2-1-2016

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Maitreyee Mukherjee
Bowling Green State University

Anirban Ray
Bowling Green State University

Anton F. Post
University of Rhode Island Narragansett Bay Campus

Robert Michael McKay
Bowling Green State University

George S. Bullerjahn
Bowling Green State University

Journal of Great Lakes Research 42 (2016) 39–49

Contents lists available at ScienceDirect

Journal of Great Lakes Research

journal homepage: www.elsevier.com/locate/jglr

Identification, enumeration and diversity of nitrifying planktonic archaea and bacteria in trophic end members of the Laurentian Great Lakes

Maitreyee Mukherjee [a], Anirban Ray [a], Anton F. Post [b], Robert Michael McKay [a], George S. Bullerjahn [a,*]

[a] Department of Biological Sciences, Bowling Green State University, 516 Life Sciences, Bowling Green, OH 43403, USA
[b] Graduate School of Oceanography, University of Rhode Island, Narragansett, RI 02882, USA

ARTICLE INFO

Article history:
Received 23 May 2015
Accepted 27 October 2015
Available online 30 November 2015

Communicated by Wendylee Stott

Index words:
Nitrification
Ammonia oxidation
Lake Superior
Lake Erie
amoA
CARD-FISH

ABSTRACT

Oligotrophic Lake Superior and mesotrophic Lake Erie are trophic end members of the hydrologically connected Laurentian Great Lakes system, and as such exhibit different profiles of dissolved nitrogen species. Nitrification in Lake Superior has led to increasing nitrate concentrations over the past century, as opposed to Erie, where nitrate inventories have declined due to denitrification. In this study, we examined the abundance and diversity of nitrifying microbes involved in the oxidation of ammonia to nitrite, and nitrite to nitrate. By *in situ* hybridization methods, we enumerated the major planktonic ammonia oxidizing bacteria (AOB) and archaea (AOA) during a July 2011 cruise from Lake Superior to Lake Erie. In Lake Superior, AOA dominated compared to AOB, typically exceeding 5×10^3 mL^{-1}, whereas in Erie, AOB were more abundant than AOA. These data parallel prior work on Lake Superior and Lake Erie sediments, in which AOA are far more abundant in Superior, but AOB dominate in Erie. The lakes were sampled during stratification, and AOA and AOB were largely restricted to the hypolimnion, consistent with the observation that ammonia oxidizers are photoinhibited in surface waters. In Lakes Superior and Erie, we also detected nitrite oxidizing bacteria (NOB) in a pattern paralleling AOA/AOB abundance. Phylogenetic analysis of archaeal 16S rRNA revealed that the planktonic archaea of Lake Superior are members of the ammonia oxidizing Group I.1a *Thaumarchaeota* most closely related to *Nitrosoarchaeum limnia*. These AOA are distinct from the Group I.1a AOA in Lake Superior sediments. The major AOB of Lake Erie form a subcluster within the genus *Nitrosospira*.

Introduction

The Laurentian Great Lakes (North America) are a hydrologically connected system harboring 20% of the world's surficial liquid fresh water. Due to differences in bathymetry, land use, human impact and climate, the lakes yield a trophic gradient from north to south. Lake Superior, the northernmost, is the largest freshwater lake in the world by surface area and it is the third largest lake in the world by volume, comprising 10% of the world's fresh water. Lake Superior is also characterized as an oligotrophic lake, with limited anthropogenic inputs due to its large volume and low population in the watershed (reviewed in Sterner, 2011). Notably, the lake is undergoing a significant change in water chemistry, as nitrate levels have increased 5 fold to 25 μM over the past century, although phosphate levels remain in the nanomolar range (Sterner et al., 2007). This nitrate increase has yielded a major stoichiometric imbalance of TN:TP (~400) in the lake which may be important in structuring the planktonic community (Sterner, 2011). The major cause of the increased nitrate level in Lake Superior strongly suggests an unbalanced microbially-driven nitrogen cycle independent of anthropogenic loadings (Finlay et al., 2007). Rates of nitrate utilization are constrained by light and nutrient (P and/or Fe) limitation (Ivanikova et al., 2007).

By contrast, Lake Erie is warmer, shallower, and strongly influenced by the large (12 million) human population in the watershed (see: www.epa.gov/greatlakes/lakeerie/). Erie is divided into three separate basins. The western and central basins are mesotrophic and affected by nutrient inputs from urban and agricultural sources. Overall, due to the warmer temperature and nutrient loadings, Lake Erie is the most biologically productive of the Great Lakes and subject to seasonal hypoxia in the central basin due to microbial respiration (Wilhelm et al., 2006). Although the major source of total inflow (about 80%) into Lake Erie is the Detroit River, the major source of nutrient inputs (nitrogen and phosphorus) to the western basin of Lake Erie is the Maumee River, delivering agricultural runoff (Baker and Richards, 2002). The high nutrient concentrations and high turbidity due to sediment plumes support algal growth in Lake Erie (Moorhead et al., 2008). In comparison to Lake Superior, nitrate levels in Lake Erie do not exhibit the decadal large increases in nitrate. In Lake Erie, nitrate levels decline from the western basin to the central basin (Winter et al., 2015), possibly due to N sinks such as denitrification in the hypoxic

* Corresponding author. Tel.: +1 419 3728527.
E-mail address: bullerj@bgsu.edu (G.S. Bullerjahn).

http://dx.doi.org/10.1016/j.jglr.2015.11.007
0380-1330/

deep waters of the central basin (Knowles et al., 1981). Winter et al. (2015) report central basin $NO_2^- + NO_3^-$ concentrations averaging below 15 μM. These differences in nitrate trends and concentrations between the two lakes prompted us to examine key taxa involved in the N cycles of these trophically distinct lakes, primarily focusing on pelagic nitrifiers.

Nitrification is a two-step process involving distinct taxa of chemolithotrophic organisms. Ammonia oxidizers (AO) obtain their energy from the oxidation of ammonia to nitrite, and nitrite oxidizing bacteria (NOB) complete the oxidation to nitrate (Abeliovich, 2006; Koops et al., 2006). Until about 15 years ago, bacteria were thought to be the only taxa that are capable of autotrophic nitrification. Ammonia oxidizing bacteria (AOB) contain a conserved functional gene (*amoA*) that encodes the large subunit of the enzyme ammonia monooxygenase (AMO), and this gene has been employed as phylogenetic marker for AOB (Rotthauwe et al., 1997). Based on genome sequences and environmental DNA from terrestrial and freshwater environments, it has shown that most AOB belong to the β-subclass of the class Proteobacteria, with a few marine gammaproteobacteria also capable of ammonia oxidation (Koops et al., 2006). However, more recent metagenomic studies of marine archaeal sequences revealed that they also possess *amoA* (Treusch et al., 2005) radically changing our view of the nitrification process in the global nitrogen cycle. Regarding the ammonia oxidizing archaea (AOA), these organisms comprise a new phylum, *Thaumarchaeota*, whose members inhabit marine, freshwater and terrestrial environments (Spang et al., 2010). Four major groups of AOA include the Thaumarchaeal marine Group I.1a (*Nitrosopumulis*), soil Group I.1b (*Nitrososphaera*), SAGMGC-1 (*Nitrosotalea*) and the *Nitrosocaldus* group. Prior work has shown that in Lake Superior sediments, the *Nitrosopumulis* and *Nitrosotalea* clusters dominate, whereas in Lake Erie, *Nitrososphaera* was most abundant (Bollmann et al., 2014).

Both these archaea and bacteria are chemolithotrophs that exhibit physiological differences with respect to their redox metabolism, and such differences are likely important in establishing dominance of AOB over AOA and vice versa. For example, AOA are more efficient scavengers of ammonia than AOB (Martens-Habbena et al., 2009), allowing AOA to thrive in lower concentrations of NH_4^+.

Nitrite oxidizing bacteria (NOB) catalyze the oxidation of nitrite (NO_2^-) to nitrate (NO_3^-). This oxidation process is the next step of nitrification in the biogeochemical nitrogen cycle. NOB strictly depends on ammonia oxidizers that convert ammonia to nitrite. NOB are phylogenetically heterogenous aerobic chemolithotrophs. They are divided into several genera in the phyla *Proteobacteria* and *Nitrospirae*: *Nitrobacter*, *Nitrococcus*, *Nitrospira*, and *Nitrospina* (Abeliovich, 2006).

This study compares the diversity and abundance of AOA, AOB and NOB in the water column of Lakes Superior and Erie during a summer survey cruise in July 2011. We hypothesized that AOA would outnumber AOB in oligotrophic Superior, and that AOB would be more abundant in Lake Erie. Patterns of NOB abundance would parallel the presence of AO in the water column. We proposed this in light of the observation that Lake Superior sediments are dominated by AOA, and AOB are more abundant in Erie sediments (Bollmann et al., 2014).

Materials and methods

Hydrographic stations and sampling

Samples were collected from multiple sites during a multi-lake research survey from Lake Superior to Lake Erie in July 2011 aboard the UNOLS R/V *Blue Heron* (Fig. 1, Table 1). Whereas our primary goal for our work was an assessment of nitrification rates and nitrifying taxa in Lake Superior (Small et al., 2013), the availability of a longer cruise enabled limited sampling in Lake Erie as a comparative study. Lake Superior stations CD1, EL0 and EL2 represent pelagic stations in the western, central and eastern regions. Lake Erie station 880 is located

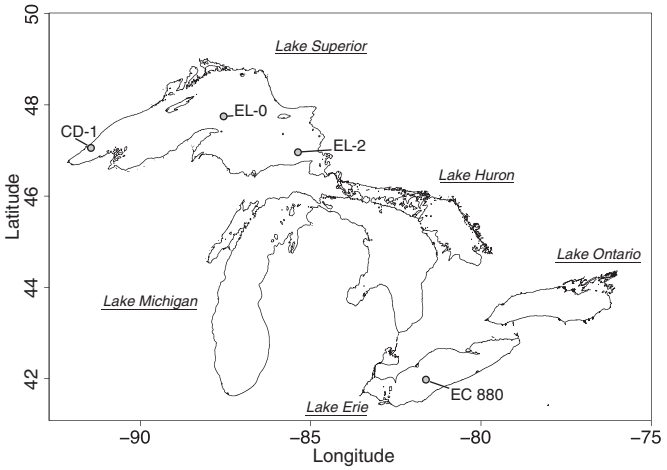

Fig. 1. Location of sampling stations for Lake Superior (top panel) and Lake Erie (bottom).

offshore in the central basin, a site prone to seasonal hypoxia in later summer (Wilhelm et al., 2006). At each station, sampling was preceded by a conductivity–temperature–depth cast, following which samples were collected from epi-, meta- and hypolimnetic depths. At all locations, water was collected into sterile polyethylene tubes and incubated for up to 7 days in freshly depolymerized and filtered (<0.22 μm) paraformaldehyde solution (2% v/v) on ice. Following sample fixation, seston were collected by filtration through white polycarbonate membranes (0.1 μm; Millipore Corporation, Billerica, MA, USA) and rinsed with double-distilled water (ddH_2O). The air-dried filters were stored at −20 °C until further processing (Sekar et al., 2003).

For analyses of environmental DNA, seston from 4 L of water was collected onto 0.22 μm Sterivex cartridges (Millipore) by peristaltic pump and frozen immediately in liquid nitrogen for shipment to the lab prior to nucleic acid extraction. DNA was routinely isolated using the DNeasy Blood & Tissue Kit (QIAGEN).

Water chemistry

Chemical parameters, including NH_4^+, particulate organic C, N and P (POC/PON/POP), and chlorophyll (Chl) *a*, were measured for all samples. For NH_4^+, 300 mL of lake water was filtered immediately following collection on pre-combusted and acid-rinsed Whatman GF/F filters and frozen. Water samples were stored in high-density polyethylene bottles, and frozen until analysis. NH_4^+ samples were analyzed with the fluorometric method using the orthophthaldehyde reagent, and corrected for matrix effects and background fluorescence following Taylor et al. (2007). POC and PON samples were collected by filtering 1 L volumes of water onto pre-combusted GF/F filters. Samples were analyzed on a Perkin Elmer 2400 CHN analyzer calibrated with an acetanilide standard. Filters similarly prepared for POP analysis were digested with potassium persulfate and total P analyzed spectrophotometrically by the ascorbic acid–molybdate method (Menzel and Corwin, 1965). TOC was measured on a Shimadzu TOC-V CSH analyzer. For Chl *a* determination, 200 mL of water was filtered onto 0.2 μm cellulose nitrate filters and immediately frozen until analysis by fluorometry following acetone extraction (Welschmeyer, 1994).

Catalyzed reporter deposition fluorescence in situ hybridization (CARD-FISH)

The protocol specified by Sekar et al. (2003) and Pernthaler et al. (2002) was routinely followed for CARD-FISH. Horseradish peroxidase (HRP)-labeled oligonucleotide probes (Table 2) were used for detection of ammonia oxidizing archaea (AOA) and bacteria (AOB) and nitrite

Table 1
Station locations and chemical analyses of samples obtained in this study (ND: no data available).

Lake	Station	Date	Lat/long	NO_3^- (μM)	NH_4^+ (μM)	Chl *a* (μg L^{-1})	POC (μM)	PON (μM)	POP (nM)	TOC (μM)
Superior	CD1 (5 m)	07/14/11	47.065 N 91.432 W	25.7	0.191	0.459	8.70	1.11	49.0	103.3
	CD1 (150 m)	07/14/11		26.6	0.433	0.219	4.90	0.53	31.0	83.5
	EL0 (5 m)	07/15/11	47.750 N 87.500 W	26.7	0.107	0.293	5.58	0.77	39.0	84.0
	EL0 (145 m)	07/15/11		26.5	0.440	0.085	ND	ND	ND	78.7
	EL2 (5 m)	07/16/11	47.000 N 85.500 W	25.9	0.150	0.251	4.23	0.59	35.8	80.3
	EL2 (150 m)	07/16/11		26.8	0.401	0.131	5.92	0.82	36.1	79.4
Erie	880 (5 m)	07/19/11	41.917 N 81.633 W	7.38	0.643	0.573	22.07	0.94	77.0	168.8
	880 (20 m)	07/19/11		3.78	4.25	0.414	10.36	0.54	110.0	149.3

oxidizing bacteria (NOB). For all AOBs, probes Nso1225 (all beta-proteobacterial AOB except *Nitrosococcus mobilis*) and Nsm156 (*Nitrosomonas* spp. and *Nitrosococcus mobilis*) FISH probes were used. In addition, probes NIT3 (*Nitrobacter* spp.) and Ntspa712 (*Nitrospira* spp.) were used for the counts of total NOB. AOA were detected by probe Cren554, which has been used as a general probe for Group I *Crenarchaeota* (now known as *Thaumarchaeota*) (Massana et al., 1997; French et al., 2012). Non-hybridizing probe NON338 was used each time as a negative control. All probes were labeled at their 5′ end with HRP. All filters hybridized with CARD-FISH probes were counterstained with DAPI.

Polymerase chain reaction (PCR)

Four liters of water from Lake Superior station CD-1 (150 m depth) and Lake Erie CCB3 (20 m) were passed through a 0.22 μm Sterivex cartridge (Millipore Corporation) to collect biomass. Environmental DNA from the filters was extracted using the QIAGEN Sterivex DNA isolation Kit (MO BIO Laboratories, Inc.). PCR amplification of the AOB *amoA* gene fragment, was performed with the primer pair amoA-1F (Rotthauwe et al., 1997) and amoA-r NEW (Hornek et al., 2006). Thermal cycling was carried out with an initial denaturation step at 94 °C for 5 min followed by 40 cycles of denaturation at 94 °C for 1 min, annealing at 53 °C for 1 min, and elongation at 72 °C for 1 min, followed by a final elongation step at 72 °C for 10 min. Positive controls containing purified DNA from lake water samples were included in all the amplification sets along with a negative control lacking DNA. Archaeal *amoA* amplification was as described in Francis et al. (2005) employing the primers Arch amoA-1F and Arch amoA-R2. PCR for bacterial 16S sequences was performed as described previously by using a universal rRNA primer pair (Juretschko et al., 1998), and AOA 16S amplification employed the primers and nested PCR conditions described previously (Stahl and Amman, 1991; Raskin et al., 1994). Positive controls containing purified DNA from lake water samples were included in all the amplification sets along with negative control (no DNA added). Primer sequences for all PCR reactions are listed in Table 3.

All 16S rDNA and *amoA* PCR products were purified using an agarose gel extraction kit and ligated according to the manufacturer's recommendations into the cloning vector (pCR4) supplied with the TOPO TA cloning kit (Invitrogen Corp., San Diego, CA, USA). Sanger sequencing was performed using the T3 primer. After sequencing and BLAST searches (Altschul et al., 1990), phylogenetic analysis of *amoA* sequences was performed in MEGA (Kumar et al., 2008). Alignment of the 16S rDNA sequences was made using CLUSTAL-W pairwise and multiple alignment tools. Bootstrap values were set to at least 1000 replicates.

Pyrosequencing of archaeal 16S reads

Archaeal V6-V4 from a single sample in the central waters of Lake Superior (EL0, 160 m) was amplified with the 517F and 1048R archaeal mixed primer set (Sogin et al., 2006). Sequencing was performed following the Roche titanium amplicon sequencing protocols. Image processing and signal calling were done using the Roche amplicon-processing pipeline (version 2.53). UChime analysis against a reference database (ChimeraSlayer GOLD) was used to eliminate chimeras (Edgar et al., 2011). The Global Alignment for Sequence Taxonomy (GAST) assigned taxonomic affiliation (Huse et al., 2008) using SILVA 111 as the reference database (Quast et al., 2013). All V6-V4 data can be accessed via the Visualization and Analysis of Microbial Population Structures website at the MBL in Woods Hole, MA, USA (http://vamps.mbl.edu).

Results

Depth-resolved whole water samples were processed for water chemistry, environmental DNA and CARD-FISH during a July 2011 research cruise from Lake Superior south to Lake Erie. Nutrients and chlorophyll were higher in Lake Erie compared to Lake Superior (Table 1; *t*-test, $p < 0.05$). Water chemistry revealed higher concentrations of chlorophyll *a*, POC, TOC and TOP in Lake Erie compared to Lake Superior, consistent with their trophic status (Table 1). Speciation of dissolved N in the two lakes was markedly different, with high nitrate levels in Lake Superior matching earlier published values (Sterner et al., 2007), and higher ammonium concentration in Lake Erie. Both lakes exhibited thermal stratification at the time of sampling, with surface temperatures in Superior and Erie of 8 °C and 24 °C, respectively (Fig. 2).

Detection and enumeration of AOA, AOB and NOB

AOA, AOB and NOB were enumerated by CARD-FISH with phylum specific, fluorescently labeled DNA probes on samples taken at three

Table 2
Oligonucleotide probe sequences used for FISH in this study.

Probe	Sequence (5′–3′)	Specificity	Reference
Cren554	TTAGGCCCAATAATCMTCCT	*Crenarchaeota*	Massana et al., 1997; Mobarry et al., 1996
Nso1225	CGCCATTGTATTACGTGTGA	Group I All β-proteobacterial ammonia oxidizing bacteria	
Nsm156	TATTAGCACATCTTTCGAT	*Nitrosomonas* spp. and *Nitrosococcus mobilis*	Mobarry et al., 1996
NIT3	CCTGTGCTCCATGCTCCG	*Nitrobacter* spp.	Wagner et al., 1996
Ntspa712	CGCCTTCGCCACCGGCCTTCC	*Nitrospira* spp.	Daims et al., 2001
NON338	ACTCCTACGGGAGGCAGC	Negative control	Wallner et al., 1993

Table 3
PCR Primers and their sequences used in this study.

Target gene	Primer set used	Sequences (5′–3′)	Reference
β-proteobacterial *amoA*	*amoA*-1F	GGGGTTTCTACTGGTGGT	Rotthauwe et al., 1997
	amoA-r NEW	CCCCTCBGSAAAVCCTTCTTC	Hornek et al., 2006
All Bacterial 16S rDNA (ammonia and nitrite oxidizers)	6116F	AGAGTTTGATYMTGGCTCAG	Juretschko et al., 1998
	630R	CAKAAAGGAGGTGATCC	Juretschko et al., 1998
Archaeal universal 16S (first PCR)	21F	TTCCGGTTGATCCYGCCGGA	DeLong et al., 1999
	958R	YCCGGCGTTGAMTCCAATT	
Archaeal 16S specific to *Crenarchaeota* (nested PCR)	344F	ACGGGGCGCAGCAGGCGCGA	Raskin et al., 1994; Stahl and Amman, 1991
	915R	GTGCTCCCCCGCCAATTCCT	
Archaeal universal 16S (pyrosequencing)	517F mix	GCCTAAAGCATCCGTAGC, GCCTAAARCGTYCGTAGC,	Sogin et al., 2006
	1048R	GTCTAAAGGGTCYGTAGC, GCTTAAAGNGTYCGTAGC, and GTCTAAARCGYYCGTAGC CGRCRGCCATGYACCWC	
Archaeal *amoA*	*amoA*F	STAATGGTCTGGCTTAGACG	Francis et al., 2005
	*amoA*R	GCGGCCATCCATCTGTATGT	

pelagic stations in Lake Superior (CD-1, EL-0 and EL-2, Fig. 1) and a single offshore station in the Lake Erie central basin (sta. 880, Fig. 1).

Lake Superior

The distribution of ammonia oxidizers in the Lake Superior water column revealed higher counts of AOA compared to AOB (Fig. 3). Except for deep water samples obtained at CD-1, AOB abundance was consistently below 2×10^3 cell mL^{-1} (Fig. 3). The AOB detected were mostly likely *Nitrosospira*-like cells, consistent with the phylogenetic data presented below. Only about 1% of the AOB-positive cells were estimated to be *Nitrosomonas*-like, by virtue of hybridization to the Nsm156 probe (Fig. 4). AOA abundances (defined as cells detected by archaeal probe Cren554) often exceeded 5×10^3 mL^{-1} in the hypolimnion (Fig. 3). AOA and AOB were more abundant in deep water, consistent with patterns observed previously during thermal stratification (Small et al., 2013). Depth-resolved distribution of NOB counts closely followed the pattern of AOs present, suggesting a tight coupling between different functional groups within the nitrifying

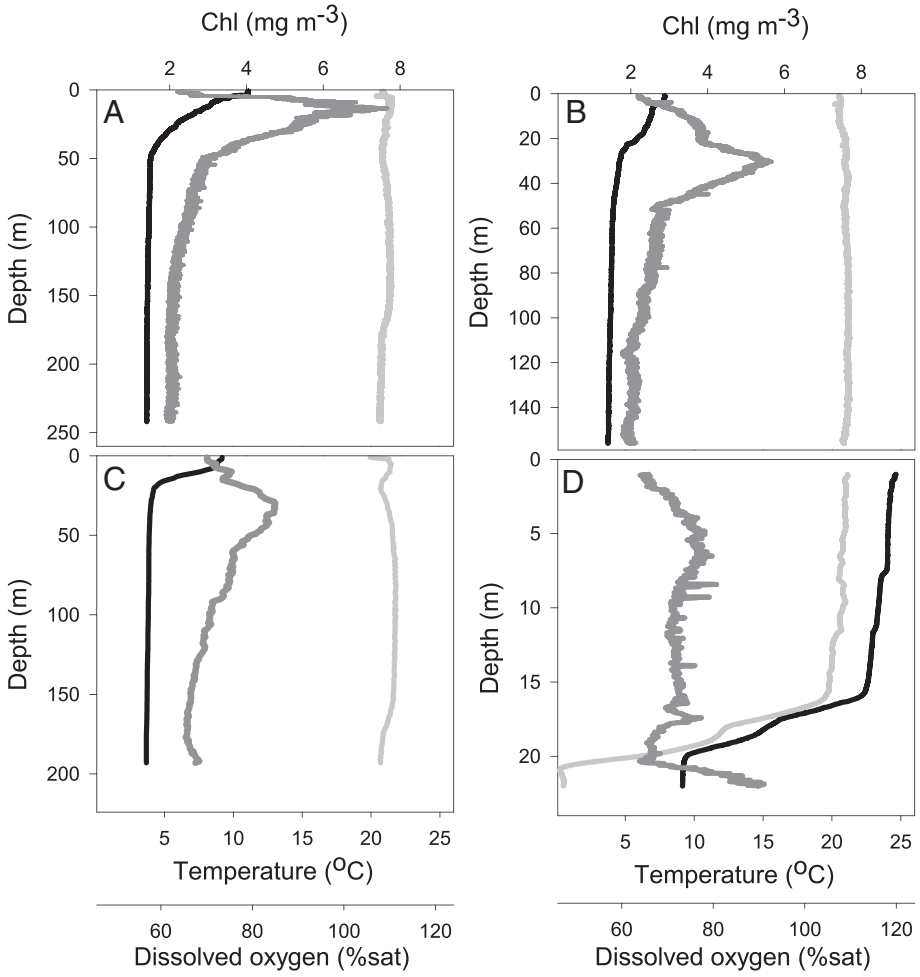

Fig. 2. Depth profiles of temperature, oxygen and chlorophyll fluorescence at Lake Superior stations CD-1, EL0, EL2 (Panels A–C), and Lake Erie station 880 (Panel D). Black line is temperature, light gray is oxygen and darker gray is chlorophyll *a* by *in situ* fluorescence.

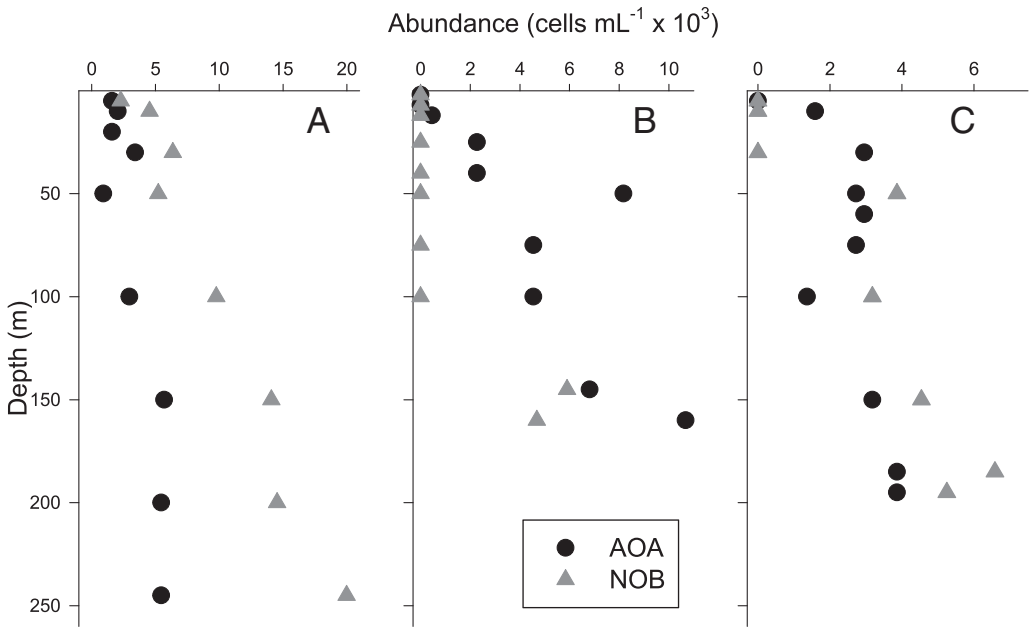

Fig. 3. Depth-resolved abundance of AOA and NOB determined by CARD-FISH at Lake Superior stations CD-1, EL0 and EL2 (Panels A–C, respectively).

community (Fig. 3). Comparative abundance of total NOB genera revealed that counts identified by the *Nitrobacter* probe (NIT3) represented 49% of NOB and 51% of NOB were detected with the *Nitrospira* probe Ntspa712.

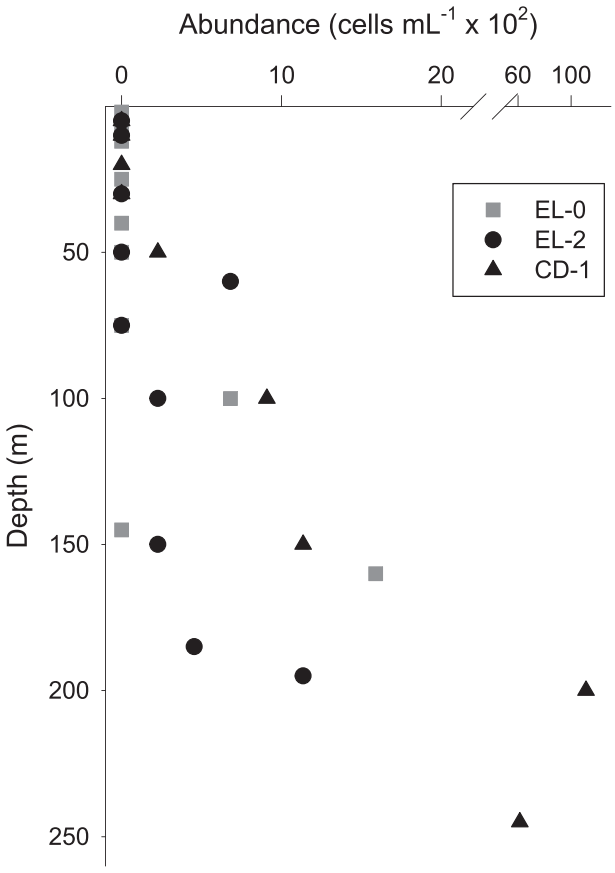

Fig. 4. Depth-resolved abundance of AOB determined by CARD-FISH at Lake Superior stations CD-1, EL0 and EL2.

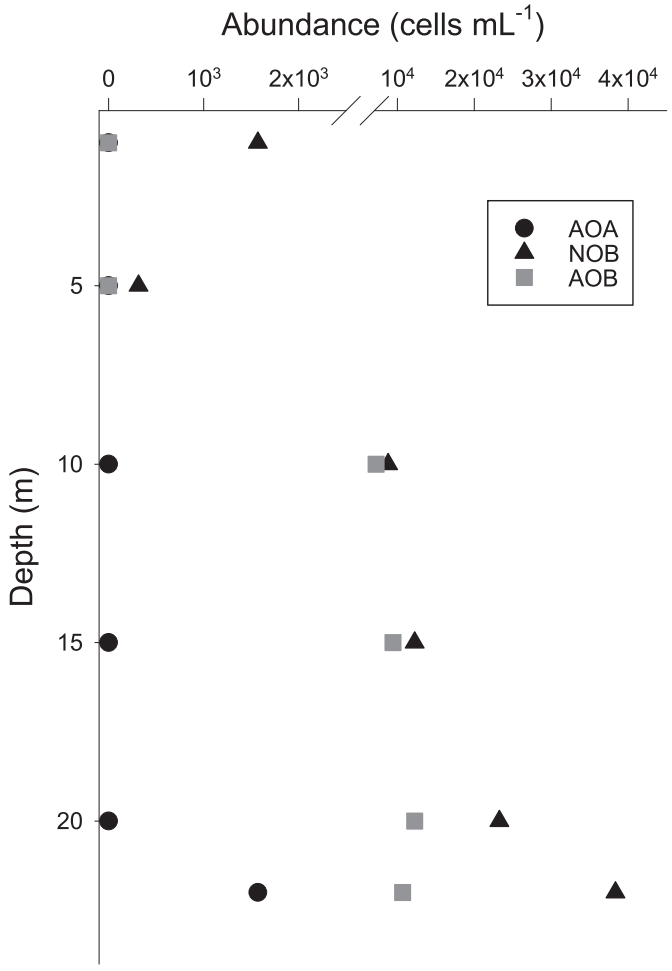

Fig. 5. Depth-resolved abundance of AOA, AOB and NOB determined by CARD-FISH at Lake Erie stations 880. Note the axis break showing AOB abundances approaching 10^4 cells mL^{-1}.

Lake Erie

In contrast to Lake Superior, central basin station 880 (maximum depth of 24 m) was dominated by AOB, exceeding 5×10^3 mL^{-1} below 10 m (Fig. 5). AOA were absent at all depths (<10 mL^{-1}) except at 20 m, where they were detected at low abundance (1.57×10^3 AOA mL^{-1}). AOA and AOB were not detected in surface waters (1–5 m depth). NOB abundance was also restricted to deeper waters, matching the depth-resolved distribution of AOB. *Nitrobacter* sp. and *Nitrospira* sp. represented 89% and 11% of the NOB counts, respectively.

AOA and AOB diversity in Erie and Superior

The CARD-FISH data were generated with the Cren554 probe that detects both *Crenarchaeota* and *Thaumarchaeota*. Prior work has documented an abundant planktonic archaeal community in Lake Superior (Reed and Hicks, 2011). As a result, the CARD-FISH counts may have identified cells that are not ammonia oxidizers. Pyrosequencing with universal Archaeal V6-V4 primers yielded a total of 2651 archaeal tags (7214 reads) from hypolimnetic environmental DNA at station EL0. All of these tags were assigned exclusively to the *Thaumarchaeota* marine Group I.1a within subcluster 1 as *Nitrosoarchaeum limnia* (Blainey et al., 2011), strongly suggesting a low overall archaeal diversity in Lake Superior, and that all archaea detected in the water column by CARD-FISH belong to the AOA group (Fig. 6).

Environmental DNA samples from the July 2011 cruise were extracted from Lake Superior (CDI 150 m) and Lake Erie (CCB3 20 m). *amoA* amplification with the archaeal and betaproteobacterial primer sets (Table 3) yielded the expected 635 base pair and 491 base pair *amoA* fragments, respectively. No PCR products were detected when gammaproteobacterial specific primers were used (Junier et al., 2008), which was not unexpected given that *Nitrosococcus* spp. are restricted to marine environments (Klotz et al., 2006). A total of 200 bacterial and archaeal *amoA* clones from each lake were randomly selected for comparative sequence analysis.

Phylogenetic analysis of *amoA* sequences revealed that the Lake Superior and Lake Erie AOA formed distinct clusters from one another, both clustering with representatives of *Thaumarchaeota* I.1a (Fig. 7). In agreement with the archaeal *amoA* data, phylogenies of 16S rRNA sequences from the Lake Superior stations yielded a similar pattern (Fig. 8). Whereas Lake Superior sediment (Bollmann et al., 2014) and water column sequences both fall within Group I.1a, they both yield distinct clusters within the group. Parallel work in the Bullerjahn laboratory has generated several enrichment cultures that reflect the AOA diversity detected in the water column (Fig. 8; Schlais M., unpublished).

All of the bacterial sequences obtained from each individual lake were highly similar to each other (≥99% sequence similarity across clone libraries). Sequence analysis of *amoA* fragments revealed that both the sequences clustered with corresponding *amoA* sequences of the *Nitrosospira* clade, consistent with the CARD-FISH data (Fig. 9). As seen in the AOA *amoA* phylogenies, Lake Erie and Lake Superior sequences clustered separately, with a single sequence dominating the Lake Superior clones. This Lake Superior *Nitrosospira* sp. likely represents a novel cluster branching closely with clusters 13–15 (Dang et al., 2010).

Discussion

Among the Great Lakes, Lake Superior and Lake Erie are two distinctly different lakes, despite the fact that they are both members of the same connected waterway. Lake Superior is oligotrophic, and has been characterized as being one of the most stoichiometrically

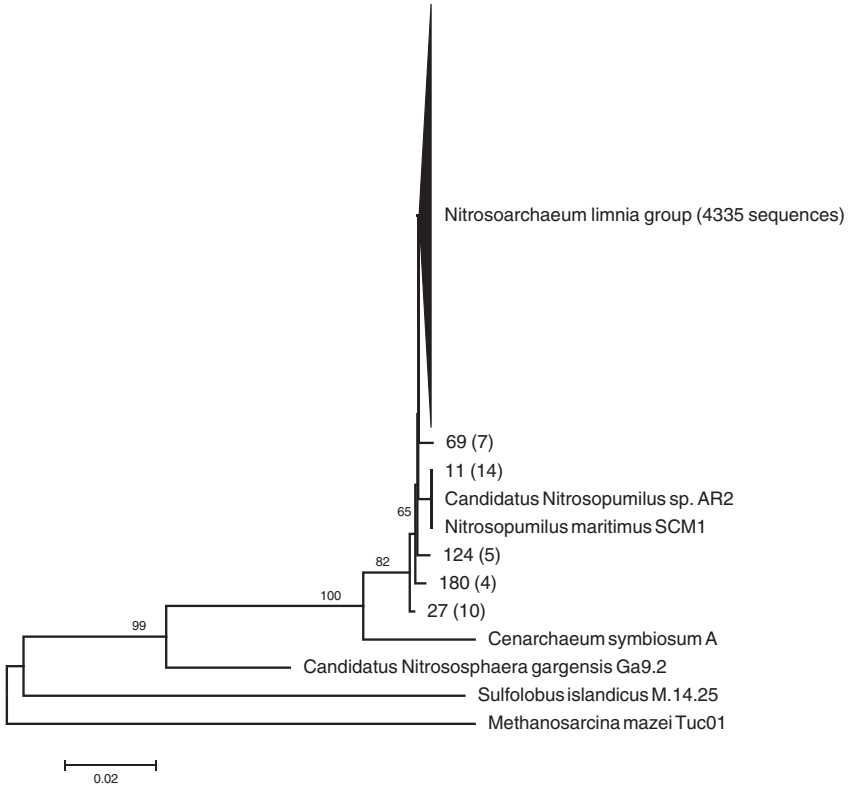

Fig. 6. Neighbor-joining phylogenetic tree of Lake Superior archaeal 16S sequences obtained by pyrosequencing of DNA from station CD-I (150 m depth). Numbers of sequences are shown in the parentheses and reflect the overall abundance of each ribotype.

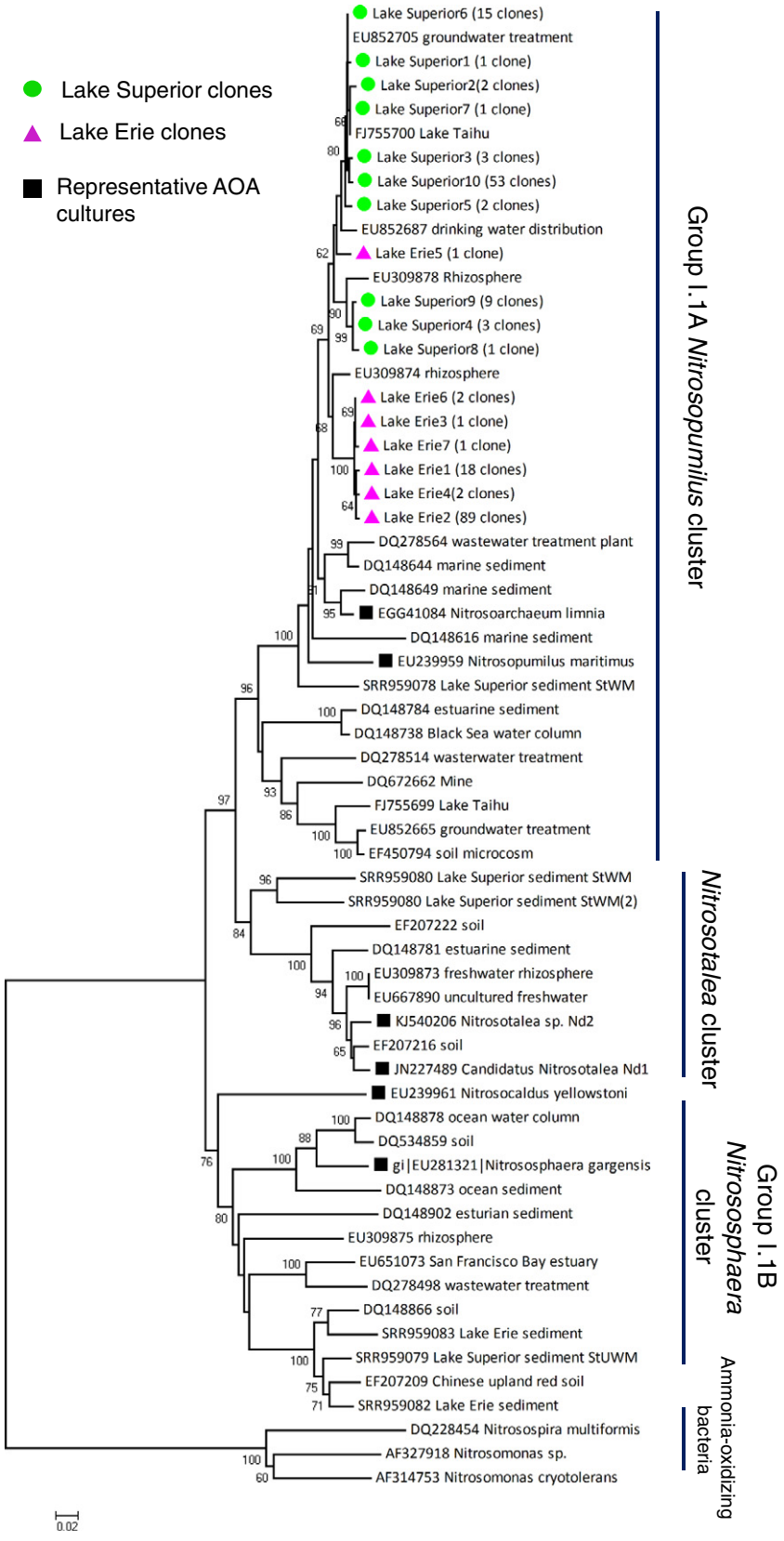

Fig. 7. Neighbor-joining phylogenetic tree of AOA *amoA* sequences obtained from Lake Superior and Lake Erie during July 2011. Clones from this study are identified with the symbols and the numbers of clones are given in parentheses.

imbalanced lakes with respect to nitrate and phosphate concentrations (Sterner et al., 2007). Dissolved nitrate concentrations are rising and currently are 25 μM, and phosphate is typically detected at nanomolar levels. In contrast, Lake Erie consists of three basins (western, central and eastern) of decreasing trophic state. The shallow (7 m) western basin is mostly eutrophic due to very high

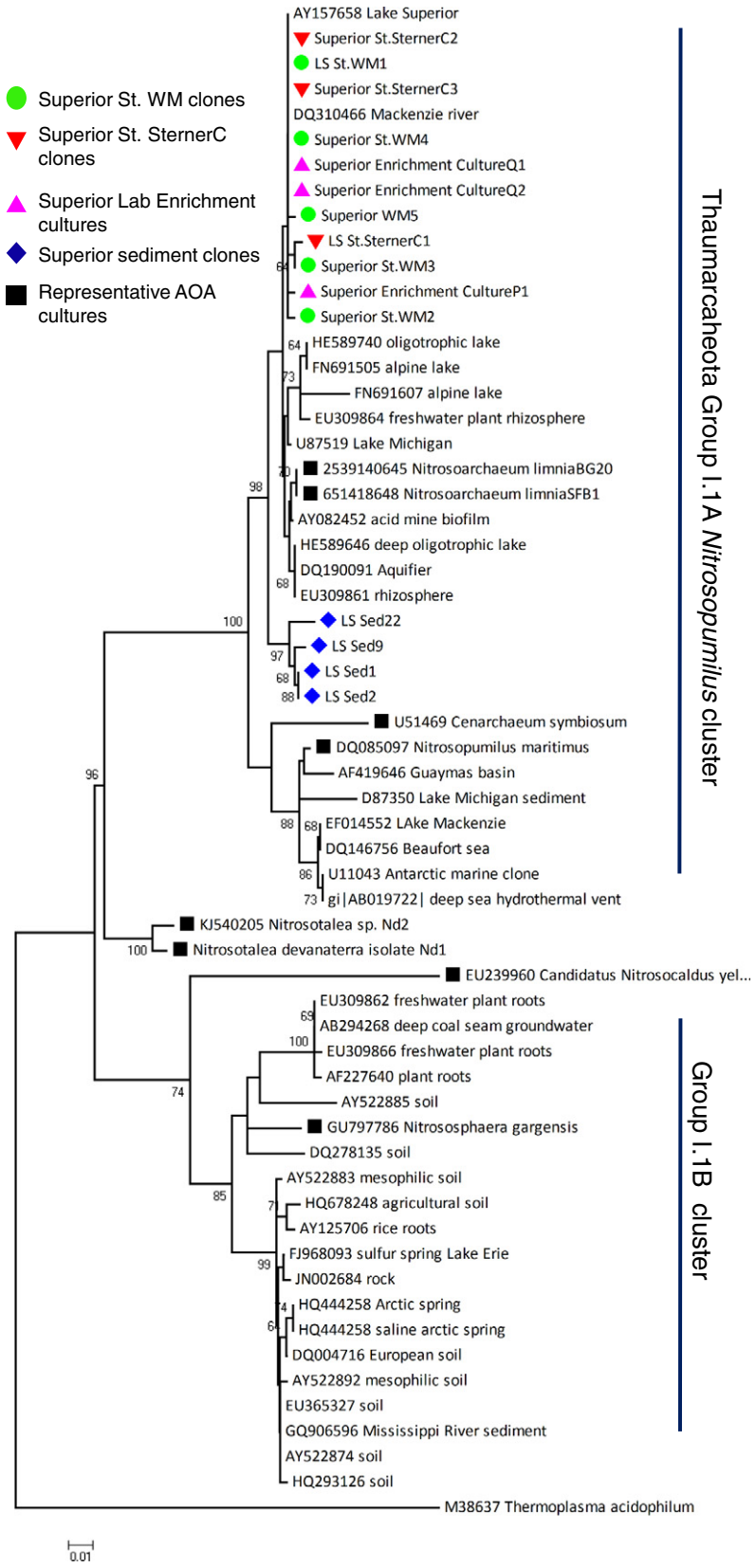

Fig. 8. Neighbor-joining phylogenetic tree of archaeal 16S sequences obtained from Lake Superior and Lake Erie during July 2011. Note that the sediment AOA sequences cluster separately from the planktonic sequences.

nutrient levels. The central basin (24 m depth) is mesotrophic, whereas the deeper (68 m) eastern basin of the lake is a low-nutrient oligotrophic environment. This is the first study on Lake Superior and Lake Erie focusing on understanding the community structure of nitrifying microbes, thereby contributing to studies that deal with how these functional groups contribute to nitrogen cycling in these lakes.

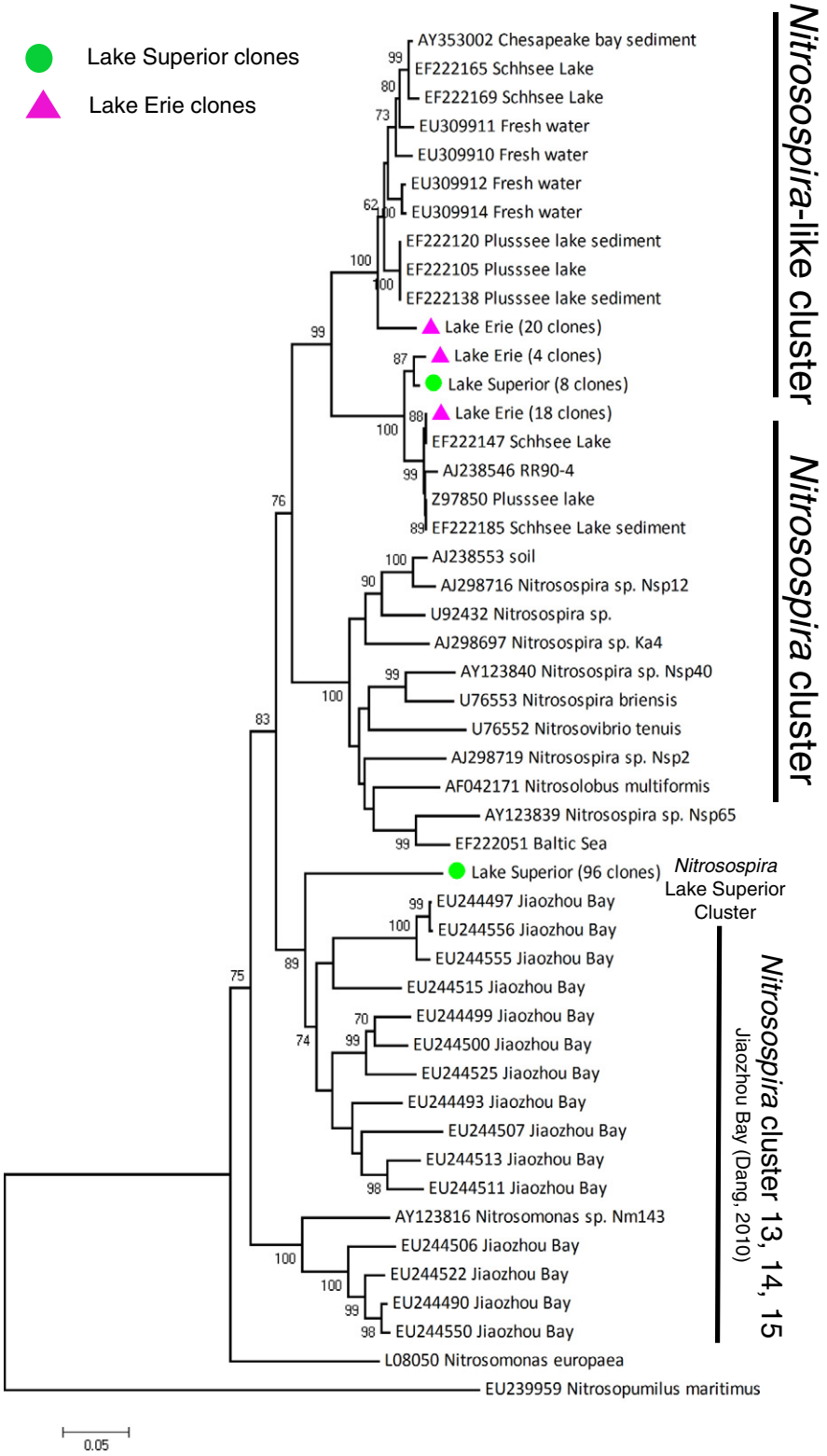

Fig. 9. Neighbor-joining phylogenetic tree of AOB *amoA* sequences obtained from Lake Superior and Lake Erie during July 2011. Clones from this study are identified with the closed circles and the numbers of clones are given in parentheses.

Lake Superior

Over the past century, the nitrate concentration in Lake Superior has increased fivefold (Sterner et al., 2007). Finlay et al. (2007) confirmed that this high level of nitrate largely arises from in-lake biological nitrification processes. The data reported here indicate that the dominant ammonia oxidizers in Lake Superior are Group I.1a *Thaumarchaeota*, as members of this group are found at all sites both in the water column and in sediments (Bollmann et al., 2014). AOB were detected in high abundance in one site, but only at depth. Overall, the higher abundance of AOA over AOB roughly parallels what is seen in Lake Superior

sediments, where AOA outnumber AOB by several orders of magnitude (Bollmann et al., 2014). However, the AOA detected in the water column cluster separately from the sediment AOA, and are closely related to the planktonic freshwater *Nitrosoarchaeum limnia* of Group I.1a subcluster 1 (Blainey et al., 2011). AOA were most abundant at depth, likely reflecting photoinhibition (French et al., 2012). Indeed, fewer nitrifiers in surface waters likely reflect light-dependent inhibition of AOA and AOB in both lakes during stratification (French et al., 2012; Merbt et al., 2012). Nonetheless, in Lake Superior AOA were detected near the surface, possibly due to the fact that the lake had been stratified for only two weeks at the time of sampling (Jay Austin, personal communication). Taking into consideration the role of AOA in water column nitrification, the average nitrification rate in Lake Superior was determined to be 24.1 nm N L^{-1} d^{-1} (Small et al., 2013). Given this rate and the average abundance of AOA (4.05×10^6 L^{-1}) in Superior, a nitrification rate of 5.9 fmol ammonia oxidized $cell^{-1}$ day^{-1} compares well with rates documented for cultured *Nitrosopumulis maritimus* and *in situ* measurements in the open sea (Könneke et al., 2005; Wuchter et al., 2006; Small et al., 2013).

The AOA counts employed the CARD-FISH probe Cren554, which is a general probe for both *Crenarchaeota* and *Thaumarchaeota*. If there were archaea present that were not AOA, the data obtained by FISH would have overestimated the nitrifying population. Pyrosequencing confirmed that all archaeal 16S sequences amplified from the Lake Superior cluster in *Thaumarchaeota* Group I.1a, strongly suggesting that the archaeal complement of the summertime microbial community is dominated by AOA (Fig. 6). These data are in agreement with those of Auguet et al. (2012) who found that the 90% of the planktonic archaea in an oligotrophic alpine lake are *Thaumarchaeota*. Furthermore, thaumarchaeotal lipids have previously been detected in the water column below the thermocline during stratification, further demonstrating the vertical partitioning of AOA to the hypolimnion (Woltering et al., 2012).

Lake Erie

Whereas both lakes have detectable populations of AOA and AOB, our data from the central basin indicate that AOB, primarily *Nitrosospira*, outnumber AOA in Lake Erie. Despite the fact that our sampling of Lake Erie is admittedly very limited, these data largely parallel what has been reported previously for Lake Erie and Lake Superior sediment nitrifiers. Bollmann et al. (2014) showed that AOA, represented by Group I.1a *Thaumarchaeota* and *Nitrosotalea*, dominate sediments in Lake Superior, and abundant *Nitrosomonas communis* and *Nitrosospira* outnumber AOA in Lake Erie. However, in our study, the AOA detected in the water column of both lakes differed from the sediment communities. In Lake Erie, sediment AOA are largely Group I.1b (*Nitrososphaera* cluster), whereas the Lake Superior sediment AOA cluster within Group I.1a, but separately from those in the water column (Fig. 8).

Prior studies examining ratios of AOA vs. AOB abundance in freshwater have documented trophic status, DOC and ammonium concentration correlating with AOA vs. AOB abundance (Herrmann et al., 2009; Hu et al., 2010; Wu et al., 2010; Fernàndez-Guerra and Casamayor, 2012; Vissers et al., 2013). Often, AOA are more abundant in oligotrophic waters with an exception being high altitude oligotrophic lakes, an observation attributed to higher tolerance of AOB to high irradiances (Hayden and Beman, 2014). Nonetheless, low ammonium availability appears to be an important driver of AOA dominance. Indeed, AOA are better scavengers for scarce ammonium, having a K_m for ammonia of 0.133 μM (Martens-Habbena et al., 2009), and freshwater enrichment cultures of AOA exhibit higher growth rates at lower [NH_4^+] (French et al., 2012). Published ammonium concentrations from oligotrophic Lake Superior and mesotrophic Lake Erie differ in ammonium concentration. Lake Superior averages 0.21 μM (Kumar et al., 2007), whereas offshore Lake Erie [NH_4^+] is consistently higher, with surface concentrations averaging 1.06 μM in spring and 0.65 μM in summer combining data from all basins (Makarewicz et al., 2000). The [NH_4^+] at the lake stations sampled in this study averaged 0.287 μM for Lake Superior and 2.45 μM for Lake Erie (Table 1), further suggesting that AOA are able to outcompete AOB at lower ammonium concentrations. This observation has been made previously, as high ammonium concentration favors AOB in creek systems and soils (e.g. Herrmann et al., 2011; Verhamme et al., 2011). Furthermore, a comparison of French lakes exhibiting different trophic status showed AOA dominance over AOB in oligotrophic low ammonium waters, and AOB abundance in a nutrient-rich lake (Hugoni et al., 2013). The influences of ammonium and other chemical factors (e.g. DOC) in dictating AOA vs. AOB in these lakes can be tested in subsequent microcosm nutrient amendment experiments and physiological studies on archaeal enrichment cultures.

Acknowledgments

The authors thank the captain and crew of the R/V *Blue Heron* for their assistance in retrieving samples for this study. Support for this study was provided by the National Science Foundation grant OCE-0927277 to RMLM and GSB.

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