# Role for urea in nitrification by polar marine Archaea

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Edited by David M. Karl, University of Hawaii, Honolulu, HI, and approved September 5, 2012 (received for review February 1, 2012)

**Despite the high abundance of Archaea in the global ocean, their metabolism and biogeochemical roles remain largely unresolved. We investigated the population dynamics and metabolic activity of *Thaumarchaeota* in polar environments, where these microorganisms are particularly abundant and exhibit seasonal growth. *Thaumarchaeota* were more abundant in deep Arctic and Antarctic waters and grew throughout the winter at surface and deeper Arctic halocline waters. However, in situ single-cell activity measurements revealed a low activity of this group in the uptake of both leucine and bicarbonate (<5% *Thaumarchaeota* cells active), which is inconsistent with known heterotrophic and autotrophic thaumarchaeal lifestyles. These results suggested the existence of alternative sources of carbon and energy. Our analysis of an environmental metagenome from the Arctic winter revealed that *Thaumarchaeota* had pathways for ammonia oxidation and, unexpectedly, an abundance of genes involved in urea transport and degradation. Quantitative PCR analysis confirmed that most polar *Thaumarchaeota* had the potential to oxidize ammonia, and a large fraction of them had urease genes, enabling the use of urea to fuel nitrification. *Thaumarchaeota* from Arctic deep waters had a higher abundance of urease genes than those near the surface suggesting genetic differences between closely related archaeal populations. In situ measurements of urea uptake and concentration in Arctic waters showed that small-sized prokaryotes incorporated the carbon from urea, and the availability of urea was often higher than that of ammonium. Therefore, the degradation of urea may be a relevant pathway for *Thaumarchaeota* and other microorganisms exposed to the low-energy conditions of dark polar waters.**

*amoA* | *ureC* | Beaufort Sea | Ross Sea | Amundsen Sea

The realization that Archaea were not strict extremophiles but widespread in the environment has become one of the most exciting findings in the recent history of microbial ecology. Since the discovery of a new group of mesophilic archaea, classified as the phylum *Thaumarchaeota* (1), that prevail in soils, oceans, and freshwater systems (2–4), the unveiling of their biogeochemical role in the environment has remained a challenge (5–7). In the oceans, *Thaumarchaeota* are very abundant (globally approximately 20% of prokaryotic cells) (8), likely influencing the oceanic biogeochemistry through contributions to the carbon and nitrogen cycles. However, the extreme difficulty in culturing representatives of this phylum has hampered elucidation of their metabolic traits.

The fact that the single planktonic marine *Thaumarchaeota* cultured to date (*Nitrosopumilus maritimus* SCM1) is a strict autotrophic ammonia oxidizer (9), and the reports on the abundance of genes encoding archaeal ammonia monooxygenases (*amoA*) in oceanic waters (4, 10), have led to the belief that marine *Thaumarchaeota* are predominantly nitrifiers. Indeed, the genetic potential for ammonia oxidation is a common feature of the other two marine *Thaumarchaeota* with sequenced genomes: *Candidatus* "Cenarchaeum symbiosum" (11) and *Candidatus* "Nitrosoarchaeum limnia SFB1" (12). However, experimental data from oceanic samples suggests that marine *Thaumarchaeota* are metabolically diverse, hinting at heterotrophic or possibly mixotrophic lifestyles (13, 14).

Consistent with the potential for heterotrophy, early single-cell activity measurements showed that the Marine Group I (MGI) *Archaea* cluster, which is the dominant thaumarchaeal group in marine waters, can incorporate organic compounds such as amino acids (15). Those initial results were confirmed in large-scale samplings across the Atlantic Ocean (13, 16, 17). However, other studies have shown that some MGI *Archaea* fix carbon autotrophically (18, 19), presumably linked to ammonia oxidation (9, 10, 20), or have provided evidence for mixed autotrophic and heterotrophic metabolisms (14, 21). Although the contribution of MGI *Archaea* to prokaryotic production and dark $CO_2$ fixation appears to be significant in the global ocean (13), their actual contribution to nitrification has not been resolved yet (22, 23).

Here we focused on the metabolism of marine *Thaumarchaeota* in polar environments, where these microorganisms are very abundant and exhibit seasonal growth (24–26). Although knowledge on the diversity of polar archaea is rapidly increasing (27–29), their in situ metabolic activities remain virtually unexplored. Two previous studies in Arctic waters obtained contradictory results, reporting high archaeal uptake of organic compounds during summer in the Chukchi Sea (30) while year-round heterotrophic activity was low in the Beaufort Sea (26). Archaeal *amo*A genes have been detected in Arctic and Antarctic waters (28), but in the Southern Ocean there are no reports of archaeal in situ activities. Hence, the main sources of energy for growth of polar *Thaumarchaeota* remain unknown. Here, we combined in situ single-cell activity measurements, quantitative PCR (qPCR), and metagenomic analyses to shed light on the metabolism of these enigmatic, uncultivated polar microorganisms.

## Results and Discussion

**Dynamics of Polar *Thaumarchaeota*.** We analyzed the dynamics of archaeal abundance along the winter-to-summer transition in surface and halocline waters of the Southeast Beaufort Sea, Western Arctic. Additionally, archaeal abundance was estimated

Author contributions: L.A.-S., A.S.W., D.R.M., P.L.Y., L.R., P.B., C.P.-A., and S.B. designed research; L.A.-S., A.S.W., D.R.M., K.B., H.F., P.L.Y., C.L., J.-É.T., M.P., F.H., M.E., and C.P.-A. performed research; L.R., P.B., and S.B. contributed new reagents/analytic tools; L.A.-S., A.S.W., D.R.M., K.B., H.F., P.L.Y., C.L., J.-É.T., M.P., F.H., M.E., L.R., P.B., C.P.-A., and S.B. analyzed data; and L.A.-S., A.S.W., D.R.M., K.B., H.F., P.L.Y., C.L., J.-É.T., M.P., F.H., M.E., L.R., P.B., C.P.-A., and S.B. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Data deposition: The metagenomic data have been deposited in the European Bioinformatics Institute (EBI) database (accession no. ERP001178); and the sequences reported in this paper have been deposited in the GenBank database (accession nos. JX512003–JX512021).

See Commentary on page 17732.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1201914109/-/DCSupplemental.

from depth profiles taken in summer in the Ross and Amundsen Seas, Antarctica (*SI Appendix*, Fig. S1). The water column in the Southeast Beaufort Sea is perennially stratified, with low-salinity Pacific waters forming a halocline between the surface Polar Mixed Layer and deeper warmer Atlantic waters. This oceanic area is oligotrophic (31), and during the sampling period, there were seasonal fluctuations in surface light availability (from 0.01 to 3.29 μmol quanta $m^{-2}$ $s^{-1}$), ice cover, and temperature (from −1.7 to −0.4 °C; *SI Appendix*, Table S1). Chl *a* values were generally low (<0.05 μg/L during the winter; *SI Appendix*, Fig. S2). The Southern Ocean sampling included a regional trophic gradient with stations located in open waters or under total ice cover in summer (*SI Appendix*, Table S1). Surface Chl *a* ranged from 0.2 to 10.4 μg/L in the Eastern Amundsen Sea and from 0.3 to 8.4 μg/L in the Ross Sea. Surface water temperature ranged from −0.21 to −1.70 °C. Different oceanographic water masses within the depth profiles were analyzed including Antarctic Surface Waters, Thermocline, deep Shelf Waters, and Circumpolar Deep Waters (CDW) to have a wide representation of Antarctic *Thaumarchaeota* from different habitats.

Our results in the Arctic confirmed previous reports of increases in the proportion of *Thaumarchaeota* in winter polar surface waters (25, 26, 32). The abundance of MGI *Archaea* in the Southeast Beaufort Sea increased from 6% of prokaryotic cells in January 2008 to 18% in March 2008, as analyzed by catalyzed reporter deposition fluorescence in situ hybridization (CARD-FISH; Fig. 1*A*). Because the winter water column remained stratified (33), our results indicate that the increase of MGI *Archaea* was due to continuous growth of these populations in situ, and not to mixing with deeper water masses, as hypothesized for Antarctic waters (28, 34). Interestingly, we detected a similar temporal trend in deeper samples from the halocline (Fig. 1*A*), where MGI *Archaea* contributed higher abundances (up to 24% of prokaryotes; *SI Appendix*, Fig. S3). In contrast to surface waters, the abundance of MGI *Archaea* in this deeper layer remained high until April (18% of prokaryotes; Fig. 1*A*).

In Arctic surface waters, the abundance of MGI *Archaea* rapidly declined once light became detectable beneath the sea ice (*SI Appendix*, Fig. S2), consistent with the idea that *Thaumarchaeota* experience photoinhibition (35). By May and June, MGI *Archaea* contributed less than 5% of cells in Arctic surface waters. Similarly, the archaeal abundance in summer Antarctic surface waters was low (< 4% of prokaryotes; Fig. 2*A*). However, *Thaumarchaeota* were abundant in deep Shelf Waters and CDW, contributing on average 10% and 19% of total cells, respectively. Thus, our data confirm that *Thaumarchaeota* are an abundant and dynamic component of polar marine microbial communities by showing growth at both surface and halocline Arctic waters during the winter.

**Single-Cell Metabolic Activity of Polar MGI *Thaumarchaeota*.** Microautoradiography combined with FISH (MAR-FISH) was used to analyze the metabolism and activity levels of polar MGI *Archaea*. Bicarbonate and leucine were used as proxies for autotrophic and heterotrophic metabolisms, respectively (13). Throughout the seasons, less than 2% of Arctic MGI archaeal cells took up leucine (Fig. 1*B*). In contrast, *Bacteria* increased their heterotrophic activity from winter to spring and summer (i.e., from 25 to 45% of *Bacteria* actively took up leucine). A similar pattern was found in Antarctic summer samples of the Ross Sea at 10- and 100-m depths, with less than 3% of MGI *Archaea* and more than 15% of bacterial cells incorporating leucine (Fig. 2*B*).

The low archaeal heterotrophic activity is consistent with results from a previous seasonal study of surface waters at a close location (Franklin Bay, Beaufort Sea), where other labile organic substrates were also tested (26). However, in summer surface waters of the Arctic Chukchi Sea, MGI *Archaea* were highly active in the uptake of a variety of organic compounds (30), suggesting regional differences in the metabolism of Arctic *Thaumarchaeota*, possibly related to differences in the composition of the archaeal communities inhabiting these two Arctic regions (27). It should be noted that the Chukchi Sea is an exceptionally nutrient-rich area and one of the most productive oceanic regions in the world (36). Therefore, we believe that our finding that polar *Thaumarchaeota* do not contribute significantly to the uptake of labile monomers such as leucine is more representative for the low to moderate productivity conditions found in most polar marine waters.

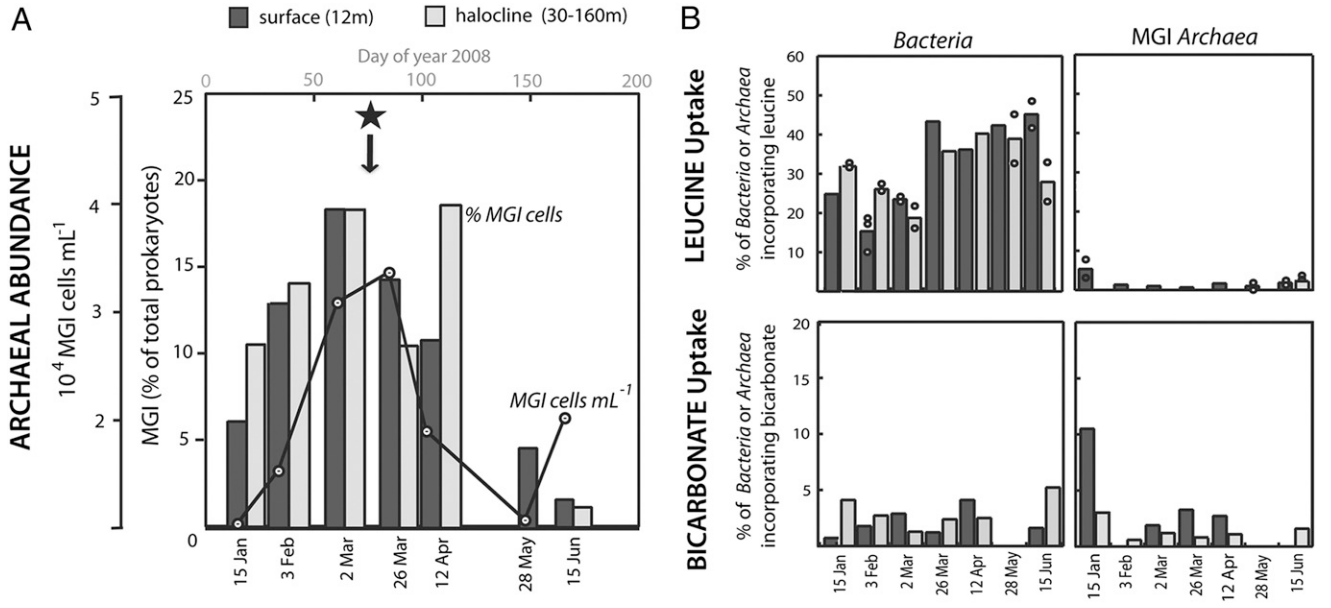


**Fig. 1.** Marine Group I (MGI) archaeal in situ abundance and metabolic activity in Arctic waters as analyzed by CARD-FISH and MAR-FISH. (*A*) Abundance of MGI *Archaea* expressed as percentage of total prokaryotes (bars) and number of cells in surface waters (line). The star symbol indicates the date at which the Arctic metagenome was retrieved. (*B*) Single-cell activity of *Bacteria* and MGI *Archaea* in the uptake of leucine (*Upper*) and bicarbonate (*Lower*). Individual dots represent replicate measurements carried out in some samples. Dark and light gray bars represent samples collected in Arctic surface and halocline waters, respectively.

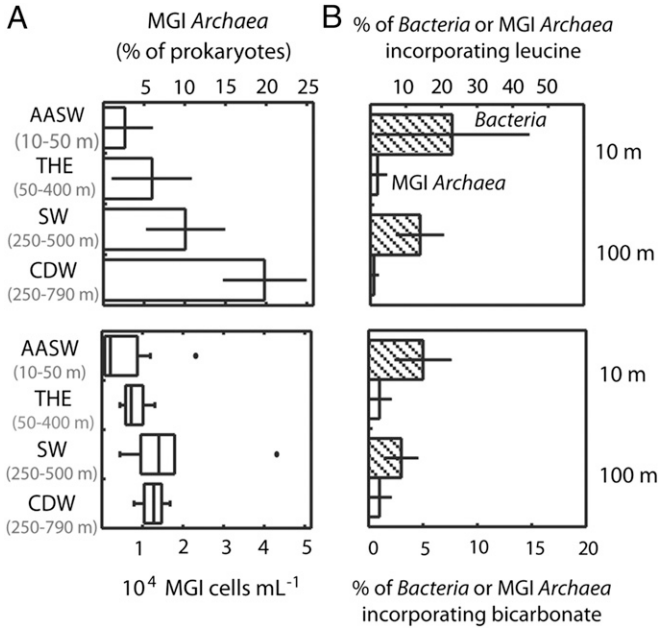


**Fig. 2.** MGI archaeal in situ abundance and metabolic activity in Antarctic waters as analyzed by CARD-FISH and MAR-FISH. (*A*) Abundance of MGI *Archaea* in waters of the Ross and Amundsen seas expressed as percentage of total prokaryotes (bars in *Upper*) and number of cells (box plots in *Lower*). Data in *Upper* are shown as averages ± SD, and *n* ranges from 4 to 18. (*B*) Single-cell activity of *Bacteria* (hatched bars) and MGI *Archaea* (open bars) in the uptake of leucine (*Upper*) and bicarbonate (*Lower*). *n* ranges from 4 to 5. AASW, Antarctic Surface Waters; CDW, Circumpolar Deep Waters; SW, Shelf Waters; THE, thermocline.

Reports of archaeal *amoA* genes in polar environments (28), and recent evidence of active nitrification in Arctic winter waters (31, 37), suggest that polar *Thaumarchaeota* may be nitrifiers. To test whether polar *Thaumarchaeota* were growing autotrophically, we measured their activity in bicarbonate uptake. With the exception of a sample collected in surface waters in January, a low proportion of Arctic *Bacteria* or MGI *Archaea* incorporated bicarbonate (<5% of active cells) even during winter (Fig. 1*B*). Similarly, low archaeal bicarbonate uptake was found in Antarctic waters down to 100 m depth (Fig. 2*B*). Interestingly, low percentages of MGI *Archaea* active in bicarbonate uptake were also generally found in summer waters of the Chukchi Sea (30). Overall, despite detecting an active growth of MGI *Archaea* in winter Arctic waters, our in situ measurements revealed paradoxically low autotrophic activities and low incorporation of leucine for this group, pointing to alternative sources for obtaining carbon and energy to sustain growth. To identify those sources and to obtain an unbiased overview of the functional potential of *Thaumarchaeotai*, we analyzed a winter Arctic metagenome.

**Metabolic Capacity of Polar *Thaumarchaeota* Analyzed via Metagenomics.** The sample for metagenomic analysis was collected under the ice during the period of highest archaeal abundance (March), when *Thaumarchaeota* made up 18% of total prokaryotic cells near the surface and in the halocline (Fig. 1*A*). Accordingly, reference genome mapping of the reads revealed that *Thaumarchaeota* comprised 14% of the total Arctic microbial community (*SI Appendix*, Fig. S4). The metagenome was collected at 65-m depth in halocline waters, where a peak in nitrite was found, and it was hypothesized that the potential for nitrification was highest (*SI Appendix*, Fig. S5).

Analysis of thaumarchaeal 16S rRNA genes in the metagenome revealed that the assemblage was represented by closely related genotypes affiliated with the cluster MGI-α, which also comprises the nitrifiers *N. maritimus* and *Ca*. N. limnia (*SI Appendix*, Fig. S4). Most (70%) of the thaumarchaeal phylotypes shared >98% similarity among their 16S rRNA genes and belonged to a cluster that included metagenomic samples retrieved from Antarctic winter waters (38, 39), and several dominant phylotypes from Arctic and Antarctic water masses (*SI Appendix*, Fig. S4). These results indicate that a single closely related clade of MGI *Archaea* dominates *Thaumarchaeota* throughout the seasons in both Arctic and Antarctic waters. At the genomic level, this clade has only 84% and 86% average nucleotide similarity with *N. maritimus* and *Ca.* N. limnia, respectively, indicating that it affiliates with hitherto unknown archaeal species (40).

From a total of 338,714 protein coding genes found in the Arctic metagenome, 5,091 were identified in contigs or reads affiliated with *Thaumarchaeota* (i.e., the Arctic thaumarchaeal metagenome). Thirty-two percent of these thaumarchaeal genes could be functionally annotated and assigned to orthologous groups (OGs) via eggNOG (*SI Appendix*). Transcriptional regulators and thioredoxins were among the most abundant thaumarchaeal OGs (*SI Appendix*, Table S2), consistent with the genome analysis of *N. maritimus* (41). Ammonia permeases (COG0004) and the enzyme glutamine synthetase (COG0174) were also well represented, suggesting the importance of nitrogen metabolism for Arctic *Thaumarchaeota*.

In the context of carbon and energy sources, we found a 6.5-kb contig containing the complete operon encoding the ammonia monooxygenase with 96% amino acid identities with *N. maritimus* (*SI Appendix*, Fig. S6). In total, the abundance of *amo* genes in the thaumarchaeal metagenome was high (i.e., 17, 19, and 12 copies of *amoA*, *amoB*, and *amoC* genes, respectively). As a reference, the thaumarchaeal metagenome had an average (±SD) of 18 (±7) copies of single-copy prokaryotic essential genes (42). Therefore, we can conclude that a majority of the metagenomic thaumarchaeal population had the ability to oxidize ammonia.

**Comparative Thaumarchaeal Metagenomic Analysis.** A genomic comparison between the Arctic thaumarchaeal metagenome and the three published MGI archaeal genomes (*N. maritimus, Ca.* C. symbiosum, and *Ca.* N. limnia) was carried out to identify common metabolic pathways and potentially unique metabolic features of polar *Thaumarchaeota* (Fig. 3). We found a functional core of 647 OGs shared by all four thaumarchaeal phylotypes that included genes involved in several central metabolic pathways (*SI Appendix*, Fig. S7), ammonia oxidation, and the main carbon fixation pathway of *Thaumarchaeaota,* i.e., the 3-Hydroxypropionate-4-hydroxybutyrate pathway (Fig. 3) (43). Among the 868 nonredundant gene families found exclusively in the Arctic thaumarchaeal metagenome, only a few could be functionally annotated (i.e., 30 OGs). Although some of these genes were involved in widespread processes such as translation or transcription, a key enzyme in fatty acids biosynthesis, which is an unusual pathway in the *Archaea* domain, was also detected in low abundance (the 3-oxoacyl-acyl-carrier-protein synthase; *SI Appendix*, Table S3).

Arctic *Thaumarchaeota* also shared some OGs exclusively with each of the MGI archaeal isolates. *Ca.* C. symbiosum was the genotype that shared the fewest number of OGs with Arctic *Thaumarchaeota* but, remarkably, most of them were involved in degradation of urea (i.e., ureases, the accessory protein *ureH* and a GTPase involved in regulation of the expression and maturation of ureases, COG0378; Fig. 3). Thaumarchaeal *ureA, ureB*, and *ureC* genes were relatively abundant in the Arctic metagenome (i.e., 8, 7, and 12 gene copies, respectively), and genes encoding urea transporters highly similar (80–90% amino acid identities) to the transporter present in *Ca.* C. symbiosum (CENSYa_0457) were also found. The presence of these genes implies that urea degradation could be a key pathway used by *Thaumarchaeota* to obtain carbon and energy in polar environments.

**Quantification of *amoA* and *ureC* Gene Abundances in Arctic and Antarctic Waters.** The prevalence of *amo*A and *ureC* genes in polar *Thaumarchaeota* was analyzed by qPCR. The copy numbers of archaeal *amo*A and 16S rRNA genes were highly correlated, and

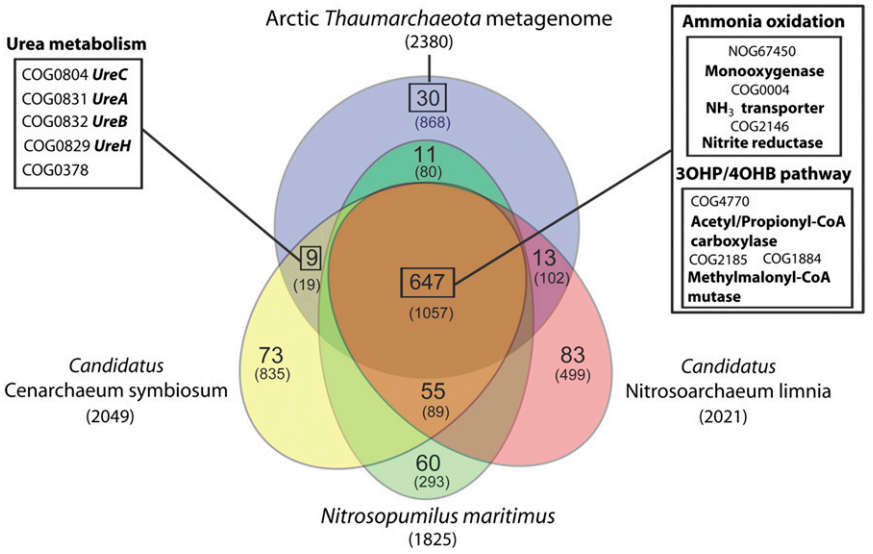


**Fig. 3.** Comparative genomic analysis between the Arctic thaumarchaeal metagenome and the three sequenced MGI *Archaea* to date. *Nitrosopumilus maritimus SCM1*, *Candidatus* N. limnia SFB1, and *Candidatus* C. symbiosum A were used to build OGs specific to *Thaumarchaeota* and, thereafter, the genes identified in Arctic *Thaumarchaeota* metagenome were assigned to these OGs. The numbers in parentheses represent the total number of OGs for the genomes or regions in the Venn. The large numbers in each region of the Venn represent OGs with functional annotation from eggNOG v2. OGs that did not occur in the Arctic thaumarchaeal metagenome and were shared among only two other genomes are hidden. Some OGs of interest have been highlighted in boxes. 3OHP/4OHB, 3 hydroxyproprionate/4 Hydroxybutyrate.

the ratio between these genes was on average 3 and 1 in Arctic and Antarctic water samples, respectively (Fig. 4 and *SI Appendix*, Tables S4 and S5), in agreement with previous reports from the Chukchi Sea (37) and off the Antarctic Peninsula (28). Our results thus indicate that a genetic potential for ammonia oxidation is widespread in polar MGI *Archaea*.

To analyze the abundance of *ureC* genes, we optimized a qPCR assay targeting polar *Thaumarchaeota ureC* genes. Full-length sequences were initially obtained by amplifying several Arctic and Antarctic samples with primers previously designed for marine thaumarchaeal *ureC* genes (44). Remarkably, most *ureC* genes retrieved from polar waters formed a tight cluster with more than 90% nucleotide identity. This polar cluster showed only approximately 75% nucleotide similarity with archaeal *ureC* genes previously found in surface and deep waters from other marine environments (44) (*SI Appendix*, Fig. S8). The qPCR analysis revealed that *ureC* genes were abundant in polar *Thaumarchaeota*, particularly in deep water layers such as the Arctic halocline or Antarctic CDW, where the average ratio of *ureC* versus MGI 16S rRNA genes was close to or greater than 1 (Fig. 4). This ratio was lower in Arctic surface samples (i.e., average ratio of 0.2), suggesting genetic differences between archaeal populations inhabiting surface and halocline waters. In Antarctic waters, the average ratio between *ureC* and MGI 16S rRNA genes was 2.7 in deeper CDW, and approximately 0.3 in other water masses. Altogether, these results indicate that the potential for urea degradation is widespread in polar *Thaumarchaeota*.

**Relevance of Urea Degradation for Polar *Thaumarchaeota*.** Relatively high concentrations of urea have been found in polar seawater and sea ice (45–49), with the principal sources being microbial

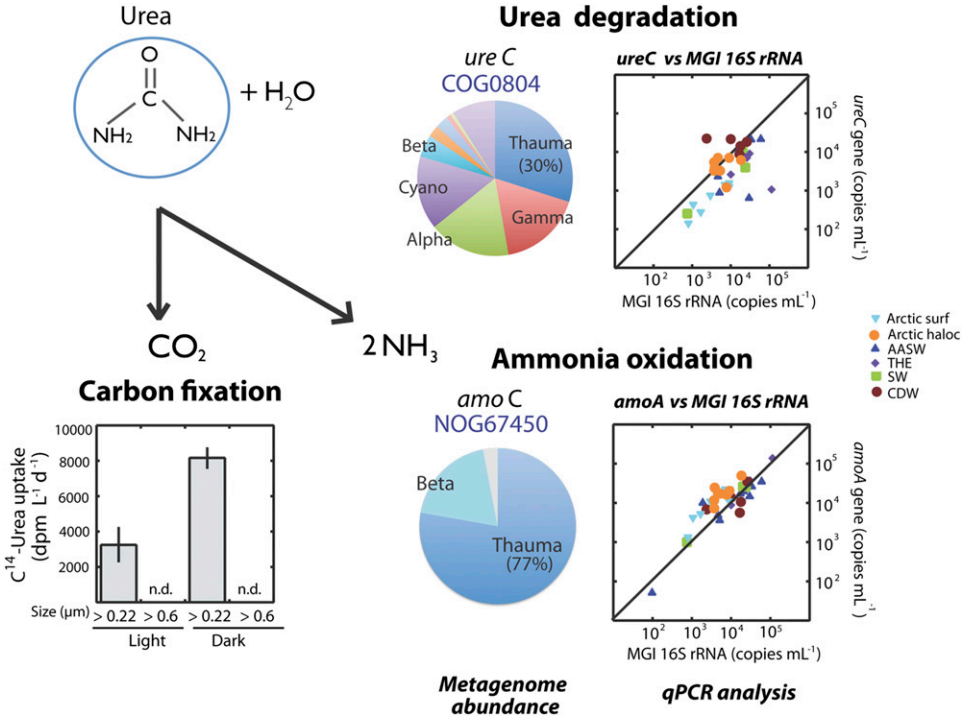


**Fig. 4.** Proposed pathway used by Arctic *Thaumarchaeota* to obtain carbon and energy from the degradation of urea. The pie charts show the phylogenetic affiliation of reads assigned to the orthologous groups COG0804 (urease) and NOG67450 (ammonia monooxygenase) within the Arctic metagenome. Plots at *Right* show the abundance of *ureC* genes (upper plot) and *amoA* genes (lower plot) versus the abundance of MGI *Archaea* 16S rRNA genes in Arctic and Antarctic waters, as analyzed by qPCR. Two samples from AASW did not show detectable amplification of *ureC* genes and were not included in the upper plot. Uptake measurements of $^{14}$C-labeled urea in surface Arctic samples of different size fractions (>0.2 and >0.6 μm) under light and dark conditions are shown on the bottom left side. AASW, Antarctic Surface Waters; CDW, Circumpolar Deep Waters; haloc, halocline; n.d, nondetected; surf, surface; SW, Shelf Waters; THE, thermocline.

metabolism of purines (50) or nitrogenous waste excretion by zooplankton and other metazoans (45). Urea is recognized as a significant source of nitrogen for polar phytoplankton (47, 48, 51), but its degradation by polar prokaryotes has received limited attention, although diverse marine microorganisms have the potential to use this compound (52). In an experiment carried out in Arctic winter surface waters, we found detectable urea utilization in the prokaryotic size fraction, but not in the size fraction corresponding to phytoplankton (i.e., >0.6 μm; Fig. 4), supporting the hypothesis that urea is relevant for the metabolism of polar prokaryotes thriving at low energy conditions.

Within *Thaumarchaeota*, the first report of ureases was in the obligate symbiont *Ca.* C. symbiosum, which may use the waste-product urea from its host, a marine sponge, to fuel ammonia oxidation (11). However, neither of the nitrifiers *N. maritimus* (41) or *Ca.* N. limnia (12) have ureases, showing that the potential to degrade urea is not ubiquitous among marine *Thaumarchaeota*. Indeed, ureases are rare in the *Archaea* domain (53). Nevertheless, we show that ureases are widespread in polar *Thaumarchaeota* and, interestingly, in the Arctic metagenome, *Thaumarchaeota* was the phylum with the highest abundance of urease genes (Fig. 4).

The potential use of urea to fuel nitrification has been reported for ammonia oxidizing bacteria (54) and, recently, for a soil nitrifying *Thaumarchaeota* isolate (55). Because synthesizing ureases and urea transporters requires energy, the utilization of urea would be advantageous only when the availability of ammonium were low or intermittent. Although urea is often assumed to be present at a lower concentration than ammonium in marine systems (53), we found the opposite pattern in summer samples collected across the Arctic Beaufort Sea. The availability of urea-N was often higher than ammonium and, remarkably, at many depths where ammonium was below detection levels, urea was still detected (*SI Appendix*, Fig. S9). Similarly, urea was often found in higher concentration than ammonium in samples collected in summer in the eastern Canadian Arctic (47).

Unfortunately, no winter measurements of ammonium are available from our study; however, this substrate was below the detection limit (<10 nM) in early spring measurements carried out on the second and seventh of April 2008. These results suggest that virtually no ammonium was available for the later part of the winter when *Thaumarchaeota* were still abundant, at least in deeper halocline waters (Fig. 1). In contrast, urea was present in several Arctic winter profiles, and the average (±SD) concentration (70 ± 80 nM) was similar to that found in summer (July, 60 ± 30 nM; *SI Appendix*, Fig. S9). Comparable results were obtained in Arctic waters at the end of March (46), and in spring Antarctic samples below the ice (56), where ammonium was below detection and urea concentration and uptake rates were relatively high. In the only station with total ice cover during our Antarctic sampling, the concentration of ammonium was also below detection limit (*SI Appendix*, Table S1). These observations suggest that urea is a more stable source of energy for ammonia oxidizers in low productive polar systems than ammonium. During polar winter, the input of photosynthetically produced organic matter is halted, but urea can be continuously supplied by other microorganisms and zooplankton (45). The ability to use urea would therefore provide an ecological advantage to nitrifying MGI *Archaea*.

We made estimates to determine whether the availability of urea would be enough to sustain the observed winter Arctic archaeal growth. The molar ratio of biomass produced per unit of ammonia molecule oxidized (C/N) in *Thaumarchaeota* was estimated 0.046 based on the growth of *N. maritimus* (9), using a biomass factor of 12 fg of C per cell (57). The net growth rate of polar MGI *Archaea* over the winter was similar in surface and halocline waters (≈0.21 $d^{-1}$) and comparable to the maximal growth rate reported for *N. maritimus* (0.72 $d^{-1}$) (9). Using a C/N ratio of 0.046, a consumption rate of 4.8 and 3.9 nM urea/d would be required to sustain the winter growth of MGI *Archaea* (from January to March) in surface and halocline waters, respectively. Assuming an average winter concentration of urea of 70 nM, the turnover time of urea would be ≈16 d, which is consistent with previous estimates from Arctic waters (47). These calculations indicate that the availability of urea in Arctic waters could support the winter archaeal growth observed, although additional energy would be required to synthesize ureases and urea transporters. We found oligopeptide transporters (COG4608) and different catabolic pathways in the thaumarchaeal metagenome (*SI Appendix*, Fig. S7). These findings suggest that polar MGI *Archaea*, in addition to ammonia, may exploit some organic compounds to supplement their growth, as found for the thaumarchaeon *Nitrososphaera viennensis* (55) or some ammonia oxidizing bacteria (58). Interestingly, urea serves as a source of nitrogen and also carbon for certain ammonia oxidizing bacteria in soil (59, 60). The detectable in situ incorporation of $^{14}$C-labeled urea shows that the carbon of urea was assimilated by polar prokaryotes (Fig. 4). The possibility that *Thaumarchaeota* were actively incorporating the carbon from urea could explain the low activity in bicarbonate uptake detected in situ.

**Role for Urea Metabolism in Marine *Thaumarchaeota*.** Based on the prevalence of *ureC* and *amoA* genes in polar *Thaumarchaeota*, we hypothesize that urea degradation may be a relevant pathway by which these populations meet their carbon and energy demands. Most of the few previous reports of marine thaumarchaeal ureases were carried out in deep Mediterranean and Pacific waters (44, 61) (*SI Appendix*, Fig. S8), where *Thaumarchaeota* also thrive under low energy conditions and urea is often present in high concentrations. In Pacific mesopelagic waters, high rates of urea degradation were measured (62). Although at the time of the latter study it was unknown that *Thaumarchaeota* were dominant members of the mesopelagic (8), it is remarkable that the degradation of urea was paralleled by high nitrification rates (62). Recently, archaeal ureases have been found in both a metagenome and a metatranscriptome of mesopelagic Pacific waters, indicating that ureases are expressed by *Thaumarchaeota* in deep oceanic waters (63).

A predominance of chemoautotrophic pathways in winter polar waters and the global dark ocean is now accepted (39), but the potential relevance of urea in this scenario has not been recognized. Recent studies suggest that the supply of ammonium is insufficient to fuel marine autotrophic microorganisms and hint at the use of alternative energy sources (22). Given the high abundance of *Thaumarchaeota* in polar and deep oceanic waters, and their genomic potential to use urea, the contribution of this substrate to the metabolic demands of these and other microorganisms living in low energy environments needs to be revised.

## Materials and Methods

Water samples for qPCR, CARD-FISH, and MAR-FISH analyses were collected near the surface and in deeper halocline waters from the Arctic Southeast Beaufort Sea during the overwintering CFL cruise and in the upper 500 m from the Antarctic Amundsen and Ross seas during the summer in the *Oden* Southern Ocean cruise (*SI Appendix*, Fig. S1). Samples were collected by using a ship-deployed rosette for open water sampling and through the ship's internal moon pool for winter ice-covered sampling. All materials and methods are described in detail in *SI Appendix*.

**ACKNOWLEDGMENTS.** We thank the captains and crew of the icebreakers *Oden* and CCGS *Amundsen* and the Swedish Research Polar secretariat for logistic support; Dan Nguyen, Roxane Maranger, and other members of the Circumpolar Flaw Lead (CFL) cruise for collecting samples; A. Niemi, B. Philippe, C. J. Mundy, A. Sallon, C. Michel, and M. Gosselin for chlorophyll data; J. Gagnon for nutrient data; P. Guillot and Y. Gratton for conductivity–temperature–depth (CTD) data; and the European Molecular Biology Laboratory Information Technology core facility and Y. Yuan for managing the high-performance computing resources. 454 pyrosequencing was supported by the K&A Wallemberg foundation. This work is a contribution to the International Polar Year–CFL system study (2007/2008) supported through grants from the Canadian International Polar Year Federal Program Office and the Natural Sciences and Engineering Research Council (NSERC) Canada. Spanish participation was funded by the Spanish Ministry of Science and Innovation Grant Boreal CLG2007-28872-E/ANT (to C.P.-A), and laboratory work was supported by the Swedish Research Council (to S.B.). L.A.-S. was supported by Marie Curie

Fellowship PIEFGA-2008-221121 and a "Juan de la Cierva" contract from the Spanish Ministry of Science and Innovation. We acknowledge support by the National Science Foundation Antarctic Organisms and Ecosystems Program Grants ANT-0741409 and ANT-08361440 (to K.B. and P.Y.); Swedish Research Council Environment, Agricultural Sciences, and Spatial Planning Grant 217-2006-342 (to L.R and H.F.); and NSERC of Canada (A.S.W., C.L., and J-É.T.).

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