitous, abundant and polar-specific crenarchaeote was the dominant ribotype in the WW and important in the upper halocline of the Arctic Ocean. Our data suggest that this organism does not contain an ammonia monooxygenase gene. In contrast to other studies where *Crenarchaeota* populations apparently lacking *amoA* genes are found in bathypelagic waters, this organism appears to dominate in well-defined, ammonium-rich, near-surface water masses in polar oceans.

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Introduction

Ammonia oxidation is the first step in nitrification, the microbially mediated pathway for the conversion of ammonia to nitrate. Although the process is quantitatively important to the global marine nitrogen cycle (Ward, 2000; Yool *et al*., 2007), ammonia oxidation has received relatively little attention in polar oceans (Yool *et al*., 2007) and only β-proteobacterial ammonia-oxidizing *Bacteria* populations (AOB) have been described there (Bano and Hollibaugh, 2000; Hollibaugh *et al*., 2002). Recent research has shown that chemoautotrophy supported by ammonia oxidation is important to the metabolism of some members of the Marine Group 1 *Crenarchaeota* (MG1C) and suggests that they play a major role in environmental ammonia oxidation (Francis *et al*., 2005; 2007; Beman and Francis, 2006; Hallam *et al*., 2006a; Leininger *et al*., 2006; Park *et al*., 2006; Wuchter *et al*., 2006; Herfort *et al*., 2007; Weidler *et al*., 2007; Beman *et al*., 2008). Other evidence (Ouverney and Fuhrman, 2000; Teira *et al*., 2004; Herndl *et al*., 2005; Hallam *et al*., 2006a; Kirchman *et al*., 2007; Agogue *et al*., 2008; De Corte *et al*., 2008) suggests that at least some MG1C populations are heterotrophic or possibly mixotrophic. Marine Group 1 *Crenarchaeota* are abundant and widely distributed in polar environments (DeLong *et al*., 1994; Massana *et al*., 1998; Murray *et al*., 1999; Church *et al*., 2003; Wells and Deming, 2003; Bano *et al*., 2004; Garneau *et al*., 2006; Wells *et al*., 2006; Gillan and Danis, 2007; Kirchman *et al*., 2007); however, their potential role in water column ammonia oxidation there has not been assessed. The purpose of this study was to compare the distributions of ammonia-oxidizing *Archaea* (AOA) and *Crenarchaeota* in two polar regions, extending studies limited so far to temperate and tropical waters and the deep sea, providing a basis for assessing their potential contribution to nitrogen cycling in polar oceans, and contributing to our understanding of their metabolic diversity.

Results and discussion

We analysed samples collected from nominal depths of 55, 131 and 235 m, spanning the permanent halocline, at stations in the central Arctic Ocean (Figs S1 and S2). Antarctic samples were collected at depths from 1 to 661 m at stations in coastal waters west of the Antarctic Peninsula (Fig. S3). Antarctic samples were assigned to

Table 1. Summary statistics for quantitative PCR estimates of gene abundance in polar data sets.

Data set and sample group	'Prokaryote' 16S rRNA genes (10^9 copies l^{-1})	*Crenarchaeota* 16S rRNA genes (10^6 copies l^{-1})	AOA *amoA* genes (10^6 copies l^{-1})	Ratio *Crenarchaeota* 16S : 'Prokaryote' 16S (%)	Ratio of AOA *amoA* to AOB *amoA*
Antarctic Coastal, SSW (1–13 m)					
n	55	55	55	55	3
Maximum	8.63	5.76	0.48	0.64	159
Minimum	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Median	2.61	0.07	< 0.01	< 0.01	61
Antarctic Coastal, WW (46–103 m)					
n	34	34	34	34	32
Maximum	2.23	240.7	51.48	26.94	371
Minimum	0.41	0.40	< 0.01	0.02	5
Median	0.92	102.3	10.59	7.95	40
Antarctic Coastal, CDW (149–661 m)					
n	22	22	22	22	20
Maximum	3.58	75.15	108.0	9.85	1 683
Minimum	0.22	0.01	< 0.01	< 0.01	< 0.01
Median	0.55	16.30	19.67	2.68	364
Antarctic Coastal, all (1–661 m)					
n	111	111	111	111	55
Maximum	8.63	240.7	108.0	26.94	1 683
Minimum	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Mean	2.03	32.45	9.19	3.70	226
Median	1.19	1.77	0.18	0.06	62
Standard deviation	2.05	56.65	16.80	6.20	387
Arctic Ocean, upper (34–55 m)					
n	11	11	11	11	9
Maximum	1.45	20.38	21.95	2.92	150
Minimum	0.19	3.16	< 0.01	0.24	< 0.01
Median	0.44	6.35	7.57	1.90	49
Arctic Ocean, middle (131 m)					
n	13	13	13	13	13
Maximum	0.93	55.35	18.09	5.98	1 805
Minimum	0.11	2.04	< 0.01	0.73	< 0.01
Median	0.20	4.74	4.86	2.85	308
Arctic Ocean, bottom (226–235 m)					
n	16	16	16	16	16
Maximum	0.32	14.60	7.08	16.85	11 071
Minimum	0.07	0.45	0.51	0.55	287
Median	0.13	2.96	3.63	1.74	1 192
Arctic Ocean, all (34–235 m)					
n	40	40	40	40	38
Maximum	1.45	55.35	21.95	16.85	11 071
Minimum	0.07	0.45	< 0.01	0.24	< 0.01
Mean	0.32	7.34	5.98	2.71	906
Median	0.22	4.75	4.92	2.07	392
Standard deviation	0.33	9.05	5.29	2.64	1 799

SSW, Summer Surface Water; WW, Winter Water; and CDW, Circumpolar Deep Water as defined in the text. The data were not normally distributed so ranges and means are reported for sample groups; means and standard deviations are given in summaries for each data set. The small *n* for the ratio of AOA to AOB in the SSW sample group is because either AOA or AOB were below detection in many samples from this group.

three water masses (Fig. S4) following Church and colleagues (2003): Summer Surface Water (SSW, a layer of low salinity water over the depth interval ~0–45 m); Winter Water (WW, a layer of cold, salty water over the depth interval ~45–105 m that is the summer remnant of the winter, surface-mixed layer); and Circumpolar Deep Water (CDW, a warmer, saltier water mass found at depths of ~105–3500 m).

Ratio of AOA to AOB

As has been reported in other studies (reviewed in Prosser and Nicol, 2008), AOA *amoA* was the most abundant form of the gene in both sets of samples (Table 1). The ratio of AOA to AOB *amoA* gene abundance ranged from 0 to 11 071 (mean 906 and standard deviation 392) and 0 to 1683 (mean 226 and standard deviation 387) in Arctic and Antarctic samples respectively. The ratio was the greatest in deep-water samples.

Abundance of prokaryotes, Crenarchaeota *and AOA*

Prokaryotes, defined here following Massana and colleagues (1998) as the sum of *Bacteria* plus *Archaea* 16S rRNA genes, were most abundant in the SSW (median 2.6×10^9 copies l^{-1}; Table 1, additional statistical analyses

in Table S1). Prokaryotes were significantly more abundant in Antarctic than in Arctic samples (median of 1.2×10^9 copies of 16S rRNA genes l^{-1} in all Antarctic samples versus 0.2×10^9 copies l^{-1} in all Arctic analysed; $\chi^2 = 57.2$, 1 d.f., $P < 0.0001$). *Crenarchaeota* 16S rRNA genes were 0.2–17% of the prokaryote 16S rRNA genes in the Arctic versus 0–27% of the population in the Antarctic. Lower relative abundance of *Crenarchaeota* in Arctic versus Antarctic samples is consistent with most literature reports (Wells and Deming, 2003; Garneau *et al*., 2006; Wells *et al*., 2006; Alonso-Sáez *et al*., 2008); however, Kirchman and colleagues (2007) reported higher *Crenarchaeota* abundances, comparable to those reported for Antarctic waters (Massana *et al*., 1998; Murray *et al*., 1998; Church *et al*., 2003), in samples from the Chukchi Sea, where water from the productive north Pacific Ocean and Bering Sea enters the Arctic Ocean.

We found that *Crenarchaeota* 16S rRNA genes and AOA *amoA* genes were below the limit of detection for many (40/55 and 17/55 respectively) SSW samples, consistent with previous reports (Massana *et al*., 1998; Murray *et al*., 1998; Church *et al*., 2003). Due to operational limits of the Arctic Ocean sampling platform (a nuclear submarine), we were unable to obtain samples consistently from the Arctic Ocean at depths equivalent to the SSW. *Crenarchaeota* were fivefold more abundant in Antarctic samples (WW and CDW) taken at depths comparable to Arctic Ocean samples (Table 1; median of 24×10^6 copies l^{-1} in SSW and CDW samples versus 4.8×10^6 copies l^{-1} in all Arctic samples; $\chi^2 = 38.2$, 1 d.f., $P < 0.0001$), while AOA *amoA* genes were 2.4-fold more abundant (median of 12 versus 4.9×10^6 copies l^{-1}; $\chi^2 = 13.2$, 1 d.f., $P = 0.0003$).

Composition of Antarctic and Arctic assemblages compared

Phylogenetic analysis of the AOA *amoA* (Fig. 1) and 16S rRNA (Fig. 2) sequences we obtained from these samples indicates that Arctic and Antarctic populations contain many of the same phylotypes; however, statistical analysis revealed that the populations were significantly different (LIBSHUFF; $P = 0.021$ and 0.0015 respectively) as a result of differences in the relative abundance of specific phylotypes.

The three major *amoA* clades we identified (Fig. 1) are represented in GenBank by closely related sequences from cultures or environmental samples. Ninety-four per cent of the AOA *amoA* gene sequences retrieved from Antarctic SSW samples and 75% of the sequences retrieved from 55 m in the Arctic Ocean were assigned to clade 'A'. The *amoA* gene sequence from *Candidatus* '*Nitrosopumilus maritimus*', a recently isolated crenarchaeote that can grow chemoautotrophically by oxidizing ammonia (Konneke *et al*., 2005), also fell into clade A, with > 90% nucleotide similarity between the *N. maritimus* gene and the most distantly related sequence in clade A. However, the majority of *amoA* sequences assigned to this clade are found in a subclade of closely related sequences (> 97% nucleotide identity) that does not contain *N. maritimus*. Most of the *amoA* sequences in clade 'B' were retrieved from the deepest samples: 235 m in the Arctic or CDW samples in the Antarctic. The clade designated as 'C' in Fig. 1 contained sequences from all depths in roughly equal proportions. Based on our analysis, AOA *amoA* genotypes A and B demonstrate depth-dependant distributions similar to those reported previously in temperate and subtropical waters (Beman and Francis, 2006; Mincer *et al*., 2007), while the vertical distribution of organisms with the clade C genotype seems to be independent of depth or of factors that co-vary with depth (Fig. 1).

Most of the 16S rRNA sequences we obtained also fell into one major clade that also contains *N. maritimus* (Fig. 2). Libraries from Antarctic WW and Arctic Ocean 55 m samples were dominated by ribotypes from this clade that were > 98.2% identical to *N. maritimus* (represented by EU199467, 83% of the WW library; and EU199667, 81% of the 55 m library). Antarctic SSW and Arctic 131 m libraries were dominated by two closely related (99.1% identity) ribotypes (EU199544, 69% of the SSW library; EU199627, 62% of the 131 m library respectively) that were > 97.5% identical to *N. maritimus*. Antarctic CDW and Arctic Ocean 235 m samples contained ribotypes from all clades. Only one ribotype (EU199638) in a clade distinct (92.8% identity) from *N. maritimus* was abundant (54% of all sequences) in the Arctic 235 m library.

amoA *gene dosage*

Crenarchaeota 16S rRNA genes were readily detected and even abundant in Antarctic WW samples; however, AOA *amoA* genes were relatively rare (Fig. 3), so that the ratio of AOA *amoA* to *Crenarchaeota* 16S rRNA genes was only 0.15 [$n = 34$, 95% confidence limit (CL): 0.11–0.18]. In contrast, we found that the ratio of AOA *amoA* to *Crenarchaeota* 16S rRNA genes was 2.0 ($n = 22$, 95% CL: 1.5–2.9) in CDW samples (Fig. 3). *amoA* gene abundance was lower in WW than CDW samples (median of 10 versus 20×10^6 copies l^{-1}; $\chi^2 = 4.47$, 1 d.f., $P = 0.034$; Fig. 3 and Table 1), but *Crenarchaeota* abundance was much greater in WW than in CDW samples (median of 102 versus 16×10^6 copies l^{-1}; $\chi^2 = 10.8$, 1 d.f., $P = 0.0010$; Fig. 3 and Table 1).

The abundance of both *Crenarchaeota* 16S rRNA and AOA *amoA* genes decreased with depth in Arctic Ocean samples (Table 1); however, in contrast to the Antarctic,

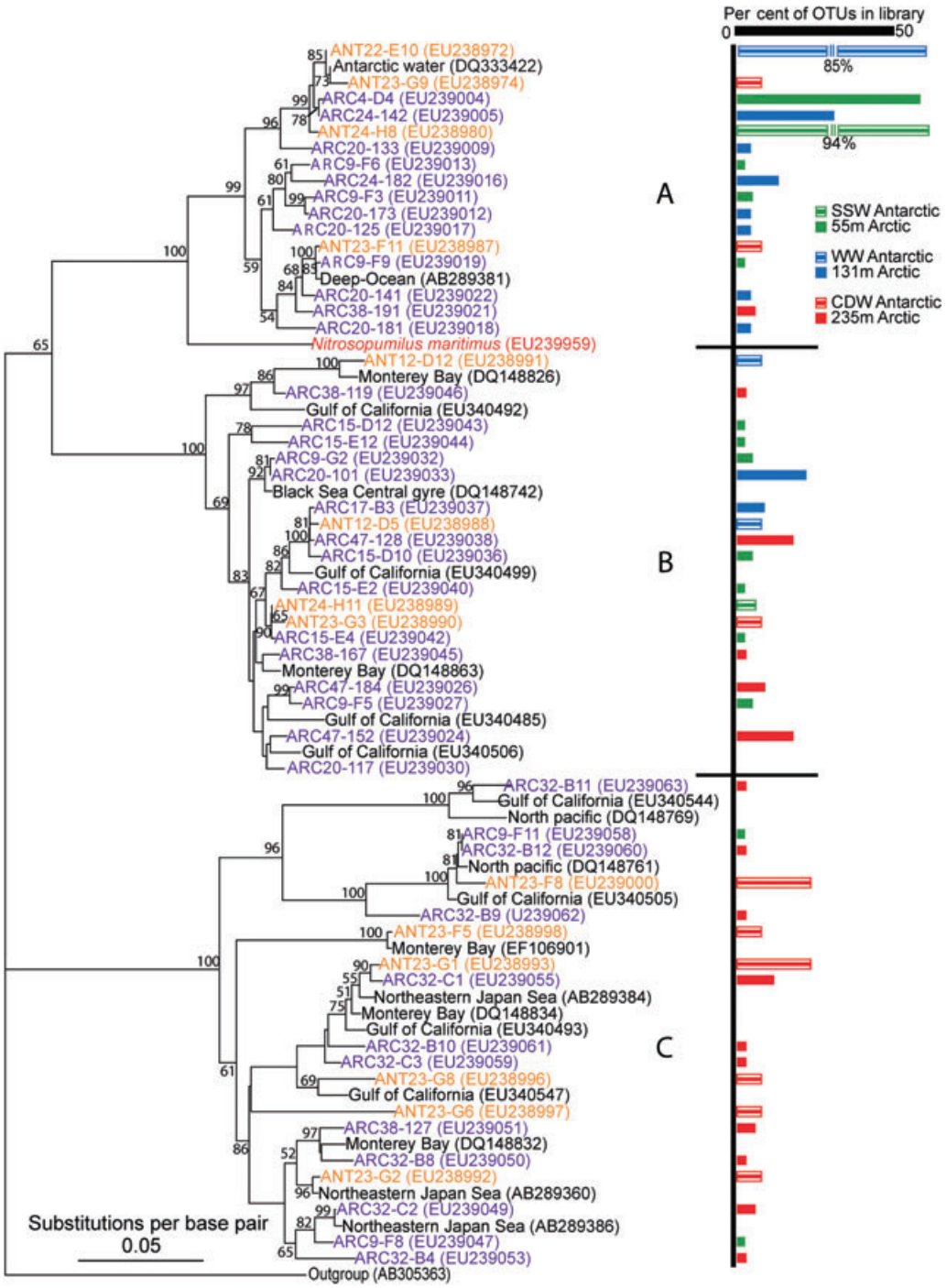

Fig. 1. Phylogenetic analysis of archaeal *amoA* gene sequences from Antarctic coastal waters and the central Arctic Ocean. Sample depth is indicated by the colour of the bar giving relative abundance in the combined library; bars are further differentiated into striped (Antarctic) or solid (Arctic) fill patterns; note breaks in the bars for dominants with percentages given next to their bars. Sequences from four different libraries from each depth/location were combined for analysis; one sequence representing groups of sequences ≥ 98% similar from each combined library is shown. In cases where two or more of the genotypes are identical, we have not combined them to preserve location and depth information. Antarctic and Arctic sequence identifiers are shown in orange and purple fonts respectively. Reference sequences are shown in black except for *Nitrosopumilus maritimus*, which is shown in red. GenBank accession numbers are given in parentheses. Major clades discussed in the text are identified by the letters A–C. This is a neighbour-joining tree based on 595 bp sequences. Bootstrap support for branches is shown if > 50% (100 iterations).

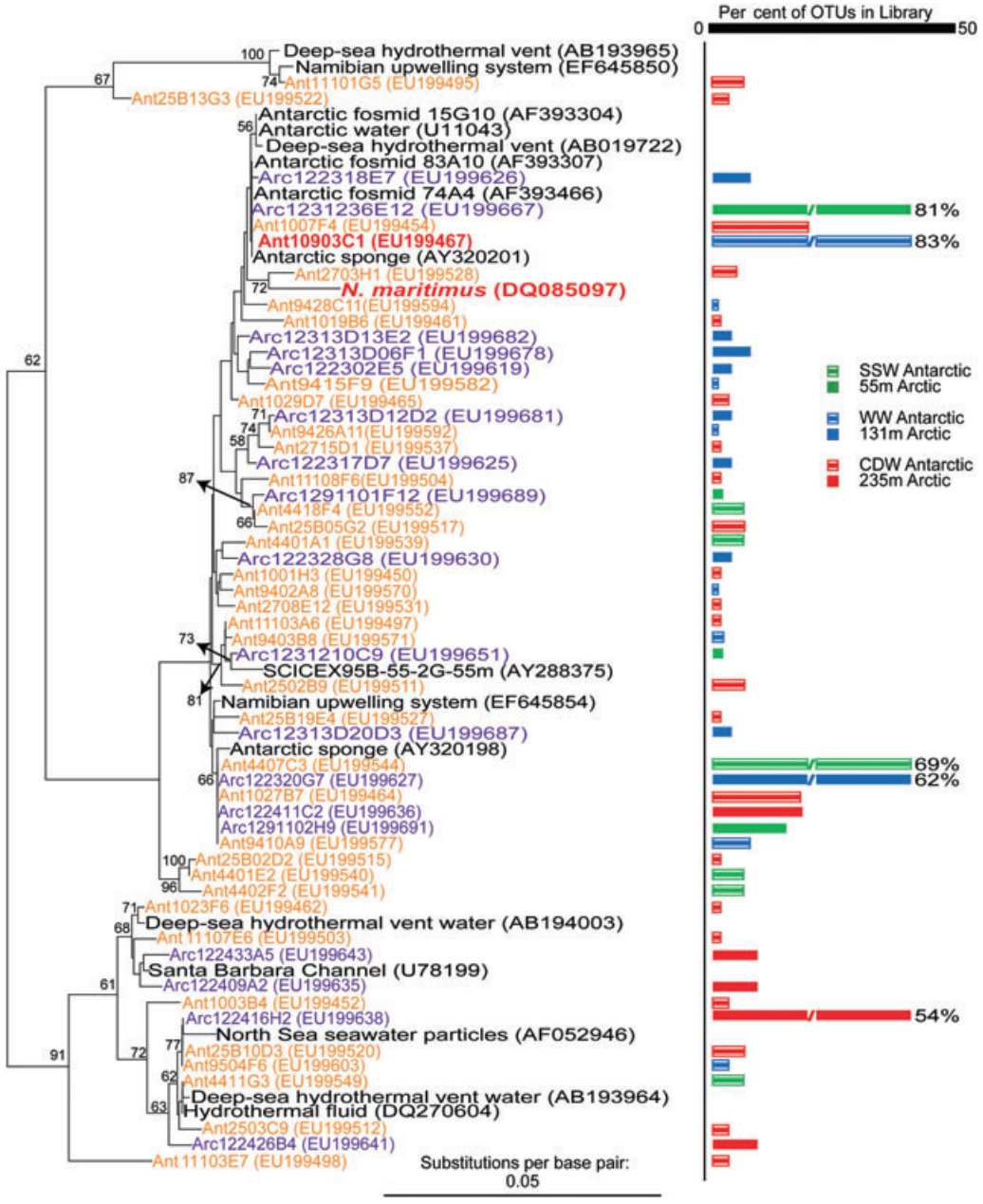

Fig. 2. Phylogenetic analysis of *Crenarchaeota* 16S rRNA gene sequences from Antarctic coastal waters and the central Arctic Ocean. Sample depth is indicated by the colour of the bar giving relative abundance in the combined library; bars are further differentiated into striped (Antarctic) or solid (Arctic) fill patterns; note breaks in the bars for dominants with percentages given next to their bars. Sequences from four different libraries from each depth/location were combined for analysis; one sequence representing groups of sequences ≥ 99.5% similar from each combined library is shown. In cases where two or more of the genotypes are identical, we have not combined them to preserve location and depth information. Antarctic and Arctic sequence identifiers are shown in orange and purple fonts respectively. Reference sequences are shown in black except *Candidatus* '*Nitrosopumilus maritimus*' and the Antarctic WW dominant, which are shown in red. GenBank accession numbers are given in parentheses. This is a neighbour-joining tree based on 876 bp sequences. Bootstrap analysis was used to estimate the reliability of phylogenetic reconstructions. Bootstrap support for branches is shown if > 50% (100 iterations).

the difference was only approximately twofold for both genes and *Crenarchaeota* 16S rRNA genes and AOA *amoA* genes were found in most samples from the shallowest depth sampled. There was no obvious trend with depth in the ratio of AOA *amoA* to *Crenarchaeota* 16S rRNA gene abundance (Fig. 4) and the correlation between *amoA* and *Crenarchaeota* 16S rRNA gene abundance was not statistically significantly different from 0 (Type II regression, $\alpha = 0.05$), either for the complete data set ($n = 40$) or for subsets parsed by depth ($n = 11$, 13 or

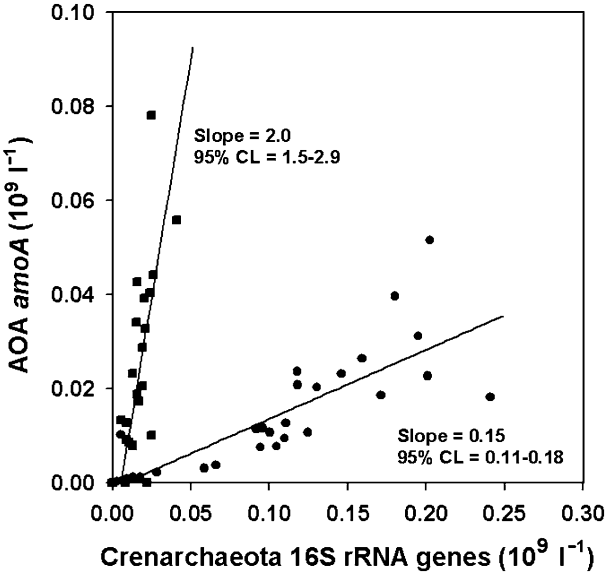

Fig. 3. Ratio of crenarchaeote *amoA* versus 16S rRNA genes in samples from the Southern Ocean off the West Antarctic Peninsula. Sample depth is indicated by symbol: circles, Winter Water (WW) samples; squares, Circumpolar Deep Water (CDW) samples. Both genes were below the limit of detection in most Summer Surface Water (SSW) samples (not shown). Regression lines, slopes and 95% confidence limits of Type II regressions are shown.

16) or by water mass ($n = 10$, 15 or 15) as shown in Fig. S2.

Most data collected to date on the relationship between AOA *amoA* gene abundance and MG1C abundance support the conclusion that MG1C are AOA (Wuchter *et al*., 2006; Francis *et al*., 2007; Herfort *et al*., 2007). Wuchter and colleagues (2006) found a strong positive correlation between AOA *amoA* gene abundance, crenarchaeotal abundance and, especially in enrichment cultures, ammonia oxidation. Their data suggest that AOA have 2.5–2.8 (North Sea), 1.2 (North Atlantic) or 1 (enrichment culture) copies of the *amoA* gene per genome. Similar ratios of *amoA* to 16S rRNA gene abundance have been reported in subsequent studies in the North Sea (Herfort *et al*., 2007), the Pacific (Monterey Bay, CA and Station ALOHA, HI; Mincer *et al*., 2007), the Gulf of California (Beman *et al*., 2008), North Atlantic mesopelagic waters (Agogue *et al*., 2008) and the Eastern Mediterranean (De Corte *et al*., 2008). The two MG1C genomes that have been sequenced (*Cenarchaeum symbiosum* and *N. maritimus*) each contain one *amoA* gene and one 16S rRNA gene (Konneke *et al*., 2005; Hallam *et al*., 2006b; http://img.jgi.doe.gov/). However, much lower ratios of these genes (< 0.05) have been reported in some bathypelagic populations (Agogue *et al*., 2008; De Corte *et al*., 2008).

The apparent absence of *amoA* genes in WW crenarchaeotes might be an artefact if the WW crenarchaeote ecotype contains a divergent *amoA* gene that is not amplified by the quantitative PCR (qPCR) primer set we used, and thus is not detected. The *amoA* qPCR primers we used are the same as those used by Agogue and colleagues (2008) and De Corte and colleagues (2008), who also identified crenarchaeote populations that apparently did not contain *amoA* genes in their samples. Following Agogue and colleagues (2008), we compared the qPCR primer sequences to sequences retrieved from our samples using primers reported in Francis and colleagues (2005). These primers are distinct from and external to the *amoA* qPCR primer sites. We found numerous instances of mismatched bases, but in most cases the mismatches were internal (Table S2). Mismatches were especially common for the reverse primer, which contained five mismatches in one instance, suggesting that this primer might lead to underestimates of *amoA* gene abundance in some cases (Table S2). However, mismatches were encountered more frequently with, and were more numerous in, sequences retrieved from CDW samples, where the expected ratio (1–2) of *amoA* to 16S rRNA gene abundance was observed (Fig. 3), than in sequences retrieved from WW samples, where *amoA* genes were under-represented. Sequences retrieved from the WW (where *amoA* genes are apparently relatively rare) contained zero or one internal mismatch with the reverse primer. While it is possible that the WW population contains an *amoA* gene that is so divergent that it is not amplified by either of these primer sets, this seems unlikely because the *Crenarchaeota* 16S rRNA genes

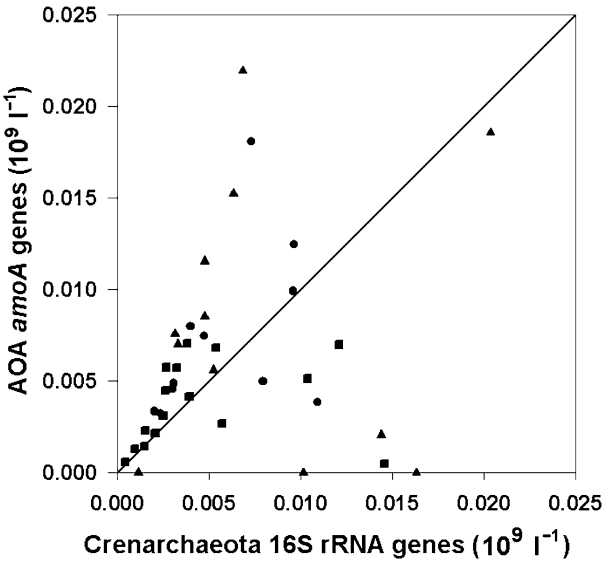

Fig. 4. Ratio of crenarchaeote *amoA* versus 16S rRNA genes in samples from the central Arctic Ocean. Sample depth is indicated by symbol: triangles, 55 m; circles, 131 m; and squares, 235 m. Type II regressions for the whole data set, for subsets parsed by depth as shown and for subsets parsed by water mass (see Fig. S2 for groupings) are not statistically significant ($\alpha = 0.05$). The 1:1 line is shown for reference.

retrieved from it were not unusual – most were > 98% identical to *Candidatus* '*N. maritimus*'.

This analysis suggests that the apparent absence of *amoA* genes from WW samples is not simply an artefact of primer bias, although both this conclusion and the further inference that this population does not oxidize ammonia remain to be verified by direct experimentation. Thus, our data suggest that there are two different populations of *Crenarchaeota* in the water column off the Antarctic Peninsula, one comprised of organisms carrying the *amoA* gene that are presumably capable of oxidizing ammonia (dominant in the CDW, background in the WW); and a second population that does not carry the *amoA* gene (dominant in WW, background in CDW).

Vertical differentiation of Antarctic Crenarchaeota *and AOA populations*

Phylogenetic analysis of *Crenarchaeota* 16S rRNA gene sequences retrieved from WW and CDW samples (Fig. S5) revealed that the same or closely related ribotypes were found in both water masses. However, comparison of library composition by LIBSHUFF revealed that different ($P = 0.001$) communities were retrieved from these water masses. In particular, the WW community was dominated (83%) by one ribotype that was > 98% similar to *Candidatus* '*N. maritimus*'. This sequence appears to be ubiquitous and important in polar regions, and possibly limited to them: a MegaBLAST (Zhang *et al*., 2000) search of the GenBank NR database on 25 February 2008 using the dominant sequence from WW samples (EU199467) as the query revealed that 323 of the top 333 entries (those with ≥ 99% identity to this sequence) were from polar regions. These sequences were retrieved in 12 different studies. All 58 hits with 100% identity (from five different studies) were from polar regions.

The relative absence of *amoA* genes from WW crenarchaeote populations coincides with the dominance of this ribotype and suggests that this organism may not be an ammonia oxidizer. A depth-dependent shift in crenarchaeote metabolism, from autotrophy to heterotrophy, has been inferred from shifts in ratios of *amoA* to 16S rRNA genes in previous reports (e.g. Herndl *et al*., 2005; Agogue *et al*., 2008; De Corte *et al*., 2008). These studies have suggested that bathypelagic populations are apparently heterotrophic while mesopelagic and near-surface populations are autotrophic ammonia oxidizers, a shift hypothesized to be driven by ammonia limitation at depth (Agogue *et al*., 2008). If this inference is correct, the metabolic differentiation between Antarctic coastal populations appears more marked than in other areas, especially considering that these Antarctic populations are separated vertically by less than 50 m. If the WW population is heterotrophic, which requires experimental verification, the distribution of crenarchaeote metabolism in the Antarctic is unique in that the putatively heterotrophic population is not bathypelagic and ammonium concentrations are much greater (Table S3) than the 5 nM cited for bathypelagic ammonium concentrations in Agogue and colleagues (2008).

What controls crenarchaeote distributions in polar regions?

We propose the following conceptual model, based on differences in the light environments of these two oceans, to explain the contrasting distributions of crenarchaeote 16S rRNA and AOA *amoA* genes in them. In one case (the coastal Antarctic), ice cover is seasonal and most of our samples were taken in open water, while the central Arctic Ocean was ice-covered when we sampled. Primary production is light-limited under ice, consequently chlorophyll concentrations were ~100-fold greater in the Antarctic versus Arctic (Table S3). Table S3 also shows that concentrations of major nutrients were similar in both oceans, and that ammonium is much higher than the concentrations postulated by Agogue and colleagues (2008) to limit growth in bathypelagic populations.

To the extent that some MG1C are heterotrophic (which we suggest to be the case for the dominant WW ribotype due to the low ratio of AOA *amoA*:*Crenarchaeota* 16S rRNA genes in WW samples), their productivity, and presumably abundance, depends on primary production to generate the organic matter they need for growth. Elevated abundances of crenarchaeotes in the WW thus reflect coupling, presumably mediated by vertical fluxes of phytodetritus, to high primary production in the overlying SSW. This implies active *Crenarchaeota* growth in the WW during summer, and minimal growth during light-limited, mid-winter conditions when this layer forms. Increased *Crenarchaeota* abundance in Antarctic surface waters during the fall and winter (Murray *et al*., 1998; Church *et al*., 2003) may thus be due to mixing of WW populations into the surface layer as water column stratification breaks down. A simple mixing model based on crenarchaeote abundances reported in Church and colleagues (2003) or on our data (Table S1) supports this analysis, as long as fall losses from the population (grazing, viral mortality etc.) are small. Lower abundances of heterotrophic *Crenarchaeota* would thus be expected in the central Arctic Ocean because permanent pack ice limits light penetration and thus photoautotrophic production. If heterotrophic and chemoautotrophic pathways dominate in different crenarchaeote ribotypes, and ammonium supply is not directly coupled to production in the overlying water (e.g. in the CDW where the water mass underlying the WW was likely advected from elsewhere), chemoautotrophic organisms might be expected

to become relatively more important where vertical fluxes of labile organic matter are lower: the CDW in the Southern Ocean and in the central Arctic Ocean.

If heterotrophic *Crenarchaeota* are dependent on primary producers as hypothesized above, why is their abundance low in the SSW where primary production is the highest? Marine Group 1 *Crenarchaeota* are generally restricted to subsurface waters (Karner *et al*., 2001; reviewed in Fuhrman and Hagström, 2008); however, they have also been reported to be abundant in surface waters at some locations (polar regions, North Sea), but only during winter or early spring. Surface water *Crenarchaeota* populations declined rapidly with the onset of spring in the Antarctic (Massana *et al*., 1998; Murray *et al*., 1998), the Arctic (Alonso-Sáez *et al*., 2008), in nearshore waters off the island of Texel, the Netherlands (53°N; Wuchter *et al*., 2006), and in the shallow, well-mixed southern North Sea between 53°N and 55°N (Herfort *et al*., 2007). The spring decrease in abundance at these locations has been attributed to competition with *Bacteria* that bloom in response to increased phytoplankton production in the spring (Massana *et al*., 1998; Murray *et al*., 1998; Church *et al*., 2003; Herfort *et al*., 2007) or to ensuing nutrient limitation (Wuchter *et al*., 2006; Herfort *et al*., 2007). This conclusion is based on the correspondence between these events, which is the strongest for the inverse correlation with phytoplankton standing crop, rather than on experimental evidence. These locations experience very low water column light levels during the winter and the increase in irradiance during spring coincides with the onset of the spring phytoplankton bloom; thus irradiance, phytoplankton productivity and phytoplankton standing crop co-vary.

No specific mechanism has been suggested to link competition or nutrient limitation with the net loss of crenarchaeotes observed during the onset of the polar/boreal spring. As an alternative hypothesis, the spring decline in *Crenarchaeota* abundance in surface waters may be due to photoinhibition, similar to that proposed to control nitrifying *Bacteria* populations (Olson, 1981; Ward *et al*., 1982), although the physiological target may be different. At least one other study (Murray *et al*., 1998) has suggested that light might play a direct role in the seasonal dynamics of polar MG1C, while Mincer and colleagues (2007) have suggested that depth distributions of crenarchaeotes and clades of *amoA* genes at lower latitudes might reflect an underlying sensitivity to photoinhibition. Coastal waters west of the Antarctic Peninsula, like most of the Southern Ocean, are not ice-covered during summer and thus are exposed to full solar irradiance, which is known to harm plankton (Meador *et al*., 2002; Pakulski *et al*., 2008). *Bacteria* abundance increases in the SSW during the summer while *Crenarchaeota* abundance declines (Massana *et al*., 1998; Murray *et al*., 1998; Church *et al*., 2003). Alonso-Sáez and colleagues (2008) report a similar pattern in Arctic Ocean coastal waters accompanying break-out of the pack ice. These observations suggest that *Bacteria* are able to respond to increased substrate availability from elevated primary production during spring and summer, where *Crenarchaeota* are not, and we infer that photoinhibition plays an important role in this differential response.

Although there are only limited data on the sensitivity of MG1C to light, this hypothesis is consistent with the loss of crenarchaeotes observed in Antarctic coastal waters during spring: abundance in the surface layer decreases as photoperiod and sun angle increase and pack ice disperses. We expect the same distribution to evolve in the central Arctic Ocean if it becomes ice-free as a result of climate change, although the lower sun angle at the higher latitude of the Arctic Ocean and the lack of a deep ozone hole as is currently found over the Antarctic during the Austral spring may limit the extent of photoinhibition in the Arctic relative to the Antarctic. From a broader perspective, this same process, photoinhibition of *Crenarchaeota* relative to *Bacteria*, may also explain the seasonal distribution of *Crenarchaeota* in the North Sea (Wuchter *et al*., 2006; Herfort *et al*., 2007) and, as suggested by Mincer and colleagues (2007) and De Corte and colleagues (2008), their vertical distribution at lower latitudes where they are restricted to deeper water (reviewed in Fuhrman and Hagström, 2008).

Experimental procedures

Sample collection

Central Arctic Ocean water samples were collected from a through-hull penetration at nominal depths of 55, 133 and 235 m during the SCICEX 97 cruise (21 August to 15 October 1997) aboard the US Navy submarine *Archerfish* as previously described (Bano and Hollibaugh, 2000). A subset of samples from 16 stations was chosen for analysis; locations are shown in Fig. S1. Depending on location, these depths sample the surface-mixed layer/upper halocline (55 m); mid-halocline (131 m); or base of the halocline/Arctic Bottom Water (235 m), as shown in Fig. S2.

Antarctic coastal water samples were collected from depths of 1–661 m with Go-Flo (General Oceanics) samplers at stations west of the Antarctic Peninsula during Palmer LTER annual cruises on the *R/V LM Gould* (cruise LMG 0501, 4 January to 1 February 2005, 14 stations, 3 depths, used for *amoA* gene sequences; and LMG 0601, 1 January to 13 February 2006, 57 stations, 2 depths per station, used for qPCR and all other analyses). Station locations are shown in Fig. S3.

Sample filtration and DNA extraction

Particulate material was collected and DNA was extracted from the particles in the same way on all cruises. Particulate

material was collected by filtration through Sterivex (Millipore, Billerica, MA) filter cartridges under pressure from a peristaltic pump. We did not utilize a pre-filter. DNA was extracted from cells in the filter cartridge as described previously (Bano and Hollibaugh, 2000). Briefly, cells were lysed and DNA was extracted using lysozyme, proteinase K and sodium dodecyl sulfate, then DNA was purified by phenol-chloroform extraction and ethanol precipitation. Details are provided in Appendix S1.

qPCR

qPCR was used to estimate the abundance of *amoA* or 16S rRNA genes from *Bacteria*, *Archaea*, *Crenarchaeota*, AOB and AOA in template DNA. Details are given in Appendix S1. Briefly, gene abundances in 1 μl of extract were measured in triplicate on an iCycler system (Bio-Rad, Hercules, CA) as described previously (see references and primers in Table 2). Abundance of *Bacteria*, *Archaea*, *Crenarchaeota* and AOB 16S rRNA genes was determined using TaqMan primers and probes. Ammonia-oxidizing *Bacteria* and AOA *amoA* genes were quantified using SYBRgreen to measure amplicon accumulation. *Bacteria* and AOB 16S rRNA standards were made from *Escherichia coli* K-12 and *Nitrosospira multiformis* ATCC 25196 genomic DNA respectively. Standards for *Archaea* and *Crenarchaeota* 16S rRNA and AOB and AOA *amoA* genes were amplicons cloned from environmental samples. Standard curves were based on a dilution series of five concentrations ranging from 10^1 to 10^7 or 10^8 copies μl^{-1} and were run in triplicate. Reaction efficiencies for controls averaged 91.7% (range 73.2–113.7%, $n = 72$; details in Table S4). Limits of detection (in gene copies per reaction) for the method under the protocol described above were: *Bacteria* 16S = 8.4×10^1; AOB 16S = 7.8×10^1; *Archaea* 16S = 7.8×10^4; *Crenarchaeota* 16S = 6.1×10^1; AOB *amoA* = 4.7×10^1; AOA *amoA* = 3×10^1.

Gene abundances in template DNA were converted to concentrations in the environment using the volume filtered (3.5–16 l, depending on the sample), the final extract volume (200 μl) and assuming a 100% extraction efficiency. These estimates were compared with direct counts as described in Appendix S1 (Figures S6 and S7) to verify that Arctic and Antarctic samples are subject to the same extraction efficiencies, that degradation of Arctic samples collected in 1997 had not compromised qPCR analyses and that our qPCR data provide a consistent estimate of the *in situ* abundance, and of the relative abundance, of the genes we assayed.

Phylogenetic analyses

Clone libraries were generated from PCR products as described previously (Bano *et al*., 2004; Wuchter *et al*., 2006) using primers listed in Table 2 and TOPO TA Cloning Kits (Invitrogen) as per the manufacturer's instructions. Some of the *amoA* gene amplicons (~20%, representatives of major clades) were sequenced in both directions to verify the sequence. All of the cloned 16S rRNA gene amplicons were sequenced in both directions, resulting in partial overlap (~600 bp) of the reads in the middle of the sequences. *Euryarchaeota* 16S rRNA gene sequences obtained were identified by BLAST (Zhang *et al*., 2000) and omitted from further analysis. *amoA* and 16S rRNA sequences obtained in this study have been deposited in GenBank (NCBI) under Accession No. EU238971 to EU239090 and EU199450 to EU199780 respectively.

Sequences were aligned using the Genetics Computer Group package (GCG Version 11.1, Accelrys, San Diego, CA). Minimum evolutionary distances were calculated with the Kimura two-parameter model. Phylogenetic trees were

Table 2. Primers and probes used in this study.

Target	Gene	Primer or probe	Use	Sequence (5′ to 3′)	Reference
Bacteria	16S rRNA	BACT1369F	Q	CGGTGAATACGTTCYCGG	Suzuki *et al*. (2000)
	16S rRNA	PROK1492R	Q	CGWTACCTTGTTACGACTT	Suzuki *et al*. (2000)
	16S rRNA	Tm1389F	Q	CTTGTACACACCGCCCGTC	Suzuki *et al*. (2000)
Archaea	16S rRNA	ARCH1-1369F	Q	CGGTGAATACGTCCCTGC	Suzuki *et al*. (2000)
	16S rRNA	ARCH2-1369F	Q	CGGTGAATATGCCCCTGC	Suzuki *et al*. (2000)
	16S rRNA	PROK1541R	Q	AAGGAGGTGATCCRGCCGCA	Suzuki *et al*. (2000)
	16S rRNA	Tm1389F	Q	CTTGTACACACCGCCCGTC	Suzuki *et al*. (2000)
	16S rRNA	21F	S	TTCCGGTTGATCCYGCCGGA	DeLong (1992)
	16S rRNA	958R	S	YCCGGCGTTGAMTCCAATT	DeLong (1992)
Crenarchaeota	16S rRNA	ARCHGI334F	Q	AGATGGGTACTGAGACACGGAC	Suzuki *et al*. (2000)
	16S rRNA	ARCHGI554R	Q	CTGTAGGCCCAATAATCATCCT	Suzuki *et al*. (2000)
	16S rRNA	Tm519AR	Q	TTACCGCGGCGGCTGGCAC	Suzuki *et al*. (2000)
AOB	16S rRNA	CTO 189fA/B	Q	GGAGRAAAGCAGGGGATCG	Kowalchuk *et al*. (1997)
	16S rRNA	CTO 189fC	Q	GGAGGAAAGTAGGGGATCG	Kowalchuk *et al*. (1997)
	16S rRNA	RT1r	Q	CGTCCTCTCAGACCARCTACTG	Hermansson and Lindgren (2001)
	16S rRNA	TMP1	Q	CAACTAGCTAATCAGRCATCRGCCGCTC	Hermansson and Lindgren (2001)
AOB	*amoA*	*amoA*-1F	S/Q	GGGGTTTCTACTGGTGGT	Rotthauwe *et al*. (1997)
	amoA	*amoA*r NEW	S/Q	CCCCTCBGSAAAVCCTTCTTC	Hornek *et al*. (2006)
AOA	*amoA*	Arch-*amoA*-for	Q	CTGAYTGGGCYTGGACATC	Wuchter *et al*. (2006)
	amoA	Arch-*amoA*-rev	Q	TTCTTCTTTGTTGCCCAGTA	Wuchter *et al*. (2006)
	amoA	Arch-*amoA*F	S	STAATGGTCTGGCTTAGACG	Francis *et al*. (2005)
	amoA	Arch-*amoA*R	S	GCGGCCATCCATCTGTATGT	Francis *et al*. (2005)

16S rRNA primer/probe names beginning with 'Tm' are Taqman probes. Under 'Use', S stands for sequencing and Q stands for qPCR.

inferred and bootstrap analysis (100 replicates) was performed with the PHYLIP package using evolutionary distances (Jukes–Cantor distances) and the neighbour-joining method. The AOA *amoA* tree was constructed with 595 bp nucleotide sequences and 16S rRNA trees were constructed with 876 bp nucleotide sequences. Clone library compositions were compared and tested for statistically significant differences with preLIBSHUFF and webLIBSHUFF v0.96 (Singleton *et al*., 2001; Upchurch, 2003; Henriksen, 2004).

Environmental data

Environmental data summarized in Table S3 were provided by others from samples and data collected on the SCICEX 97 and LMG 0601 cruises. Dr T. Whitledge provided nutrient and chlorophyll data; Dr G. Steward provided bacterioplankton abundance; and hull-mounted instrumentation collected dissolved oxygen, temperature and salinity data on the SCICEX 97 cruise. Dr M. Vernet provided nutrient data and H. Ducklow provided data on bacterioplankton abundance for the LMG 0601 cruise, while dissolved oxygen, temperature and salinity data were collected by instruments mounted on the Niskin rosette sampler. Table S3 reports summary statistics for all samples collected on both cruises; a subset of these samples was used in our analysis of *amoA* gene distributions.

Statistical analyses

Data were not normally distributed, thus non-parametric analyses were used to test relationships, although parametric summary statistics were calculated and are reported in Table S1. The SAS procedure NPAR1WAY was used to test the statistical significance of differences between subsets of data using Wilcoxon rank sums and Kruskal–Wallis tests. The slope of the relationship between paired variables was determined with Type II regressions (Legendre and Legendre, 1998; routines available at http://www.bio.umontreal.ca/legendre/index.html) using the Major Axis (qPCR data) or Standard Major Axis (qPCR data versus flow cytometer or direct counts) procedures with a permutation test of significance (999 iterations) to determine 95% CL of slopes.

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Supporting information

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

Appendix S1. Supporting information – details of analytical methods, supplementary tables and figures.

Fig. S1. Location of central Arctic Ocean stations where samples analysed in this study were collected. Stations were sampled during the SCICEX 97 cruise.

Fig. S2. T/S diagram showing the relationship of samples to water masses encountered in Arctic Ocean samples. Green symbols are from 55 m (upper halocline/surface-mixed layer); blue symbols indicate samples from 131 m (mid-halocline); and red symbols indicate samples from 235 m (bottom of the halocline/top of the Arctic Bottom Water). Grey crosses are T/S plots of deep hydrocasts (to 1500 m) taken at three different locations. Ovals enclose samples grouped by water mass (versus depth) for statistical analysis.

Fig. S3. Location of stations sampled west of the Antarctic Peninsula (WAP) during LMG 0601. Samples were collected on lines 200–600. Station map courtesy of Palmer Station LTER.

Fig. S4. T/S diagram showing the relationship of samples to water masses encountered west of the Antarctic Peninsula. Green symbols are in the SSW water mass, blue symbols indicate samples from the WW water mass and red symbols indicate samples from the CDW water mass; water mass nomenclature from Church and colleagues (2003).

Fig. S5. Phylogenetic analysis of *Crenarchaeota* 16S rRNA gene sequences retrieved from Winter Water (WW) and Circumpolar Deep Water (CDW) samples. Sequences from four separate samples of each water mass were combined for the analysis. One sequence was used to represent groups of sequences (ribotypes) that are $\geq$ 99.5% similar. Bar lengths indicate the percentage of the combined library for WW (blue) or CDW (red) represented by that sequence. This is a neighbour-joining tree based on 876 bp sequences. Bootstrap support for branches is shown if > 50% (100 iterations).

Fig. S6. Comparison of flow cytometer counts of 'prokaryotes' with qPCR estimates of 'prokaryote' rRNA gene abundance in Antarctic samples. The regression line shown is a Type II regression; the slope and its 95% confidence limits are given. Samples were stained with SYTO-13 for flow cytometry. Flow cytometry data courtesy H. Ducklow, Palmer Station LTER. The r^2 value for a Type I regression of this same data set was 0.36 and the slope was 2.6 (95% CL: 1.7–3.6).

Fig. S7. Comparison of SYBR green epifluorescence counts (Steward *et al*., 2007) of 'prokaryotes' with qPCR estimates of 'prokaryote' rRNA gene abundance in Arctic samples. The regression line shown is a Type II regression; the slope and its 95% confidence limits are given. The r^2 value for a Type I regression of this same data set was 0.66 and the slope was 3.3 (95% CL: 2.5–4.1).

Table S1. Summary statistics for quantitative PCR estimates of gene abundance in polar samples.

Table S2. Alignment of *amoA* gene qPCR primer sequences from Wuchter and colleagues (2006) with the priming sites in longer sequences retrieved from our samples using *amoA* gene primers from Francis and colleagues (2005).

Table S3. Summary of environmental variables measured on Antarctic (LMG 0601) and Arctic (SCICEX 97) cruises.

Table S4. Amplification efficiencies of qPCR primers and/or probes.

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