

# Regional patterns in ammonia-oxidizing communities throughout Chukchi Sea waters from the Bering Strait to the Beaufort Sea

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**ABSTRACT:** The shallow Chukchi Sea is a highly productive region of the Arctic Ocean, fed by Pacific water transported north through the Bering Strait. Nitrification in Chukchi Sea bottom waters oxidizes significant amounts of regenerated ammonium, which increases the pool of nitrate available for denitrification and changes the nutrient balance of this water prior to export to the Canada Basin. However, little is known about the ammonia-oxidizing microbial communities in Chukchi Sea waters. We used quantitative polymerase chain reaction assays to determine the abundance and transcriptional activity of both ammonia-oxidizing *Archaea* (AOA) and ammonia-oxidizing *Bacteria* (AOB) along coastal Chukchi Sea bottom waters, as well as waters of Atlantic-origin over the Beaufort slope. While AOA significantly outnumbered AOB in deeper Beaufort slope waters, AOB were more abundant at most coastal stations, potentially due to a greater concentration of regenerated ammonium trapped in coastal bottom waters. Quantification of 2 marine AOA ecotypes, Water Column A (WCA) and Water Column B (WCB), showed a surprisingly high abundance of WCB, generally considered a deep-water ecotype, in shallow coastal waters as well as deep slope waters, likely due to transport via shelfbreak upwelling. Transcript abundances also suggested WCA and WCB in coastal waters were transcriptionally active at comparable levels. The relatively high abundance of AOB and WCB suggests the Chukchi Sea has unique ammonia-oxidizing communities compared to most shallow coastal oceans, highlighting the importance of regional biogeochemical processes (ammonium regeneration) and physical processes (upwelling) in structuring coastal microbial communities.

**KEY WORDS:** *Thaumarchaeota* · Ammonia oxidizers · Arctic · Ammonium · Ecotypes · Nitrification

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## INTRODUCTION

The Chukchi Sea is a highly productive region of the Arctic Ocean (Grebmeier et al. 2006, Pabi et al. 2008, Yun et al. 2016), with high phytoplankton biomass in open water and under sea ice (Arrigo & van Dijken 2011, Arrigo et al. 2012). Newly produced

organic matter is remineralized in deeper waters and shelf sediments (Anderson et al. 1988, Henriksen et al. 1993), where the resulting particulate organic matter drives high rates of biogeochemical cycling. For example, Chukchi Sea shelves, despite their relatively small areal size, account for a significant fraction of global marine nitrogen loss due to high

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denitrification rates (Devol et al. 1997, Chang & Devol 2009, Mills et al. 2015). Chukchi Sea water flows northward and feeds into the nutrient maximum at the upper halocline of the central Arctic Ocean (Jones & Anderson 1986, Cooper et al. 1997), eventually exiting the Arctic through the Canadian Arctic Archipelago and Fram Strait. Because microbial processes in the Chukchi Sea have such pronounced ramifications for organic matter and nutrient cycling both in the Arctic and in the global oceans (Dittmar & Kattner 2003, Pedrós-Alió et al. 2015), there is longstanding interest in the ecology and biogeochemical impacts of microbial populations throughout the Arctic Ocean.

Several studies have suggested nitrification, the microbial oxidation of ammonia to nitrite and nitrate, is an important process in the Chukchi Sea water column. By comparing nutrient concentrations, oxygen drawdown, and nitrate isotopic values along the northward flow path of the Chukchi Sea, Brown et al. (2015) found evidence of active nitrification in both the water column and the sediments, suggesting a high proportion of bottom water nitrate in the Chukchi Sea came from local organic matter remineralization and nitrification. This conclusion matched previous studies from this region, which had reported high bottom water nitrification rates in the coastal Chukchi Sea driven at least partly by ammonium diffusing from the sediments and being oxidized in the water column (Henriksen et al. 1993, Souza et al. 2014). Measurements taken at Point Barrow also showed high nitrification in winter and spring compared to summer (Christman et al. 2011, Baer et al. 2014). Thus, water column nitrification appears to play an important role in the biogeochemistry of the Chukchi Sea. However, little is known about the distribution of ammonia oxidizers in Chukchi Sea waters.

Ammonia oxidation, the initial (and rate-limiting) step of nitrification, is catalyzed by distinct groups of ammonia-oxidizing *Archaea* (AOA) and ammonia-oxidizing *Bacteria* (AOB). The *amoA* gene, coding for the  $\alpha$ -subunit of the ammonia monooxygenase enzyme, is a common functional gene used to study AOA and AOB (e.g. Francis et al. 2005, O'Mullan & Ward 2005). The importance of marine AOB has been long recognized (Carlucci & McNally 1969, Ward et al. 1982), but AOA, belonging to Marine Group I (MGI) *Crenarchaeota* (Fuhrman et al. 1992, DeLong 1992) and now called *Thaumarchaeota* (Brochier-Armanet et al. 2008), were relatively recently discovered and shown to chemoautotrophically oxidize ammonia (Könneke et al. 2005). Francis

et al. (2005) identified 2 major marine water column AOA clades, designated Water Column A (WCA) and Water Column B (WCB), which numerically dominate offshore ammonia-oxidizing communities (Francis et al. 2005, Wuchter et al. 2006, Mincer et al. 2007, Kalanetra et al. 2009, Santoro et al. 2010, Biller et al. 2012, Newell et al. 2013) and appear to represent distinct shallow and deep ecotypes (Hallam et al. 2006, Wuchter et al. 2006, Beman et al. 2008, Biller et al. 2012, Sintes et al. 2013, Tolar et al. 2013, Smith et al. 2016). However, relatively few studies have quantified, these ecotypes in the sea. Clade-specific WCA and WCB quantitative polymerase chain reaction (qPCR) primer sets have revealed high abundance of WCA in surface waters and WCB in deeper waters throughout the Pacific Ocean (Beman et al. 2008, 2010, Mosier & Francis 2011, Robidart et al. 2012, Santoro et al. 2013, 2017, Smith et al. 2014a, 2016, Shiozaki et al. 2016), and surveys of the coastal Arctic and northern Atlantic Ocean using different clade-specific primer sets also confirmed depth-specific patterns between thaumarchaeal ecotypes (Sintes et al. 2013, 2016). Unfortunately, relatively little is known about the distribution or activity of these ecotypes in the Arctic Ocean.

Prior to the discovery of AOA, initial work found AOB in the central Arctic Ocean were largely *Nitrosospira*-like, whereas waters influenced by the Chukchi Sea also contained *Nitrosomonas*-like AOB (Bano & Hollibaugh 2000). Later work from the same samples showed AOA outnumbered AOB, with clone library data indicating typical depth partitioning between WCA and WCB (Kalanetra et al. 2009). Throughout the coastal Canadian Arctic, AOA gene and transcript abundances were highest in waters originating from the Arctic halocline (Galand et al. 2009b, Pedneault et al. 2014), and AOA abundance in the Canadian Arctic also varied substantially between winter and spring (Alonso-Sáez et al. 2012). In two recent studies that investigated the WCA and WCB ecotypes in the Chukchi Sea, AOA (predominantly WCA) outnumbered AOB at a station near the Bering Strait (Shiozaki et al. 2016), and surface waters off Point Barrow had higher abundances of both AOA (including both WCA and WCB) and AOB in winter compared to summer, when nitrification rates were also high (Christman et al. 2011). However, these 2 studies had limited spatial sampling within the Chukchi Sea (only 1 station each), hindering our ability to assess ammonia-oxidizing communities across this dynamic region of the Arctic.

We investigated the abundance and transcriptional activity of AOA (including WCA and WCB) and AOB

throughout bottom waters of the eastern Chukchi Sea from the Bering Strait to the Beaufort Sea and compared these communities to those in the deeper Atlantic-origin waters of the adjacent Beaufort slope to determine the differences between waters originating from the Pacific and Atlantic oceans. Additionally, quantitatively mapping *amoA* gene and transcript abundances of WCA and WCB in the Arctic Ocean increased our knowledge of the distribution and transcriptional activity of these ecotypes in polar oceans.

## MATERIALS AND METHODS

### Sample collection

Water samples were collected on the HLY1101 cruise (June 25–July 29, 2011) onboard the US Coast Guard Cutter 'Healy'. Descriptions of sampling and measurements for oceanographic parameters (including nutrient concentrations) have been reported elsewhere (Arrigo et al. 2014, Brown et al. 2015, Lowry et al. 2015, Mills et al. 2015). Discrete data were used if both discrete and sensor-based data

were available (e.g. salinity and dissolved oxygen). Sample depth was calculated from pressure as described by Sea-Bird Scientific ([www.seabird.com/document/an69-conversion-pressure-depth](http://www.seabird.com/document/an69-conversion-pressure-depth)).

At a subset of HLY1101 stations (Fig. 1), discrete samples from the bottom of the water column (Brown et al. 2015) were collected from Niskin bottles mounted on the rosette and sampled for microbial biomass by filtering up to 1 l through a 0.2  $\mu\text{m}$  pore size Supor polyethersulfone disc filter (Pall) using a vacuum pump (pressure less than  $-5$  inches of mercury). Filters were immediately frozen on liquid nitrogen and stored at  $-80^\circ\text{C}$  until analysis. Stations were selected to include both Pacific-origin waters over the Chukchi shelf and Atlantic-origin waters in the Beaufort Sea. All Chukchi Sea microbial samples came from the bottom of the water column (within 3 m of the seafloor) (Table S1 in the Supplement at [www.int-res.com/articles/suppl/a079p273\\_supp.pdf](http://www.int-res.com/articles/suppl/a079p273_supp.pdf)). All Beaufort slope stations were deeper and had higher salinity ( $>34$ ) and potential density ( $\sigma_{\theta}$ ;  $>27.5$ ) than Chukchi Sea stations (Fig. S1 and Table S1 in the Supplement). Instead of sampling bottom waters, Beaufort slope stations were sampled at depths targeting waters of Atlantic origin, below the Arctic halocline but above Canada Basin deep waters (Codispoti et al. 2005, Jackson et al. 2010). Most Beaufort slope stations were above the slope itself, except for Stns 132 and 133, which were just inside the Canada Basin.

### Nucleic acid extraction and gene/transcript quantification

DNA and RNA were coextracted from single filters using the mirVana miRNA Isolation Kit (Thermo Fisher) and DNeasy Blood & Tissue Kit (Qiagen), with RNA treated with the Ambion TURBO DNA-free Kit (Thermo Fisher) to remove DNA and purified using the RNeasy MinElute Kit (Qiagen), all following previously published protocols (Smith et al. 2014a). cDNA was synthesized using random hexamers with the Invitrogen SuperScript III First-Strand Synthesis System (Thermo Fisher), including negative controls for each RNA sample where nuclease-free water was

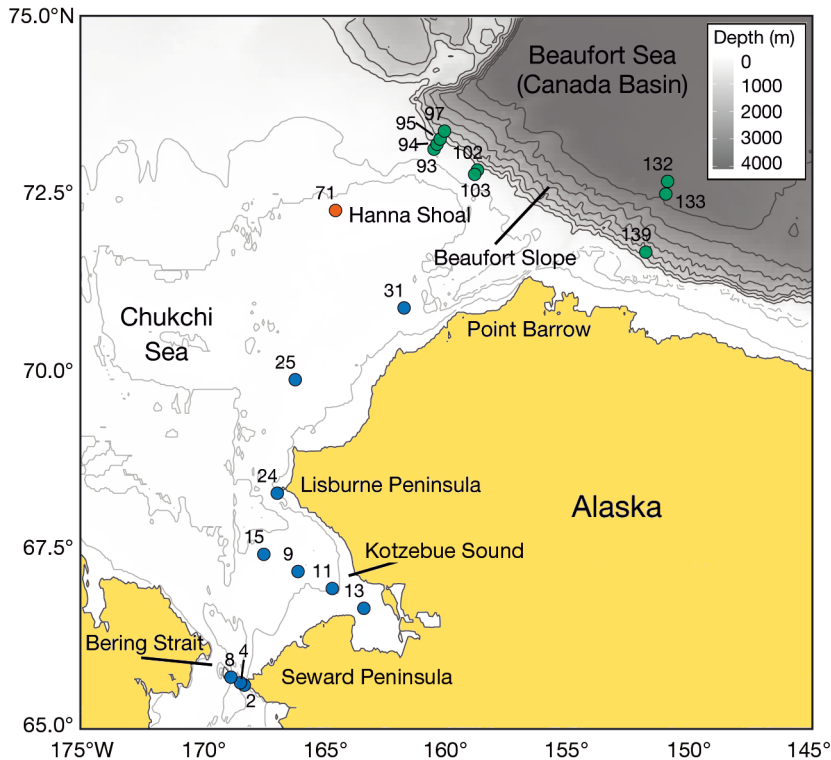


Fig. 1. Sampling locations in the Chukchi Sea and Beaufort slope. Gray lines indicate bathymetry, and gray shading represents depth. Stations are color coded by region: coastal stations are blue, Beaufort slope stations are green, and the northern Chukchi Sea station is orange

substituted for the reverse transcriptase enzyme. Contaminating DNA was assessed by quantifying bacterial 16S rRNA genes in the reverse transcription negative control reactions (Suzuki et al. 2000) (Table S2 in the Supplement).

Gene and transcript abundances were quantified with qPCR assays using an Applied Biosystems Step OnePlus Real-Time PCR System (Thermo Fisher). SYBR assays were used with universal archaeal *amoA* primers to estimate total AOA (Francis et al. 2005) and bacterial *amoA* for AOB (Rotthauwe et al. 1997, Hornek et al. 2006), while TaqMan assays were used for MGI (e.g. thaumarchaeal) 16S rRNA (Miner et al. 2007, Lund et al. 2012) and clade-specific WCA and WCB *amoA* (Mosier & Francis 2011). To distinguish them from clade-specific abundance data, qPCR data using the Francis et al. (2005) primers are referred to as total AOA *amoA*. Samples were run in triplicate, with the mean value reported. Standard curves were generated via serial dilution of linearized plasmids from previously sequenced clones for each gene and were run in triplicate with at least 5 dilutions per assay. Table S2 in the Supplement shows details on primers, reaction conditions, and standard curve data for each assay.

### Statistical analyses

Statistical analyses were completed using R (R Core Team 2015). Statistical significance of differences between coastal and Beaufort slope samples was assessed using Welch's 2-tailed *t*-tests. Pairwise correlations were estimated using Spearman's rank correlation coefficient ( $r_s$ ). Principal component analysis (PCA) was used to compare *z*-transformed environmental parameters (Legendre & Legendre 2012) via the *vegan* package in R (Oksanen et al. 2013) and are presented using scaling 1, where the projection of a point onto a vector reflects its approximate position on the vector, and distances between points reflect their Euclidean distances (Legendre & Legendre 2012).

## RESULTS

### Oceanographic setting

Using environmental data, we grouped samples into 3 distinct regions: coastal Chukchi Sea, northern Chukchi Sea, and Beaufort slope (Fig. 2). The first principal component in this analysis clearly splits coastal samples from slope and northern Chukchi

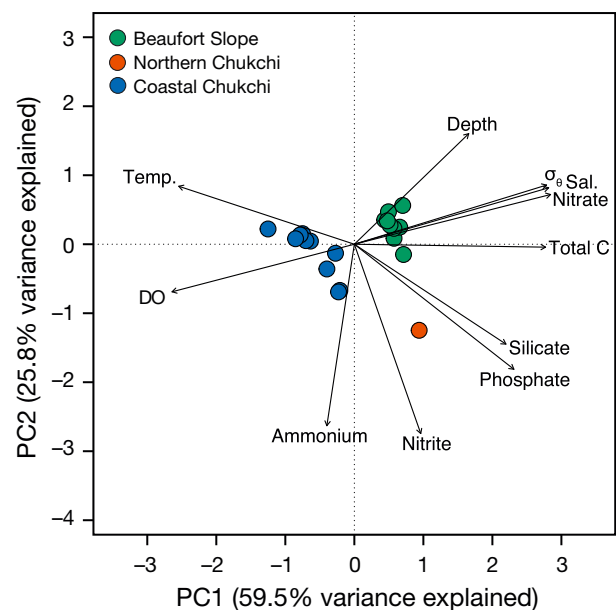


Fig. 2. Biplot representation (scaling 1) of the principal component (PC) analysis of *z*-transformed environmental data. Samples are colored by region, with clusters showing significant regional differences in oceanographic conditions.  $\sigma_\theta$ : potential density anomaly; DO: dissolved oxygen; Sal.: salinity; Temp.: temperature; Total C: total carbon

Sea samples, largely due to differences in salinity and its covariates ( $\sigma_\theta$ , nitrate, dissolved oxygen, and temperature). The second principal component mostly reflected intraregional variations in ammonium, nitrite, phosphate, and silicate, although the northern Chukchi Sea sample was also clearly separated from other regions along this axis (Fig. 2).

The northern Chukchi Sea region included only 1 sample (Stn 71), which had low temperature and high chl *a*, silicate, phosphate, ammonium, and nitrite (Table S1 in the Supplement). Comparison of coastal and Beaufort slope waters showed coastal bottom waters were significantly warmer, fresher, and less dense than Beaufort slope waters and had significantly more ammonium. Beaufort slope samples were enriched in other nutrients except nitrite, which was not significantly different between these regions (Table 1, Table S1 in the Supplement).

### Ammonia oxidizer gene abundance

Thaumarchaeal *amoA* genes were detected in all samples (Fig. 3A). The range of total AOA genes was  $5.8 \times 10^4$  genes  $l^{-1}$  (Stn 2) to  $6.6 \times 10^7$  genes  $l^{-1}$  (Stn 94), with significantly higher abundances in Beaufort slope waters (average of  $5.8 \times 10^7$  genes  $l^{-1}$ )

Table 1. Regional differences between the coastal Chukchi Sea and the Beaufort slope. Values are regional averages  $\pm$  SD; df from *t*-tests were corrected to account for unequal variance and rounded to the nearest integer. **Bold** indicates significant value ( $p < 0.05$ ; determined using Welch's 2-tailed *t*-tests).  $\sigma_\theta$ : potential density anomaly; AOA: ammonia-oxidizing *Archaea*; AOB: ammonia-oxidizing *Bacteria*; DO: dissolved oxygen; MGI: Marine Group I; Total C: total carbon; WCA: Water Column A; WCB: Water Column B

	Coastal average	Beaufort slope average	df	p
<b>Environmental parameters</b>				
Temperature ( $^{\circ}\text{C}$ )	2.2 $\pm$ 1.6	0.3 $\pm$ 0.3	10	<b>0.006</b>
Salinity	31.6 $\pm$ 1.0	34.8 $\pm$ 0.1	9	<b>&lt;0.001</b>
$\sigma_\theta$ ( $\text{kg m}^{-3} - 1000$ )	25.3 $\pm$ 0.8	27.9 $\pm$ 0.1	9	<b>&lt;0.001</b>
DO ( $\mu\text{mol l}^{-1}$ )	373.7 $\pm$ 23.9	295.6 $\pm$ 8.2	11	<b>&lt;0.001</b>
Silicate ( $\mu\text{mol l}^{-1}$ )	7.2 $\pm$ 2.7	10.9 $\pm$ 2.2	17	<b>0.004</b>
Phosphate ( $\mu\text{mol l}^{-1}$ )	0.8 $\pm$ 0.2	1.0 $\pm$ 0.1	12	<b>0.006</b>
Nitrate ( $\mu\text{mol l}^{-1}$ )	0.9 $\pm$ 1.2	13.2 $\pm$ 0.3	10	<b>&lt;0.001</b>
Nitrite ( $\mu\text{mol l}^{-1}$ )	0.03 $\pm$ 0.04	0.02 $\pm$ 0.03	17	0.512
Ammonium ( $\mu\text{mol l}^{-1}$ )	0.9 $\pm$ 0.9	0.1 $\pm$ 0.1	9	<b>0.011</b>
Total C ( $\text{mmol l}^{-1}$ )	2.1 $\pm$ 0.1	2.2 $\pm$ 0.1	17	<b>&lt;0.001</b>
<b>Gene abundances<sup>a</sup></b>				
WCA (copies $\text{l}^{-1}$ )	3.9 $\times 10^4 \pm 3.2 \times 10^4$	2.0 $\times 10^6 \pm 1.9 \times 10^6$	8	<b>0.015</b>
WCB (copies $\text{l}^{-1}$ )	3.0 $\times 10^4 \pm 1.8 \times 10^4$	4.8 $\times 10^7 \pm 2.9 \times 10^7$	8	<b>0.001</b>
AOA (copies $\text{l}^{-1}$ )	1.5 $\times 10^5 \pm 7.4 \times 10^4$	3.0 $\times 10^7 \pm 2.3 \times 10^7$	8	<b>0.005</b>
MGI 16S rRNA (copies $\text{l}^{-1}$ )	1.6 $\times 10^5 \pm 1.3 \times 10^5$	5.4 $\times 10^7 \pm 3.0 \times 10^7$	8	<b>&lt;0.001</b>
AOB (copies $\text{l}^{-1}$ )	5.1 $\times 10^5 \pm 3.6 \times 10^5$	4.7 $\times 10^4 \pm 5.4 \times 10^4$	9	<b>0.003</b>
AOA:AOB	0.5 $\pm$ 0.4	1.6 $\times 10^3 \pm 1.8 \times 10^3$	8	<b>0.027</b>
<b>Transcript abundances<sup>a</sup></b>				
WCA (copies $\text{l}^{-1}$ )	3.0 $\times 10^4 \pm 3.0 \times 10^4$	5.9 $\times 10^4 \pm 9.0 \times 10^4$	10	0.392
WCB (copies $\text{l}^{-1}$ )	3.1 $\times 10^4 \pm 1.4 \times 10^4$	8.8 $\times 10^5 \pm 1.4 \times 10^6$	8	0.096
AOA (copies $\text{l}^{-1}$ )	8.2 $\times 10^4 \pm 5.3 \times 10^4$	1.7 $\times 10^5 \pm 1.8 \times 10^5$	9	0.193
MGI 16S rRNA (copies $\text{l}^{-1}$ )	1.9 $\times 10^5 \pm 2.4 \times 10^5$	1.4 $\times 10^7 \pm 2.3 \times 10^7$	8	0.112

<sup>a</sup>Quantitative polymerase chain reaction data are for *amoA* genes or transcripts except for MGI 16S rRNA data. AOA is total AOA abundance using the universal primer set (Francis et al. 2005)

compared to coastal waters (average of  $1.5 \times 10^5$  genes  $\text{l}^{-1}$ ) (Table 1). MGI 16S rRNA genes were also detected in all samples (Fig. 3B) and were significantly more abundant in Beaufort slope samples (mean of  $5.4 \times 10^7$  genes  $\text{l}^{-1}$ ) than coastal stations ( $1.6 \times 10^5$  genes  $\text{l}^{-1}$ ) (Table 1). There was a strong correlation between MGI 16S rRNA genes and total AOA *amoA* genes, falling close to the 1:1 line (Table 2; see Fig. 5B).

AOB *amoA* genes ranged from  $4.3 \times 10^3$  genes  $\text{l}^{-1}$  (Stn 97) to  $1.2 \times 10^6$  genes  $\text{l}^{-1}$  (Stn 15), both of which were about 1 order of magnitude less than the extreme values for AOA genes. AOB genes were significantly more abundant in coastal waters than in Beaufort slope waters (Table 1). They were also low at Stn 13, the station furthest inland in Kotzebue Sound, and were slightly higher at some shallower Beaufort slope stations (93 and 103) compared to deeper stations (Fig. 3C). For all genes, abundances

at Stn 71 (in the northern Chukchi Sea) were more similar to coastal waters than Beaufort slope waters (Fig. 3).

Clade-specific AOA assays showed a general increase in both WCA and WCB gene abundance going from coastal to Beaufort slope stations, similar to the total AOA pattern (Fig. 4, Table 1). Minimum gene abundances were comparable for WCA ( $6.7 \times 10^3$  genes  $\text{l}^{-1}$  at Stn 31) and WCB ( $7.7 \times 10^3$  genes  $\text{l}^{-1}$  at Stn 13); however, WCB greatly outnumbered WCA genes in Beaufort slope samples, reflected by the much greater mean WCB abundance ( $4.8 \times 10^7$  genes  $\text{l}^{-1}$ ) compared to mean WCA abundance ( $2.0 \times 10^6$  genes  $\text{l}^{-1}$ ) (Table 1, Fig. 4). The sum of WCA and WCB genes was highly correlated with both total thaumarchaeal *amoA* genes (Fig. 5A) and MGI 16S rRNA genes (Table 2). At coastal and northern Chukchi Sea stations (except Stns 2 and 24), total AOA gene abundance was higher than WCA + WCB abundance ( $3.78 \pm 3.06$  [SD] fold higher, on average; Welch's 2-tailed *t*-test,  $p = 0.007$ ).

Abundances were significantly different between coastal and Beaufort slope samples for all genes (Table 1). AOB abundance was greater in

coastal waters, while all other genes were more abundant over the Beaufort slope. The ratio of AOA:AOB *amoA* genes was also significantly greater over Beaufort slope waters (Table 1) and had a range from 0.06 (Stn 2) to 5949 (Stn 133) (Fig. 3D). AOA and AOB genes were significantly negatively correlated ( $r_s = -0.725$ ,  $p < 0.001$ ), reflecting their different spatial abundance patterns.

We selected  $\sigma_\theta$  and ammonium to compare distributions of various gene abundances, as these variables had a strong influence on the first and second principal component, respectively, in our PCA (Fig. 2). AOB abundance was highly positively correlated with ammonium and negatively correlated with  $\sigma_\theta$  (Table 3). In contrast, total AOA, WCA, and WCB gene abundances were positively correlated with  $\sigma_\theta$  and negatively correlated with ammonium, with particularly strong correlations between  $\sigma_\theta$  and both AOA and WCB (Table 3).



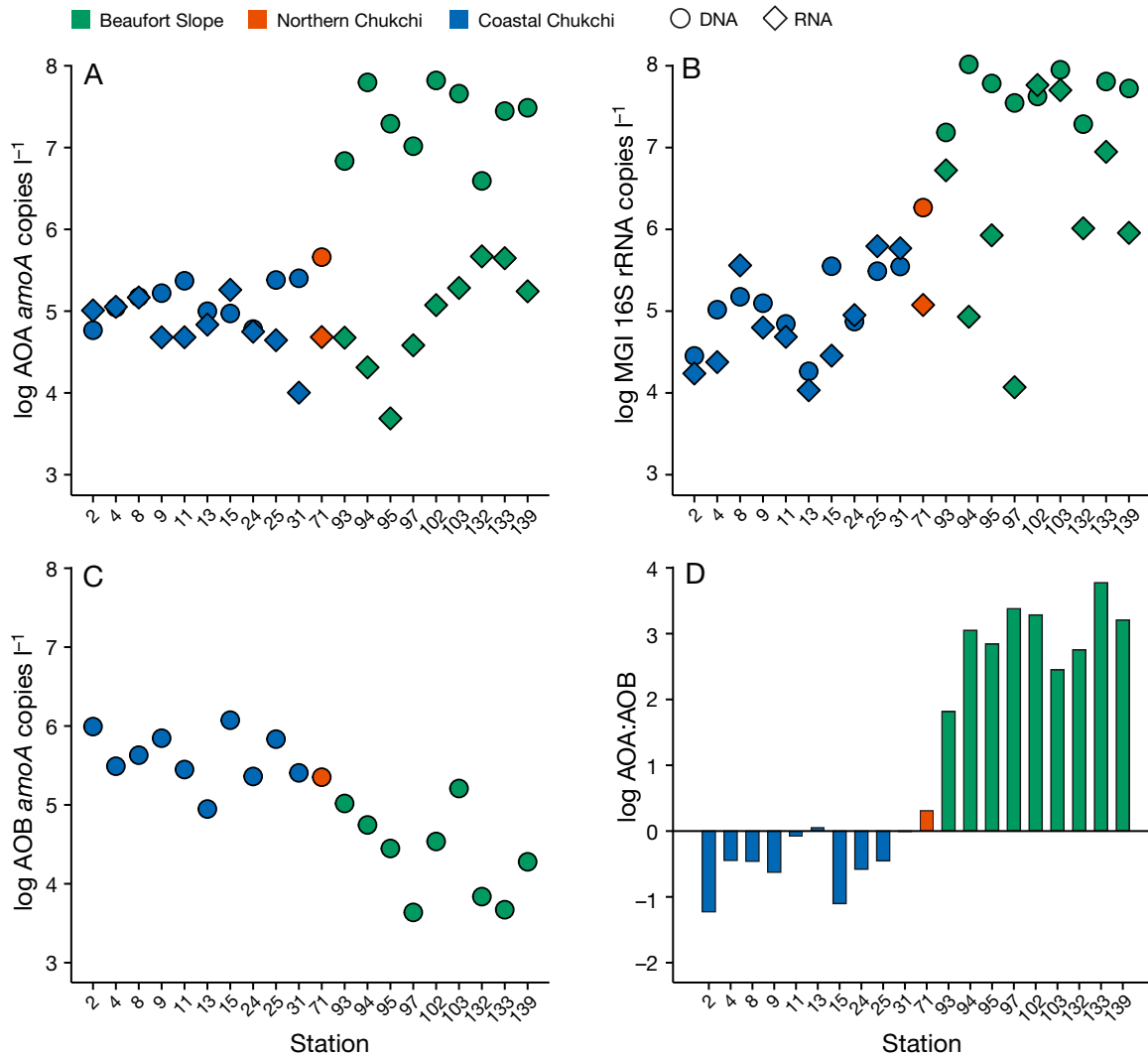


Fig. 3. Gene and transcript abundance of (A) ammonia-oxidizing *Archaea* (AOA) *amoA*, (B) Marine Group I (MGI) 16S rRNA, and (C) ammonia-oxidizing *Bacteria* (AOB) *amoA*. (D) Log ratio of AOA to AOB genes. Data are color coded by region. In (A) and (B), circles are gene abundances and diamonds are transcript abundances

**Ammonia oxidizer transcript abundance**

AOA transcript abundance had a more restricted range and much lower maximal values than gene abundance: total AOA *amoA* transcripts were detected at all stations and present from  $4.88 \times 10^3$  transcripts l<sup>-1</sup> (Stn 95) to  $4.67 \times 10^5$  transcripts l<sup>-1</sup> (Stn 132) (Fig. 3A). WCA transcripts were detected at 19 of 20 stations and present from  $7.62 \times 10^2$  transcripts l<sup>-1</sup> (Stn 2) to  $2.73 \times 10^5$  transcripts l<sup>-1</sup> (Stn 103) (Fig. 4A), and WCB transcripts were detected at 18 of 20 sta-

Table 2. Spearman's rank correlation coefficients for ammonia-oxidizing *Archaea* (AOA) quantitative polymerase chain reaction data. The top panel shows correlations between different AOA assays, while the bottom panel shows correlations between gene and transcript abundances for individual AOA assays; p-values are shown in parentheses. MGI: Marine Group I; WCA: Water Column A; WCB: Water Column B

Correlations between AOA assays				
	WCA + WCB		MGI 16S	
Total AOA (genes)	0.805 (<0.001)		0.902 (<0.001)	
Total AOA (transcripts)	0.562 (0.011)		0.274 (0.242)	
Correlations between genes and transcripts				
Total AOA	WCA	WCB	WCA + WCB	MGI 16S
-0.30 (0.901)	0.051 (0.837)	0.787 (<0.001)	0.514 (0.022)	0.647 (0.003)

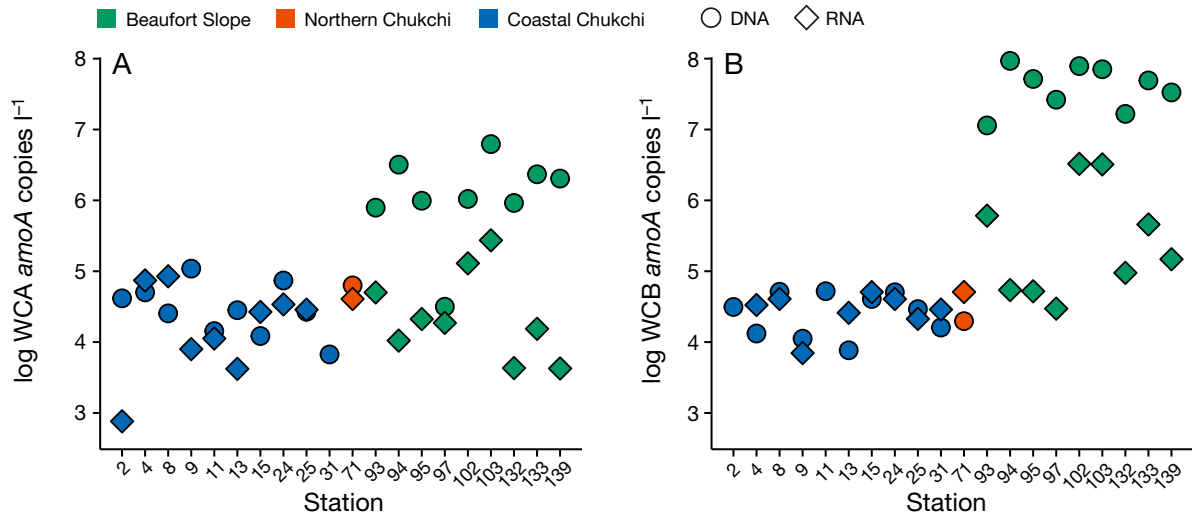


Fig. 4. Thaumarchaeal ecotype-specific gene and transcript abundance of (A) Water Column A (WCA) *amoA* and (B) Water Column B (WCB) *amoA*. Data are color coded by region. Circles are gene abundances, and diamonds are transcript abundances

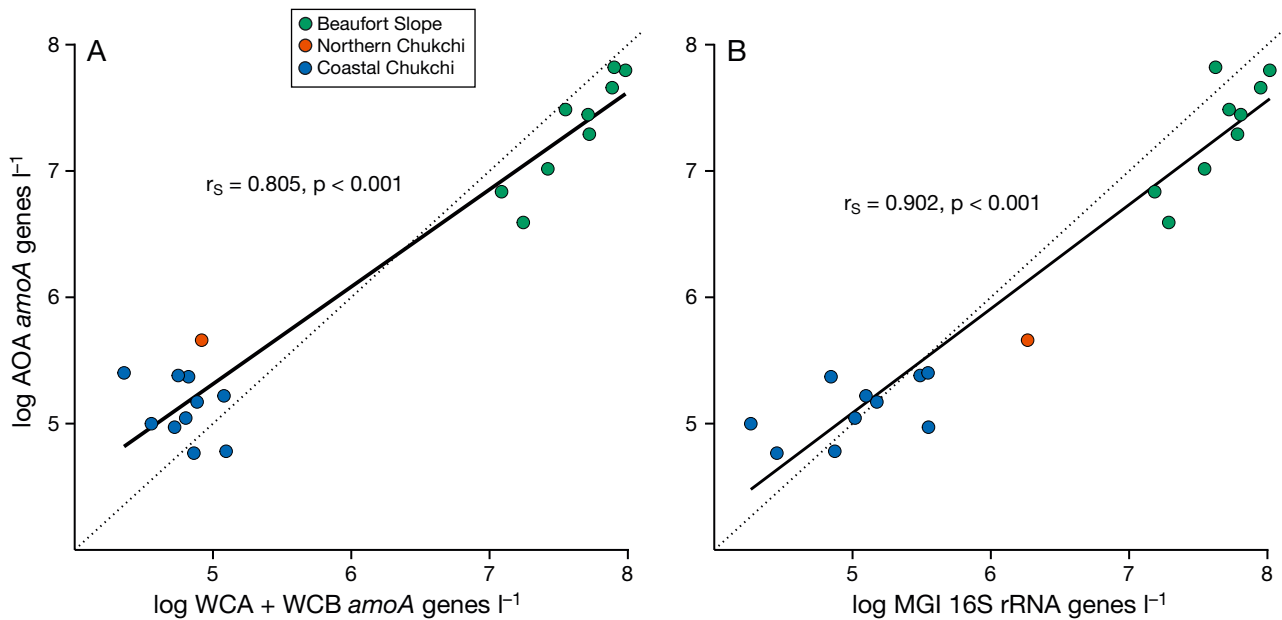


Fig. 5. Comparison of the abundances of total ammonia-oxidizing *Archaea* (AOA) *amoA* genes and (A) Water Column A (WCA) + Water Column B (WCB) *amoA* genes or (B) Marine Group I (MGI) 16S rRNA genes. Points are color coded by region. The solid line is the fit of a simple linear regression, and the dotted line shows a 1:1 relationship.  $r_s$ : Spearman's rank correlation coefficient

tions and present from  $7.00 \times 10^3$  transcripts  $l^{-1}$  (Stn 9) to  $3.28 \times 10^6$  transcripts  $l^{-1}$  (Stn 102) (Fig. 4B). Unlike gene abundance, which showed significant regional differences, transcript abundance was not significantly different between coastal and Beaufort slope waters (Table 1).

Table 3. Spearman's rank correlation coefficients for correlations between *amoA* gene abundance and ammonium or potential density anomaly ( $\sigma_\theta$ ); p-values are shown in parentheses. AOA: ammonia-oxidizing *Archaea*; AOB: ammonia-oxidizing *Bacteria*; WCA: Water Column A; WCB: Water Column B

	Total AOA	AOB	WCA	WCB
Ammonium	-0.517 (0.020)	0.789 (<0.001)	-0.635 (0.003)	-0.612 (0.004)
$\sigma_\theta$	0.889 (<0.001)	-0.674 (0.002)	0.565 (0.011)	0.779 (<0.001)

While thaumarchaeal transcript abundance showed no consistent regional variation across our samples, Stns 102 and 103 had elevated WCA and WCB transcript abundance, as well as MGI 16S rRNA copies, compared to the rest of the Beaufort slope samples (Figs. 3B & 4). Total AOA *amoA* transcripts were correlated with WCA + WCB transcripts (though this relationship was weaker than the correlation between total AOA *amoA* and WCA + WCB genes) but were not significantly correlated with MGI 16S rRNA copies (Table 2). Correlations between *amoA* genes and transcripts were significant for WCB and MGI 16S rRNA but not for total AOA or WCA (Table 2).

Attempts to quantify AOB *amoA* transcripts led to very high transcript abundances, often greater than AOB genes by over an order of magnitude (data not shown). However, amplicon sequencing of a cDNA clone library revealed significant non-specific amplification (including 16S rRNA genes). Therefore, AOB transcript data were not analyzed further. In reverse transcription negative control reactions, bacterial 16S rRNA genes were below the detection limit (100 copies reaction<sup>-1</sup>), suggesting carryover of contaminating DNA was not a major issue.

## DISCUSSION

### Physicochemical setting

Our samples clearly represent 3 oceanographically distinct water masses (Arrigo et al. 2014, Brown et al. 2015, Lowry et al. 2015, Mills et al. 2015), referred to as coastal Chukchi Sea waters, northern Chukchi Sea waters, and Beaufort slope waters (Figs. 1 & 2, Fig. S1 in the Supplement). Chukchi Sea waters originate from the northern Pacific Ocean (Roach et al. 1995), whereas the high salinities and temperatures in our Beaufort slope samples indicated Atlantic Ocean origin (Fig. S1 in the Supplement) (Jackson et al. 2010). Most coastal samples were warm, fresh, and nutrient poor and thus appeared to be a mix of Chukchi Summer Water and Alaska Coastal Water (Fig. S1) (Brown et al. 2015, Gong & Pickart 2015) in the flow path of the Alaskan Coastal Current (Walsh et al. 1989, Gong & Pickart 2015). Stn 8 (in the Bering Strait) was substantially enriched in silicate, nitrate, and ammonium (Table S1 in the Supplement), a clear signal of waters originating from the Gulf of Anadyr (Cooper et al. 1997). Stns 13, 11, 9, and 15 transected Kotzebue Sound (Fig. 1), with warmer, fresher water at inland stations (Table S1), likely due to freshwater runoff.

### Regional differences in ammonia oxidizer populations in the Chukchi Sea

To our knowledge, our data are the first quantitative mapping of clade-specific WCA and WCB gene and transcript abundance in the Arctic Ocean. Our quantification of gene abundance at many different stations allowed for a wide regional comparison of the distribution of these ecotypes. In coastal waters, WCA and WCB were generally present at comparable levels, with neither group more than an order of magnitude greater than the other (Fig. 4). Abundance of both ecotypes decreased moving north from Kotzebue Sound, but no other clear spatial patterns were evident in the coastal samples. However, both WCA and WCB abundances were far lower in coastal Chukchi Sea waters compared to Beaufort slope waters (Fig. 4, Table 1), with WCB definitively outnumbering WCA over the Beaufort slope (Table 1). This pattern was reflected by the strong correlation between  $\sigma_\theta$  and WCB (Table 3). Our Beaufort Slope samples therefore support the idea that WCA and WCB are commonly associated with shallow and deep marine waters, respectively (Beman et al. 2008, Kalanetra et al. 2009, Sintès et al. 2013, Smith et al. 2014a, 2016, Tolar et al. 2016a, Santoro et al. 2017), as these samples were all collected at >230 m. Overall, the presence of unique ammonia-oxidizing communities between the Chukchi Sea and the Beaufort slope matches previous findings of significant differences in microbial communities between Arctic Ocean basins (Ferrari & Hollibaugh 1999, Maranger et al. 2015, Pedrós-Alió et al. 2015).

Total AOA abundance (quantified using universal primers) was highly correlated with the sum of WCA and WCB abundances (Fig. 5A), suggesting that these 2 clades represent the majority of the *Thaumarchaeota* in the Arctic regions sampled here. Similar results have been documented throughout the eastern Pacific Ocean (Beman et al. 2008, 2010, Smith et al. 2016), suggesting WCA and WCB are the numerically dominant ammonia oxidizers throughout much of the sea. Many studies of *amoA* diversity using clone libraries have implicated WCA and WCB as important ammonia oxidizers in various marine regions (e.g. Francis et al. 2005, Wuchter et al. 2006, Santoro et al. 2010) and in the Arctic specifically (Kalanetra et al. 2009, Christman et al. 2011, Pedneault et al. 2014). Our quantitative mapping of thaumarchaeal ecotypes confirms these results throughout the Chukchi Sea and Beaufort slope. Deviation from this pattern was found at some coastal stations, where total abundances were



higher than the sum of WCA and WCB, indicating the possible presence of additional thaumarchaeal clades. Because coastal stations were sampled just above the sediment surface, benthic ammonia oxidizers may be transported to the water column by sediment resuspension, although the distribution of AOA in Chukchi Sea sediments is currently unknown.

### **Role of physical forcing in structuring ammonia-oxidizing communities**

Since coastal Chukchi Sea waters are shallow, the comparable abundance of WCA and WCB genes throughout this region is somewhat perplexing, as WCB are typically considered a deep-water ecotype. A previous study of Point Barrow surface waters found both WCA and WCB sequences in clone libraries (Christman et al. 2011), suggesting that both clades were also present in these shallow (2 m depth) coastal waters. Upwelling of deeper water (from either the North Pacific or the Canada Basin) into the Chukchi Sea could transport WCB into this shallow region. Chukchi Sea waters originate in the shallow Bering Sea (Coachman 1986, Cooper et al. 1997), and salinity fluctuations through the Bering Strait have been linked to upwelling intensity in the southern Bering Sea (Roach et al. 1995, Woodgate et al. 2005). Theoretically, North Pacific waters upwelled into the Bering Sea and transported north may bring WCB to the Chukchi Sea, since WCB are abundant in the subsurface North Pacific (Shiozaki et al. 2016, Smith et al. 2016). However, Shiozaki et al. (2016) did not find WCB in the Bering Sea, decreasing the likelihood of this hypothesis.

Alternatively, shelfbreak upwelling along the Beaufort slope could transport deep Canada Basin microbial communities to the Chukchi Sea shelf. Driven by strong easterly winds, shelfbreak upwelling periodically transports deep waters from the Canada Basin into the northern Chukchi Sea, advecting salt and nutrients (and likely microbes) from the halocline to shallower onshore waters (Carmack & Kulikov 1998, Pickart et al. 2013b, Spall et al. 2014). Since the HLY1101 cruise followed a period of prolonged upwelling (Spall et al. 2014), we contend that shelfbreak upwelling was the likely cause of the high WCB gene abundances in shallow Chukchi Sea waters. In the past 2 decades, upwelling events along the Beaufort and Chukchi Sea shelves have increased in both occurrence and severity (Pickart et al. 2013a, Spall et al. 2014), suggesting an increased

influence of upwelling on the microbial communities in this region.

Our study aimed to sample a geographically disparate set of stations throughout the Chukchi Sea and Beaufort slope to assess the dynamics of the thaumarchaeal communities in shallow bottom waters in the Chukchi Sea and 1 density layer along the Beaufort slope. A drawback to this approach is the lack of resolution throughout the water column. Previous work has shown the microbial communities of the Arctic Ocean differ significantly based on depth (Bano & Hollibaugh 2002, Bano et al. 2004, Kirchman et al. 2007, Galand et al. 2009a, Amano-Sato et al. 2013), including *Thaumarchaeota* (Kalanetra et al. 2009, Pedneault et al. 2014). These patterns are not surprising, given the highly stable density stratification persistently present through much of the Arctic (Aagaard et al. 1981). In many marine regions with relatively stratified water masses, thaumarchaeal communities are similarly stratified with depth (Beman et al. 2008, Yakimov et al. 2011, Santoro et al. 2013, Sintès et al. 2013, Tolar et al. 2016a, Smith et al. 2016), and previous studies in polar oceans have found that the different water masses harbor distinct microbial communities (e.g. Murray et al. 1998, Bano et al. 2004, Galand et al. 2010, Tolar et al. 2016a), suggesting that the physical processes separating water masses strongly affect microbial community composition.

Unlike the central Arctic Ocean, however, the Chukchi Sea does not have a strong halocline and convectively mixes every winter (Aagaard et al. 1981, Jones & Anderson 1986), so microbial communities in this region may be less stratified. Stronger mixing and a shallow water column may homogenize the microbial communities throughout the Chukchi Sea and therefore contribute to the presence of typical deep ecotypes (e.g. WCB) in shallower waters, as these microbes would be mixed throughout the water column following transport via upwelling. Unlike stratified polar waters, upwelling and mixing may have transported common deep-water microbes (such as WCB) into the relatively shallow coastal Chukchi Sea waters. These physical processes play an important role in the distribution of microbial communities in the coastal ocean in general, as demonstrated by genetic or metagenomic diversity in dynamic coastal regions (Murray et al. 1999, Naudin et al. 2001, Allen et al. 2012, Smith et al. 2014a, Lindh et al. 2015). In the Chukchi Sea, the lack of the typical divide between shallow- and deep-water thaumarchaeal ecotypes is likely due to physical forcings.

### Determining if remineralized ammonium selects for AOB-rich communities in the Chukchi Sea

Unlike AOA, AOB were more abundant in coastal bottom waters than Beaufort slope waters (Table 1). These contrasting spatial patterns reflected Atlantic-origin Beaufort slope waters enriched in AOA (including both WCA and WCB) and depleted in AOB compared to the Pacific-origin waters in the Chukchi Sea (Figs. 3 & 4). Beaufort slope samples were all collected well below the euphotic zone, confirming previous reports of AOA outnumbering AOB in deep Arctic Ocean waters (Kalanetra et al. 2009), a pattern common to deep waters throughout the global ocean (e.g. Karner et al. 2001, Church et al. 2010, Santoro et al. 2010, Newell et al. 2011, Sintes et al. 2013, Tolar et al. 2013, Smith et al. 2016).

Marine ammonia-oxidizing communities typically have *Thaumarchaeota* vastly outnumbering AOB, including in the northern Pacific Ocean (Martens-Habbena et al. 2015, Shiozaki et al. 2016, Tolar et al. 2016b). Our finding that AOB outnumbered *Thaumarchaeota* in the majority of coastal Chukchi Sea bottom waters (Fig. 3D) is therefore quite striking. Our samples were collected after summer phytoplankton blooms had begun (Brown et al. 2015, Mills et al. 2015). During this season, ammonium often builds up on the Chukchi Sea shelf due to remineralization (Cooper et al. 1997, Brown et al. 2015). Ammonium concentrations in our coastal samples were higher over the shelf than over the Beaufort slope and were highly correlated with AOB abundance (Tables 1 & 3, Table S1 in the Supplement). Numerous studies have suggested AOB can respond more rapidly to ammonium inputs than AOA and can outcompete AOA at higher ammonium concentrations due to higher maximum growth rates (Bollmann et al. 2005, Martens-Habbena et al. 2009, Bouskill et al. 2012, Carey et al. 2016); the surprisingly high AOB abundance in coastal Chukchi Sea bottom waters may therefore be due to their ability to rapidly oxidize the high flux of regenerated ammonium and/or sustain rapid growth due to relatively high ammonium availability. Whether these dynamics are reflected in ammonia oxidation rates in this region remains to be seen. In an analogous study, nitrification rates increased dramatically following phytoplankton death in a low-temperature coastal seawater pond (Collos et al. 1988), suggesting regenerated ammonium can drive high rates of nitrification even in cold waters.

In contrast to AOB, physiological and genomic studies of marine *Thaumarchaeota* have suggested

an oligotrophic lifestyle and an extremely high affinity for ammonium (Martens-Habbena et al. 2009, Walker et al. 2010, Santoro et al. 2015), indicating at least some AOA in the ocean can thrive in low-ammonium waters; these data match our negative correlation between AOA abundance and ammonium (Table 3). Additionally, marine AOA in coastal waters are often negatively correlated with proxies of phytoplankton growth: studies in Monterey Bay found larger WCA populations in less productive water masses (Robidart et al. 2012, Smith et al. 2014a), and experimental evidence showed a significant decrease in AOA *amoA* transcript abundance (and nitrification rates) during a simulated phytoplankton bloom (Smith et al. 2014b). Relatively low AOA abundance in our samples may therefore reflect the timing of our sampling (following bloom initiation); without seasonal sampling in this region, it is difficult to determine the interactions, if any, between phytoplankton and ammonia oxidizers in the productive Chukchi Sea.

### Transcriptional activity of *Thaumarchaeota* in the Arctic Ocean

The detection of AOA *amoA* mRNA in all Arctic samples indicated that AOA in these waters were transcriptionally active. Marine AOA *amoA* transcripts are often far less abundant than genes (Labrenz et al. 2010, Pitcher et al. 2011, Smith et al. 2014a, 2016). While our Beaufort slope samples match this pattern, the ratio of AOA *amoA* transcripts to genes was relatively high in shallower Chukchi Sea waters (Figs. 3 & 4). A similar pattern was observed in coastal Antarctic waters, where the ratio of AOA *amoA* transcripts to genes was higher in shallow compared to deep waters (Tolar et al. 2016a). Relatively high fractions of the thaumarchaeal community are therefore active in some shallow polar regions, setting these waters apart from lower-latitude regions where *Thaumarchaeota* are generally most active in the upper mesopelagic but not in shallower waters (e.g. Pitcher et al. 2011, Smith et al. 2016).

As with genes, WCA and WCB transcripts represented the majority of transcriptionally active AOA throughout the Arctic Ocean. Stn 2 (in the eastern Bering Strait) was the only station where total transcript abundance far exceeded WCA and WCB transcript abundance (Figs. 3 & 4), suggesting other thaumarchaeal clades may be active at this station. Although their gene abundances have been fairly

well studied (e.g. Francis et al. 2005, Hallam et al. 2006, Beman et al. 2008, Santoro et al. 2013, 2017, Sintes et al. 2013, Smith et al. 2014a), only a few studies have measured WCA and WCB transcript abundances in the ocean. In the northeastern Pacific Ocean, WCA transcripts outnumbered WCB transcripts in the upper water column but declined with depth, suggesting activity linked with ammonium regeneration; WCB genes and transcripts in this region were more constant with depth (Smith et al. 2016). Our data, however, suggest a different mechanism may be driving WCA and WCB activity across the Arctic Ocean: WCA transcript abundance remained relatively constant throughout the sampled coastal Chukchi and Beaufort slope waters, while WCB transcript abundance increased at a number of Beaufort slope stations (e.g. 93, 102, and 103; Fig. 4) which were relatively shallow and had higher ammonium concentrations compared to the other stations in this region (Table S1 in the Supplement). These data suggest the WCB upwelled from deeper oligotrophic waters were highly active at these stations, which we hypothesize may be due to relatively greater ammonium availability over the slope. This transcriptional response of WCB to changing environmental conditions matches observations from coastal Monterey Bay, where Smith et al. (2014a) postulated WCB were more transcriptionally responsive to changing environmental conditions than WCA based on a strong correlation between WCA genes and transcripts (e.g. a constant fraction of transcripts per WCA cell) and no corresponding correlation for WCB (Smith et al. 2014a). Across our Arctic Ocean samples, WCB genes and transcripts were correlated, while those of WCA were not (Table 2). However, this statistical relationship was largely driven by the high WCB (and not WCA) transcript abundances at some Beaufort slope stations, where both WCA and WCB gene abundances were also high. We therefore hesitate to ascribe any physiological meaning to this correlation. Yet, the increase in WCB transcript abundances at some higher-ammonium Beaufort Sea slope stations suggests this ecotype may be capable of rapid responses to ammonium availability.

## CONCLUSIONS

Ammonia-oxidizing communities were distinct between Chukchi Sea and Beaufort slope waters, reflecting the different oceanographic setting between warm and ammonium-rich Pacific-origin

waters in the coastal Chukchi Sea and cold, salty, and nitrate-rich waters deeper over the slope. Previous evidence of nitrification in these waters indicates ammonia oxidizers in these regions have a significant impact on regional biogeochemistry. While AOA vastly outnumbered AOB in the deeper slope waters, their abundances were surprisingly similar in the coastal Chukchi Sea. The high relative abundance of AOB may reflect a rapid response to regenerated ammonium. WCA and WCB made up the majority of AOA (although other clades may be present in coastal waters). The high abundance of the deep-water WCB clade in coastal waters was unexpected but likely due to transport from deeper regions via upwelling. Both ecotypes were transcriptionally active across nearly all stations, suggesting both WCA and WCB are important throughout the Chukchi Sea. Overall, ammonia oxidizers in Chukchi Sea bottom waters are regionally distinct and likely have a substantial impact on nitrogen cycling throughout the Arctic Ocean.

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