LETTERS

Major gradients in putatively nitrifying and non-nitrifying Archaea in the deep North Atlantic

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Aerobic nitrification of ammonia to nitrite and nitrate is a key process in the oceanic nitrogen cycling mediated by prokaryotes[1]. Apart from Bacteria belonging to the β- and γ-Proteobacteria involved in the first nitrification step, Crenarchaeota have recently been recognized as main drivers of the oxidation of ammonia to nitrite in soil as well as in the ocean, as indicated by the dominance of archaeal ammonia monooxygenase (*amoA*) genes over bacterial *amoA*[2,3]. Evidence is accumulating that archaeal *amoA* genes are common in a wide range of marine systems[3–6]. Essentially, all these reports focused on surface and mesopelagic (200–1,000 m depth) waters, where ammonia concentrations are higher than in waters below 1,000 m depth. However, Crenarchaeota are also abundant in the water column below 1,000 m, where ammonia concentrations are extremely low. Here we show that, throughout the North Atlantic Ocean, the abundance of archaeal *amoA* genes decreases markedly from subsurface waters to 4,000 m depth, and from subpolar to equatorial deep waters, leading to pronounced vertical and latitudinal gradients in the ratio of archaeal *amoA* to crenarchaeal 16S ribosomal RNA (rRNA) genes. The lack of significant copy numbers of *amoA* genes and the very low fixation rates of dark carbon dioxide in the bathypelagic North Atlantic suggest that most bathypelagic Crenarchaeota are not autotrophic ammonia oxidizers: most likely, they utilize organic matter and hence live heterotrophically.

Planktonic Archaea consisting of the two major groups, Crenarchaeota and Euryarchaeota, might account for about one-third of all prokaryotic cells in the global ocean[7,8]. Crenarchaeota are generally more abundant than Euryarchaeota in marine waters[7–9] and mainly comprise the marine Crenarchaeota group I (MCGI) and the deep-branching pSL12-like clade[4]. Until recently, only members the bacterial domain, γ- and β-Proteobacteria were known to oxidize NH_4^+ to NO_2^-, and γ-, β-, δ-Proteobacteria and *Nitrospira* species to oxidize the intermediate product NO_2^- to NO_3^- (ref. 1). Recent genomic studies suggest that mesophilic Crenarchaeota might play a role in the ammonia oxidation, as the *amoA* gene encoding a subunit of one of the key enzymes in the ammonia oxidation, ammonia monooxygenase, has been detected[2,10,11]. The only two mesophilic marine crenarchaeal isolates (that is, *Cenarchaeum symbiosum* and *Nitrosopumilus maritimus*) also carry this *amoA* gene[12,13]. Recent quantitative studies showed that MCGI oxidize ammonia as their energy source and that archaeal *amoA* genes are at least one order of magnitude more abundant than bacterial *amoA* in various marine and soil environments[3–5,14,15]. Moreover, Mincer *et al.*[4] recently suggested that the pSL12-like clade may also contain *amoA* genes. Taken together, there is evidence that at least some of the MCGI are chemoautotrophic ammonia oxidizers although other studies indicate that Crenarcheaota might be heterotrophic as well[8,16–19].

All the studies quantifying archaeal *amoA* gene abundance in the pelagic realm, however, focused on the euphotic and mesopelagic zones, where ammonia is generated by remineralization processes such as in the oxygen minimum zone[3–5,11]. In addition, the only-free living isolate, *Nitrosopumilus maritimus*, has been obtained from an ammonia-rich tropical aquarium[12]. However, Crenarchaeota are abundant in bathypelagic (1,000–4,000 m depth) waters as well[20,21], where ammonia concentrations are below 5 nM (M. Woodward, personal communication) and only detectable with highly sensitive methods, thus making it unlikely that ammonia is readily available as an energy source in these deep waters.

The contributions of putatively ammonia-oxidizing Archaea (AOA) and Bacteria (AOB) to the total archaeal and bacterial communities were determined by quantitative PCR (qPCR) in the main water masses of the Atlantic from 65° N to 5° S covering a depth range from 100 m to 4,000 m. The abundance of specific groups of planktonic Crenarchaeota (MCGI and the pSL12-like clade) based on 16S rRNA gene fragments was determined and compared with the abundance of AOA and AOB using two specific primer sets targeting archaeal and bacterial *amoA* genes at 10 stations, comprising different water masses with specific physical and chemical characteristics (for details see Supplementary Fig. 1 and Supplementary Tables 1 and 2).

The highest MCGI abundance was found in the mesopelagic layer in almost all the stations, particularly in the oxygen minimum layer (that is, between 200 and 600 m depth; Fig. 1a and Supplementary Tables 1 and 3). The abundance of the pSL12-like clade was about 1.5 orders of magnitude lower than MCGI (Supplementary Table 3). In most of the samples (40 out of 55), the contribution of the pSL12-like clade to total crenarchaeal abundance was less than 5% (Supplementary Table 3) but followed a similar vertical distribution pattern as MCGI. The pSL12-like clade was more abundant just below the euphotic zone (that is, 250 m depth) than in deeper water masses (Supplementary Table 3). A similar trend was observed by Mincer *et al.*[4], who found more pSL12-related members below the euphotic zone of the North Pacific subtropical gyre. The distribution of the copy numbers of the 16S rRNA genes (MCGI and pSL12-clade) obtained by qPCR corresponds to the distribution pattern of Crenarchaeota with depths reported previously using fluorescent *in situ* hybridization[7,8,20,21]. Overall, marine Crenarchaeota seem to be particularly associated with the oxygen minimum zone[4,5,11,22].

Copy numbers of archaeal *amoA* genes were always highest at the base of the euphotic zone, ranging from 2.5×10^3 to 25×10^3 ml^{-1} and decreasing with depth (Fig. 1b and Supplementary Table 3). Although no latitudinal trend in the abundance of the archaeal *amoA* genes was detectable at the base of the euphotic zone, in deeper waters, archaeal *amoA* copy numbers decreased by two to three orders of magnitude from the subpolar to the subtropical and equatorial Atlantic (Fig. 1b and Supplementary Table 3). The higher abundance of archaeal *amoA* genes in the meso- and bathypelagic waters in the northern part of the Atlantic coincides with the higher

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ammonia concentrations there[21], as these deep waters have been recently formed and therefore are younger than in the southern part of the North Atlantic[23]. In contrast to the high number of archaeal *amoA* copy numbers, β-proteobacterial *amoA* copy numbers were at least one order of magnitude lower than archaeal *amoA* genes in the individual depth layers (Supplementary Table 3). γ-Proteobacterial *amoA* genes were not quantified in this study because ammonia-oxidizing γ-Proteobacteria were not detected in this region of the Atlantic by qPCR in a previous study[3] nor in Arctic or North Pacific samples[4,24]. Taken together, our data confirm previous findings that crenarchaeal *amoA* genes are far more abundant than bacterial *amoA* genes in marine systems[3,5,15].

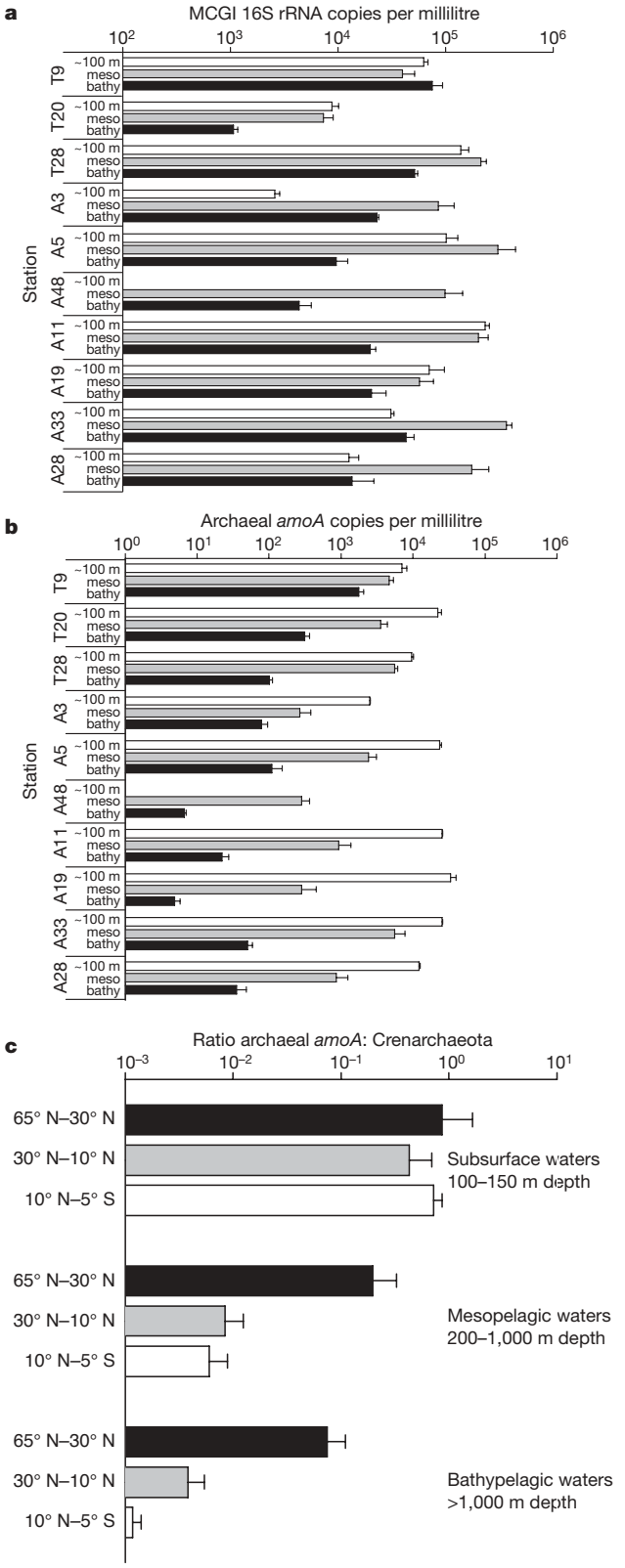

Figure 1 | Latitudinal and depth distribution of crenarchaeal 16S rRNA and archaeal *amoA* copy numbers in the eastern North Atlantic. **a**, **b**, Abundance of MCGI (**a**) and AOA (**b**) in the subsurface (~100 m) ($n = 9$), mesopelagic (meso) ($n = 24$) and bathypelagic (bathy) ($n = 22$) waters. **c**, Ratio of archaeal *amoA* copies to crenarchaeal (MCGI + pSL12-like clade) 16S rRNA copies per millilitre of marine water ($n = 55$). Bars indicate standard errors.

The average ratio of archaeal *amoA* copy numbers compared with crenarchaeal 16S rRNA genes in the subsurface waters (100–150 m depth) was close to 1, particularly in the northern and equatorial North Atlantic, whereas it was less than 0.01 in the bathypelagic waters of the subtropical and equatorial region of the North Atlantic (Fig. 1c and Supplementary Fig. 2). Our ratios for the surface waters are similar to those of Mincer *et al.*[4], who reported a ratio between archaeal *amoA* and archaeal 16S rRNA genes of about 1, whereas Wuchter *et al.*[3] and Beman *et al.*[5] reported a ratio of 2.8 and 2.5, respectively. Genomic studies on the two mesophilic crenarchaeal isolates obtained thus far revealed that *Cenarchaeum symbiosum* and *Nitrosopumilus maritimus* contain only one *amoA* gene copy per cell[13]. In the 100–150 m depth range, the average ratios between archaeal *amoA* and crenarchaeal 16S rRNA genes were 0.9, 0.4 and 0.7 for the northern, subtropical and equatorial regions, respectively (Fig. 1c). We conclude, therefore, that in subsurface waters most of the Crenarchaeota are putatively capable of ammonia oxidation throughout the North Atlantic. In the northern part of the Atlantic (65–30° N), the ratio of archaeal *amoA* to crenarchaeal 16S rRNA genes decreased only by about one order of magnitude from the surface waters to the bathypelagic realm (Fig. 1c). In the subtropical and equatorial regions of the Atlantic (30° N – 5° S), however, this ratio decreased by at least two orders of magnitude from the subsurface to the bathypelagic waters (Fig. 1c). Hence, AOA are apparently only abundant throughout the water column in the northern North Atlantic, with its newly formed and therefore young deep waters[23,25,26]. The major fraction of the abundant Crenarchaeota in the subtropical and tropical North Atlantic deep waters most likely use energy sources other than ammonia.

To assess the diversity of AOA in the meso- and bathypelagic waters of the North Atlantic, archaeal *amoA* clone libraries were established for two mesopelagic samples where qPCR analyses revealed relatively high numbers of AOA, and for one bathypelagic sample where the AOA abundance was very low (Supplementary Table 3). The bathypelagic clone library exhibited the lowest observed and estimated operational taxonomic unit richness (Supplementary Fig. 3 and Supplementary Table 4). The richness levels of the two mesopelagic clone libraries were comparable or higher than those observed for archaeal *amoA* clone libraries from other marine pelagic environments[11] (Supplementary Table 4). In a DNA-based phylogenetic tree, the obtained sequences fell into two distinct groups: cluster A ('shallow' cluster) and cluster B ('deep' cluster)[5,11,27] (Fig. 2). Based on phylogenetic analyses of PCR clone libraries, individual regions of the mesopelagic North Atlantic harbour specific archaeal *amoA* sequences (that is, North Atlantic and equatorial subclusters (Fig. 2)). More than half of the sequences of the clone library from the mesopelagic North Atlantic formed a coherent cluster closely related to sequences obtained from the same region[3]. Similarly, specific sequences recovered from the bathypelagic clone library fell into bathypelagic subcluster 1 within cluster A and into bathypelagic subcluster 2 of the phylogenetically distinct cluster B (Fig. 2). About 60% of the obtained archaeal *amoA* sequences were affiliated to the North Atlantic subcluster harbouring *amoA* sequences from a diverse range of marine habitats[3–5,11,22] (Fig. 2). Taken together, our phylogenetic analyses indicate latitudinal and vertical differences among archaeal *amoA* sequences with generally rather low diversity in equatorial bathypelagic waters (Supplementary Fig. 3) coinciding with the rather low abundance of AOA.

There is, however, still the possibility that deep ocean Crenarchaeota harbour specific versions of *amoA* genes not targeted by the available primer sets.

An archaeal 16S rRNA clone library was also established for one mesopelagic sample and three bathypelagic samples (for details see Supplementary Information). In a DNA-based phylogenetic tree, the obtained crenarchaeal sequences fell into the MCGI and pSL12-like groups (Supplementary Fig. 4). The phylogenetic analyses of the archaeal 16S rRNA genes also strengthen the idea that specific water masses harbour specific ecotypes and that the presence of operational taxonomic unit 2, closely related to *Nitrosopumilus maritimus*, especially in the TRANSAT sample, might explain the high abundance of *amoA* genes in this specific water mass (for details see Supplementary Information).

Ammonia-oxidizing Archaea are generally chemoautotrophic, using acetyl-coenzyme A carboxylase to fix inorganic carbon[28].

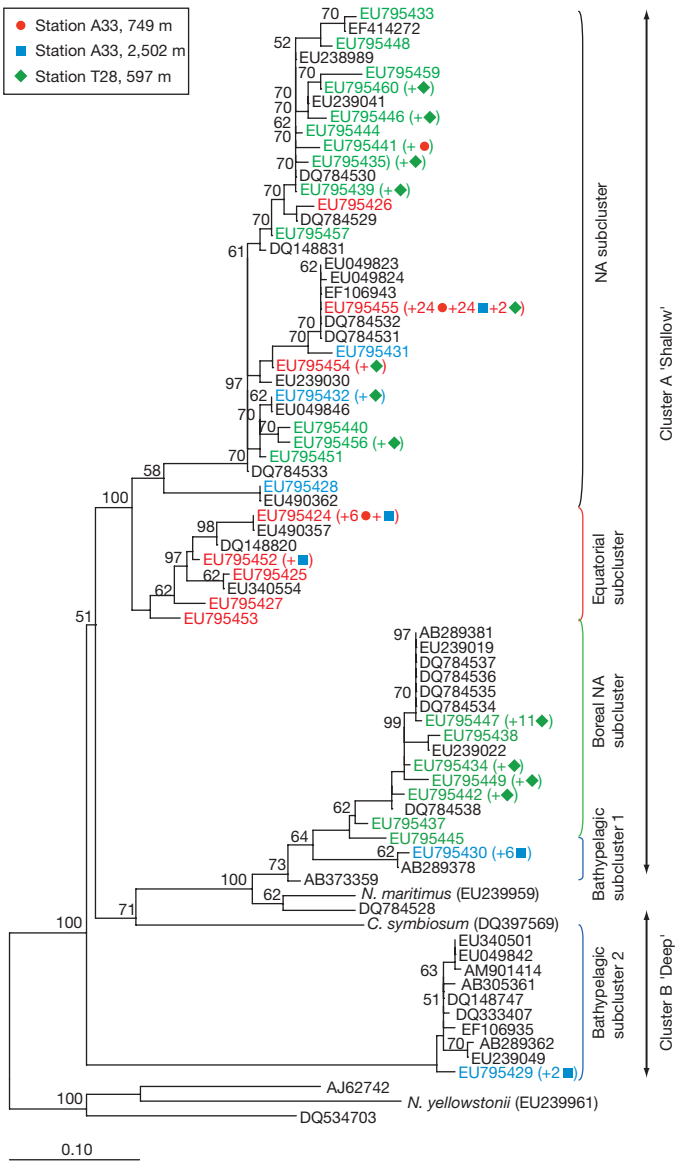

Figure 2 | Phylogenetic tree of archaeal *amoA* sequences recovered from the North Atlantic. Neighbour-joining bootstrap tree of long *amoA*-like nucleotide sequences to which we added our partial sequences (217 base pairs (bp), EU795424–EU795460) and the sequences of Wuchter *et al.*[3] (217 bp, DQ784528–DQ784537) using the ARB parsimony tool (NA, North Atlantic). One representative of sequence group ≥99% identical is shown (in colour); the number in front of the respective symbol indicates the number of clones. Database sequences are shown in black. Bootstrap values (>50%) are indicated at each branch point.

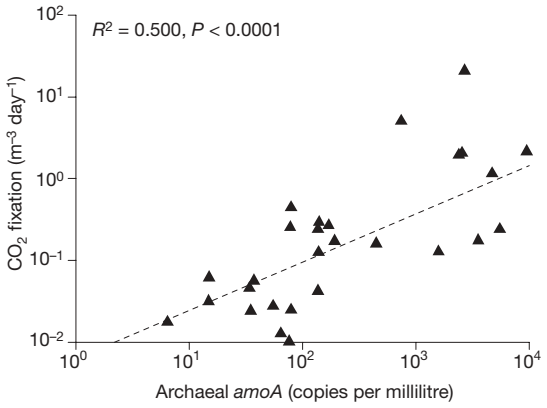

Figure 3 | Archaeal *amoA* copy numbers versus CO_2 fixation. Relation between archaeal *amoA* gene copy numbers and CO_2 fixation across all the stations and depths (except station T9; $n = 31$). Regression equation: $y = 0.0063x^{0.5905}$.

Hence, if AOA are the dominating nitrifiers, as suggested by the much higher archaeal than bacterial *amoA* copy numbers, one would expect a tight relation between archaeal *amoA* copy numbers and bulk CO_2 fixation rates. Relating the dark CO_2 fixation rates obtained at these specific stations and depths by Herndl *et al.*[8] and T. Reinthaler and G.J.H. (unpublished observations) to the archaeal *amoA* copy numbers determined in this study, it appears that about 50% of the variation in CO_2 fixation rates can be explained by archaeal *amoA* abundance (Fig. 3). This provides further independent evidence that most bathypelagic Crenarchaeota are not chemoautotrophic nitrifiers, but most likely use organic substrates as carbon and energy sources, as also indicated by microautoradiography combined with fluorescence *in situ* hybridization and genomic analyses[8,16–18,21,27]. This conclusion is further supported by recent comparative metagenomic analyses of uncultivated Archaea of various meso- and bathypelagic oceanic regions by Martin-Cuadrado *et al.*[29]. In none of the nine fosmid libraries of MCGI from 500 to 3,000 m depth were *amoA* genes detected[29].

In summary, it appears that only in the northern part of the North Atlantic mesophilic Archaea predominantly oxidize ammonia as an energy source throughout the water column including the bathypelagic realm. The abundance of putatively ammonia-oxidizing Crenarchaeota gradually decreases in the meso- and bathypelagic waters from the north to the equator in the Atlantic Ocean, coinciding with an increasing age of these water masses and, concomitantly, a decrease in the diversity of archaeal *amoA* sequences. In the subtropical and equatorial bathypelagic waters, AOA contribute less than 1% to the crenarchaeal community. Hence, only a minor fraction of the bathypelagic Crenarchaeota putatively oxidize ammonia in the temperate and subtropical North Atlantic. Consequently, a heterotrophic life mode for bathypelagic Crenarchaeota seems to become increasingly important from north to south in the Atlantic's interior.

METHODS SUMMARY

Sampling was conducted aboard the RV *Pelagia* during two cruises (TRANSAT-1 and ARCHIMEDES-2) following the North Atlantic Deep Water from 65° N to 5° S in the eastern basin of the Atlantic Ocean. Specific water masses were identified and sampled based on their physical and chemical characteristics.

Using qPCR, the copy numbers of MCGI 16S rRNA, pSL12-like 16S rRNA, archaeal *amoA* and β-proteobacterial *amoA* genes were determined using specific primers and SYBR Green.

Archaeal *amoA* clone libraries were generated from two stations: station A33 at two distinct depths (749 and 2,502 m) and station T28 at 597 m depth by PCR amplification using specific archaeal *amoA* primers. Archaeal 16S rRNA gene clone libraries were generated from three stations: station T9 at 2,071 m, station A3 at two distinct depths (249 and 2,750 m) and one additional station in the Romanche

Fracture Zone (−0.21° N, −18.43° E) near station A33 (1° N, −20.60° E) at 7,155 m depth by PCR amplification using specific archaeal primers.

Dark archaeal CO_2 fixation was measured by the incorporation of [^{14}C]bicarbonate. The resulting mean disintegrations per minute of the samples were converted into organic carbon produced over time and corrected for the natural dissolved inorganic carbon concentration.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions The manuscript was written by H.A. and G.J.H. DNA extractions were performed by H.A. and M.B., qPCR by H.A., M.B. and J.D. and phylogenetic analyses by M.B. Measurements of dissolved inorganic carbon incorporation were done by G.J.H.

Author Information The archaeal sequences are deposited in GenBank under accession numbers EU650236–EU650270 (station 3, ARCHIMEDES-2), FJ002858–FJ002876 (station 23, ARCHIMEDES-3), FJ150794–FJ150834 (Station 9, TRANSAT-1) for 16S rRNA genes and EU795424–EU795460 and EU810209–EU810235 for *amoA* genes. Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to G.J.H. (herndl@nioz.nl).

METHODS

Study sites and sampling. Sampling was conducted aboard the RV *Pelagia* during two cruises (TRANSAT-1 and ARCHIMEDES-2) following the North Atlantic Deep Water from 65° N to 5° S in the eastern basin of the Atlantic Ocean. Specific water masses were identified and sampled based on their physical and chemical characteristics (Supplementary Table 1). At each station, six depths were sampled with 10-l NOEX samplers mounted on a conductivity–temperature–depth (CTD) frame. Ten stations were used for qPCR analysis (Supplementary Fig. 1).

DNA extraction of marine samples. Extraction of total DNA was performed using an UltraClean soil DNA and Mega soil DNA isolation kits (Mobio) for TRANSAT-1 and ARCHIMEDES-2 samples, respectively.

qPCR. The copy numbers of MCGI 16S rRNA, pSL12-like 16S rRNA, archaeal *amoA* and β-proteobacterial *amoA* genes were determined in triplicate on the non-diluted samples and in two different dilutions per sample using specific primers (Supplementary Table 2) and SYBR Green. The four real-time approaches yielded highly reproducible standard curves with DNA of the plasmid 088exp4, the fosmid HF770_041/11, *Nitrosopumilus maritimus* and a mix of four β-Proteobacteria, for MCGI, pSL12-like, archaeal *amoA* and β-proteobacterial *amoA* standards, respectively. All reactions were performed in duplicate for the standards and in triplicate for the samples; an average value was calculated for each sample. Two different methods were used to determine the presence of non-specific amplified products. No unspecific products were found in any of the analysis (see Supplementary Information for details).

Archaeal *amoA* clone libraries. Archaeal *amoA* clone libraries were generated from two stations: station A33 at two distinct depths (749 and 2,502 m) and station T28 at 597 m depth by PCR amplification using specific archaeal *amoA* primers (Supplementary Fig. 1 and Supplementary Table 2). The obtained sequences were compared with sequences from public databases and analysed using the ARB phylogenetic package (see Supplementary Information for details).

Archaeal 16S rRNA gene clone libraries. Archaeal 16S rRNA gene clone libraries were generated from three stations: station T9 at 2,071 m, station A3 at two distinct depths (249 and 2,750 m) and one additional station in the Romanche Fracture Zone (−0.21° N, −18.43° E) near station A33 (1° N, −20.60° E) at a depth of 7,155 m by PCR amplification using specific archaeal primers (Supplementary Fig. 1 and Supplementary Table 2). The obtained sequences were compared with sequences from public databases and analysed using the ARB phylogenetic package (see Supplementary Information for details).

Archaeal CO_2 fixation. Dark CO_2 fixation was measured by the incorporation of [^{14}C]bicarbonate (100 μCi, Amersham) in 40-ml samples in triplicate and triplicate formaldehyde-fixed blanks incubated in the dark at *in situ* temperature for 60–72 h. Subsequently, the incubations were terminated by adding formaldehyde (2% final concentration) to the samples, filtering onto 0.2-μm filters (Millipore, polycarbonate), rinsing three times with 10 ml of ultrafiltered water (30 kDa molecular mass cut off) and then exposing the filters to a fume of concentrated HCl for 12 h. Thereafter, the filters were placed in scintillation vials, 8 ml of scintillation cocktail (FilterCount, Canberra-Packard) was added and, after about 18 h, counted on board in a liquid scintillation counter (LKB Wallac). The instrument was calibrated with internal and external standards. The resulting mean numbers of disintegrations per minute of the samples were corrected for the mean disintegrations per minute of the blanks, converted into organic carbon produced over time and corrected for the concentration of natural dissolved inorganic carbon.

CORRIGENDUM

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The *y* axis in Fig. 3 of this Letter was incorrectly labelled 'CO_2 fixation (m^{-3} day^{-1})'. It should read 'CO_2 fixation (μmol C m^{-3} day^{-1})'.