Limited accessibility of nitrogen supplied as amino acids, amides, and amines as energy sources for marine *Thaumarchaeota*

Julian Damashek[1,5], Barbara Bayer[2,6], Gerhard J. Herndl[2,3], Natalie J. Wallsgrove[4], Tamara Allen[4], Brian N. Popp[4], James T. Hollibaugh[1]

[1]Department of Marine Sciences, University of Georgia, Athens, GA, USA

[2]Department of Limnology and Bio-Oceanography, University of Vienna, Vienna, Austria

[3]Department of Marine Microbiology and Biogeochemistry, NIOZ, Royal Netherlands Institute for Sea Research, Utrecht University, Utrecht, The Netherlands

[4]Department of Geology and Geophysics, University of Hawai'i at Manoa, Honolulu, HI, USA

[5]Present address: Department of Biology, Utica College, Utica, NY, USA

[6]Present address: Department of Ecology, Evolution, and Marine Biology, University of California, Santa Barbara, CA

Corresponding authors:

Julian Damashek, 1600 Burrstone Road, Utica, NY 13502 • T: (315) 223-2326, F: (315) 792-3831 • judamash@utica.edu

James T. Hollibaugh, 325 Sanford Drive, Athens, GA 30602 • T: (706) 542-7671, F: (706) 542-5888 • aquadoc@uga.edu

Running title: DON oxidation by marine *Thaumarchaeota*

Keywords: *Thaumarchaeota*, nitrification, dissolved organic nitrogen, polyamines, reactive oxygen species, archaea

SUMMARY

Genomic and physiological evidence from some strains of ammonia-oxidizing *Thaumarchaeota* demonstrate their additional ability to oxidize nitrogen (N) supplied as urea or cyanate, fueling conjecture about their ability to conserve energy by directly oxidizing reduced N from other dissolved organic nitrogen (DON) compounds. Similarly, field studies have shown rapid oxidation of polyamine-N in the ocean, but it is unclear whether *Thaumarchaeota* oxidize polyamine-N directly or whether heterotrophic DON remineralization is required. We tested growth of two marine *Nitrosopumilus* isolates on DON compounds including polyamines, amino acids, primary amines, and amides as their sole energy source. Though axenic cultures only consumed N supplied as ammonium or urea, there was rapid but inconsistent oxidation of N from the polyamine putrescine when cultures included a heterotrophic bacterium. Surprisingly, axenic cultures oxidized ^{15}N-putrescine during growth on ammonia, suggesting co-metabolism or accelerated breakdown of putrescine by reactive metabolic byproducts. Nitric oxide, hydrogen peroxide, or peroxynitrite did not oxidize putrescine in sterile seawater. These data suggest that the N in common DON molecules is not directly accessible to marine *Thaumarchaeota*, with thaumarchaeal oxidation (and presumably assimilation) of DON-N requiring initial heterotrophic remineralization. However, reactive byproducts or enzymatic co-metabolism may facilitate limited thaumarchaeal DON-N oxidation.

INTRODUCTION

While chemoautotrophy supported by ammonia (NH_3) oxidation is the primary metabolism of most *Thaumarchaeota* (Francis *et al.*, 2007; Stahl and de la Torre, 2012; Santoro *et al.*, 2019), there has also been speculation on their additional capacity to oxidize nitrogen (N) from a variety of dissolved organic nitrogen (DON) compounds. Numerous experiments have demonstrated that some *Thaumarchaeota* can grow via oxidation of N supplied as urea: following the discovery of urease and urea transporter genes in the sponge symbiont *Cenarchaeum symbiosum* (Hallam *et al.*, 2006), thaumarchaeal urease genes were documented throughout the ocean (Yakimov *et al.*, 2011; Alonso-Saez *et al.*, 2012; Smith *et al.*, 2016), rate measurements using ^{15}N tracers showed moderate urea-N oxidation in coastal waters (Tolar *et al.*, 2017; Damashek *et al.*, 2019a; Kitzinger *et al.*, 2019; Laperriere *et al.*, 2021), and marine *Thaumarchaeota* capable of growth via stoichiometric oxidation of urea-N to nitrite (NO_2^-) were isolated (Qin *et al.*, 2014; Bayer *et al.*, 2016; Carini *et al.*, 2018). In addition to urea, a thaumarchaeote isolated from a hot spring contains a cyanate hydratase gene (*cynS*) and can grow via oxidation of cyanate-N (Palatinszky *et al.*, 2015). Oxidation of both urea- and cyanate-N by *Thaumarchaeota* appears to drive NO_2^- production in the northern Gulf of Mexico (Kitzinger *et al.*, 2019). These studies provide ample evidence of widespread thaumarchaeal oxidation of urea-N, and likely cyanate-N, throughout the ocean.

In addition to urea and cyanate, there is interest in whether *Thaumarchaeota* can directly oxidize N from other common DON compounds. Early studies of marine microbial assemblages demonstrated thaumarchaeal amino acid assimilation (Ouverney and Fuhrman, 2000; Teira *et al.*, 2006; Kirchman *et al.*, 2007), and recent experiments in the coastal Pacific Ocean demonstrated assimilation of N but not carbon (C) from amino acids, prompting speculation about the ability

of *Thaumarchaeota* to oxidize amino acid-N directly (Dekas *et al.*, 2019). However, data on thaumarchaeal DON-N oxidation rates are scarce. ^{15}N-tracer experiments in the coastal South Atlantic Bight, where *Thaumarchaeota* are the dominant ammonia oxidizers (Hollibaugh *et al.*, 2011; Hollibaugh *et al.* 2014; Tolar *et al.*, 2017; Liu *et al.*, 2018; Damashek *et al.*, 2019a), not only measured detectable oxidation of N supplied as urea and L-glutamic acid (L-GLU), but found oxidation rates of N from the polyamine putrescine (1,4-diaminobutane; PUT) to be far higher than oxidation rates of urea- or amino acid-N (Damashek *et al.*, 2019a). Polyamines consist of a reduced C backbone with at least two amine substitutions (Tabor and Tabor, 1984). Ubiquitously high intracellular concentrations (~mM) of polyamines such as PUT, spermine, and spermidine (Tabor and Tabor, 1985; Liu *et al.*, 2016) and rapid turnover rates in seawater (Lee and Jørgensen, 1995; Liu *et al.*, 2015) suggest fast microbial cycling of polyamines in the marine environment, including oxidation of polyamine-N (Damashek *et al.*, 2019a). However, it is unknown whether *Thaumarchaeota* can conserve energy by oxidizing polyamine- or amino acid-N directly, or if prior heterotrophic remineralization to ammonium (NH_4^+) is required.

We tested the ability of two thaumarchaeal strains originally isolated from the northern Adriatic Sea (*Nitrosopumilus piranensis* D3C and *N. adriaticus* NF5; Bayer *et al.*, 2016; Bayer *et al.*, 2019a) to grow using a variety of DON compounds as sole energy sources. Given prior evidence of rapid PUT-N oxidation in the field, our primary focus was polyamines. Axenic thaumarchaeal cultures were unable to grow when supplied with single DON compounds, other than the expected growth on urea by *N. piranensis* D3C. However, experiments with ^{15}N-labeled compounds demonstrated that axenic cultures of both strains oxidized a significant amount of PUT-N when growing on NH_3, suggesting PUT may be co-metabolized or broken down by reactive byproducts produced during thaumarchaeal growth. Furthermore, enrichment cultures

88 containing a heterotrophic bacterium occasionally showed rapid PUT-N oxidation, indicating an
89 important role for heterotrophic DON remineralization in this process. Our data indicate that
90 oxidation of reduced N by marine *Nitrosopumilaceae* is restricted to well-known substrates, but
91 suggest breakdown of DON and oxidation of its liberated N may occur as an indirect effect of
92 thaumarchaeal growth or due to tight coupling between DON remineralization by heterotrophs
93 and subsequent thaumarchaeal oxidation of the resulting NH_4^+.
94
95 **RESULTS**
96 GROWTH EXPERIMENTS
97 Growth experiments were conducted with axenic cultures of *Nitrosopumilus* strains D3C
98 and NF5 (Bayer *et al.*, 2019a) grown in NH_4^+-free Synthetic Crenarchaeota Medium (SCM;
99 Könneke *et al.*, 2005) amended with a variety of single DON compounds (1 or 2 mM added N;
100 Table 1) as their sole energy and N source. Small amounts of NO_2^- (10-50 μM) were linearly
101 produced over 70 days in all treatments (including negative controls with no added NH_4^+ or
102 DON), presumably due to NH_4^+ contamination or breakdown and oxidation of media
103 components such an antibiotics. Both strains converted NH_3 into NO_2^- stoichiometrically within
104 7 days, and D3C converted urea-N into NO_2^- within ~14 days (Table 1; Fig. S1), consistent with
105 previous data from these isolates (Bayer *et al.*, 2016; Bayer *et al.*, 2019a). A small fraction (20-
106 30%) of the glutamine-N amendment in both strains was converted to NO_2^- linearly with time.
107 None of the other DON amendments led to NO_2^- production (Table 1; Fig. S1).

Table 1 NO_2^- produced (mean ± standard deviation of triplicates) by axenic thaumarchaeal strains in media containing single organic N compounds as the sole energy source. Bolded text shows treatments with at least one isolate accumulating NO_2^- to a level greater than the negative control treatments.

			D3C		NF5	
Substrate	Amendment (μM)	N Addition (μM)	NO_2^- Produced (μM)	Available N Oxidized (%)	NO_2^- Produced (μM)	Available N Oxidized (%)
Ammonium	1000	1000	**951.8 ± 10.4**	**95.2 ± 1.0**	**982.1 ± 19.3**	**98.2 ± 1.9**
Urea	500	1000	**1034.5 ± 16.9**	**103.5 ± 1.7**	8.2 ± 7.0	0.8 ± 0.7
1,2-diaminoethane	500	1000	16.7 ± 4.1	1.7 ± 0.4	17.2 ± 1.4	1.7 ± 0.1
1,3-diaminopropane	500	1000	23.4 ± 2.3	2.3 ± 0.2	23.4 ± 3.0	2.3 ± 0.3
Putrescine	500	1000	43.9 ± 1.9	4.4 ± 0.2	16.0 ± 3.1	1.6 ± 0.3
Cadaverine	500	1000	21.7 ± 1.2	2.2 ± 0.1	23.4 ± 2.2	2.3 ± 0.2
1,7-diaminoheptane	500	1000	23.4 ± 0.6	2.3 ± 0.1	22.1 ± 0.8	2.2 ± 0.1
Spermine	500	2000	48.3 ± 3	2.4 ± 0.2	23.9 ± 7.6	1.2 ± 0.4
Spermidine	500	1000	45.9 ± 1.8	4.6 ± 0.2	13.7 ± 3.6	1.4 ± 0.4
Methylamine	1000	1000	39.9 ± 1.8	4.0 ± 0.2	13.5 ± 3.8	1.4 ± 0.4
L-Glutamate	1000	1000	43.9 ± 3	4.4 ± 0.3	13.0 ± 5.7	1.3 ± 0.6
L-Glutamine	500	1000	**299.1 ± 6.1**	**29.9 ± 0.6**	**212.5 ± 4.4**	**21.3 ± 0.4**
L-Arginine	500	2000	48.7 ± 3.1	2.4 ± 0.2	26.8 ± 0.7	1.3 ± 0.0
L-Asparagine	500	1000	32.3 ± 2.5	3.2 ± 0.3	45.2 ± 2.1	4.5 ± 0.2
L-Glycine	1000	1000	32.5 ± 2.8	3.3 ± 0.3	42.8 ± 1.4	4.3 ± 0.1
L-Ornithine	500	1000	27.1 ± 2	2.7 ± 0.2	38.0 ± 1.3	3.8 ± 0.1
Acetamide	1000	1000	28.2 ± 2.4	2.8 ± 0.2	41.2 ± 1.4	4.1 ± 0.1
Formamide	1000	1000	45.1 ± 0.7	4.5 ± 0.1	52.8 ± 0.8	5.3 ± 0.1
Nicotinamide	1000	2000	33.1 ± 2.3	1.7 ± 0.1	41.2 ± 1.8	2.1 ± 0.1
Propionamide	1000	1000	34.7 ± 3.4	3.5 ± 0.3	37.9 ± 1.8	3.8 ± 0.2
Butyramide	1000	1000	39.4 ± 5.4	3.9 ± 0.5	37.3 ± 4.9	3.7 ± 0.5
Water	0	0	42.3 ± 3.4		41.9 ± 2.4	

Further growth experiments were conducted with enrichment cultures of *Nitrosopumilus* strains D3C and NF5 containing ~5-15% bacterial 16S rRNA genes (Table 2) belonging to the heterotrophic bacterium *Oceanicaulis alexandrii* (Bayer *et al.*, 2016). Growth on NH_3 or urea proceeded rapidly and matched published rates (Bayer *et al.*, 2016). NO_2^- accumulation during growth of enrichments on

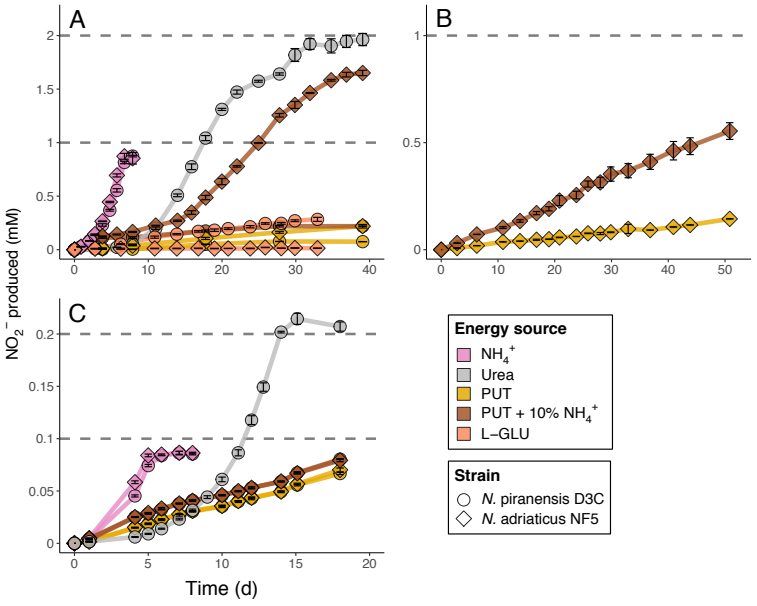

Fig. 1 NO_2^- accumulation by enrichment cultures of *N. piranensis* D3C (circles) and *N. adriaticus* NF5 (diamonds) over time. Color denotes the compound included as sole energy source in media. Points show the average of triplicate incubation bottles while error bars show standard deviation. Dashed lines show maximal NO_2^- production given added N (two lines are present to show total added N when NH_4^+ and urea were included). **A)** Growth with either 1 mM NH_4^+, 1 mM urea, 1 mM PUT, 1 mM PUT plus 100 μM NH_4^+, or 1 mM L-GLU. **B)** Growth with either 1 mM PUT or 1 mM PUT plus 100 μM NH_4^+ (*N. adriaticus* NF5 only). **C)** Growth with either 100 μM NH_4^+, 100 μM urea, 100 μM PUT, or 100 μM PUT plus 10 μM NH_4^+. Bacterial and thaumarchaeal 16S rRNA gene quantities for selected experiments (panels A, C) are shown in Table 2.

120 NH_3-free SCM amended with either 500 µM PUT or 500 µm PUT + 50 µM NH_4^+ was often
121 linear and relatively slow (Fig. 1), but occasionally resembled a typical microbial growth curve
122 (e.g., *N. adriaticus* NF5 grown with PUT and NH_4^+ amendment; Fig. 1 A). During growth on
123 NH_3, thaumarchaeal and bacterial 16S rRNA genes in selected treatments (those shown in Fig. 1
124 A, C) generally retained the same relative abundance as the respective inoculum (~90-95%
125 thaumarchaeal genes) and attained abundances comparable to the initial inoculum by the end of
126 the experiment (Table 2). Thaumarchaeal and bacterial genes gradually increased in treatments
127 containing PUT, with bacterial genes often reaching higher abundances than the initial inoculum
128 (Table 2). Nitrite concentration increased rapidly when *N. adriaticus* NF5 was amended with
129 PUT + NH_4^+ (Fig. 1 A). Both populations of genes increased in this treatment; notably, bacterial
130 genes increased from
131 6.7×10^6 genes mL^{-1} in
132 the inoculum (8.2% of
133 16S genes) to 1.4–
134 4.5×10^8 genes mL^{-1}
135 (79.3–81.0% of 16S
136 genes) during the
137 incubation (Table 2).
138
139

Table 2 Quantification of bacterial and thaumarchaeal 16S rRNA genes via qPCR for selected experiments with enrichment cultures. NO_2^- accumulation in these experiments is shown in Fig. 1 A, C.

				16S rRNA genes mL^{-1}		
Strain	**Substrate**	**Nutrients**	**Time (d)**	**Bacterial**	**Thaumarchaeal**	***% Thaumarchaeota***
D3C	All D3C treatments	Fig. 1 A	Inoculum	1.5×10^7	9.4×10^7	86.3
NF5	All NF5 treatments	Fig. 1 A	Inoculum	6.7×10^6	7.5×10^7	91.8
NF5	NH_4^+	Fig. 1 A	8	7.8×10^6	5.8×10^7	88.1
NF5	NH_4^+	Fig. 1 A	8	6.6×10^6	6.5×10^7	90.9
NF5	Putrescine + NH_4^+	Fig. 1 A	37	4.5×10^8	1.2×10^8	20.7
NF5	Putrescine + NH_4^+	Fig. 1 A	37	1.4×10^8	3.4×10^7	19.0
D3C	All D3C treatments	Fig. 1 C	Inoculum	1.5×10^6	1.3×10^7	94.2
D3C	NH_4^+	Fig. 1 C	8	8.7×10^5	1.4×10^7	96.6
D3C	NH_4^+	Fig. 1 C	8	1.3×10^6	1.5×10^7	95.5
D3C	Urea	Fig. 1 C	8	1.3×10^6	7.3×10^6	91.9
D3C	Urea	Fig. 1 C	8	1.7×10^6	7.9×10^6	90.1
D3C	Urea	Fig. 1 C	14	1.1×10^6	3.4×10^7	98.4
D3C	Urea	Fig. 1 C	14	1.2×10^6	3.7×10^7	98.4
D3C	Urea	Fig. 1 C	18	1.1×10^6	3.1×10^7	98.3
D3C	Urea	Fig. 1 C	18	1.3×10^6	4.0×10^7	98.3
D3C	Putrescine	Fig. 1 C	8	1.0×10^6	6.1×10^6	91.4
D3C	Putrescine	Fig. 1 C	8	1.8×10^6	8.9×10^6	90.9
D3C	Putrescine	Fig. 1 C	14	1.2×10^6	8.6×10^6	93.6
D3C	Putrescine	Fig. 1 C	14	1.1×10^6	8.3×10^6	93.6
D3C	Putrescine	Fig. 1 C	18	3.6×10^6	1.2×10^7	87.0
D3C	Putrescine	Fig. 1 C	18	1.4×10^6	1.4×10^7	95.0
NF5	All NF5 treatments	Fig. 1 C	Inoculum	1.5×10^6	1.6×10^7	95.1
NF5	NH_4^+	Fig. 1 C	8	1.0×10^6	1.4×10^7	96.1
NF5	NH_4^+	Fig. 1 C	8	1.5×10^6	1.5×10^7	94.8
NF5	Putrescine	Fig. 1 C	8	1.6×10^6	6.4×10^6	89.1
NF5	Putrescine	Fig. 1 C	8	1.6×10^6	5.9×10^6	88.3
NF5	Putrescine	Fig. 1 C	14	1.0×10^6	7.2×10^6	93.6
NF5	Putrescine	Fig. 1 C	14	1.1×10^6	7.7×10^6	93.2
NF5	Putrescine	Fig. 1 C	18	3.0×10^6	9.7×10^6	86.7
NF5	Putrescine	Fig. 1 C	18	2.5×10^6	1.1×10^7	89.8

ISOTOPE EXPERIMENTS

Both axenic *Nitrosopumilus* strains oxidized 100 µM NH_3 to NO_2^- within 6 days. When 1.25 µM ^{15}N-NH_4^+ was added to the growth media containing 100 µM unlabeled NH_4^+, all of the ^{15}N was converted to ^{15}N-NO_2^- by stationary phase (NF5: 1.20 ^{15}N-NO_2^- µM produced; D3C: 1.09 µM; Fig. 2). Oxidation of polyamine-N was tested by adding ^{15}N-labeled 1,2-diaminoethane (DAE), 1,3-diaminopropane (DAP), or PUT to the growth media. L-GLU was used as a control for remineralization, given its rapid catabolism and NH_4^+ remineralization by marine heterotrophs (Hollibaugh, 1978; Goldman *et al.*, 1987; Goldman and Dennett, 1991). Growth with ^{15}N-labeled DAE, DAP, or GLU did not produce ^{15}N-NO_2^-, with values similar to the negative control (^{14}N-NH_4^+ amendment, no added ^{15}N). During growth with ^{15}N-PUT, ~35% of the added ^{15}N was oxidized to ^{15}N-NO_2^- as NH_3 was consumed (NF5: 0.45 ^{15}N-NO_2^- µM produced; D3C: 0.43 µM; Fig. 2).

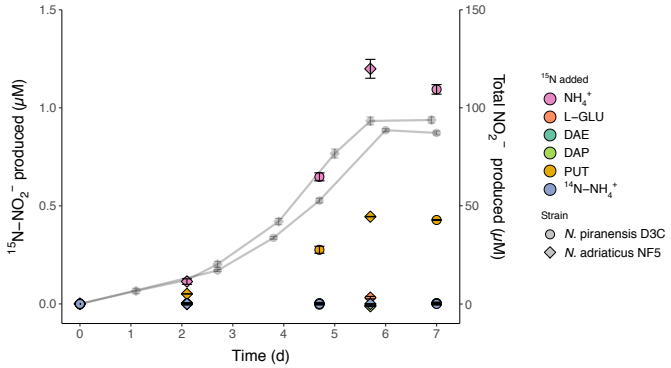

Fig. 2 ^{15}N-NO_2^- production from ^{15}N-labeled organic N compounds during growth of axenic cultures of *N. piranensis* D3C (circles) and *N. adriaticus* NF5 (diamonds) on NH_3. Gray points show total NO_2^- (^{14}N and ^{15}N; right y-axis) while points with color show concomitant production of ^{15}N-NO_2^- (left y-axis). All points represent the midpoint of duplicate incubation bottles, with error bars representing the range. The ^{14}N-NH_4^+ treatment was a negative control containing no added ^{15}N.

To test whether this ^{15}N-PUT oxidation was due to abiotic reactions with short-lived reactive metabolic intermediates, ^{15}N-PUT was incubated in filtered-sterilized, oligotrophic seawater or filtered-sterilized SCM dosed with nitric oxide (NO), peroxynitrite ($ONOO^-$), or hydrogen peroxide (H_2O_2). Amendment with only NO_2^- or PUT provided controls with no reactive compounds (Fig. 3 F). There was no significant difference in $\delta^{15}N_{NOx}$ values between

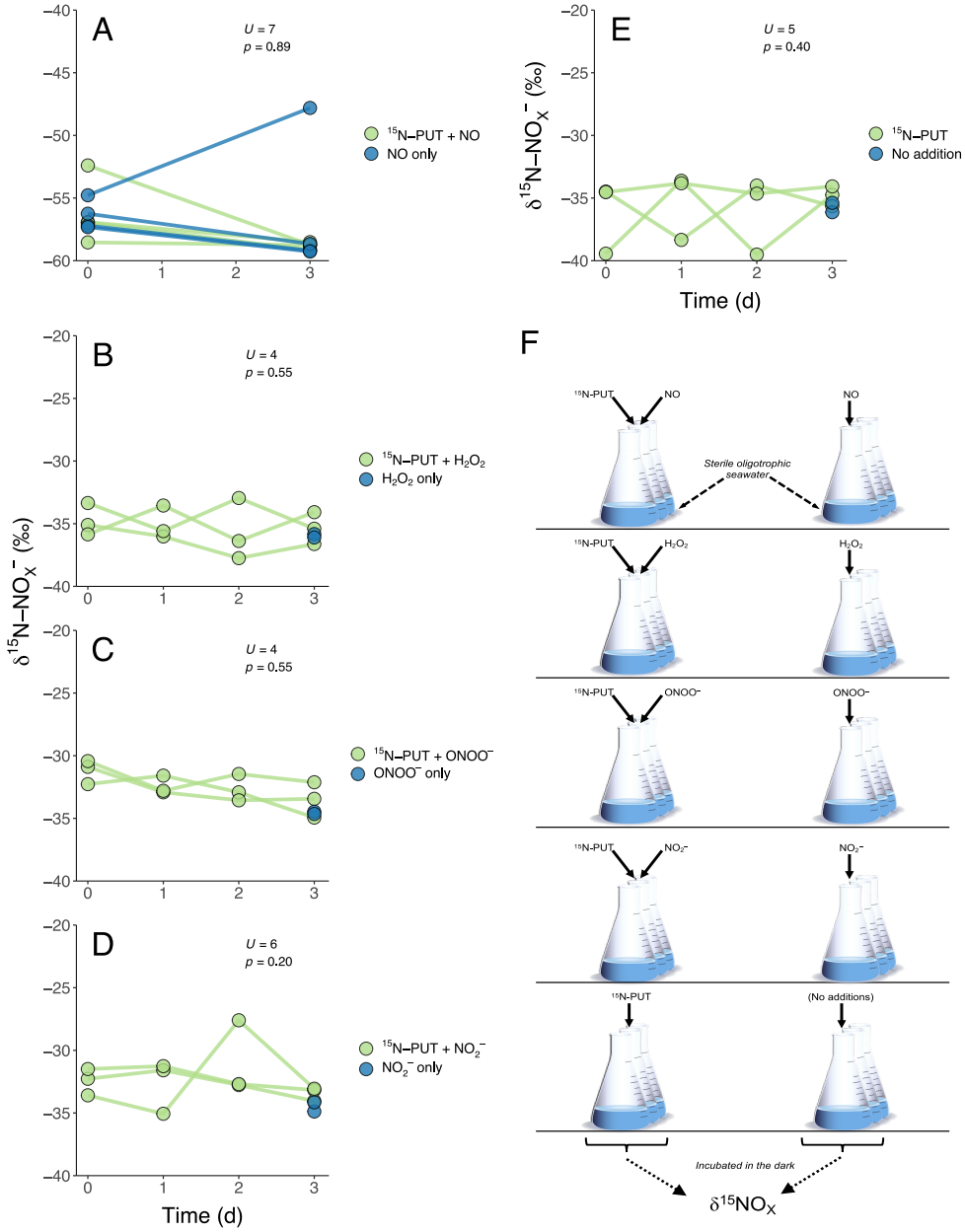

Fig. 3 A-E) $\delta^{15}NO_x$ (‰) over time in abiotic incubations containing ^{15}N-labeled PUT and a single reactive compound (blue) or a reactive compound only (green). Results of a Mann-Whitney *U*-test are shown in each panel. **F)** Schematic of abiotic incubation experiments. The left column shows treatments including ^{15}N-labeled PUT and a reactive compound while the right column shows control treatments containing a reactive compound and no ^{15}N-labled PUT.

157 incubations of culture medium or filtered seawater receiving NO additions (U=26, p=0.574);

158 therefore, NO addition experiments using either matrix were combined in data analyses.

159 There was no significant difference in the ratio of $^{15}N/^{14}N$ in the nitrate plus nitrite (NO_X) pool

160 ($\delta^{15}N_{NOx}$) at the termination of any of the incubations containing ^{15}N-PUT versus those without

^{15}N-PUT (Mann-Whitney *U*-tests, all $p \geq 0.2$; Fig. 3 A-E). Furthermore, there was no increase in $\delta^{15}N_{NOx}$ values when NO gas was directly mixed with a variety of ^{15}N-labeled polyamines, L-GLU, or NH_4^+ in sterile oligotrophic seawater (Mann-Whitney *U*-test, p=0.64; Fig. 4). NO_2^- in NO-amended incubation endpoints was likely produced via spontaneous NO oxidation (Lewis and Deen, 1994), as NO_2^- was not detectable in incubations without added NO (regardless of ^{15}N addition). Therefore, NO_2^- concentration in incubation endpoints likely reflects the concentration of the initial NO amendment, and was fairly well correlated to $\delta^{15}N_{NOx}$ values (Spearman's $\rho = -0.70$, p=0.02; Fig. 4).

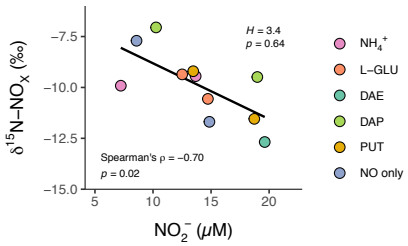

Fig. 4 $\delta^{15}NO_x$ (‰) as a function of NO_2^- concentration following 24 hours of abiotic incubation of sterile seawater amended with ^{15}N-labeled compounds and NO gas. NO (1% in N_2) was directly bubbled into seawater. Color denotes ^{15}N addition. Results of a Kruskal-Wallis test (comparing $\delta^{15}NO_x$ between treatment groups) and Spearman's correlation coefficient are shown. NO_2^- in these incubation endpoints was likely due to abiotic NO oxidation and therefore reflects the amount of added NO.

DISCUSSION

Organic N oxidation by Thaumarchaeota *relies on remineralization*

Polyamines are ubiquitous in cells (Tabor and Tabor, 1985; Nishibori and Nishijima, 2004; Liu *et al.*, 2016) and highly labile in aqueous environments (Lu *et al.*, 2015; Liu *et al.*, 2015; Krempaska *et al.*, 2017; Madhuri *et al.*, 2019). The amine groups at the ends of polyamines are essentially NH_3 with an aliphatic C chain substituted for a hydrogen, and since their pK_a values (~10.5, depending on chain length and temperature) are close to that of NH_3 (~9.25), they are similarly protonated in seawater. Thus, recent field evidence of rapid oxidation of polyamine-N in *Thaumarchaeota*-rich ocean waters (Damashek *et al.*, 2019a) begs the question of whether these archaea can use polyamine-N directly as an energy source.

184 We tested this hypothesis using two *Nitrosopumilus* strains phylogenetically similar to
185 the *Thaumarchaeota* typical of South Atlantic Bight inshore waters (Hollibaugh *et al.*, 2011;
186 Hollibaugh *et al.*, 2014; Liu *et al.*, 2018; Damashek *et al.*, 2019b), where previously-measured
187 polyamine-N oxidation rates were high (Damashek *et al.*, 2019a). Efforts to grow *N. piranensis*
188 D3C and *N. adriaticus* NF5 with single polyamines as their sole energy source conclusively
189 demonstrated that they cannot grow by oxidizing N from PUT or other common polyamines
190 (Fig. S1). Previous experiments with the same strains found only minimal [^{3}H]leucine
191 incorporation, particularly when archaea were starved of NH_3, similarly suggesting no ability to
192 use leucine as a carbon or energy source (Bayer *et al.*, 2019a). There is long-standing
193 biogeochemical and genomic evidence that some marine *Thaumarchaeota* may use some organic
194 compounds as C sources (e.g., Ouverney and Fuhrman, 2000; Teira *et al.*, 2004; Swan *et al.*,
195 2014; Seyler *et al.*, 2018; Dekas *et al.*, 2019), and potentially even as an energy source (in
196 addition to NH_3; Dekas *et al.*, 2019). As with polyamines, we found no evidence of growth by
197 either *Nitrosopumilus* strain on a wide variety of common amino acids, amines, or amides (Table
198 1; Fig. S1). This suggests that NH_3, urea, and cyanate remain the only known growth substrates
199 of marine *Thaumarchaeota*.
200 The lack of evidence for direct oxidation of DON-N by pure cultures suggests that
201 observed rates of DON-N oxidation (and potentially assimilation) by *Thaumarchaeota* in mixed
202 communities are not a consequence of direct oxidation by *Thaumarchaeota* alone, but instead
203 couple heterotrophic DON-N remineralization to NH_4^+ with subsequent thaumarchaeal oxidation
204 of this newly-produced NH_4^+. Strong evidence for this conclusion comes from growth
205 experiments with enrichment cultures: the presence of a heterotrophic bacterium led to a
206 substantial accumulation of NO_2^- in the medium and an increase in the abundance of bacterial

16S rRNA genes, especially when cultures were amended with both PUT and NH_4^+ (Fig. 1; Table 2). In contrast, pure cultures of the same *Thaumarchaeota* were unable to oxidize N supplied as PUT in the absence of NH_3 (Fig. S1). In addition to indicating a reliance of thaumarchaeal DON-N oxidation on external remineralization, these findings may have similar implications for experiments measuring DON assimilation by *Thaumarchaeota* in mixed communities.

Polyamines are highly labile and energy-rich, as their backbone contains multiple fully-reduced C atoms. It is not surprising that adding this rich C source led to rapid bacterial growth in enrichment cultures, similar to the stimulation of heterotrophic communities documented in field studies (Mou *et al.*, 2011; Mou *et al.*, 2015). Notably, the C:N ratio of PUT (2) is lower than that of natural marine microbial cells (typically ~4-5; Goldman *et al.*, 1987; White *et al.*, 2019), suggesting PUT catabolism by marine bacteria will lead to production of excess NH_4^+ (Hollibaugh, 1978; Goldman and Dennett, 1991). Therefore, NO_2^- production in enrichment cultures could result from bacterial catabolism of PUT, followed by oxidation of the resulting NH_4^+ by *Thaumarchaeota*, typically leading to slow and linear NO_2^- accumulation (Fig. 1). The occasional rapid accumulation of NO_2^- when NH_4^+ was added alongside PUT (Fig. 1 A) may indicate the bacterium in this enrichment was unable to use PUT alone as an N source (i.e., needed free NH_4^+ to catabolize PUT), or may suggest bacterial dependence on *Thaumarchaeota* (growing on the low concentration of NH_3) as a source of nutrients or organic substrates (e.g., Doxey *et al.*, 2015; Heal *et al.*, 2018; Bayer *et al.*, 2019b).

Since many DON compounds are highly labile C sources, it is imperative to systematically account for rapid heterotrophic remineralization of N from DON in incubations of mixed communities before concluding that *Thaumarchaeota* are capable of direct oxidation or

assimilation of DON, ideally by using pure cultures to test field hypotheses. Urea is a clear exception, since pure cultures of some marine *Thaumarchaeota* can grow with urea-N as their only energy and N source (Qin *et al.*, 2017a; Bayer *et al.*, 2019a). Direct cyanate-N oxidation/assimilation may also occur in marine *Thaumarchaeota*, but it is unclear how marine *Thaumarchaeota* could directly oxidize cyanate-N. While pure cultures of *Nitrosopumilus maritimus* SCM1 produced ^{15}N-NO_2^- when ^{15}N-cyanate was added to their growth media, this only happened when they were already growing on NH_3 (Kitzinger *et al.*, 2019; see discussion below). The pathway of cyanate oxidation (or NH_4^+ production from cyanate) is unclear since their genomes lack canonical cyanase genes (Kitzinger *et al.*, 2019).

Of the myriad polyamines, amino acids, amines, and amides we tested as thaumarchaeal growth substrates, the only compound leading to appreciable NO_2^- accumulation was glutamine: when both axenic strains were supplied with glutamine as their sole energy source, there was a slow, linear accumulation of NO_2^- (Fig. S1). Since the increase in NO_2^- concentration over time did not resemble a growth curve, it is unlikely that the archaea were directly using glutamine-N as a growth substrate. Although the mechanism is not clear, glutamine was probably slowly remineralized to NH_4^+, which was then oxidized. A simple explanation is abiotic glutamine deamination, which occurs relatively rapidly in liquid media containing phosphate and bicarbonate at neutral pH (Gilbert *et al.*, 1949), such as the SCM used here. However, our data cannot rule out a biological explanation. Glutamine is a common biochemical amino/amido donor, and *Thaumarchaeota* encode many genes involved in these reactions (e.g., glutamine amidotransferases or transaminases in amino acid and cofactor biosynthesis pathways; Walker *et al.*, 2010; Kerou *et al.*, 2016) as well as a variety of putative amino acid transporters (Offre *et al.*, 2014). If excess glutamine is transported into thaumarchaeal cells, some may be recycled to

253 NH_4^+ for either assimilatory or dissimilatory use. This amino acid "recycling" is common in
254 energy-starved microbes (Lever *et al.*, 2015) and was recently hypothesized to play a role in
255 thaumarchaeal survival in deep sea sediments (Kerou *et al.*, 2021). It is possible that energy-
256 starved *Thaumarchaeota* in our experiment transported glutamine into their cells but were unable
257 to incorporate it into biomass, so they hydrolyzed the glutamine amide N and shunted the
258 resulting NH_4^+ into energy production, leading to slow growth. Direct tests of thaumarchaeal
259 glutamine uptake and transformation compared to abiotic breakdown in SCM would be needed
260 to uncover the mechanism behind the slow glutamine oxidation documented here.
261
262 *Potential co-metabolism or abiotic breakdown of polyamines*
263 Although axenic cultures of marine *Nitrosopumilus* were unable to grow using PUT as
264 their sole energy and N source, the significant fraction of ^{15}N-PUT oxidized during growth on
265 NH_3 (Fig. 2), combined with the correlation between PUT-N and NH_3 oxidation rates in the
266 ocean (Damashek *et al.* 2019a), suggests PUT may be co-metabolized by the archaeal ammonia
267 monooxygenase enzyme (AMO) or decomposed by reactive intermediates produced during NH_3
268 oxidation (e.g., Martens-Habbena *et al.*, 2015; Kim *et al.*, 2016). Therefore, *Thaumarchaeota* in
269 environments with high polyamine availability and enough NH_3 for rapid growth may indirectly
270 oxidize a significant amount of PUT-N to NO_2^- despite their inability to use PUT-N as a direct
271 energy source, increasing the flux of N from DON into the NO_X pool and potentially
272 contributing to measured PUT-N oxidation rates in the ocean (Damashek *et al*., 2019a).
273 Co-metabolism of a variety of compounds due to non-specific oxidation by ammonia
274 monooxygenase is well documented in ammonia-oxidizing bacteria (AOB). Some AOB can, for
275 example, co-metabolize methane (Hyman and Wood, 1983; Ward, 1987), and some AOB and

276 *Thaumarchaeota* can co-metabolize a variety of organic compounds (e.g., Rasche *et al.*, 1991;
277 Wright *et al.*, 2020). Furthermore, co-metabolism of some DON compounds has been
278 documented: two AOB strains and the thaumarchaeote *Nitrososphaera gargensis* can co-
279 metabolize the tertiary amines mianserin and ranitidine during growth on NH_3 (Men *et al.*,
280 2016). Given the close structural and chemical similarity between NH_3 and the primary amine
281 groups on PUT-N, it seems conceivable that archaeal ammonia monooxygenase may similarly be
282 able to oxidize PUT-N.

283 There has been recent recognition of the role played by reactive metabolic intermediates
284 (primarily NO and H_2O_2) in thaumarchaeal physiology, and of the importance of chemical
285 transformations catalyzed by these reactive compounds in culture experiments. For example,
286 pure thaumarchaeal isolates produce H_2O_2 (Kim *et al.*, 2016; Bayer *et al.*, 2019c) but lack
287 catalase to facilitate its rapid detoxification. Exposure of isolates or field populations to high
288 concentrations of H_2O_2 therefore arrests their growth and activity (Kim *et al.*, 2016; Tolar *et al.*,
289 2016; Qin *et al.*, 2017b; Bayer *et al.*, 2019c). NO, an obligate intermediate in the archaeal
290 ammonia oxidation pathway, accumulates during growth of thaumarchaeal cultures (Martens-
291 Habbena *et al.*, 2015; Kozlowski *et al.*, 2016; Sauder *et al.*, 2016; Hink *et al.*, 2017) and is found
292 in high concentrations in marine regions with high nitrification rates or abundant
293 *Thaumarchaeota* (Ward and Zafiriou, 1988; Lutterbeck *et al.*, 2018). NO is a reactive radical that
294 rapidly forms reactive nitrogen oxide species (RNOS; e.g., NO_2 or N_2O_3) upon exposure to O_2 or
295 superoxide (Wink and Mitchell, 1998). RNOS are highly destructive of many biological
296 molecules; relevant to our data, RNOS react with primary amines to produce nitrosamines that
297 are subsequently deaminated to NH_4^+ (Ridnour *et al.*, 2004). These reactions may be a
298 mechanism for liberating NH_4^+ from polyamines due to NO production during thaumarchaeal

299 growth on NH_3. Similarly, reactions of NO with various compounds have been posited to explain
300 chemical transformations during thaumarchaeal growth: the abiotic reaction between NO and
301 reduced iron in growth media is thought to form nitrous oxide in cultures (Kozlowski *et al.*,
302 2016), and NO may react with cobalamin within thaumarchaeal cells, leading to nitrocobalamin
303 production (Heal *et al.*, 2018).

304 Given the capability of NO-derived RNOS to react with primary amines, speculation
305 about the reactivity of NO in thaumarchaeal cultures, and the documented ability of polyamines
306 to scavenge oxygen radicals (Khan *et al.*, 1992; Ha *et al.*, 1998; Chattopadhyay *et al.*, 2003), we
307 hypothesized that NO produced during NH_3 oxidation could react abiotically with ^{15}N-PUT to
308 produce the $^{15}N\text{-}NO_2^-$ we observed (Fig. 2). However, experiments with cell-free artificial media
309 and sterilized seawater showed no evidence of reaction between NO and ^{15}N-polyamines (Fig. 3
310 A, Fig. 4). NO_2^- was produced rapidly in treatments with added NO since NO auto-oxidizes to
311 NO_2^- and H^+ in oxic water (Lewis and Deen, 1994), but this NO_2^- did not contain the ^{15}N
312 initially added as ^{15}N-PUT. The correlation between NO_2^- and $\delta^{15}N_{NOx}$ values shown in Fig. 4
313 thus reflects the δ^{15}N value of spontaneously oxidized NO, with no apparent ^{15}N enrichment due
314 to oxidation of any ^{15}N-labeled substrates. We then tested whether ^{15}N-polyamines react with
315 H_2O_2 or $ONOO^-$ (formed by reacting NO_2^- and H_2O_2; Robinson and Beckman, 2005) to yield
316 $^{15}N\text{-}NO_2^-$, as these reactive compounds are also produced during thaumarchaeal growth (Kim *et*
317 *al.*, 2016; Heal *et al.* 2018; Bayer *et al.*, 2019b), but found no detectable $^{15}N\text{-}NO_2^-$ in these
318 treatments either (Fig. 3). This suggests that some PUT oxidation seen in field experiments
319 (Damashek *et al.* 2019a), with mixed cultures (Fig. 1), or during growth on NH_3 (Fig. 2) may be
320 explained by enzymatic co-metabolism of PUT, or due to reactions with reactive species that we
321 did not test (e.g., superoxide or other RNOS compounds).

Our isotope experiments were restricted to PUT, DAP, and DAE due to limited commercial availability of ^{15}N-labeled polyamines. Of these, only PUT is commonly found in high concentrations in phytoplankton and bacterial cells or marine waters (Nishibori *et al.*, 2001; Lu *et al.*, 2014; Liu *et al.*, 2016; Lin and Lin, 2019). It remains unknown whether other common polyamines (e.g., spermine, spermidine, norspermine, or norspermidine) are comparably oxidized during thaumarchaeal NH_3 oxidation. However, the oxidation of cyanate-N by marine *Thaumarchaeota* may be analogous, given that axenic *N. maritimus* SCM1 cultures only oxidized ^{15}N-cyanate while growing on NH_3 (Kitzinger *et al.*, 2019), comparable to our results with ^{15}N-PUT. Kitzinger *et al*. (2019) hypothesized that *N. maritimus* could break down cyanate extracellularly, since ^{15}N-NH_4^+ was produced in cultures amended with ^{15}N-cyanate despite no known cyanate hydratase genes existing in the *N. maritimus* SCM1 genome. Whether due to abiotic reactions with metabolic intermediates (intra- or extracellularly), some as yet undiscovered mechanism of extracellular remineralization, or co-metabolism by ammonia monooxygenase, the dual evidence of oxidation of PUT-N (Fig. 2) and cyanate (Kitzinger *et al.*, 2019) during thaumarchaeal growth on NH_3 suggests some DON compounds can be oxidized indirectly by growing *Thaumarchaeota*, but definitive demonstrations of mechanisms remain elusive.

CONCLUSIONS

Given the ability of *Thaumarchaeota* to grow using reduced N supplied as urea and cyanate, there has been interest in their potential to access other forms of organic N, spurred by numerous field studies reporting putative thaumarchaeal DON assimilation or oxidation. Our experiments with two axenic isolates suggest marine *Thaumarchaeota* cannot directly access N

345 supplied as polyamines, amino acids, amides, or primary amines as an energy source. Inclusion
346 of a heterotrophic bacterium in enrichment cultures of *Thaumarchaeota* resulted in the oxidation
347 of PUT-N, demonstrating the importance of DON remineralization to NH_4^+ by heterotrophs for
348 *Thaumarchaeota* to oxidize N supplied as DON. Therefore, claims of DON use by
349 *Thaumarchaeota* in mixed communities must strictly account for heterotrophic remineralization.
350 Despite lacking the ability to grow on PUT alone, the surprising finding that both pure strains
351 oxidized ^{15}N supplied as PUT while growing on NH_3 suggests some DON may be co-
352 metabolized or broken down abiotically, possibly mediated by reactive species produced during
353 NH_3 oxidation. Abiotic experiments ruled out some of the known reactive oxygen and nitrogen
354 intermediates of thaumarchaeal metabolism as oxidants, but did not identify the mechanism
355 leading to ^{15}N-PUT oxidation in cultures. This study suggests that oxidation of most DON-N for
356 energy conservation by marine *Thaumarchaeota* requires initial DON remineralization to NH_4^+
357 by heterotrophs, but also indicates a potential role for co-metabolism or reactive metabolic
358 byproducts in thaumarchaeal DON-N oxidation.
359
360 **EXPERIMENTAL PROCEDURES**
361 GROWTH EXPERIMENTS
362 Axenic cultures of *Nitrosopumilus piranensis* D3C and *N. adriaticus* NF5 (Bayer *et al.*,
363 2019a) were grown in SCM amended with an array of single DON compounds as sole energy
364 and N sources, including NH_4^+, urea, amino acids, primary amines (including polyamines), or
365 amides added to 1 or 0.5 mM final concentration (1 or 2 mM N; Table 1). All treatments were
366 run in triplicate for each strain and included pyruvate (200 µM) to scavenge reactive oxygen
367 species (Kim *et al.*, 2016) and 50 µg/mL each of streptomycin and kanamycin. NH_4Cl was added

368 to positive controls but was not included in other treatments. Experiments were initiated using a
369 10% (v/v) transfer of stationary phase cultures (with NH_4^+ completely consumed) to ensure
370 against NH_4^+ carryover. Growth experiments were conducted in the dark at 29°C for ~70 days
371 and subsampled for NO_2^- determination as described above. Culture purity was tested throughout
372 the duration of the experiment by adding Marine Broth 2216 to aliquots of the culture (10% v/v)
373 and monitoring for bacterial growth by measuring OD_{600}.
374 Growth experiments were also conducted with early enrichment cultures of *N. piranensis*
375 strain D3C and *N. adriaticus* strain NF5, in which approximately 5-15% of the cells were the
376 heterotrophic bacterium *Oceanicaulis alexandrii* (Bayer *et al.*, 2016; Bayer *et al.*, 2019c).
377 Triplicate cultures were grown in SCM containing either 1 mM NH_4^+, 1 mM urea, 1 mM PUT,
378 and 1 mM PUT + 100 µM NH_4^+ as N or energy sources. Experiments were conducted at 29°C in
379 the dark and subsampled over time for immediate NO_2^- determination using standard methods
380 (Griess reagent; Strickland and Parsons, 1972). In selected rounds of these experiments,
381 subsamples were taken for qPCR analysis by mixing 0.8 mL of a culture with 0.8 mL 2X lysis
382 buffer (1.5 M sucrose, 80 mM EDTA, 100 mM Tris; pH 8.3) and freezing immediately at -80°C.
383 DNA was extracted using standard phenol/chloroform techniques (Tolar *et al.*, 2013; Damashek
384 *et al.*, 2019a). Bacterial and thaumarchaeal 16S rRNA genes were quantified using primers
385 BACT1369F/PROK1492R/TM1389F and GI_334F/GI_554R/TM519AR (Suzuki *et al.*, 2000),
386 respectively (see Table S1 for amplification conditions). Reactions were run in triplicate on a
387 C1000 Touch Thermal Cycler/CFX96 Real-Time System (Bio-Rad) using the Platinum qPCR
388 SuperMix-UDG (Thermo Fisher). Standard curves consisted of a dilution series of a linearized
389 plasmid containing a previously-sequenced amplicon.
390

391 ISOTOPE EXPERIMENTS

392 The ability of *Thaumarchaeota* to oxidize DON during growth on NH_3 was assessed
393 using ^{15}N isotope tracers (98-99% ^{15}N, Cambridge Isotope Laboratories). Axenic cultures were
394 grown on SCM as described above except using 100 µM ammonium chloride and 20 µM
395 pyruvate. Culture purity was tested as described above. Triplicate bottles per substrate were
396 amended to 1.25 µM of ^{15}N-labeled substrate: DAE, DAP, PUT, ammonium chloride, L-GLU, or
397 ^{14}N ammonium chloride (a negative control with no added ^{15}N). Both amine groups of DAE,
398 DAP, and PUT were ^{15}N-labeled, leading to total ^{15}N additions of 2.5 µM in these treatments.
399 Growth was estimated by measuring NO_2^- production over time. At multiple timepoints,
400 subsamples were frozen at -80°C in polypropylene tubes for isotopic analysis.

401 $\delta^{15}NO_x$ values were measured using the bacterial denitrifier method (Sigman *et al.*,
402 2001). Briefly, NO_X was converted to nitrous oxide by *Pseudomonas aureofaciens* and its N
403 isotopic ratio was measured using a Finnigan MAT-252 isotope ratio mass spectrometer coupled
404 with a modified GasBench II interface (Casciotti *et al.*, 2002; Beman *et al.*, 2011; McIlvin and
405 Casciotti, 2011). The concentration of ^{15}N-NO_2^- (µM) was calculated by multiplying the NO_X
406 atom fraction ^{15}N by the NO_2^- concentration (nitrate was not detectable).

407 Abiotic oxidation of ^{15}N-PUT by multiple reactive compounds was tested by amending
408 triplicate flasks containing 75 mL of 0.22-µm filtered aged surface water from the Gulf Stream
409 or Station ALOHA (NH_4^+ and NO_X below the limit of detection), or sterile SCM, with ^{15}N-PUT
410 and a single reactive compound (NO, H_2O_2, or $ONOO^-$; Fig. 3 F). Flasks were then incubated at
411 23°C in the dark. Two control treatments were run that did not contain reactive compounds: one
412 amended with NO_2^-, and one with no addition. For each treatment (including the two negative
413 controls), parallel replicated incubations were conducted with and without added ^{15}N-PUT (Fig.

3 F). NO was generated from the NO donor (Z)-1-[N-(3-aminopropyl)-N-(n-propyl)amino]diazen-1-ium-1,2-diolate (PAPA NONOate; Cayman Chemical, Ann Arbor). 100 µM of PAPA NONOate was added to each flask to produce 200 µM NO (Hrabie *et al.*, 1993). The PAPA NONOate stock was prepared by slowly injecting 5.7 mL 0.01 M NaOH into an airtight vial containing 50 mg PAPA NONOate. This stock was wrapped in foil and stored at 4°C prior to use within hours. NO addition experiments were also conducted using blank SCM media (no added N, as described above). In H_2O_2 (Millipore Sigma, Burlington, MA), $ONOO^-$, NO_2^-, and no addition control treatments, 1 µM of the respective compound was added to flasks containing 100 nM ^{15}N-PUT, while NO treatment flasks contained 1.25 µM ^{15}N-PUT. $ONOO^-$ was generated by mixing NO_2^- and H_2O_2 (Robinson and Beckman, 2005). At the beginning and end of each incubation, subsamples were frozen at -80°C for $\delta^{15}NO_x$ determination (as described above). Twenty µM sodium nitrate with a known $\delta^{15}N_{NOx}$ value was added to samples prior to conversion of NO_X to nitrous oxide to enable isotopic measurements in experiments with no NO_2^- accumulation (H_2O_2, $ONOO^-$, NO_2^-, and no addition control).

In tests of the oxidative ability of NO on multiple ^{15}N-labeled compounds, NO gas (1% v/v in N_2; Airgas, Radnor Township, PA) was directly bubbled through a syringe into Erlenmeyer flasks containing filtered aged surface water from the Gulf Stream (NH_4^+ and NO_X below the limit of detection). ^{15}N-labeled NH_4^+, L-GLU, DAE, DAP, and PUT were added to duplicate flasks (as well as a negative control with no added ^{15}N) and incubated at 23°C in the dark. After 24 hours, subsamples were frozen at -80°C for $\delta^{15}NO_x$ determination (as described above).

DATA ANALYSIS

Mann-Whitney *U*-tests were conducted using R (R Core Team, 2019) to determine whether $\delta^{15}NO_x$ values differed between abiotic incubation endpoints of treatments with and without ^{15}N-PUT (shown in Fig. 3) and between abiotic incubation endpoints of treatments containing different ^{15}N-labeled substrates (shown in Fig. 4). Correlations were determined by calculating Spearman's rank correlation coefficient (ρ) in R. Plots were made using the ggplot2 R package (Wickham, 2016).

ACKNOWLEDGMENTS

This work was funded by NSF OCE grants 1538677 (to JTH) and 1537995 (to BNP). BB was supported by the Uni:docs Fellowship of the University of Vienna. A contribution number from the University of Hawai'i School of Earth Science and Technology will be provided upon manuscript acceptance. The authors declare no conflicts of interest.

REFERENCES

Alonso-Saez, L., Waller, A.S., Mende, D.R., Bakker, K., Farnelid, H., Yager, P.L., et al. (2012) Role for urea in nitrification by polar marine Archaea. Proc Natl Acad Sci **109**: 17989–17994.

Bayer, B., Vojvoda, J., Offre, P., Alves, R.J.E., Elisabeth, N.H., Garcia, J.A., et al. (2016) Physiological and genomic characterization of two novel marine thaumarchaeal strains indicates niche differentiation. ISME J **10**: 1051–1063.

Bayer, B., Vojvoda, J., Reinthaler, T., Reyes, C., Pinto, M., and Herndl, G.J. (2019a) *Nitrosopumilus adriaticus* sp. nov. and *Nitrosopumilus piranensis* sp. nov., two ammonia-oxidizing archaea from the Adriatic Sea and members of the class *Nitrososphaeria*. Int J Sys Evol Microbiol **69**: 1892–1902.

Bayer, B., Hansman, R.L., Bittner, M.J., Noriega-Ortega, B.E., Niggemann, J., Dittmar, T., and Herndl, G.J. (2019b) Ammonia-oxidizing archaea release a suite of organic compounds potentially fueling prokaryotic heterotrophy in the ocean. Environ Microbiol **21**: 4062–4075.

Bayer, B., Pelikan, C., Bittner, M.J., Reinthaler, T., Könneke, M., Herndl, G.J., and Offre, P. (2019c) Proteomic response of three marine ammonia-oxidizing archaea to hydrogen peroxide and their metabolic interactions with a heterotrophic alphaproteobacterium. mSystems **4**: e00181–19.

Beman, J.M., Chow, C.-E., King, A.L., Feng, Y., Fuhrman, J.A., Andersson, A., et al. (2011) Global declines in oceanic nitrification rates as a consequence of ocean acidification. Proc Natl Acad Sci **108**: 208–213.

Carini, P., Dupont, C.L., and Santoro, A.E. (2018) Patterns of thaumarchaeal gene expression in culture and diverse marine environments. Environ Microbiol **20**: 2112–2124.

Casciotti, K.L., Sigman, D.M., Hastings, M.G., Böhlke, J.K., and Hilkert, A. (2002) Measurement of the oxygen isotopic composition of nitrate in seawater and freshwater using the denitrifier method. Anal Chem **74**: 4905–4912.

Chattopadhyay, M.K., Tabor, C.W., and Tabor, H. (2003) Polyamines protect *Escherichia coli* cells from the toxic effect of oxygen. Proc Natl Acad Sci **100**: 2261–2265.

Damashek, J., Tolar, B.B., Liu, Q., Okotie Oyekan, A.O., Wallsgrove, N.J., Popp, B.N., and Hollibaugh, J.T. (2019a) Microbial oxidation of nitrogen supplied as selected organic nitrogen compounds in the South Atlantic Bight. Limnol Oceanogr **64**: 982–995.

Damashek, J., Edwardson, C.F., Tolar, B.B., Gifford, S.M., Moran, M.A., and Hollibaugh, J.T. (2019b) Coastal ocean metagenomes and curated metagenome-assembled genomes from Marsh Landing, Sapelo Island (Georgia, USA). Microbiol Resour Announc **8**: e00934–19.

Dekas, A.E., Parada, A.E., Mayali, X., Fuhrman, J.A., Wollard, J., Weber, P.K., and Pett-Ridge, J. (2019) Characterizing chemoautotrophy and heterotrophy in marine archaea and bacteria with single-cell multi-isotope NanoSIP. Front Microbiol **10**: 2682.

Doxey, A.C., Kurtz, D.A., Lynch, M.D., Sauder, L.A., and Neufeld, J.D. (2015) Aquatic metagenomes implicate *Thaumarchaeota* in global cobalamin production. ISME J **9**: 461–471.

Francis, C.A., Beman, J.M., and Kuypers, M.M.M. (2007) New processes and players in the nitrogen cycle: the microbial ecology of anaerobic and archaeal ammonia oxidation. ISME J **1**: 19–27.

Gilbert, J.B., Price, V.E., and Greenstein, J.P. (1949) Effect of anions on the non-enzymatic desamidation of glutamine. J Biol Chem **180**: 209–218.

Goldman, J.C., Caron, D.A., and Dennett, M.R. (1987) Regulation of gross growth efficiency and ammonium regeneration in bacteria by substrate C: N ratio. Limnol Oceanogr **32**: 1239–1252.

Goldman, J.C. and Dennett, M.R. (1991) Ammonium regeneration and carbon utilization by marine bacteria grown on mixed substrates. Mar Biol **109**: 369–378.

Ha, H.C., Sirisoma, N.S., Kuppusamy, P., Zweier, J.L., Woster, P.M., and Casero, R.A. (1998) The natural polyamine spermine functions directly as a free radical scavenger. Proc Natl Acad Sci **95**: 11140–11145.

Hallam, S.J., Mincer, T.J., Schleper, C., Preston, C.M., Roberts, K., Richardson, P.M., and DeLong, E.F. (2006) Pathways of carbon assimilation and ammonia oxidation suggested by environmental genomic analyses of marine *Crenarchaeota*. PLoS Biol **4**: e95.

Heal, K.R., Qin, W., Amin, S.A., Devol, A.H., Moffett, J.W., Armbrust, E.V., et al. (2018) Accumulation of NO_2-cobalamin in nutrient-stressed ammonia-oxidizing archaea and in the oxygen deficient zone of the eastern tropical North Pacific. Environ Microbiol Rep **10**: 453–457.

Hink, L., Lycus, P., Gubry-Rangin, C., Frostegård, Å., Nicol, G.W., Prosser, J.I., and Bakken, L.R. (2017) Kinetics of NH_3-oxidation, NO-turnover, N_2O-production and electron flow during oxygen depletion in model bacterial and archaeal ammonia oxidisers. Environ Microbiol **19**: 4882–4896.

Hollibaugh, J.T. (1978) Nitrogen regeneration during the degradation of several amino acids by plankton communities collected near Halifax, Nova Scotia, Canada. Mar Biol **45**: 191–201.

Hollibaugh, J.T., Gifford, S., Sharma, S., Bano, N., and Moran, M.A. (2011) Metatranscriptomic analysis of ammonia-oxidizing organisms in an estuarine bacterioplankton assemblage. ISME J **5**: 866–878.

Hollibaugh, J.T., Gifford, S.M., Moran, M.A., Ross, M.J., Sharma, S., and Tolar, B.B. (2014) Seasonal variation in the metratranscriptomes of a Thaumarchaeota population from SE USA coastal waters. ISME J **8**: 685–698.

Hrabie, J.A., Klose, J.R., Wink, D.A., and Keefer, L.K. (1993) New nitric oxide-releasing zwitterions derived from polyamines. J Org Chem **58**: 1472–1476.

Hyman, M.R. and Wood, P.M. (1983) Methane oxidation by *Nitrosomonas europaea*. Biochem J **212**: 31–37.

Kerou, M., Offre, P., Valledor, L., Abby, S.S., Melcher, M., Nagler, M., et al. (2016) Proteomics and comparative genomics of *Nitrososphaera viennensis* reveal the core genome and adaptations of archaeal ammonia oxidizers. Proc Natl Acad Sci **113**: E7937–E7946.

Kerou, M., Ponce-Toledo, R.I., Zhao, R., Abby, S.S., Hirai, M., Nomaki, H., et al. (2021) Genomes of Thaumarchaeota from deep sea sediments reveal specific adaptations of three independently evolved lineages. ISME J https://doi.org/10.1038/s41396-021-00962-6.

Khan, A.U., Mei, Y.H., and Wilson, T. (1992) A proposed function for spermine and spermidine: protection of replicating DNA against damage by singlet oxygen. Proc Natl Acad Sci **89**: 11426–11427.

Kim, J.-G., Park, S.-J., Sinninghe Damsté, J.S., Schouten, S., Rijpstra, W.I.C., Jung, M.-Y., et al. (2016) Hydrogen peroxide detoxification is a key mechanism for growth of ammonia-oxidizing archaea. Proc Natl Acad Sci **113**: 7888–7893.

Kirchman, D.L., Elifantz, H., Dittel, A.I., Malmstrom, R.R., and Cottrell, M.T. (2007) Standing stocks and activity of Archaea and Bacteria in the western Arctic Ocean. Limnol Oceanogr **52**: 495–507.

Kitzinger, K., Padilla, C.C., Marchant, H.K., Hach, P.F., Herbold, C.W., Kidane, A.T., et al. (2019) Cyanate and urea are substrates for nitrification by Thaumarchaeota in the marine environment. Nat Microbiol **4**: 234–243.

Kozlowski, J.A., Stieglmeier, M., Schleper, C., Klotz, M.G., and Stein, L.Y. (2016) Pathways and key intermediates required for obligate aerobic ammonia-dependent chemolithotrophy in bacteria and Thaumarchaeota. ISME J **10**: 1836–1845.

Könneke, M., Bernhard, A.E., de la Torre, J.R., Walker, C.B., Waterbury, J.B., and Stahl, D.A. (2005) Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature **437**: 543–546.

Krempaska, N., Horňák, K., and Pernthaler, J. (2017) Spatiotemporal distribution and microbial assimilation of polyamines in a mesotrophic lake. Limnol Oceanogr **63**: 816–832.

Laperriere, S.M., Morando, M., Capone, D.G., Gunderson, T., Smith, J.M., and Santoro, A.E. (2021) Nitrification and nitrous oxide dynamics in the Southern California Bight. Limnol Oceanogr **66**: 1099–1112.

Lee, C. and Jørgensen, N.O.G. (1995) Seasonal cycling of putrescine and amino acids in relation to biological production in a stratified coastal salt pond. Biogeochemistry **29**: 131–157.

Lever, M.A., Rogers, K.L., Lloyd, K.G., Overmann, J., Schink, B., Thauer, R.K., et al. (2015) Life under extreme energy limitation: a synthesis of laboratory- and field-based investigations. FEMS Microbiol Rev **39**: 688–728.

Lewis, R.S. and Deen, W.M. (1994) Kinetics of the reaction of nitric oxide with oxygen in aqueous solutions. Chem Res Toxicol **7**: 568–574.

Lin, H.-Y. and Lin, H.-J. (2019) Polyamines in microalgae: something borrowed, something new. Mar Drugs **17**: 1.

Liu, Q., Lu, X., Tolar, B.B., Mou, X., and Hollibaugh, J.T. (2015) Concentrations, turnover rates and fluxes of polyamines in coastal waters of the South Atlantic Bight. Biogeochemistry **123**: 117–133.

Liu, Q., Nishibori, N., Imai, I., and Hollibaugh, J.T. (2016) Response of polyamine pools in marine phytoplankton to nutrient limitation and variation in temperature and salinity. Mar Ecol Prog Ser **544**: 93–105.

Liu, Q., Tolar, B.B., Ross, M.J., Cheek, J.B., Sweeney, C.M., Wallsgrove, N.J., et al. (2018) Light and temperature control the seasonal distribution of thaumarchaeota in the South Atlantic bight. ISME J **12**: 1473–1485.

Lu, X., Zou, L., Clevinger, C., Liu, Q., Hollibaugh, J.T., and Mou, X. (2014) Temporal dynamics and depth variations of dissolved free amino acids and polyamines in coastal seawater determined by high-performance liquid chromatography. Mar Chem **163**: 36–44.

Lu, X., Sun, S., Hollibaugh, J.T., and Mou, X. (2015) Identification of polyamine-responsive bacterioplankton taxa in South Atlantic Bight. Environ Microbiol Rep **7**: 831–838.

Lutterbeck, H.E., Arévalo-Martínez, D.L., Löscher, C.R., and Bange, H.W. (2018) Nitric oxide (NO) in the oxygen minimum zone off Peru. Deep-Sea Res Pt II **156**: 148–154.

Madhuri, S., Wang, K., Bade, D., and Mou, X. (2019) Concentration and turnover of dissolved free polyamines on the South Coast of Lake Erie. Limnol Oceanogr **64**: 1641–1650.

Martens-Habbena, W., Qin, W., Horak, R.E.A., Urakawa, H., Schauer, A.J., Moffett, J.W., et al. (2015) The production of nitric oxide by marine ammonia-oxidizing archaea and inhibition of archaeal ammonia oxidation by a nitric oxide scavenger. Environ Microbiol **17**: 2261–2274.

McIlvin, M.R. and Casciotti, K.L. (2011) Technical updates to the bacterial method for nitrate isotopic analyses. Anal Chem **83**: 1850–1856.

Men, Y., Han, P., Helbling, D.E., Jehmlich, N., Herbold, C., Gulde, R., et al. (2016) Biotransformation of two pharmaceuticals by the ammonia-oxidizing archaeon *Nitrososphaera gargensis*. Environ Sci Technol **50**: 4682–4692.

Mou, X., Vila-Costa, M., Sun, S., Zhao, W., Sharma, S., and Moran, M.A. (2011) Metatranscriptomic signature of exogenous polyamine utilization by coastal bacterioplankton. Environ Microbiol Rep **3**: 798–806.

Mou, X., Jacob, J., Lu, X., Vila-Costa, M., Chan, L.-K., Sharma, S., and Zhang, Y.-Q. (2015) Bromodeoxyuridine labelling and fluorescence-activated cell sorting of polyamine-transforming bacterioplankton in coastal seawater. Environ Microbiol **17**: 876–888.

Nishibori, N., Yuasa, A., Sakai, M., Fujihara, S., and Nishio, S. (2001) Free polyamine concentrations in coastal seawater during phytoplankton bloom. Fish Sci **67**: 79–83.

Nishibori, N. and Nishijima, T. (2004) Changes in polyamine levels during growth of a red-tide causing phytoplankton *Chattonella antiqua* (Raphidophyceae). Eur J Phycol **39**: 51–55.

Offre, P., Kerou, M., Spang, A., and Schleper, C. (2014) Variability of the transporter gene complement in ammonia-oxidizing archaea. Trend Microbiol **22**: 665–675.

Ouverney, C.C. and Fuhrman, J.A. (2000) Marine planktonic archaea take up amino acids. Appl Environ Microbiol **66**: 4829–4833.

Palatinszky, M., Herbold, C., Jehmlich, N., Pogoda, M., Han, P., Bergen, von, M., et al. (2015) Cyanate as an energy source for nitrifiers. Nature **524**: 105–108.

582 Qin, W., Amin, S.A., Martens-Habbena, W., Walker, C.B., Urakawa, H., Devol, A.H., et al. (2014) Marine
583 ammonia-oxidizing archaeal isolates display obligate mixotrophy and wide ecotypic variation. Proc Natl Acad
584 Sci **111**: 12504–12509.
585 Qin, W., Heal, K.R., Ramdasi, R., Kobelt, J.N., Martens-Habbena, W., Bertagnolli, A.D., et al. (2017a)
586 *Nitrosopumilus maritimus* gen. nov., sp. nov., *Nitrosopumilus cobalaminigenes* sp. nov., *Nitrosopumilus*
587 *oxyclinae* sp. nov., and *Nitrosopumilus ureiphilus* sp. nov., four marine ammonia- oxidizing archaea of the
588 phylum *Thaumarchaeota*. Int J Sys Evol Microbiol **67**: 5067–5079.
589 Qin, W., Meinhardt, K.A., Moffett, J.W., Devol, A.H., Virginia Armbrust, E., Ingalls, A.E., and Stahl, D.A. (2017b)
590 Influence of oxygen availability on the activities of ammonia-oxidizing archaea. Environ Microbiol Rep **9**:
591 250–256.
592 R Core Team (2019) R: A language and environment for statistical computing. R Foundation for Statistical
593 Computing, Vienna, Austria. https://www.R-project.org/.
594 Rasche, M.E., Hyman, M.R., and Arp, D.J. (1991) Factors limiting alpihatic chlorocarbon degradation by
595 *Nitrosomonas europaea*: cometabolic inactivation of ammonia monooxygenase and substrate specificity. Appl
596 Environ Microbiol **57**: 2986–2994.
597 Ridnour, L.A., Thomas, D.D., Mancardi, D., Espey, M.G., Miranda, K.M., Paolocci, N., et al. (2004) The chemistry
598 of nitrosative stress induced by nitric oxide and reactive nitrogen oxide species. Putting perspective on stressful
599 biological situations. Biol Chem **385**: 1–10.
600 Robinson, K.M. and Beckman, J.S. (2005) Synthesis of peroxynitrite from nitrite and hydrogen peroxide. In, Packer,
601 L. and Cadenas, E. (eds), Methods in Enzymology, Vol. 396. Elsevier, pp. 207–214.
602 Santoro, A.E., Richter, R.A., and Dupont, C.L. (2019) Planktonic marine archaea. Annu Rev Mar Sci **11**: 131–158.
603 Sauder, L.A., Ross, A.A., and Neufeld, J.D. (2016) Nitric oxide scavengers differentially inhibit ammonia oxidation
604 in ammonia-oxidizing archaea and bacteria. FEMS Microbiol Lett **363**: fnw052.
605 Seyler, L.M., McGuinness, L.M., Gilbert, J.A., Biddle, J.F., Gong, D., and Kerkhof, L.J. (2018) Discerning
606 autotrophy, mixotrophy and heterotrophy in marine TACK archaea from the North Atlantic. FEMS Microbiol
607 Ecol **94**: fiy014.
608 Sigman, D.M., Casciotti, K.L., Andreani, M., Barford, C., Galanter, M., and Böhlke, J.K. (2001) A bacterial method
609 for the nitrogen isotopic analysis of nitrate in seawater and freshwater. Anal Chem **73**: 4145–4153.
610 Smith, J.M., Damashek, J., Chavez, F.P., and Francis, C.A. (2016) Factors influencing nitrification rates and the
611 abundance and transcriptional activity of ammonia-oxidizing microorganisms in the dark northeast Pacific
612 Ocean. Limnol Oceanogr **61**: 596–609.
613 Stahl, D.A. and de la Torre, J.R. (2012) Physiology and diversity of ammonia-oxidizing archaea. Annu Rev
614 Microbiol **66**: 83–101.
615 Strickland, J. and Parsons, T.R. (1972) A Practical Handbook of Seawater Analysis, 2nd ed. Fisheries Research
616 Board of Canada, Ottawa.
617 Suzuki, M.T., Taylor, L.T., and DeLong, E.F. (2000) Quantitative analysis of small-subunit rRNA genes in mixed
618 microbial populations via 5'-nuclease assays. Appl Environ Microbiol **66**: 4605–4614.
619 Swan, B.K., Chaffin, M.D., Martinez-Garcia, M., Morrison, H.G., Field, E.K., Poulton, N.J., et al. (2014) Genomic
620 and metabolic diversity of Marine Group I Thaumarchaeota in the mesopelogic of two subtropical gyres. PLoS
621 ONE **9**: e95380.
622 Tabor, C.W. and Tabor, H. (1984) Polyamines. Annual Review of Biochemistry **53**: 749–790.
623 Tabor, C.W. and Tabor, H. (1985) Polyamines in microorganisms. Microbiol Rev **49**: 81–99.
624 Teira, E., Reinthaler, T., Pernthaler, A., Pernthaler, J., and Herndl, G.J. (2004) Combining catalyzed reporter
625 deposition-fluorescence in situ hybridization and microautoradiography to detect substrate utilization by
626 bacteria and archaea in the deep ocean. Appl Environ Microbiol **70**: 4411–4414.
627 Teira, E., van Aken, H., Veth, C., and Herndl, G.J. (2006) Archaeal uptake of enantiomeric amino acids in the meso-
628 and bathypelagic waters of the North Atlantic. Limnol Oceanogr **51**: 60–69.
629 Tolar, B.B., King, G.M., and Hollibaugh, J.T. (2013) An analysis of Thaumarchaeota populations from the Northern
630 Gulf of Mexico. Front Microbiol **4**: 72.
631 Tolar, B.B., Powers, L.C., Miller, W.L., Wallsgrove, N.J., Popp, B.N., and Hollibaugh, J.T. (2016) Ammonia
632 oxidation in the ocean can be inhibited by nanomolar concentrations of hydrogen peroxide. Front Mar Sci **3**:
633 237.
634 Tolar, B.B., Wallsgrove, N.J., Popp, B.N., and Hollibaugh, J.T. (2017) Oxidation of urea-derived nitrogen by
635 thaumarchaeota-dominated marine nitrifying communities. Environ Microbiol **19**: 4838–4850.

Walker, C.B., de la Torre, J.R., Klotz, M.G., Urakawa, H., Pinel, N., Arp, D.J., et al. (2010) *Nitrosopumilus maritimus* genome reveals unique mechanisms for nitrification and autotrophy in globally distributed marine crenarchaea. Proc Natl Acad Sci **107**: 8818–8823.

Ward, B.B. (1987) Kinetic studies on ammonia and methane oxidation by . Arch Microbiol **147**: 126–133.

Ward, B.B. and Zafiriou, O.C. (1988) Nitrification and nitric oxide in the oxygen minimum of the eastern tropical North Pacific. Deep Sea Res Pt A **35**: 1127–1142.

White, A.E., Giovannoni, S.J., Zhao, Y., Vergin, K., and Carlson, C.A. (2019) Elemental content and stoichiometry of SAR11 chemoheterotrophic marine bacteria. Limnol Oceanogr Lett **4**: 44–51.

Wickham, H. (2016) ggplot2: Elegant Graphics for Data Analysis Springer, New York.

Wink, D.A. and Mitchell, J.B. (1998) Chemical biology of nitric oxide: insights into regulatory, cytotoxic, and cytoprotective mechanisms of nitric oxide. Free Radic Biol Med **25**: 434–456.

Wright, C.L., Schatteman, A., Crombie, A.T., Murrell, J.C., and Lehtovirta-Morley, L.E. (2020) Inhibition of ammonia monooxygenase from ammonia-oxidizing archaea by linear and aromatic alkynes. Appl Environ Microbiol **86**: e02388–19.

Yakimov, M.M., La Cono, V., Smedile, F., DeLuca, T.H., Juárez, S., Ciordia, S., et al. (2011) Contribution of crenarchaeal autotrophic ammonia oxidizers to the dark primary production in Tyrrhenian deep waters (Central Mediterranean Sea). ISME J **5**: 945–961.

SUPPLEMENTAL TABLES AND FIGURES

Table S1

Primers, cycling information, and assay data for qPCR reactions

Fig. S1 NO_2^- accumulation in axenic cultures of *N. piranensis* D3C (circles) and *N. adriaticus* NF5 (diamonds) over time. Colors denote the compounds added to the media as sole energy and N sources. Points show the average of triplicate incubation bottles while error bars show standard deviation.