

1 **Novel insights into the *Thaumarchaeota* in the deepest oceans: their metabolism**
2 **and potential adaptation mechanisms**

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18 Running title: *Thaumarchaeota* in the deepest oceans

19

20 **Abstract**

21 **Background:** Marine Group I (MGI) *Thaumarchaeota*, which play key roles in the
22 global biogeochemical cycling of nitrogen and carbon (ammonia oxidizers), thrive in
23 the aphotic deep sea with massive populations. Recent studies have revealed that MGI
24 *Thaumarchaeota* were present in the deepest part of oceans - the hadal zone (depth >
25 6,000 m, consisting almost entirely of trenches), with the predominant phylotype being
26 distinct from that in the “shallower” deep sea. However, little is known about the
27 metabolism and distribution of these ammonia oxidizers in the hadal water.

28 **Results:** In this study, metagenomic data were obtained from 0-10,500 m deep seawater
29 samples from the Mariana Trench. The distribution patterns of *Thaumarchaeota* derived
30 from metagenomics and 16S rRNA gene sequencing were in line with that reported in
31 previous studies: abundance of *Thaumarchaeota* peaked in bathypelagic zone (depth
32 1,000 – 4,000 m) and the predominant clade shifted in the hadal zone. Several
33 metagenome-assembled thaumarchaeotal genomes were recovered, including a near-
34 complete one representing the dominant hadal phylotype of MGI. Using comparative
35 genomics we predict that unexpected genes involved in bioenergetics, including two
36 distinct ATP synthase genes (predicted to be coupled with H⁺ and Na⁺ respectively),
37 and genes horizontally transferred from other extremophiles, such as those encoding
38 putative di-*myo*-inositol-phosphate (DIP) synthases, might significantly contribute to
39 the success of this hadal clade under the extreme condition. We also found that hadal
40 MGI have the genetic potential to import a far higher range of organic compounds than
41 their shallower water counterparts. Despite this trait, hadal MDI ammonia oxidation
42 and carbon fixation genes are highly transcribed providing evidence they are likely
43 autotrophic, contributing to the primary production in the aphotic deep sea.

44 **Conclusions:** Our study reveals potentially novel adaptation mechanisms of deep-sea
45 thaumarchaeotal clades and suggests key functions of deep-sea *Thaumarchaeota* in
46 carbon and nitrogen cycling.

47 **Keywords:** *Thaumarchaeota*, Mariana Trench, Hadal zone, Metagenomics,
48 Comparative genomics, Sodium bioenergetics

49

50 **Introduction**

51 Concepts of the carbon cycle in deep sea (depth > 200 m) have been challenged due to
52 recent re-evaluation of the imbalance between the quantity of sinking organic carbon
53 from surface and the consumption by deep-sea heterotrophic microorganisms.
54 Chemolithoautotrophs are thought to be partially responsible for this puzzling
55 phenomenon [1]. The deep ocean environment, devoid of sunlight, is one of the few
56 ecosystems on Earth where primary production is mainly driven by
57 chemolithoautotrophy rather than photosynthesis [2, 3]. Marine *Thaumarchaeota* are
58 chemolithoautotrophs and considered to be important participants in this dark primary
59 production process [4]. *Thaumarchaeota* were initially known as mesophilic
60 *Crenarchaeota* [5] and most studied members of this phylum are ammonia-oxidizing
61 archaea (AOA) [6, 7]. AOA are thought to be the most numerous living organisms in
62 the dark ocean, representing up to 40% of all prokaryotic cells [8]. The average depth
63 of Earth's oceans is about 3,682 m [9], and the aphotic zones occupy approximately 95%
64 of the volume of all the world's oceans. Therefore studies of the piezotolerant and
65 abundant ammonia oxidizers could significantly advance our understanding of global
66 nitrogen and carbon cycles.

67 Few marine *Thaumarchaeota* strains have been isolated in pure culture, all of which
68 belong to the family *Nitrosopumilaceae* [10, 11]. Most other reported *Thaumarchaeota*
69 are from enrichment cultures [12-16] or are symbiotically associated with marine
70 sponges [17]. However, no thaumarchaeotal culture (neither pure culture nor
71 enrichment) has been retrieved from the deep sea. Early studies of the deep-sea
72 planktonic *Thaumarchaeota* were mainly based on environmental marker genes such
73 as the 16S rRNA gene and the *amoA* gene encoding the subunit A of the ammonia

74 monooxygenase [18, 19], while recent development of sequencing technologies has
75 enabled genomic level studies based on single amplified genomes (SAGs) and
76 metagenome-assembled genomes (MAGs) [20-24].

77 A number of studies have indicated that distinct phylogenetic clades of
78 *Thaumarchaeota* dominate in different water depths: shallow waters are typically
79 dominated by AOA associated with the cultivated genus *Nitrosopumilus* (member of
80 the alpha AOA) and the beta AOA clade (e.g. *Candidatus Nitrosopelagicus brevis*) [19-
81 21] (nomenclature of the alpha, beta and gamma clades is based on a study by Massana
82 and colleagues [25]); the gamma AOA clade, also known as DMGI (Deep Marine
83 Group I), represents an uncharacterized lineage within Group 1.1a *Thaumarchaeota*
84 and is present over a broad range of ocean depths. Recent studies suggest that several
85 members of genus *Nitrosopumilus* are also present in deep seawater; these
86 representatives predominate in the deep-sea hydrothermal plume of the Guaymas Basin
87 [22] and the deep hypersaline anoxic basins of the Red Sea [26]. At a depth of 7,000 m
88 in the Atlantic and Challenger Deep, West Pacific, studies based on *amoA* or 16S rRNA
89 genes reported that the dominant clade was closer to the genus *Nitrosopumilus* than the
90 gamma AOA [27, 28]. Hence we also termed these alpha AOA in hadal zone the ‘hadal
91 MGI’ (HMGI) in this study. The previous published SAG “*Candidatus Nitrosopumilus*
92 sp. PRT-SC01” from the Puerto Rico Trench shed the first light on the potential lifestyle
93 of the *Thaumarchaeota* in hadal water [29], but the incompleteness of this SAG and the
94 absence of many key genes, such as ammonia monooxygenase (AMO) genes (including
95 four subunits *amoA*, *amoB*, *amoC* and “*amoX*”), made its metabolic potential and role
96 in the global nitrogen and carbon cycles unknown. Recently, the distribution of deep-
97 sea archaeal ecotypes was analyzed in the Mariana and the Ogasawara Trenches by the
98 retrieval of several MAGs and SAGs, indicating the presence of AMO containing alpha
99 AOA in the deep sea [24]. Distribution of these ammonia oxidizers in the deep-sea
100 water column might be more complex than previously thought and further research is
101 needed at the genomic level to understand the reasons underpinning their distribution

102 patterns.

103 Our recent work in the Mariana Trench reported the predominance of heterotrophic
104 hydrocarbon-degrading bacteria in the bottom water [30]. Metagenomic data in these
105 samples were revisited to extend our understandings of autotrophic ammonia oxidizers
106 in the deepest oceans. Here, we present a novel near-complete genome (100%
107 completeness based on CheckM [31] but with seven gaps between contigs) representing
108 the *Nitrosopumilus*-associated clade in the hadal zone and demonstrate, for the first time,
109 the transcriptional activity of ammonia oxidation genes and a key gene participating
110 inorganic carbon fixation in these archaea from > 10 km deep Challenger Deep samples,
111 within the Mariana Trench, Earth's oceans deepest known site. Comparative genomic
112 approaches were employed to determine the potential mechanisms required for the
113 success of this unique archaeal clade in such an extreme trench environment and the
114 transcriptional activity of the mechanisms were confirmed. This study therefore
115 provides a new perspective on the adaptation strategies of archaea in the hadal zone and
116 their involvement in the nitrogen and carbon cycling in the deep sea.

117 **Results and Discussion**

118 *Sampling and physicochemical characteristics at Mariana Trench*

119 The depth transect at the Challenger Deep of Mariana Trench was sampled on two
120 cruises at 0, 2,000, 4,000, 8,000, 9,600, 10,400 and 10,500 m depths. Ammonia
121 concentration was uniform across the transect and ranged from 17.5 to 26.7 nM
122 (Additional file 1: Table S1). Likewise, nitrite concentration was low and constant over
123 the depth, never exceeding 0.11 μ M (Additional file 1: Table S1). There was an increase
124 in nitrate concentration with increasing depth, i.e., nitrate ranged between 34-39 μ M
125 at >2,000 m, while in the surface its concentration was 0.01-0.32 μ M. There was a slight
126 decrease in pH from the surface (8.24) to the bottom (7.8) of the trench. Salinity
127 remained constant throughout the different sampling depths. Temperature generally
128 decreased with seawater depth and ranged from 29°C at the surface to approximately 1

129 °C at the bottom of the trench. There was a marked increase in silicate concentration
130 over depth and the concentration ranged between 0.42 and 159 μM .

131 *Diversity and distribution of archaea along the depth transect*

132 A total of 190 Gbp raw metagenomic data was retrieved at various depths (0, 2,000,
133 4,000, 8,000, 9,600, 10,400 and 10,500 m) from two cruises in the Challenger Deep.
134 Binning and assembly of these data resulted in hundreds of bins including four
135 thaumarchaeal MAGs (MTA1, MTA4, MTA5 and MTA6 [short for Mariana Trench
136 Archaea]) representing four distinct deep-sea clades of AOA (Table 1). Phylogenetic
137 analyses were conducted based on 16S rRNA, *amoA* genes (found in metagenomes)
138 and 60 concatenated ribosomal proteins in order to investigate the evolutionary
139 relationships between these deep-sea *Thaumarchaeota* (Fig. 1a and Additional file 1:
140 Figure S1). Relative abundances of different thaumarchaeotal clades were also
141 examined through metagenomic *amoA* genes to determine the differences in their
142 distribution patterns in various samples along the vertical transect (Fig. 1b).
143 Furthermore, sequencing of the environmental 16S rRNA genes was conducted using
144 two different primer sets to elucidate the distribution of these ammonia oxidizers (Fig.
145 1c).

146 Primers targeting both *Archaea* and *Bacteria* were used in 16S rRNA gene sequencing.
147 However, results of the two primer sets showed apparent differences, likely indicating
148 a PCR bias, e.g. relative abundance of *Thaumarchaeota* estimated by the 341F/802R
149 primers was three times greater than that by the 515F/806R primers at 8,000 m (Fig.
150 1c). Nevertheless, similar patterns were shown in the vertical distribution of
151 *Thaumarchaeota* estimated from the metagenomic *amoA* genes and 16S rRNA gene
152 amplicons with both primer sets (Fig. 1b and 1c). For example, both 16S rRNA gene
153 primers retrieved almost no thaumarchaeotal sequences in 0 m samples, which was
154 consistent with previous results [28] (Depth 0 m metagenomics analysis where very
155 few sequences were present, Fig. 1b). Furthermore, both methods predicted the highest
156 relative abundance of AOA in 2,000 m samples, ranging from 5.9% to 14.9% of total

157 prokaryotes. Previous estimation based on different methods (such as DAPI nucleic
158 acid staining or primers targeting 16S rRNA genes) indicated that 20-75% of total
159 sequences belonged to *Thaumarchaeota* at a similar depth [8, 28]. Between the two
160 primers sets, the 341F/802R set is more likely to reflect the real distribution pattern of
161 AOA, because the results from this primer sets are more consistent both with previously
162 published studies and with our metagenomic dataset. Although our results gained using
163 two methods (16S rRNA and *amoA* genes) predicted the abundance to be lower than in
164 previous studies, considering the existence of 16S rRNA PCR bias and the highly
165 conservative estimation method of metagenomic *amoA* genes (explained in Material
166 and Methods), the inconsistency between these results is moderately small and within
167 an acceptable range.

168 The thaumarchaeal community exhibited a pronounced change over the depth transect
169 in both methods (16S rRNA and *amoA* genes), in agreement with previous studies [24,
170 28]. As expected, the thaumarchaeal community of shallower depths (2,000 to 4,000
171 m) were dominated (95.92 %) by the gamma AOA with only a small proportion
172 (1.56 %) of the thaumarchaeal community at 2,000 m being beta AOA, which have
173 been previously reported in shallower waters [24, 28]. The beta AOA have been
174 reported to exist predominantly in lower epipelagic and upper mesopelagic zone (depth
175 50 ~ 500 m) [14, 24, 28]. These AOA were detected in our deep sea samples at low
176 relative abundances (0.91 %) suggesting that they might not be native to these depths.
177 Again, in agreement with earlier reports, the abundance of alpha AOA was considerably
178 higher at the greatest depths and accounted for approximately 70% of all archaea at
179 8,000 m depth. The gamma AOA were also relatively abundant (39.09 %) in these
180 >6,000m samples. Unexpectedly, our study also retrieved sequences most likely related
181 to the thermophilic AOA clade, which includes the genus *Candidatus Nitrosocaldus*
182 typically found in hot springs [32-34] (*amoA* gene of MTA5 was clustered with *Ca.*
183 *Nitrosocaldus* in Additional file 1: Figure S1b, Fig. 1a). The sequences related to *Ca.*
184 *Nitrosocaldus* were predominantly found in the 2,000 m samples, which is surprising

185 given that the temperature at 2,000 m in Mariana Trench is ~ 2.3 °C (Additional file 1:
186 Table S1). This is, to our knowledge, the first time, that sequences related to *Ca.*
187 *Nitrosocaldus* have been reported in either a saline environment or an ecosystem with
188 a high hydrostatic pressure.

189 Microorganisms in water samples can be divided into free-living (0.2 ~ 3 μm) and
190 particle-associated (>3 μm) fractions by membrane filter sizes. Microorganisms
191 abundant in free-living fraction are usually considered to be planktonic, while those
192 found in particle-associated fraction might attach to particulate organic matter.
193 According to the relative abundance estimates from samples below 200 m,
194 *Thaumarchaeota* were consistently less abundant in the particle-associated samples
195 than in the free-living samples, suggesting that most *Thaumarchaeota* through the water
196 column are planktonic. However, the gamma AOA in 10,400 and 10,500 m are equally
197 abundant in the particle-associated samples and in the free-living samples, indicating
198 that several members of the gamma AOA clade might have undiscovered interactions
199 with particulate organic matter.

200 Four thaumarchaeotal MAGs (MTA1, MTA4, MTA5 and MTA6) were retrieved from
201 our samples. In addition to these MAGs, other thaumarchaeal fragments (short contigs
202 or scaffolds) binned with other *Bacteria* or *Archaea* were also detected, resulting in a
203 highly “contaminated” bin (a bin merging sequences from different strains or species).
204 MAG MTA1 harbors a near-complete genome sequence belonging to alpha AOA,
205 which predominate the hadal thaumarchaeotal community. MAG MTA4, recovered
206 from binning of 2,000 m water samples, is a member of the gamma AOA. Most
207 previous studies of deep-sea thaumarchaeotal SAGs have mainly focused on this clade
208 [20, 21], which are also present in all of our deep-sea samples (especially abundant in
209 2,000 and 4,000 m samples). Binning of samples from other depths did not result in
210 higher quality assemblies of gamma AOA genomes, thus only MTA4 was analyzed to
211 examine the potential functions of this clade. However, due to the low completeness
212 and quality of MAG MTA4, previously published high-quality SAGs of the same clade

213 were used for the subsequent comparative genomics analyses. MAG MTA6 is nearly
214 identical to *Ca. Nitrosopelagicus brevis* CN25 [14] with ANI \approx 98% and affiliated with
215 the beta AOA clade.

216 Intriguingly, our study retrieved a MAG (MTA5) representing the thermophilic
217 thaumarchaeotal clade, which contains the archaeal genus *Ca. Nitrosocaldus* [32-34].
218 This was very surprising given that organisms belonging to this clade have been
219 previously reported exclusively in fresh water hot springs. The phylogenetic placement
220 of MAG MTA5 corresponds to the *amoA* gene and ribosomal proteins, suggesting that
221 this is not a chimeric genome of multiple lineages nor a result of assembly or binning
222 errors (Fig. 1a and Additional file 1: Figure S1). All AMO subunits were present in the
223 MAG MTA5, indicating that this organism is a putative ammonia oxidizer. However,
224 the sequencing coverage was low ($\times 10$) and further studies will be required to
225 investigate the presence, metabolism and ecological function of this clade of AOA in
226 the deep sea.

227 Although the gamma AOA were more abundant than the alpha AOA in shallower
228 samples (2,000 and 4,000 m in Fig. 1b and 1c), it was difficult to recover high-quality
229 genomic bins belonging to the gamma AOA from these samples (only one gamma AOA
230 MAG (MTA4) was recovered with a low completeness of 24.84%). The greater species
231 diversity within the gamma AOA might explain this result and accordingly, both ANI
232 and tetranucleotide frequency correlation coefficient values (TETRA) [35] indicate that
233 the alpha AOA may consist of a single phylotype, whereas the gamma AOA have
234 multiple phylotypes (Additional file 1: Figure S2). A recent study also suggested that
235 the genomes of the alpha AOA might experience less gene flow due to presence of
236 genes encoding a thrombospondin-like extracellular structure [24]. This structure
237 contains five Ca^{2+} -binding domains and may regulate the cellular structure for adhesion,
238 thus leading to the smaller divergence of the alpha AOA [24]. Furthermore, the
239 phylogenetic distances of other genes (such as the *amoA* and the ribosomal protein
240 genes) among the gamma AOA were greater than those of the alpha AOA. It is

241 interesting to note that another highly redundant merged bin with > 400%
242 contamination was generated in our binning process. This bin contained fragments of
243 the gamma AOA and multiple *amoA* genes (Table 1). It is likely that multiple strains or
244 species of gamma AOA were too similar to be distinguished and thus were placed into
245 this bin. This would also explain why no high quality gamma AOA MAG was recovered
246 in our study even if gamma AOA were abundant in the samples. The contaminated
247 metagenomic bin was omitted from subsequent analyses due to its poor quality.

248 *Archaeal MTA1 MAG from the hadal zone*

249 MAG MTA1 is one of the first high-quality draft thaumarchaeotal genome from the
250 hadal zone which meets the recently proposed quality standards for MAGs and SAGs
251 (completeness > 90%, contamination < 5%, containing all three rRNA genes and
252 enough tRNA genes) [36]. The MTA1 MAG is 100% complete and belongs to the alpha
253 AOA, the most abundant free-living archaeal clade at 8,000m depth in the Mariana
254 Trench. Given the vast abundance of these archaea in the hadal zone and the major gaps
255 in our knowledge of their lifestyle and environmental adaptation, we focused
256 subsequent analyses on this MAG. MAG MTA1 was therefore used to predict
257 adaptations and metabolism of archaea in the hadal zone and key predictions were
258 validated by examining the transcriptional activity of genes in the predicted pathways.

259 The estimated size of a closed circular genome of MTA1 is ~1.3 Mb, which is among
260 the smallest thaumarchaeotal genomes reported, and is similar to that of *Ca. N. brevis*
261 CN25 (1.23 Mb), *Ca. Nitrosomarinus catalina* SPOT01 (1.36 Mb), and several near
262 complete SAGs of the gamma AOA. All of these deep-sea AOA genomes are
263 streamlined compared to other thaumarchaeotal strains (other complete marine
264 *Thaumarchaeota* are >1.6 Mb; Table 1).

265 To get a better overview of the MTA1 MAG, genes were annotated with Archaeal
266 Clusters of Orthologous Genes database (arCOG) [37], and a comparison of arCOG
267 categories was conducted with several other *Thaumarchaeota*, including

268 representatives of epipelagic *Nitrosopumilus* and *Ca. Nitrosopelagicus* strains and of
269 the gamma AOA clade (Additional file 1: Figure S3). MTA1 MAG has fewer genes
270 associated with cell wall, membrane and envelope biogenesis (category M) than either
271 *Ca. N. brevis* CN25 or *Ca. N. catalina* SPOT01. Other categories with relatively high
272 gene number reductions are categories R and S, which both represent genes with
273 unknown functions.

274 While gamma AOA are the dominant clade in the “ordinary” deep sea, the alpha AOA
275 emerge and dominate the archaeal community in most samples from the greatest depths
276 (>8,000 m; at least in Mariana Trench). A comparison between the gamma AOA and
277 the alpha AOA was performed to examine their unique genes based on arCOG
278 categories (Additional file 1: Figure S4). In most categories the gamma AOA possessed
279 more unique genes than the alpha AOA, especially in the categories M and R (M: cell
280 wall, membrane and envelope biogenesis; R: general function predicted only),
281 indicating their larger genomic inventories.

282 *Central metabolism of alpha AOA in the hadal zone*

283 The potential metabolic pathways of MAG MTA1 were examined (Fig. 2).
284 Unsurprisingly, the overall predicted metabolic map of MTA1 is similar to that of other
285 previously described representatives of the genus *Nitrosopumilus*, such as the type
286 strain of this genus, *Nitrosopumilus maritimus* SCM1 [38] (Additional file 1: Table S2).
287 For the core pathways that enable *Thaumarchaeota* to grow chemolithoautotrophically,
288 ammonia oxidation and carbon fixation by the modified 3-hydroxypropionate/4-
289 hydroxybutyrate (3-HP/4-HB) cycles are considered essential. Like many other marine
290 *Thaumarchaeota*, MAG MTA1 contains a set of genes involved in the utilization of
291 urea. Various *Nitrosopumilus* strains can grow on urea as their sole energy source [11,
292 39, 40] and urea is a common molecule in the sea water. The genetic potential of MAG
293 MTA1 predicts that ammonia is oxidized in the periplasm by AMO, and electrons
294 produced in this step are transferred by blue copper-containing proteins to a quinone
295 reductase and then to the main electron transfer chain. Carbon fixation is carried out by

296 the modified 3-HP/4-HB pathway, which has two major parts: one contains two
297 carboxylation reactions (consuming two bicarbonate molecules) transforming acetyl-
298 CoA via 3-hydroxypropionate to succinyl-CoA, and the other transforms succinyl-CoA
299 to 4-hydroxybutyrate and then back to two acetyl-CoA via multiple enzymes including
300 4-hydroxybutyryl-CoA dehydratase (*hcd*), a key enzyme in this pathway. This pathway
301 is thought to be the most energy-efficient one in carbon fixation under aerobic
302 conditions, and perfectly suits the lifestyles of archaea under low energy supplies [41].
303 Other ubiquitous pathways of marine AOA, such as the incomplete tricarboxylic acid
304 cycle and non-oxidative pentose phosphate pathway, are also conserved in MTA1.

305 *Synteny between MTA1 and the type strain of Thaumarchaeota*

306 An alignment between the MTA1 genome and the type strain *Nitrosopumilus maritimus*
307 SCM1 was performed to assess the genome arrangement and the conservation of
308 synteny (Fig. 3). Although the MTA1 genome is not closed, the gene organisation
309 within the contigs is robust due to the high sequencing depth ($\times 97$). The genome
310 organisation of MTA1 is largely similar to that of SCM1 and the order of MTA1 contigs
311 could be inferred from the SCM1 genome (Fig. 3). There are three large insertions on
312 the MTA1 genome as well as multiple minor genomic rearrangements compared to the
313 SCM1 genome (Fig. 3). Interestingly, several unique genes are located near the
314 insertion sites, including the glycine cleavage system on contig 2. In addition, multiple
315 unique genes were located near the gaps between the contigs, e.g. the set of atypical A-
316 type ATPase genes.

317 *Unusual bioenergetics of archaea in the hadal zone*

318 The MTA1 MAG contains two sets of A-type ATP synthase genes, which was
319 considered unusual among published marine AOA genomes until very recently (Fig. 4).
320 The first four steps of the electron transfer chain are conserved between MTA1 and
321 other marine *Thaumarchaeota*, but the complex V, the archaeal-type ATP synthase, is
322 atypical for most marine AOA. The atypical ATP synthase of MTA1 falls within the

323 same phylogenetic cluster as sequences for the gamma AOA, the terrestrial acidophilic
324 AOA *Ca. Nitrosotalea* [42], neutrophilic *Ca. Nitrosocosmicus* [43-45] and several
325 acidophilic or hyperthermophilic archaea in other phyla. In contrast, the typical ATP
326 synthase set in MTA1 is conserved in most other *Thaumarchaeota* and *Crenarchaeota*
327 (Fig. 4c). During the review of this current manuscript, Wang and colleagues published
328 a study demonstrating that the distinct, atypical ATP synthase found in the deep sea
329 AOA, and in AOA genera *Ca. Nitrosotalea* and *Ca. Nitrosocosmicus*, is a key
330 adaptation to low pH and, most likely, also to elevated pressures [46].

331 Wang and colleagues confirmed that the transcriptional activity of the atypical ATP
332 synthase is elevated at low pH and that the heterologous expression of this operon
333 confers to *E. coli* the ability to grow faster at low pH. This strongly suggests that this
334 operon is a V-type ATPase involved in pumping out protons and maintaining pH
335 homeostasis [46]. Interestingly, the related euryarchaeal ATPase / ATP synthase
336 sequences (Fig. 4c) couple the gradient of Na⁺ to ATP synthesis instead of proton
337 pumping [47] and the subunit *c* of ATPase / ATP synthase contains the ion binding
338 motifs which determine the preference for H⁺ or Na⁺. Analyses of the subunit *c*
339 sequences of the MTA1 imply that the two distinct ATPase / ATP synthase sets are
340 coupled to Na⁺ or H⁺, respectively (Fig. 4b) [47]. A combination of sodium and proton
341 motive force is present in many marine bacteria, e.g. *Vibrio* species found in the deep
342 sea [48] and the Marine Group II *Euryarchaeota*, which are ubiquitous in the marine
343 environment, have putative Na⁺-coupling ATP synthases [49]. However, there is no
344 direct experimental evidence for the coupling of ATP synthesis to either H⁺ or Na⁺
345 gradients in *Thaumarchaeota* and the findings by Wang and colleagues favour the
346 explanation that this protein is involved in proton extrusion.

347 Previous phylogenetic analysis suggested these ATP synthases are spread among
348 archaea and bacteria through horizontal gene transfer (HGT) [50]. The gene synteny
349 surrounding the typical ATP synthase of MTA1 is conserved in other *Thaumarchaeota*
350 (Fig. 4a), and the phylogeny of the subunit A of this ATP synthase is congruent with

351 that of the 16S rRNA and ribosomal proteins genes. In contrast, the downstream and
352 upstream genes of the atypical ATPase / ATP synthase set in MTA1 are different from
353 other *Thaumarchaeota* (Fig. 4a). Furthermore, linear regression results of
354 tetranucleotide frequency divergencies indicate that the atypical ATPase / ATP synthase
355 was likely acquired through a horizontal gene transfer (Additional file 1: Figure S5). If
356 these ATPases / ATP synthases were horizontally acquired, it is most likely that they
357 originated from the gamma AOA. The topology of the phylogenetic tree (Fig. 4c)
358 implies that the ATPases / ATP synthases of all the *Thaumarchaeota* were transferred
359 horizontally from *Euryarchaeota*. This is in agreement with the conclusions by Wang
360 and colleagues who suggested that the ATPase operon has been horizontally transferred
361 between TACK and DPANN superphyla and *Euryarchaeota* [46].

362 Intriguingly, genes putatively associated with Na⁺ bioenergetics are relatively common
363 in the MTA1 MAG. In addition to ubiquitous transporters, such as Na⁺/Ca⁺ antiporters,
364 NhaP-type Na⁺(K⁺)/H⁺ antiporters and Na⁺-dependent bicarbonate transporters, present
365 in other epipelagic *Nitrosopumilus* genomes, a subset of unique transporters was found
366 only in the alpha AOA and gamma AOA (Additional file 1: Table S3). For example, a
367 putative transporter similar to the NhaD-type Na⁺/H⁺ antiporter was present in MAG
368 MTA1 and closely related SAGs of the same AOA clade [22]. In addition, a unique
369 putative Na⁺/solute symporter gene (Na⁺/glucose symporter superfamily, similar to the
370 PutP-type Na⁺/proline symporter) was present in MTA1. These genes are all predicted
371 to require a Na⁺ gradient or other monovalent cations across the membrane, although
372 these predictions are pending experimental validation in *Thaumarchaeota*. Likewise,
373 functionally similar Na⁺/H⁺ antiporter and Na⁺/solute symporter genes are present in
374 the genomes of the genus *Candidatus Nitrosotalea* [51]. However, the identities
375 between these genes in *Ca. Nitrosotalea* and MTA1 genes are too low (only
376 approximately 20%) for them to be considered homologues.

377 *Adaptation of archaea to the extreme pressure in the hadal zone*

378 For organisms living in the hadal zone, one of the major challenges is to adapt to the

379 extremely high hydrostatic pressure. Under high hydrostatic pressure, proteins from
380 organisms accustomed to ambient atmospheric pressures undergo denaturation [52].
381 Osmoprotectants, also called osmolytes or compatible solutes, are produced as one of
382 the major mechanisms to adapt to extreme pressures [53]. Some representatives of the
383 genus *Nitrosopumilus* have the genetic potential to synthesize the osmolyte ectoine [22,
384 38]. Mannosylglycerate has also been reported as an osmolyte in the hot spring AOA
385 *Nitrososphaera gargensis* [54]. In contrast to some of the previously published AOA
386 genomes, no genes involved in biosynthesis of these osmoprotectants could be detected
387 in the MTA1 MAG.

388 The MTA1 MAG harbours an extra genomic island associated with inositol-1-
389 phosphate cytidyltransferase (*IPCT*) and di-myo-inositol phosphate phosphate
390 synthase (*DIPPS*), which may be involved in adaptation to high hydrostatic pressure.
391 These genes participate in the biosynthesis of di-myo-inositol phosphate (DIP), which
392 is a key osmoprotectant previously found in many hyperthermophilic archaea and
393 bacteria [55, 56]. Coding sequences for these two enzymes have merged into a single
394 open reading frame in the MTA1 MAG and an additional inositol-1-monophosphatase
395 (*IMPA*) gene copy is located in the vicinity of the merged gene. The *IMPA* gene is
396 usually present as a single copy in other previously sequenced archaeal genomes and is
397 normally responsible for the hydrolysis of *myo*-inositol monophosphate to generate
398 phosphate and *myo*-inositol, a usual osmoprotectant and a precursor of DIP. These two
399 genes, in addition to two other genes annotated as encoding a TATA-box binding protein
400 and an AsnC family transcriptional regulator, respectively, formed a small genomic
401 island in MAG MTA1 and a previously published SAG which belongs to the same AOA
402 clade (Additional file 1: Figure S6). Production of *myo*-inositol has been previously
403 postulated as a key adaptation mechanism of archaea to the deep sea [24, 29] but there
404 is no prior evidence that these genes are transcribed and required for the survival under
405 high pressure. To validate this prediction, the *DIPPS/IPCT* transcripts were quantified
406 by RT-qPCR in this study and were shown to be relatively abundant (up to ~3,000

407 copies per liter) in our cold sea water samples at 4,000 m to 10,500 m depths. Indeed,
408 these transcripts were most abundant in 8,000 m deep samples where the abundance of
409 alpha AOA was also the highest (temperature ~1.96 °C, Fig. 5). This provides novel
410 evidence that (i) these archaeal populations are active in the hadal zone and (ii) the
411 production of the osmolyte *myo*-inositol may be required for the survival under high
412 hydrostatic pressure. The unexpected finding of these *DIPPS/IPCT* homologues in both
413 thermophiles and the MTA1 MAG implies that microbes adapt to different harsh
414 environmental factors through similar mechanisms.

415 The MTA1 MAG has a glycine cleavage system along with the genes involving in
416 lipoylation, which could also play a role in osmoregulation [57]. Glycine cleavage
417 system and lipoate-related genes are present in several gamma AOA SAGs, indicating
418 that the accumulation or utilization of glycine might be ubiquitous in deep-sea archaeal
419 clades (Additional file 1: Table S4). The glycine cleavage system was also recently
420 reported in alpha, gamma and delta AOA lineages in the Mariana and Ogasawara
421 Trenches [24]. Apart from osmoprotectants, chaperones may help proteins fold properly
422 and maintain their functions under high hydrostatic pressure [58]. In most marine
423 *Thaumarchaeota*, there are only two gene copies of thermosomes (group II
424 chaperonins) [59, 60]. MAG MTA1 has an additional thermosome encoding gene
425 located near the unique Na⁺/solute symporter and urease genes (Additional file 1:
426 Figure S7b). The extra thermosome gene is phylogenetically distinct (Additional file 1:
427 Figure S7a), suggesting a distinct function compared to the typical thermosomes and
428 potential unique advantages in protein folding and proper functioning under high
429 hydrostatic pressure.

430 *Autotrophy vs heterotrophy in deep-sea archaea*

431 Over the years there has been a continuous debate as to whether the lifestyle of marine
432 archaea is primarily autotrophic, mixotrophic or heterotrophic [4, 61, 62]. There is
433 evidence that some marine archaea can take up and utilize organic compounds [61-63],
434 while ammonia-oxidizing archaea in the marine environment are typically considered

435 autotrophs able to fix their own inorganic carbon. Trench environments are particularly
436 interesting in this respect as these habitats are considered less oligotrophic than the
437 upper layers of the ocean and their primary production is thought to be driven by the
438 sinking organic nutrients [53]. To gain a better understanding of the preferred lifestyles
439 of deep-sea archaea and their capacity for mixotrophy and the uptake of organic
440 compounds, we compared the amino acid and inorganic ion transporter genes between
441 alpha AOA and gamma AOA clades (Additional file 1: Table S5). Interestingly, the
442 genomes from the alpha AOA clade contained a greater number (57% more) of
443 transporters for the uptake of organic compounds than those belonging to gamma AOA
444 clade. The presence of these additional transporter genes in the alpha AOA would be
445 parsimonious with a less oligotrophic lifestyle and the suggestion that primary
446 production in the deepest seas is driven by sinking organic carbon. This would also be
447 an attractive explanation for the different distribution patterns of the alpha AOA and the
448 gamma AOA between the hadal zone and upper layers. However, it is not clear how this
449 would fit together with the presence of the 3-HP/4-HB pathway for autotrophic carbon
450 fixation in the alpha AOA.

451 *Evidence of autotrophy in MTA1*

452 Considering the presence of both the inorganic carbon fixation pathway and the large
453 complement of predicted transporters for organic compounds in the MTA1 MAG, we
454 further investigated whether the lifestyle of archaea in the hadal zone is autotrophic. To
455 address this question we monitored the abundance and transcription of key autotrophy
456 marker genes, *amoA* and *hcd*, from alpha AOA by q-PCR on DNA and cDNA (Fig. 6).
457 The *amoA* gene encodes for the α subunit of ammonia monooxygenase, whilst *hcd*
458 encodes the key enzyme of the archaeal carbon fixation 3-HP/4-HB pathway and both
459 are required for autotrophic growth in AOA. Consistent with the metagenomics data
460 (Fig. 1b), the *amoA* and *hcd* gene transcripts of alpha AOA were most abundant in
461 samples at 8,000 m. Furthermore, the abundance of *amoA* and *hcd* gene transcripts
462 mirrored their gene abundance levels, *i.e.* most of these genes were in samples at 4,000

463 to 10,500 m and were absent in samples shallower than 2,000 m. Given such high *amoA*
464 and *hcd* gene transcript levels in the hadal zone (Fig. 6), it is most likely that MTA1
465 AOA and, moreover, the alpha AOA, are important autotrophic ammonia oxidizers in
466 these aphotic waters. *Thaumarchaeota* have been previously demonstrated to drive dark
467 carbon fixation at 3,000 m depth in the Mediterranean Sea [4], but to our knowledge
468 this is the first report documenting the transcription of the key genes in the
469 thaumarchaeal carbon fixation pathway at >10,000 m depth and in the trench
470 environment. It is also worth noting that previously characterized marine AOA have an
471 extremely high affinity for NH_4^+ and the ammonium concentration remained constantly
472 above the reported K_m throughout the depth transect in our dataset (Additional file 1:
473 Table S1) [64]. AOA in the hadal zone are therefore unlikely to be limited for
474 ammonium. Collectively, this suggests that *Thaumarchaeota* in the hadal zone grow
475 autotrophically and may play important, understudied roles in nitrogen and carbon
476 cycling in the deep ocean. In addition, these deep-sea archaea have the genetic potential
477 for uptake of many organic compounds, suggesting that under certain conditions they
478 may be able to metabolize organic carbon.

479 **Conclusions**

480 The aim of this study was to gather information on the metabolism and cellular
481 adaptations of archaea in the deep sea. We postulate that genes involved in bioenergetics
482 and osmoprotectant biosynthesis are important in the adaptation of ammonia-oxidizing
483 archaea to the high hydrostatic pressure in the deep sea and we further demonstrated
484 the transcriptional activity of the *myo*-inositol production pathway in these archaea.
485 Furthermore, we demonstrated that the key enzymes of ammonia oxidation and carbon
486 fixation were transcriptionally active, strongly suggesting an autotrophic lifestyle.
487 Nevertheless, genes associated with the transport of organic compounds in the alpha
488 AOA would also be compatible with the different distribution patterns of the alpha and
489 gamma AOA clades in trenches and upper layers of the sea. Given the vast number of
490 thaumarchaeal cells in the world's oceans and transcriptional activity of their carbon

491 fixation pathway, the role of archaea in dark primary production warrants future
492 investigation. Metagenomic and single-cell approaches only generate predictions based
493 on genetic information. Experiments with the cultures of deep-sea archaea are
494 necessary to ultimately prove these predictions and to understand the adaptation
495 mechanisms in detail. The enrichment and isolation of pure cultures is still a major
496 bottleneck for the studies of *Thaumarchaeota* in the deep sea. Nevertheless, this current
497 study provides a framework for future culture trials and represents a major step forward
498 in understanding the environmental adaptation and metabolism of *Thaumarchaeota* in
499 the deep sea.

500 Based on the facts that: firstly, most of the alpha AOA in trenches represent the same
501 phylotype, and secondly, their ANIs between other species are below the species
502 threshold (< 95%), we propose a specific name provisionally here for this
503 *Nitrosopumilus*-related species.

504 *'Candidatus Nitrosopumilus hadaliensis'* sp. nov.

505 Etymology. hadaliensis (Neo-Latin feminine adjective name): from hadal, originally
506 from Greek Hades, referring to the oceanographic zone deeper than 6,000 meters; -
507 ensis: belonging to. This name implies that the organism mainly thrives in hadal zones.

508

509 **Material and Methods**

510 A whole flow processing diagram is shown in Additional file 1: Figure S8.

511 *Sampling*

512 Water samples at depths of 0, 4,000, 9,600, 10,400 and 10,500 m were collected at
513 Challenger Deep of Mariana Trench aboard the R/V *Dong Fang Hong 2* in Sep. 2016,
514 and samples at 0, 2,000, 4,000 and 8,000 m were collected at the same station in Mar.
515 2017 as described in our recent work [30]. These samples were brought up to the surface
516 by Niskin bottles. Microorganisms were sequentially collected by 3 µm and 0.2 µm

517 polycarbonate membranes and stored at -80 °C prior to processing for sequencing.
518 Water physicochemical attributes (Additional file 1: Table S1) were measured by a CTD,
519 while the nutrients (e.g. NH₄⁺) were analyzed using spectrophotometric and
520 colorimetric methods [65].

521 *DNA and RNA extractions and sequencing*

522 DNA and RNA extractions, reverse transcription, sequencing and reads quality control
523 were the same as described in our recent work [30]. Metagenomic sequencings for 2016
524 and 2017 cruises were conducted by BGI (Shenzhen, China) and Novogene
525 Bioinformatics Technology Co., Ltd. (Beijing, China) with the same platform (Illumina
526 HiSeq X-Ten), respectively, while the 16S rRNA gene sequencing for relative
527 abundance estimation was performed by Majorbio (Shanghai, China).

528 *Assembly, binning, reassembly and gene annotations*

529 In this study, IDBA-ud 1.1.2 was used to assemble the quality-controlled reads into
530 scaffolds [66] and SPAdes 3.11.0 was chosen to re-assemble mapped reads [67].
531 Metagenomic reads recruitment (mapping) processes were conducted by BMap 37.56
532 and bwa 0.7.5a [68, 69].

533 MetaBAT 2.12.1 [70] was used to do binning, which is a process to divide the assembled
534 scaffolds into different “bins” based on parameters of the scaffolds, like for example,
535 their tetranucleotide frequency patterns and differential sequencing coverages in
536 various samples. Assembling qualities and initial phylogenetical positions of these bins
537 were measured by CheckM 1.0.7 [31]. Annotations of these genomes were based on
538 arCOG using Prodigal 2.6.3, BLAST+ 2.2.30 and HMMER 3.1b2 [37, 71-73]. Coding
539 sequences were predicted by Prodigal with default settings, and then searched against
540 the arCOG database by both BLAST and HMMER using recommended thresholds
541 (expect value <1e-5). Furthermore, to make sure that the annotation is robust, we also
542 used another automatic online pipeline service RAST with default settings [74]. Genes
543 with ambiguous or uncertain annotations were checked again using InterPro and

544 NCBI's conserved domain database on their online service [75, 76].

545 Except for MTA6, all other MTAs were generated by binning. The initial version of
546 MTA1 was from the four deepest merged samples: particle-associated and free-living
547 10,400 and 10,500 m samples. To increase the completeness of MTA1, reads from 8,000
548 and 9,600 m samples were also extracted from referential reads mapping with 97%
549 identities. In the final step, to ensure such MTA1 was not a mixture of different samples,
550 we assembled the reads with 97% identity mapped on initial MTA1 derived only from
551 the 8,000 m free-living sample (MTA1 was most abundant in this sample compared to
552 other samples), and all the analyses in this study were based on this final assembly of
553 the metagenome which originated from a single sample. Two other MTAs resulted
554 directly from binning of one single sample (2,000 m depth sample). MTA6 was a
555 reference-based assembly from the reads mapped on *Ca. N. brevis* CN25 with 97%
556 identity because we found one *amoA* gene at 2,000 m depth which was almost identical
557 to the *amoA* in this strain. There were no other *amoA* genes (like *amoA* of the ammonia
558 oxidizing bacteria) in all of these samples.

559 *Phylogenetic trees and relative abundance estimate*

560 Phylogenetic trees were built by MEGA7.0.26 [77], and subsequently rendered using
561 iTOL [78]. Relative abundances of MTAs and other *Thaumarchaeota* in our samples
562 were estimated by the following formula. Sequencing coverages were calculated by the
563 ratio of mapped reads total length to the length of the chosen gene. We chose the *amoA*
564 gene to represent the *Thaumarchaeota* in the deep sea because during the read
565 recruitment process we observed its sequencing coverages to be similar to the whole
566 genomes or the 16S rRNA genes (data not shown). Three single-copied phylogenetic
567 marker genes *rplB*, *rpsC* and *rpoB* downloaded from RDP's FunGene [79] were used
568 as templates to estimate the total number of genomes in the samples. All these genes
569 (protein sequences) were searched in our non-redundant protein database with
570 HMMER. After that, all *amoA* sequences were checked manually (after manual check,
571 their e-values were approximately $> 1e-50$), while e-value thresholds of those single-

572 copy markers were set to 1e-5 in order to cover short fragments of these markers. Hence
573 this is a highly conservative estimation. After deriving the proteins, reads were mapped
574 on the DNA sequences of these proteins with 97% identity (BBMap) and total
575 sequencing coverages of each gene were calculated according to the following formula.

$$576 \quad \text{Relative Abundance of MGI} = \frac{\text{Coverage of } amoA}{\text{Average coverage of the markers}}$$

577 *Primer design for qPCR*

578 Specific primers targeting alpha AOA clade were designed using Primer-BLAST [80].
579 Due to the high sequence conservation of *amoA* gene, sequences of several other
580 epipelagic *Nitrosopumilus* sequences were also targeted, but all showed a distinct
581 difference from those of gamma AOA or other currently known taxa of AOA. The
582 standard curves were generated by plasmids containing target sequences. All the
583 plasmids were sequenced and validated carefully to ensure they were identical to our
584 targets. All primers and PCR conditions are listed in Additional file 1: Table S6. The
585 detection limit of the assays was 1 gene copy per reaction. Results of 0 and 2,000 m
586 samples were all below the detection threshold. Three technical replicates were used
587 for each sample in qPCR and the results shown are means of these replicates.

588 *Synteny analysis between MTA1 and Nitrosopumilus maritimus SCM1*

589 Whole genome bidirectional alignments based on BLASTp [71] were performed with
590 thresholds (30% identity, 1e-5 e-value, one best match). Translated proteins of MTA1
591 were aligned against SCM1 proteins (SCM1 as template) and vice versa. Results of the
592 two alignments were combined; thus a best match bijection was established between
593 homologous proteins of MTA1 and SCM1. The location information of homologous
594 protein genes was recorded while performing BLASTp. The figure was drawn using
595 Circos [81] based on the location information. Start position of SCM1 chromosome was
596 adjusted in the figure to match the start position of MTA1 contig 3. All the RNA (rRNA
597 and tRNA) genes, unique genes or genes which were not the best match were omitted
598 from this analysis.

599 **Additional files**

600 Additional file 1: Supplementary figures and tables.

601 **DECLARATIONS**

602 **Consent for publication**

603 Not applicable.

604 **Ethics approval and consent to participate**

605 Not applicable.

606 **Availability of data and materials**

607 The quality-controlled reads from the 2017 cruise are stored in NCBI's Sequence Read
608 Archive (SRA) with accession number SRR8404393 to SRR8404400, while those from
609 2016 are from our recent work [30]. Sequences of 16S rRNA genes from two different
610 primers are stored in SRA with accession number SRR9029131 to SRR9029144. All
611 four MTAs are documented in NCBI's GenBank with accession number
612 SHMJ00000000, SHMK00000000, SHML00000000, SHMM00000000.

613 **Competing interests**

614 The authors declare that they have no competing interests.

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623 **Authors' contributions**

624 X-HZ designed the experiments and analyzed the data. HZ and HL performed the
625 metagenomic binning and following analyses. HZ, DS and YZ did the RT-qPCR. JL
626 and YZ collected the water samples and analyzed the environmental sequence data. YZ
627 extracted the community DNA. DS extracted the RNA from seawater. JT designed the
628 cruise and the large-volume water sampler. LLM and JDT provided critical ideas for
629 the analyses and experimental design. HZ, X-HZ, LLM, JDT, JL, YZ and HL wrote the
630 manuscript. All authors edited and approved the final manuscript.

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870

871 **Figure legends**

872 **Fig. 1** Diversity and distribution of *Thaumarchaeota* in the Challenger Deep, Mariana
873 Trench. Clade classification is based on Massana *et al.*, 2000 and Nunoura *et al.*, 2015
874 [25, 28] **a** Phylogenetic tree based on 60 ribosomal proteins (inferred amino acid tree).
875 This is a maximum likelihood tree with Poisson model and universal rates on all sites.
876 Sites presented in less than half of taxa were deleted. All branches gave 100% bootstrap
877 support after 100 tests except where indicated with the values indicated next to the
878 branch. There were 8602 positions in the final alignment. Ribosomal proteins used in
879 this phylogenetic analysis are documented in Additional file 1: Table S7. **b** AOA
880 relative abundance at various depths based on the ratio of the coverage of the *amoA*
881 gene to the average of the single-copy marker genes in metagenomes. Alternative *amoA*
882 gene-based classification is based on Francis *et al.*, 2005 [18]. Abundance of the clades
883 was estimated by calculating the *amoA* gene abundance from these clades directly in

884 our environmental samples. Since MTA4 does not have the *amoA* gene due to its
885 incompleteness, an *amoA* gene from the same clade (gamma AOA) was used instead. **c**
886 AOA relative abundance at various depths based on 16S rRNA sequencing in 2017
887 samples. PCR primers used in the 16S rRNA sequencing are listed in Additional file 1:
888 Table S6.

889 **Fig. 2** Predicted metabolism of hadal zone archaea based on the MTA1 MAG. Genes
890 in these pathways are listed in Additional file 1: Table S2. The urea transporter is absent
891 in the MTA1 MAG but present in other alpha AOA, thus it is shown as a dotted line.

892 **Fig. 3** Genome synteny between the MTA1 MAG and *Nitrosopumilus maritimus* SCM1.
893 Only the aligned genes of the two genomes are shown. Important core genes are marked
894 on the SCM1 genome and unique genes discussed in this study are illustrated on the
895 MTA1 genome (marked with an insertion symbol (^)). Abbreviations: GHS, glycine
896 cleavage system; DIPPS+IPCT, di-*myo*-inositol phosphate phosphate synthase and
897 inositol-1-phosphate cytidylyltransferase'; AMO, ammonia monooxygenase; *hcd*: 4-
898 hydroxybutyryl-CoA dehydratase.

899 **Fig. 4** The two ATP synthase gene islands in MTA1 **a** Gene order of the ATP synthase
900 gene islands. **b** Ion binding position of subunit c. **c** Neighbor joining phylogenetic tree
901 of A-type ATP synthase subunit A. Black dots on the branches in part C indicate
902 bootstrap support is higher than 90% after 100 tests.

903 **Fig. 5** Transcript abundance of DIPPS+IPCT genes at various water depths. After
904 calculation, if the copy numbers of genes are lower than 1 copy per reaction tube, we
905 consider them to be 0. Results of 0 and 2 km samples were all lower than this threshold,
906 while others were much higher. DIPPS: di-*myo*-inositol phosphate phosphate synthase;
907 IPCT: inositol-1-phosphate cytidylyltransferase; these two genes merged into one in
908 MTA1.

909 **Fig. 6** Transcript abundance of the autotrophy markers *amoA* and *hcd* estimated by RT-
910 qPCR over the depth transect.

911 * As in Fig. 5, the transcript copy numbers in the samples with <1 copy per reaction
912 were considered to be zero.

913

Table 1 Assemblage information of four MTA MAGs and reference genomes

	Completeness	Contamination	Strain heterogeneity	Size (Mbp)	Contigs	GC (%)	Sequencing coverages	Sampling spot (and depth)	Note
Metagenomic assembled “bins”:									
MTA1	100	0	0	1.28	7	33.2	97×	Mariana Trench 4,000 – 10,500 m (predominantly 8,000 m)	Predominant phylotype in hadal zone; alpha AOA
MTA4	24.84	0.49	0	0.42	159	34.6	-	Mariana Trench 2,000 – 10,500 m (predominantly 2,000 m)	Member of the gamma AOA
MTA5	82.94	1.94	0	1.59	380	34.9	10×	Mariana Trench 2,000 m	<i>amoA</i> gene phylogenetically clustered with thermophilic <i>Thaumarchaeota</i>
MTA6	98.22	0	0	1.07	39	33.4	5×	Mariana Trench 2,000 m	Nearly identical to CN25
Merged bin of DMGI	-	~400	-	-	-	32.1	400×	Mariana Trench 2,000 – 10,500 m (predominantly 2,000 m)	Highly merged bin of gamma AOA
Other representative SAGs (partial):									
PRT-SC01	32.69	1.94	33.33	0.61	140	33.1	-	Puerto Rico Trench, Atlantic 8,000 m	Predominant phylotype in hadal zone; alpha AOA
AAA282-K18	77.99	0	0	1.04	40	33.4	-	Dark ocean depth > 200 m	Similar to MTA1 and PRT-SC01 alpha AOA

AB-629-I23	95.87	9.47	0	1.31	133	35.7	-	Dark ocean depth > 200 m	Member of the gamma AOA
AAA007-O23	96.84	0	0	1.09	4	35.6	-	Mesopelagic zone (200 – 1,000 m)	Member of the gamma AOA
AAA799-D07	41.18	1.46	0	0.44			-	Red sea brine pool 2,000 m	Member of the gamma AOA
AAA799-E16	85.11	2.59	75.00	1.45			-	Red sea brine pool 2,000 m	closed related to <i>Nitrosopumilus maritimus</i> SCM1
AAA799-N04	84.47	0.24	0	1.33			-	Red sea brine pool 2,000 m	closed related to <i>Nitrosopumilus maritimus</i> SCM1
Pure culture strains:									
SCM1	100	1.94	0	1.65	1	34.2	-	Sea water aquarium	Type strain
Enrichment strains									
CN25	100	2.91	0	1.23	1	33.2	-	Open ocean 25 m	Streamlined, similar size to MTA1
SPOT01	100	0.97	0	1.36	1	31.4	-	San Pedro Ocean Time-Series site 75 m	Streamlined, similar size to MTA1
D3C	99.51	0	0	1.71	1	33.8	-	Northern Adriatic Sea water off Piran depth 0.5 m	
NF5	100	0	0	1.8	1	33.4	-	Northern Adriatic Sea water off Piran depth 0.5 m	
BG20	99.03	5.83	83.33	1.85	343	32.5	-	Low-salinity sediments of the San Francisco Bay	

								estuary
SFB1	98.06	0	0	1.77	1	32.6	-	Low-salinity sediments of the San Francisco Bay estuary
AR2	97.09	0	0	1.69	1	33.6	-	Sediments from Svalbard in the Arctic Circle
AR1	94.66	0	0	1.64	1	34.2	-	Sediments from Svalbard in the Arctic Circle
BD31	92.39	1.94	0	1.57	171	33.8	-	Coastal/estuarine sediments in San Francisco Bay 1 cm sediments

Qualities of these assemblies were examined by CheckM based on 145 single-copy markers for *Thaumarchaeota*.

For SAGs and MAGs completeness > 90%, contamination < 5%, containing all the rRNA and tRNA genes are considered to be high quality [36]. Currently in deep-sea AOA only MTA1 meet these standards (Several SAGs or MAGs lack rRNA or tRNA genes, although their completeness is high enough).