Large lakes harbor streamlined free-living nitrifiers

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Running title: Nitrifiers across the Laurentian Great Lakes

Abstract

Microbial nitrification is a critical process governing nitrogen availability in aquatic systems and provides energy for aphotic carbon fixation. Nitrifiers are phylogenetically and physiologically diverse, but the factors controlling this diversity, and its biogeochemical implications, are poorly understood, particularly in freshwater systems. Here, we characterized the taxonomic, genomic, and metabolic diversity of free-living nitrifiers across Earth's largest freshwater system, the Laurentian Great Lakes. Surprisingly, ammonia oxidizing Bacteria (AOB) related to *Nitrosospira* dominated over ammonia oxidizing Archaea (AOA) at most stations, even in oligotrophic lakes with extremely low substrate concentrations. Nitrite oxidizing Bacteria (NOB) *Ca. Nitrotoga* and *Nitrospira* each dominated a subset of stations, even switching within a lake. Among four ecotypes of *Nitrosospira* we identified, one encodes proteorhodopsin, which could enhance survival of this AOB in transparent, low nutrient lakes. Genome reconstructions for free-living AOA, AOB, and NOB indicate small genomes across all taxa and diverse adaptations to sunlight and oxidative stress. Our findings expand the known functional diversity of nitrifiers and provide the first integrated picture of freshwater nitrifier genomics and ecology. As lakes worldwide undergo rapid environmental change, understanding the relationship between microbial biodiversity and nitrogen cycling is essential for predicting future ecosystem health and services.

Introduction

The oxidation of ammonia to nitrate powers the growth of nitrifying microorganisms and represents a critical flux in the global nitrogen cycle. In aquatic systems, microbial nitrification of ammonia released from sinking organic matter produces nitrate, which can then be removed from the system by denitrification (1). As chemolithoautotrophs, nitrifiers are also a major source of dark carbon fixation (2), which may contribute significant organic carbon to the microbial food web of the ocean's interior (3–5) and of deep freshwater lakes (6).

Microbial nitrifiers encompass wide diversity in phylogeny, physiology, and ecology, and understanding the drivers and consequences of this diversity across ecosystems is a fundamental challenge. Ammonia-oxidizing Archaea (AOA) in the phylum *Thaumarchaeota* dominate the mesopelagic oceans (7,8), likely due to their high affinity for ammonia (9,10) and streamlined genomes (11). In freshwater systems, AOA have been found to dominate oligotrophic lakes, while ammonia-oxidizing Bacteria (AOB) affiliated with the *Nitrosomonadaceae* (Betaproteobacteria) dominate more eutrophic systems (12–17). Complicating this picture, however, there is considerable genomic and physiological variation within each group. Some lineages, such as the thaumarchaeal *Nitrosopelagicus* (11) and bacterial *Nitrosomonas* cluster 6a (18,19), seem to be more specialized for nutrient scarcity than other related lineages. Within the AOA, there are also distinct ecotypes that appear to segregate with depth in the water column, in both marine (8,20) and freshwater systems (12). In freshwaters especially – which are poorly characterized compared to the oceans – it remains difficult to predict which ammonia-oxidizing taxa are likely to dominate in a given system.

For aquatic nitrite oxidizers, which span the phyla *Nitrospira*, *Nitrospinae*, and *Proteobacteria*, niche differentiation is even less clear. The oceans are dominated by exclusively marine lineages (2,21,22), consistent with ancient salinity-driven divergence. Cultivated strains of NOB show variation in substrate affinity and physiology (21,23,24), but the phylogenetic conservation of these traits, and their influence on environmental distributions, are

poorly understood. Moreover, recent studies have discovered that NOB are capable of alternative energy metabolisms (25,26) and can access nitrogen from cyanate and urea (27–29), expanding their ecological potential. In freshwater systems, the NOB *Ca. Nitrotoga* (Betaproteobacteria) was only recently discovered to be widespread (30), and the diversity of this genus and factors favoring its success are unknown.

Here, we use the Laurentian Great Lakes as a model system to examine niche partitioning among planktonic freshwater nitrifiers. The Great Lakes hold 20% of Earth's surface freshwater and more than half of this volume receives little to no light (< 1% surface irradiance). This system, while hydrologically connected, spans strong trophic and chemical gradients: ultraoligotrophic Lake Superior supports rates of primary production and nitrification comparable to the ocean gyres (31,32), while Lake Erie supports greater production (33) and more than 70-fold higher nitrification rate (34). Between these extremes, Lake Ontario has low ambient ammonium concentrations like Lake Superior (35) but nitrification rates up to four times higher (36). While previous studies reported that AOA and AOB dominate Lakes Superior and Erie, respectively (16,32), recent community profiling has revealed broader diversity in both ammonia-oxidizing and nitrite-oxidizing lineages (37–39). We sought to link taxonomic, genomic, and metabolic diversity of nitrifiers with the varied biogeochemistry of the Great Lakes, using genome reconstructions and abundance profiling. Our results uncover novel lineages and metabolic capabilities and provide the first large-scale assessment of freshwater nitrifier genomics.

Materials and Methods

Sample collection

Water samples were collected from the Laurentian Great Lakes aboard the R/V *Lake Guardian*, during the biannual Water Quality Surveys conducted by the U.S. EPA Great Lakes National Program Office (40). Data presented here were collected in April and August 2012.

Samples were collected using a CTD rosette sampler (Sea-Bird Scientific) at the surface (2 m), deep chlorophyll maximum (if present), the mid-hypolimnion, and near the bottom of the water column (10 m above the lake bottom at most stations, 1 m above bottom at shallow stations). For each sample, 5-8L of water was pre-filtered through a GF/A glass fiber filter (Whatman 1820-047; nominal pore size 1.6 μm) to exclude eukaryotic phytoplankton and particle associated microbes, and cells were collected on 0.22 μm Sterivex filters (Millipore SVGP01050). Filters were stored at -80°C . For dissolved nutrient analysis, 0.22 μm filtrate was collected in 125 ml acid-clean HDPE bottles (Nalgene) and stored at -20°C . Samples for single cell amplified genomes (SAG) were collected in August 2014. For each sample, 1 ml of raw water was incubated with 100 μl of glycerol-TE buffer (20 ml 100X TE pH 8 + 100 ml glycerol + 60 ml water; final concentration after sample addition is 10mM Tris, 1mM EDTA, 5% glycerol) for 10 minutes in the dark, then flash frozen in liquid nitrogen and stored at -80°C until processing.

Physicochemical data

CTD profiles, water chemistry and chlorophyll-*a* data were collected by the U.S. EPA according to standard protocols (41) and retrieved from the Great Lakes Environmental Database (<https://cdx.epa.gov/>) for 2012 and 2013. In addition, we measured dissolved nitrogen species from August 2013 samples. Ammonium concentrations were measured using the OPA method in a 96-well plate (42). Nitrate and nitrite concentrations were measured using the Greiss reaction method in a 96-well plate (43).

16S rRNA analysis

The full 16S rRNA amplicon dataset was described by Paver and colleagues (37). Here we focus on data from the V4-V5 region (primers 515F-Y, 926R; ref. 44), collected in 2012 in tandem with metagenome samples from select stations. We classified sequences using the Silva v. 132 database (45) and the wang method (46) as implemented by mothur (47).

Sequences classified to each detected family of nitrifiers (ammonia oxidizer families *Nitrosomonadaceae* and *Nitrosopumilaceae*; nitrite oxidizer families *Gallionellaceae* and *Nitrospiraceae*) with a mothur-assigned confidence score above 90 were delineated into taxonomic units using minimum entropy decomposition with a minimum substantive abundance of 10 (48).

Metagenome and single-cell genome analysis

One station per lake in Superior, Michigan, Huron, and Ontario, and two stations in Erie, were selected for metagenome sequencing. DNA was extracted using a modified phenol:chloroform extraction protocol (37) and libraries prepared according to the Illumina TruSeq protocol. Samples from spring 2012 were sequenced at the Joint Genome Institute using Illumina HiSeq (2x150bp). Samples from summer 2012 were sequenced at the University of Chicago Functional Genomics Core Facility using Illumina HiSeq 2500 (2x250bp). Separate assemblies of quality filtered reads were carried out for each metagenome using metaSPAdes 3.1.11 (49). Detailed QC, assembly, contig binning, and annotation are described in Supplementary Methods.

To confirm the presence of proteorhodopsin, we analyzed a single cell amplified genome from *Nitrosospira* collected from Lake Michigan and sequenced by the Joint Genome Institute. Quality filtered reads were downloaded from JGI IMG/ER and normalized using bbnorm.sh with target=100 and mindepth=2. Normalized reads were assembled using SPAdes in single cell mode (50) with flags --sc and --careful. Resulting scaffolds were annotated identically to MAGs as described in Supplementary Methods.

Phylogenomic trees and individual gene trees for proteorhodopsin, cyanase, and photolyase were constructed as described in Supplementary Methods.

Results

Niche partitioning of nitrifiers across the Great Lakes

To map free-living (here defined as cells that pass through a 1.6µm filter) nitrifiers across the Great Lakes, we searched our recent 16S rRNA datasets for known nitrifying taxa (37). We detected putative AOB in the genus *Nitrosospira* (Betaproteobacteria) and AOA similar to *Nitrosoarchaeum*, along with putative NOB in the genera *Ca. Nitrotoga* (Betaproteobacteria) and *Nitrospira* (Nitrospirae). We did not detect 16S rRNA amplicons from *Nitrosococcus*, *Nitrococcus*, *Nitrospina* or *Nitrobacter*. The relative abundance of nitrifiers was negatively correlated with photosynthetically active radiation (PAR; Spearman's rho = -0.89, $p < 2.2e-16$) and reached a maximum below the depth of 1% PAR in each lake, up to 20% of amplicon sequences (Fig S1a). The relative abundances of ammonia- and nitrite-oxidizing taxa were strongly correlated (Spearman's rho = 0.918, $p < 2.2e-16$; Fig S1b). Free-living nitrifiers were rare (<0.1% relative abundance) in bottom water samples from the southern basin of Huron (HU15M) and western basin of Erie (HU91M); these two stations are the shallowest in our dataset and have relatively high light penetration to the bottom (~1% PAR). Chlorophyll-a concentration was also negatively correlated with the relative abundance of nitrifiers (Spearman's rho = -0.677, $p < 1.7e-7$; Fig S1c). These findings are consistent with previous work demonstrating photoinhibition of nitrification (51–54), as well as potential competition with phototrophs for ammonium (55).

The taxonomic assemblage of nitrifiers, along with nitrogen availability, differed across lakes and even across stations within a lake (Fig 1, Tables S1-S3). AOB (*Nitrosomonadaceae*) were observed across all lakes. In contrast, AOA (*Nitrosoarchaeum*) sequences only exceeded 0.5% relative abundance at the three deepest stations (SU08M, MI41M, ON55M) where the ratio of AOB:AOA ranged from 10:1 to 1:3 (Table S1). A single dominant oligotype accounted for more than 85% of *Nitrosoarchaeum* sequences in all samples, while four major oligotypes of

Nitrosomonadaceae were observed (data not shown). We found pronounced shifts in the dominant NOB across stations (Fig 1), and all stations except those in Lake Ontario showed strong dominance (greater than ten-fold) of either *Ca. Nitrotoga* or *Nitrospira*. *Nitrospira* was the only nitrite oxidizer detected in Superior and the dominant nitrite oxidizer in parts of Michigan (MI41M, MI18M). By contrast, *Ca. Nitrotoga* was the only nitrite oxidizer observed in Erie and Huron and the dominant nitrite oxidizer at the shallowest station in Michigan (MI27M). In all but one sample, a single dominant oligotype of *Ca. Nitrotoga* and *Nitrospira* accounted for >74% and >84% of sequences from each taxon, respectively.

Ecotypic variation in abundant streamlined Nitrosospira

We reconstructed 15 genomes of the AOB *Nitrosospira*, substantially expanding genome descriptions for this genus (56–58). Based on a phylogenomic tree, free-living Great Lakes *Nitrosospira* fall into two major clades, both of which are distinct from published species; each of these clades also includes MAGs recovered from lakes Biwa and Baikal, suggesting novel globally distributed freshwater lineages (Fig S2). One clade, which we call NspGL1, has a reduced genome (average 1.4 Mb) and low G+C content (41%) (Fig 2, Table S4), typical of streamlined genomes (59). The second clade was resolved into three subclades (denoted NspGL2a, 2b, and 3; Fig S2) based on phylogeny and average nucleotide identity (ANI), all with small genome sizes of 1.5–1.8 Mb and 50% G+C content (Fig 2, Table S4). Based on short read mapping, these subclades are ecologically distinct: NspGL1 and NspGL2b -- with the smallest genomes -- are the dominant AOB in the upper oligotrophic lakes, while NspGL2a is only abundant in Ontario and NspGL3 is only abundant in Erie (Fig 2). Hereafter we refer to these subclades as ecotypes due to their phylogenetic and ecological separation.

We compared the genomes of our Great Lakes *Nitrosospira* to 87 reference AOB genomes belonging to the family *Nitrosomonadaceae*, revealing distinctive patterns of gene content. Great Lakes *Nitrosospira* genomes encode fewer two-component signal transduction

systems, transposases, motility genes, pilus and secretion genes, and defense-related genes, and their complement of transporters is distinct from most reference genomes (Table S5). They also lack functions related to biofilm formation such as polysaccharide matrix production (e.g. *pel* genes) and extracellular protein targeting (exosortase and PEP-CTERM motifs). These features are in sharp contrast to *Nitrosospira* isolates from soil (56,58) and to oligotrophic *Nitrosomonas* isolates (60) and are consistent with a passive planktonic lifestyle in extremely low-nutrient systems.

We next compared metabolic potential among Great Lakes AOB ecotypes to understand their ecological preferences for upper lakes (NspGL1, NspGL2b), Ontario (NspGL2a), and Erie (NspGL3). Surprisingly, all seven NspGL1 MAGs contain a cluster of genes encoding proteorhodopsin and retinal biosynthesis, including 15,15'-Beta-carotene dioxygenase (*blh*), lycopene cyclase (*crtY*), phytoene dehydrogenase (*crtI*), phytoene synthase (*crtB*), and GGPP synthase (*crtE*) (Fig 3a). We also identified proteorhodopsin in a single cell amplified genome (SAG) representing NspGL1 from Lake Michigan (Fig 3a), demonstrating that it is not an artifact of metagenome assembly. To our knowledge, this is the first example of a nitrifier with proteorhodopsin. All NspGL1 proteorhodopsins share residues H95, D127 and E138 along with a short beta-turn (G111-P116) between helices B and C, which are characteristic features of proteorhodopsin as distinct from sensory and other rhodopsins (61), and the presence of leucine at position 135 suggests green light tuning (62) (Fig 3b). All of the genes in this module have highest similarity to homologs from *Polynucleobacter*, but are flanked by Nitrosomonadaceae-like genes, suggesting recent horizontal gene transfer (Fig 3a). The predicted NspGL1 proteorhodopsins cluster with *Polynucleobacter*, *Methylopumilus*, and other freshwater Betaproteobacteria in Supercluster III as defined by MicRhoDE (63) (Fig S3). We compared the homologous genome region in two highly similar MAGs from Lakes Biwa and Baikal (Fig S2); these contigs lack the proteorhodopsin module but appear to flank a variable region where the contig assembly ends (Fig S4). A proteorhodopsin photosystem could support

survival of NspGL1 in the presence of sunlight, which has been shown to inhibit ammonia oxidation (51,64). In the upper lakes where NspGL1 is abundant, light penetration is high well below the thermocline in stratified periods (65), and deep water taxa are seasonally advected to the surface by water column mixing (37). In addition to proteorhodopsin, NspGL1 – but not the other three ecotypes of Great Lakes *Nitrosospira* – encode a class I cyclopyrimidine dimer photolyase, which uses light energy to repair UV-induced DNA damage, and the catalase- peroxidase *katG*, suggesting that the NspGL1 ecotype is adapted to relatively shallow depths in the water column (Fig 2).

Great Lakes *Nitrosospira* carry a reduced, ecotype-specific complement of nitrogen metabolism genes compared to reference AOB (Fig 2; Table S5). All are presumed to have the core ammonia oxidation enzymes ammonia monooxygenase and hydroxylamine dehydrogenase; these genes were assembled and binned as expected in some MAGs and were manually identified on short unbinned contigs in other cases. Surprisingly, all Great Lakes *Nitrosospira* lack the copper protein nitrosocyanin, whose precise function is unknown but so far has been found in all AOB except one member of the *N. oligotropha* clade (60). The lack of nitrosocyanin extends beyond the Great Lakes MAGs to closely related freshwater and marine strains, along with five more members of the *N. oligotropha* clade (Fig S2); its absence may be related to the divergence of these clades. Only NspGL1 and NspGL2b encode NO-forming nitrite reductase (NirK), which confers nitrite tolerance (66); this result is surprising given that these two clades dominate the upper lakes where productivity and reduced N are lowest. None of the Great Lakes MAGs encode NO reductase (NorCBQD), and NspGL1 lacks cytochrome P460 family proteins; these genomic patterns may influence N₂O emissions across the Great Lakes. Nitrogen acquisition is also distinct among Great Lakes AOB: NspGL1 lacks an apparent ammonium transporter, but encodes urease structural and accessory genes (*ureABCEFG*) and a high-affinity urea transporter (*urtABCDE*). Further, all Great Lakes ecotypes encode a high-affinity amino acid transporter (*livFGHM*) and a secreted non-specific nuclease; these genes are

rare (<5%) in reference genomes and could supply reduced nitrogen and/or organic carbon. Finally, NspGL1 and NspGL3 have genes for producing cyanophycin, an intracellular storage compound for nitrogen (58,67). Together, the minimal and distinctive gene complements present in Great Lakes *Nitrosospira* illustrate the variability and adaptability of AOB gene content across habitats.

As with nitrogen metabolism, carbon metabolism is also distinct between Great Lakes and reference AOB, and among Great Lakes ecotypes (Fig 2; Table S5). Unlike most reference AOB, Great Lakes *Nitrosospira* lack two key enzymes of the oxidative pentose phosphate pathway, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. All ecotypes except Erie-specific NspGL3 also lack genes for glycogen synthesis and degradation. These findings suggest continuous autotrophy, rather than “feast or famine” dynamics of carbon storage and utilization, or diel periodicity as in photoautotrophs. The key enzyme for carbon fixation, RuBisCO, has evolved several kinetically distinct forms whose distribution likely reflects ecological pressures (68). NspGL1 and NspGL3 both contain Form IA RuBisCO, while NspGL2a and NspGL2b contain Form IC RuBisCO (68,69) (Fig 2). NspGL1 genomes also possess an alpha carboxysome-like *cso* operon, similar to *Nitrosomonas eutropha* C91 (69), though our draft assembly lacks the expected carbonic anhydrase (*csoS3/csoSCA*). Carboxysome-associated RuBisCO may allow NspGL1, the ecotype most strongly adapted to energy and nutrient limitation, to more efficiently fix CO₂ by minimizing the wasteful oxygenation reaction and reducing the cellular nitrogen allocation to RuBisCO (68). The ranges of kinetic properties observed in other autotrophs for Form IA_q (found in NspGL3) and Form IC (found in NspGL2a and NspGL2b) overlap, and therefore more work is needed to understand the fitness advantages, if any, that this RuBisCO diversity confers on Great Lakes nitrifiers.

Streamlined freshwater Thaumarchaeota

We reconstructed three similar genomes (>99% ANI) of *Nitrosoarchaeum* (NarchGL; Fig S5, Fig S6, Table S4) from three separate samples (two from Superior, one from Ontario), consistent with our low observed 16S rRNA diversity for *Thaumarchaeota*. These NarchGL genomes are very similar (~99% ANI) to two genomes reconstructed from Lake Baikal, located thousands of kilometers away (70). Their next closest relatives are also from freshwater environments, and phylogenetic clustering suggests that salinity is an important driver of divergence throughout the *Nitrosopumilaceae* (Fig S5). Using our reconstructed genomes as probes for metagenomic read recruitment, NarchGL were detected in Superior, Michigan, and Ontario; they represented roughly one-third of ammonia oxidizers in the mid-hypolimnion of station SU08M, reaching relative abundance of approximately 0.5% (Fig 2).

NarchGL share nearly 90% of their predicted proteins with close relatives including *Ca. Nitrosoarchaeum limnia*; at the same time, they show distinctive patterns in gene content that pinpoint the key selective pressures of deep lakes (Fig 2, Table S6). All three NarchGL genomes encode urease and a urea transporter, implicating urea as a vital source of nitrogen for energy and/or biosynthesis. Consistent with phosphorus scarcity in much of the Great Lakes (71), NarchGL encode high affinity transport systems for phosphate and potentially phosphonates, though we did not identify a phosphonate lyase. In addition to both CRISPR/Cas enzymes cas1 and cas4, NarchGL genomes contain several phage proteins, suggesting viral infection and integration events may be common. DNA photolyases, which have been found in epipelagic clades of marine *Thaumarchaeota* (72–74), are present in all low salinity *Nitrosoarchaeum* including NarchGL, suggesting NarchGL are exposed to sunlight due to high water clarity (65) and/or annual mixing in the Great Lakes. NarchGL also lack the common tRNA modification 4-thiouridylation (indicated by IscS K04487 and ThiI-NFACT PF02568-PF18297; ref. 75); we propose that the absence of this modification, which is susceptible to near-UV radiation (75), is also related to sunlight exposure.

While aquatic AOA are typically adapted to oligotrophic conditions, NarchGL appear especially streamlined. Their genomes reveal striking reduction in environmental sensing, response, and regulatory functions, relative to other *Nitrosoarchaeum* and *Nitrosopumilaceae* (Table S6). NarchGL encode fewer variants of the general archaeal transcription factors, TATA binding protein (TBPs, PF00352) and transcription factor B (TFBs, PF00382 and PF08271), combinations of which influence differential gene expression (76). NarchGL also encode fewer two-component systems that transmit environmental signals to control gene expression or protein activity (domains: cache PF02743, HAMP PF00672, His kinase A PF00512, receiver PF00072). Further, they are depleted in ArsR family transcription factors (PF01022), P-II proteins for regulation of nitrogen metabolism (PF00543), and other potential regulatory domains (CBS PF00571, USP PF00582). This extremely limited regulatory capacity in NarchGL stands in sharp contrast to closely related *Ca. N. limnia*, but instead parallels the oceanic minimalist *Ca. Nitrosopelagicus brevis* (11) (Table S6).

Expanded diversity of Ca. Nitrotoga with reduced genomes

Despite the broad distribution of *Ca. Nitrotoga* in freshwater systems and beyond, only six genomes are available, derived from rivers heavily impacted by urban and agricultural influence, a wastewater treatment plant, and coastal sediment (30,77,78). Hence the metabolic and phylogenetic diversity of this group is virtually unexplored. We reconstructed six new MAGs of *Ca. Nitrotoga*, which form two clusters with >99% ANI within each cluster and ~97% ANI between clusters (NtogaGL1a and NtogaGL1b; Fig S2). These new *Ca. Nitrotoga* MAGs are far smaller than published genomes (1.5 Mb compared to 2.6-3.0 Mb; Fig 4, Table S4), lacking functions such as motility and chemotaxis, pilus biogenesis, and DNA repair (*mutLS*) (Table S7). Great Lakes *Ca. Nitrotoga* also encode markedly fewer two-component systems for sensing and responding to environmental cues, defense-related genes (restriction-modification, toxin-antitoxin, and Crispr-Cas systems), transposases, and efflux transporters (Table S7).

While incomplete assembly of hypervariable genome regions may explain some of these absences, the overall genome properties are consistent with a relatively stable low-nutrient environment and planktonic lifestyle.

The reduced genomes of *NtogaGL1a/b* help clarify core features of the genus *Nitrotoga*, along with accessory functions that may enable local adaptation in specific populations. To date, sequenced *Ca. Nitrotoga* including *NtogaGL1a/b* encode similar electron transport pathways, including NADH dehydrogenase-Complex I, succinate dehydrogenase-Complex II, and Alternative Complex III, along with high-affinity *cbb3*-type cytochrome oxidases suggesting adaptation to low oxygen conditions. They also share the Calvin cycle for carbon fixation, a complete TCA cycle, and an evolutionarily distinct nitrite oxidoreductase (NXR) from other NOB (30,77,78). All *Ca. Nitrotoga* to date also share transporters for amino acids and peptides, potential sources of C and/or N. *Ca. Nitrotoga* can also potentially access reduced sulfur compounds for energy via sulfite dehydrogenase, suggesting metabolic flexibility beyond nitrite oxidation.

Beyond these similarities, the small genomes of *NtogaGL1a/b* are distinct from previously described *Ca. Nitrotoga* in many ways. *NtogaGL1a/b* lack NiFe hydrogenase to use hydrogen as an energy source. They also lack nitrogen metabolism functions including assimilatory nitrite reductase (*nirBD*), nitrite reductase to NO (*nirK*), and nitric oxide reductase (*norQD*). Based on gene content, *NtogaGL1a/b* appear unable to use hexoses like glucose, since they lack the glycolytic enzyme phosphofructokinase and the Entner-Doudoroff pathway, similar to *Nitrobacter winogradskyi* (79). Consistent with this, they also lack genes for storage and breakdown of glycogen (Table S7). All but one of the *NtogaGL1a/1b* genomes encode cyanate lyase (*cynS*), which is found in other NOB but no *Ca. Nitrotoga* to date (27,80,81). The *cynS* gene, adjacent to *glnK-amtB* for ammonium sensing and transport, likely functions in N assimilation, as recently described for *Nitrospinae* (22). While cyanase has been shown to mediate reciprocal feeding between some NOB and ammonia oxidizers (27), this interaction is

unlikely in the free-living (<1.6µm) size-fraction and dilute environment sampled here. Notably, cyanase from NtogaGL1a/1b, along with predicted *Nitrospirae* proteins from Lake Baikal and soil, form a distinct phylogenetic cluster from most nitrifier cyanase proteins observed to date (Fig S7).

The two ANI-based clusters we detected, NtogaGL1a and NtogaGL1b, appear to be phylogenetically and ecologically distinct ecotypes. Based on short-read mapping, NtogaGL1b dominates Erie, while NtogaGL1a dominates all other Ca. Nitrotoga-containing samples (Fig 4). We found several metabolic genes that differentiate the two ecotypes. ER-specific NtogaGL1b genomes share a region encoding thiosulfate dehydrogenase (*tsdA*), cytochromes, transport of sulfur-containing compounds, lactate dehydrogenase (*ldh*), a two-component system, and a Crp-family transcription factor (Fig S8). This region may be involved in oxidizing thiosulfate as an energy source, and sensing and responding to redox changes that accompany seasonal hypoxia in Lake Erie. The corresponding region in NtogaGL1a encodes an integrase and photolyase, consistent with greater DNA photodamage in the more transparent waters of Michigan, Huron, and Ontario where NtogaGL1a is abundant. NtogaGL1a also lacks superoxide oxidase (*cybB*) found in NtogaGL1b and reference strains, suggesting these two ecotypes experience differential oxidative stress.

Great Lakes *Nitrospira* reveal adaptations to sunlit oxic environment

We reconstructed six closely related genomes of *Nitrospira* (~99% ANI; Fig S9, Table S4), representing the predominant NOB throughout Lake Superior and in parts of Michigan, Ontario, and Huron (Fig. 4; Table S1). These genomes, which we refer to as NspiraGL, fall within lineage II (Fig S9), which is broadly distributed across soil, freshwater, and engineered habitats (21); however, genome analyses to date have focused on strains from wastewater and engineered systems, leaving major blind spots. NspiraGL share core features of *Nitrospira* metabolism, including a periplasmic-facing NXR that is advantageous under substrate-limiting

conditions, multiple cytochrome *bd*-like oxidases, and the reverse TCA cycle for carbon fixation (80). However, as with *Ca. Nitrotoga*, the *Nitrospira* genomes we reconstructed in the Great Lakes are markedly smaller (~1.8 Mb) than published reference genomes (Fig. 4; Table S4), with reduced capacity for environmental sensing, transport, defense, and transposition, and a lack of pilus or flagellar motility (Table S8). NspiraGL encode just five sigma factors, compared to 18 in *Nitrospira moscoviensis*, consistent with genome streamlining theory (59). Further, NspiraGL genomes encode a single NXR, while *N. moscoviensis* carries five copies that are differentially regulated (29,82). NspiraGL also lack the *glnE* gene for glutamine synthetase (GS) adenylyltransferase, suggesting that GS activity is not repressed by this mechanism. Together, these features suggest limited regulatory and ecological flexibility, consistent with a relatively constant, oligotrophic environment.

Compared to other *Nitrospira*, NspiraGL exhibit limited energetic flexibility, but can access diverse nitrogen sources (Fig. 4, Table S8). We predict that NspiraGL are unable to grow on hydrogen or formate as alternative energy sources (25,29), as they lack NiFe-hydrogenase and formate dehydrogenase. The glycolysis and oxidative TCA cycles appear to be incomplete, lacking phosphofructokinase and citrate synthase, respectively; this suggests a limited capacity for organic carbon utilization. Regarding nitrogen metabolism, NspiraGL lack *nirK*, encoding NO-forming nitrite reductase, but maintain nitric oxide reductase (*norQD*), implying that cellular NO is formed by other mechanisms. To obtain nitrogen for biosynthesis, NspiraGL encode a high-affinity nitrate/nitrite/cyanate transporter (*nrtABC*), assimilatory nitrite reductase (*nirA*), and cyanase (*cynS*), along with two *amt* family ammonium transporters. Although none of the NspiraGL MAGs include urease (*ureCBA*), one does contain urease accessory proteins (*ureEFGD*) and two contain a urea transporter (*urtABCD*), suggesting incomplete assembly of the urea utilization pathway. As with *Ca. Nitrotoga*, we suggest that cyanase, along with urease where present, functions in nitrogen assimilation rather than cross-feeding, given the dilute environment and free-living planktonic cells.

Beyond energy, carbon, and nitrogen metabolism, we discovered striking differences between NspiraGL and reference *Nitrospira* related to DNA repair. NspiraGL encode two additional photolyase-related proteins, along with a class I cyclopyrimidine dimer (CPD) photolyase found in most reference *Nitrospira* (Fig 5, Fig S10). Photolyases use blue light energy to repair DNA lesions caused by UV radiation (83). The two additional genes in NspiraGL are adjacent and share best hits with Betaproteobacteria, suggesting recent horizontal transfer (Fig 5). One likely encodes an FeS-BCP photolyase, which repairs (6-4) dipyrimidine lesions (84,85). The other shares an FAD-binding domain with photolyases but the C-terminal region has no recognizable domains (Fig 5b). This protein is widespread in aquatic bacteria and has not been functionally characterized, though an actinobacterial homolog was suggested to be involved in light sensing and regulation (86). Beyond photolyases, NspiraGL also encode uracil-DNA glycosylase (UNG), which removes misincorporated uracil from DNA. Uracil results from deamination of cytosine, which can occur spontaneously or be induced by NO (87). In addition to the photolyases and UNG that repair DNA lesions, NspiraGL encode translesion DNA polymerase V (*umuCD*) which enables replication to proceed past lesions. Together, these genes indicate that *Nitrospira* in the Great Lakes experience significant DNA damage, including UV-induced damage that also requires light for the repair process, in hypolimnion waters with high transparency (65) and/or during seasonal mixing.

Other major differences between NspiraGL and reference *Nitrospira* are related to reactive oxygen species (ROS) (Table S8). Surprisingly, despite their oxic habitat, NspiraGL lack superoxide dismutase (SOD), monofunctional catalase (*katE*), classic regulators of oxidative stress response (*oxyR* and *perR*), and bacterioferritin, which limits the Fenton reaction by sequestering free iron. However, all six NspiraGL MAGs, but few reference genomes (7% of 75), have recently acquired bifunctional catalase-peroxidase *katG*; interestingly we also observed *katG* in Great Lakes *Ca. Nitrotoga* and *Nitrosospira* (Fig 2, Fig 4). The absence of SOD suggests that NspiraGL does not produce damaging levels of endogenous superoxide,

perhaps because NspiraGL lack the major respiratory and non-respiratory flavoproteins that produce ROS in other SOD-containing *Nitrospira* (88). Unlike superoxide, H₂O₂ can cross membranes, and is known to be produced by both photooxidation of dissolved organic matter and dark heterotrophic activity (89). The lakes where NspiraGL dominate have high water clarity (65) and low productivity, consistent with abiotic photochemistry as the primary source of exogenous ROS; this stress may have selected for *katG* as a defense, though without the canonical *oxyR* regulation. The absence of bacterioferritin could be related to iron limitation (71). NspiraGL also lack cytochrome c peroxidase, which is found in 70 of 75 reference genomes; this protein is proposed to function in anaerobic respiration of H₂O₂ (90) and therefore its absence in NspiraGL is consistent with a constant oxic environment. Together, these results indicate that *Nitrospira* in the Great Lakes face distinct ROS pressures that have shaped their gene content.

Discussion

The Laurentian Great Lakes harbor nitrifiers that are phylogenetically related, but markedly different in genome size and functional capacity, from their well-studied relatives inhabiting wastewater systems, soils, and even other freshwater systems. By examining the entire nitrifier assemblage at once, we detected common features across taxa that illuminate the selective pressures faced by microbes in deep lakes. All the lineages we describe show small genome sizes (1.3-1.8 Mb), reduced capacity for environmental sensing and response, and adaptation to a passive (i.e. non-motile) planktonic lifestyle, expanding the paradigm of streamlining observed for oceanic AOA (11) to AOB, *Nitrospira*, and *Ca. Nitrotoga* in oligotrophic freshwaters. Within the cosmopolitan *Nitrosospira*, we found ecotypes with a gradient of genome reduction that maps onto their habitats' trophic gradient: from NspGL1 (1.4 Mb, low GC, upper lakes) to NspGL2b (1.5 Mb, upper lakes) to NspGL2a (1.6 Mb, Ontario) to NspGL3 (1.8 Mb, Erie) (Fig 2). The thaumarchaeal NarchGL have markedly reduced regulatory

capacity like the open ocean strain *Nitrosopelagicus brevis* (11). The NOB NspiraGL have genomes 50-60% smaller than described *Nitrospira* and dominate, along with the AOB NspGL1 and the AOA NarchGL, the deeper more oligotrophic basins, while *Ca. Nitrotoga* favor shallower more productive basins. The emergence of Erie-specific ecotypes of both *Nitrosospira* (NspGL3) and *Ca. Nitrotoga* (NtogaGL1b) demonstrates how distinct this habitat is compared to the other lakes.

Nitrifiers inhabiting the transparent waters of the upper Great Lakes show distinctive adaptations to light including diverse photolyases, ROS detoxification, and even proteorhodopsin. This discovery is surprising, given that nitrifiers are rare in the surface mixed layer of the Great Lakes (Fig. S1) and that photoinhibition of ammonia oxidation and nitrifier growth is well documented (51-54,64). We propose that proteorhodopsin could be used to augment energy metabolism when ammonia oxidation is photoinhibited. Water clarity has increased over the past several decades in Lakes Michigan and Huron, now surpassing that of Lake Superior (65). High light penetration along with seasonal mixing likely exposes deep water cells to damaging levels of light and oxidative stress. Future cultivation and physiological studies will document photoinhibition and potential phototrophy in Great Lakes nitrifiers.

The capacity for nitrification is found across multiple phyla, and our work unveils new clues to understanding the ecological and evolutionary potential of these diverse lineages. This collective nitrifier diversity undoubtedly influences the cycling of carbon and nitrogen across this ecosystem, and future work will explore the differential contributions to nitrification by the distinct lineages we described here. Understanding what controls the diversity of nitrifiers and other key functional groups, and the consequences of this diversity for biogeochemistry, are essential for forecasting the effects of rapid environmental change across the large lakes of the world (e.g. ref. 91) and predicting impacts on the critical ecosystem services they provide (92).

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Figure Legends

Figure 1. Dissolved inorganic nitrogen availability and distribution of nitrifiers across the Great Lakes. **(a)** Oxidized nitrogen concentrations. Values include NO_x concentrations from published studies (n=128; ref. 16,36,38,93,94), US EPA Water Quality Surveys in 2012 and 2013 (n=1626 from GLENDa database), and this study (n=20) (Table S2). **(b)** Ammonium concentrations. Values are derived from the literature as in (a) (n=118) and from this study (n=20) (Table S3). **(c)** Distribution of nitrifiers across the Great Lakes. Top panel: map of sampling stations. Stars indicate stations chosen for metagenome analysis. Bottom panel: relative abundance of ammonia-oxidizing (green) and nitrite-oxidizing (pink) families based on 16S rRNA V4-V5 amplicon sequencing, sampled in the mid-hypolimnion (except western Erie, sampled 1m from bottom). Data is plotted roughly west to east as indicated on the map.

Figure 2. Genome properties and cross-lake distribution of ammonia oxidizing organisms, showing both Archaea (top) and Betaproteobacteria (bottom). Rows highlighted in green represent clusters of genomes reconstructed from the Great Lakes; expanded phylogenetic trees are shown in Figures S2 and S5. For catalase, “E” indicates monofunctional catalase *katE*; “G” indicates bifunctional catalase-peroxidase *katG*. For carbon fixation, RuBisCO type is shown in parentheses (68); HP/HB, 3-Hydroxypropionate/4-Hydroxybutyrate cycle; CBB, Calvin-Benson-Bassham cycle. Bubble plot shows composition of ammonia oxidizers in hypolimnion samples, using MAGs as probes to recruit metagenomic reads (values sum to 100% for each lake column). Genes identified in only a subset of genomes are shown as (+).

Figure 3. Evidence for proteorhodopsin (PR) in *Nitrosospira* from the Great Lakes. (a) Gene neighborhood surrounding PR in *Nitrosospira* MAG MC17_S15_bin_110 and SAG 207399. Genes are colored according to the best BLAST hit taxonomy in the NCBI nr database. (b) Alignment of predicted *Nitrosospira* PR with reference sequences. Diagnostic features are

highlighted (61,62): blue boxes, diagnostic residues for PR; pink box, residue indicative of blue or green tuning; green underline, shorter beta-sheet region in PR. Sequence accession numbers: bacteriorhodopsin P02945, actinorhodopsin A0A1D9E0H1, sensory rhodopsin P42196, *Dokdonia* PR EAQ40507.1, SAR86 blue-tuned PR Q4PP54, SAR86 green-tuned PR Q9F7P4, SAR11 PR A6YQL7.

Figure 4. Genome properties and cross-lake distribution of nitrite oxidizing taxa *Nitrospira* (top) and *Ca. Nitrotoga* (Betaproteobacteria; bottom). Rows highlighted in pink represent clusters of genomes reconstructed from the Great Lakes. Expanded phylogenetic trees are shown in Figures S2 and S6. rTCA, reductive tricarboxylic acid cycle; CBB, Calvin-Benson-Bassham cycle; ONR, octaheme nitrite reductase. Values in parentheses indicate RuBisCO type (68). Bubble plot shows composition of NOB per lake based on metagenomic read mapping. Genes identified in only a subset of genomes are indicated by (+).

Figure 5. Distinct photolyase proteins in NspiraGL. **a)** Phylogenetic tree showing families of photolyases. Three families are found in NspiraGL: CPD class I photolyase, FeS-BCP / CRYPro family, and an uncharacterized CPF-related family found in diverse Bacteria. CPD Class I photolyases are also found in other nitrifiers including *Ca. Nitrotoga* NtogaGL1a, *Nitrosospira* NspGL1, and *Nitrosoarchaeum* NarchGL. **b)** Domain structure of the three photolyase families present in NspiraGL.

Acknowledgments

Sequencing support for this project was provided by the DOE Joint Genome Institute Community Sequencing Program (CSP #1565, #503460). Computational resources were provided by the UChicago Research Computing Center. Funding for this work was provided by Illinois-Indiana Sea Grant (Grant # NA14OAR170095), UChicago Women's Board, and the National Science Foundation (OCE-1830011 to MLC). We thank Glenn Warren and the science staff in the Great Lakes National Program Office of the US EPA, and the captain and crew of the R/V *Lake Guardian*, for facilitating sample collection. We thank members of the Coleman and Waldbauer labs for assistance with sample collection and processing, and for discussion and comments on the manuscript.

Data Availability

The metagenome-assembled genomes presented here are available via NCBI BioProject PRJNA636190. 16S rRNA data is available at NCBI BioProject PRJNA591360. Metagenomes sequenced by JGI are available at <http://genome.jgi.doe.gov>, Project IDs 1045056, 1045059, 1045062, 1045065, 1045068, 1045071. Single cell amplified genome is available at img.jgi.doe.gov, IMG Genome ID 3300033241.

Competing Interests

The authors declare no competing interests.

Figure 1

Figure 2

Figure 3

Figure 4

Supplementary Information

Extended Methods

Metagenome QC and Assembly

Raw reads for spring surface samples were quality controlled at the Joint Genome Institute, using `bbduk.sh` for adapter trimming (`ktrim=r`, `minlen=40`, `minlenfraction=0.6`, `mink=11`, `tbo`, `tpe`, `k=23`, `hdist=1`, `hdist2=1`, `ftm=5`) and quality filtering (`maq=8`, `maxns=1`, `minlen=40`, `minlenfraction=0.6`, `k=27`, `hdist=1`, `trimq=12`, `qtrim=rl`). Raw reads for summer hypolimnion samples were adapter trimmed, quality filtered, and interleaved using `bbduk` (parameters: `ktrim=r`, `mink=8`, `hdist=2`, `k=21`, `forcetrimleft=10`, `forcetrimright=199`, `minlen=150`) using BBTools suite version 35.74 (<https://sourceforge.net/projects/bbmap/>). Separate assemblies of quality filtered reads were carried out for each metagenome using `metaSPAdes 3.1.11 --meta` mode using default k sizes of 21, 33, 55 (1). For mapping, forward and reverse reads were merged using `bbmerge` in BBtools, using `qtrim2=r trimq=10,13,16` and `adapter=default`. Merged short reads from all 12 metagenomes were mapped onto each assembly using `bowtie2` in `--sensitive` mode (2), and together this information was used to bin assembled contigs. Binning was performed using `MetaBAT2` (3), `Binsanity` (4) and `CONCOCT` (5) using default parameters. The resulting bins were scored, aggregated, and de-replicated using `DAS_Tool` (6) followed by manual curation using `Anvi'o` (7). We assessed genome completion and contamination of manually curated bins using `CheckM lineage_wf` (8), and all genomes presented here are greater than 70% complete and have less than 10% contamination (Table S1). Potential nitrifiers were screened by searching for ammonia monooxygenase, hydroxylamine oxidoreductase and nitrite oxidoreductase within reconstructed genomes using `blastp` (9). For bins where any of these genes were detected, we identified bacterial single copy core genes (10) or archaeal single copy core genes (11) using `HMMER` (12), as implemented in `Anvi'o`. Single copy core genes were queried against proteins predicted from bacterial and archaeal

genomes in RefSeq (13), and taxonomic identity of these core genes was ascertained based on a least common ancestor approach using a 0.1% window around the bit score of the best hit using KronaTools (14). Taxonomic assignment was further validated using GTDB-tk (15). Grouping of MAGs into clades and subclades based on ANI was carried out using fastANI (16).

Annotation and gene clustering

Genes from putative nitrifier genomes were annotated using InterProScan 5.30-69.0 (17) and eggno-mapper against the bactNOG database (18). Gene cluster analysis was carried out using the Anvi'o pangenome pipeline (19), using blastp to determine sequence similarity, ITEP to eliminate weak similarity (20) and MCL to cluster, using a minbit of 0.5, MCL inflation of 2 and minimum gene occurrence of 1 (21). Assignment of KEGG Orthology to gene clusters was carried out using GhostKOALA (22). Sigma factors were tallied by identifying gene clusters annotated with the following PFAMs: PF00309, PF03979, PF00140, PF04542, PF04539, PF04545, PF08281. Prokka (23) was used to generate GenBank file format files from MAGs and SAGs, and genoPlotR (24) was used to generate gene neighborhood maps

Gene tree construction

The NspGL1 proteorhodopsin sequence was inserted into the MicRhoDE rhodopsin tree using pplacer (25) through the MicRhoDE Galaxy pipeline (26). We then constructed a more targeted phylogenetic tree using aligned reference sequences of Supercluster III from MicRhoDE, filtered to exclude fragments shorter than 220 amino acids. To this alignment, we added NspGL1 sequences using MAFFT (27) along with high similarity sequences from NCBI nr that were not present in MicRhoDE. The tree was inferred using RaxML with model PROTGAMMALG (28). The tree was visualized in iTOL (29) and more distant clusters were collapsed for clarity.

A cyanase phylogenetic tree was created using sequences drawn from querying NtogaGL cyanase against NCBI nr using blastp, as well as sequences from references (30–32).

Sequences were aligned using MAFFT (27) and the tree was inferred using RaxML with model PROTGAMMALG (28). Tree was visualized in iTOL (29) and branches were colored based on the taxonomy of the parent genome.

Photolyase-related proteins in GL MAGs were identified by searching for the following features: K01669, COG0415, PF03441, PF00875, PF04244, SSF48173, SSF52425. Reference proteins (n=56) spanning the previously defined families of photolyases and cryptochromes (33) were obtained from UniProt, along with aquatic bacterial sequences described by Maresca and colleagues (34). The reference sequences were aligned using MAFFT (27), and sequences from GL MAGs were added using the MAFFT --addfragments option. The tree was estimated using IQ-TREE 2 (35) and visualized using iTOL (29).

Phylogenomic tree construction

All *Nitrospirae*, *Thaumarchaeota*, *Gallionellaceae* and *Nitrosomonadaceae* genomes were downloaded from Genbank (36) and included in phylogenetic trees for their respective family. Single copy core genes were extracted as described above and individually aligned at the protein level using muscle (37). Core proteins were then concatenated for each genome. Concatenated alignments were trimmed using Gblocks (38) and analyzed by RAxML 8.2.12 (28) to create a phylogenetic tree using the PROTGAMMALG model and 50 bootstraps. Trees were visualized in iTOL (29).

Proteorhodopsin assembly verification

We used several approaches to validate the presence of proteorhodopsin in assembled *Nitrosospira* genomes, to rule out the possibility of chimeric assemblies from different species. We note that proteorhodopsin-containing contigs were independently assembled and binned together with core *Nitrosospira* contigs from seven different samples (i.e. each sample was

assembled and binned separately, rather than co-assembled). In five of seven cases, proteorhodopsin and retinal biosynthesis genes were assembled together with core *Nitrosospira* genes on the same contig. To rule out a systematic reproducible error in assembly and/or binning, we compared these seven MAGs to a single cell amplified *Nitrosospira* genome (SAG) from Lake Michigan, obtained as part of another project with the JGI. This SAG was processed through JGI's standard decontamination pipeline and manually investigated to ensure lack of contamination. We found no evidence of contaminating core genes, as all core genes had best hits to either *Nitrosospira* or more generally *Nitrosomonadaceae* in nr. SAG contigs were matched to homologous contigs from NspGL1 MAGs to determine if any SAG contigs were unique using FastANI (16) with --visualize flag. All contigs from this *Nitrosospira* SAG were found within an NspGL1 MAG. Bandage (39) was used to manually inspect the assembly graph around the contig that contained the NspGL1 *Nitrosospira* proteorhodopsin to ensure that the assembled contig did not represent a chimeric contig or inappropriate scaffolding. We verified that a single, unique path exists from the beginning to the end of the NspGL1 contig containing proteorhodopsin (Fig 3, Fig S4). Further, we verified that consistent coverage across this contig existed by mapping short reads from the original sample using bowtie2 (2) and viewing results using Integrated Genomics Viewer (40). A closely related assembly of the same genomic region from Lake Biwa did not show evidence of proteorhodopsin; to confirm this difference between the Biwa and Great Lakes MAGs, we mapped reads from Lake Biwa (41) (BioProject PRJDB6644) onto the assembled contig described above (Fig 3, Fig S4) using bowtie2 (2). This analysis demonstrated that while a large fraction of the NspGL1 contig in question recruited reads from Lake Biwa at high identity (98-99%), starting upstream of proteorhodopsin and retinal biosynthesis, this contig no longer recruited reads from Lake Biwa.

Manual identification of key nitrification genes

Despite recovery of 15 high completion MAGs in NspGL1/2a/2b/3, many of these MAGs

lacked key nitrification genes in *amo* and *hao* operons. This was largely due to the fact that *amo* and *hao* operons were often assembled on small contigs below the minimum size cutoff we imposed for binning contigs. Difficulty in assembling these contigs was likely in part due to the several *amo* and *hao* operons with extremely high identity to one another in each genome, a phenomenon which has been observed in other *Nitrosospira* (42). Manual assembly graph inspection with Bandage (39) supported this hypothesis, as did assessment of abundance of short reads associated with *amo* operons from NspGL and comparison of abundance of short reads associated with core gene *rpoB* from NspGL, using ROcker (43). Still, an exemplar MAG from at least one representative of each ecotype (NspGL1/2a/2b/3) was found with both *amo* and *hao* operons. Further, manual inspection of unbinned contigs confirmed that *amo* and *hao* operons existed on contigs in every sample from which a MAG for a particular ecotype was recovered. That is, for every time that an NspGL1 MAG was recovered from a sample, we were able to determine that an *amo* and *hao* operon which could be affiliated with NspGL1 existed, even if it was not correctly binned. Affiliation for these unbinned key nitrification genes was carried out by alignment of *amoAB* and *haoAB* sequences to *amoAB* and *haoAB* sequences correctly binned in NspGL ecotypes. This process was also carried out for two NtogaGL1a MAGs for *nxrAB*, which were poorly assembled in those two samples.

Data analysis and plots

All data were analyzed in R version 3.5.3 (44) and plots were generated using ggplot2 (45).

Figure S1. Relationships between environmental factors and relative abundance of nitrifiers in the Great Lakes. Relative abundance based on 16S rRNA V4-V5 region amplicon sequencing from Summer 2012. Nitrifier abundance includes sequences assigned to the families *Nitrosopumilaceae* (AOA), *Nitrosomonadaceae* (AOB), *Nitrospiraceae* (NOB), and *Gallionellaceae* (NOB). (a) Relative abundance of nitrifiers (% of total community) compared to photosynthetically active radiation (PAR). (b) Correlation between the relative abundances of nitrite oxidizers and ammonia oxidizers in each sample. A 1:1 line (solid) and linear regression fit (dashed) are shown for reference. (c) Relative abundance of nitrifiers (% of total community) compared to chlorophyll *a* concentration. PAR and chlorophyll data from the EPA Great Lakes Environmental Database (<https://cdx.epa.gov/>).

Figure S2. Phylogenomic tree of the *Nitrosomonadales* order of *Betaproteobacteria*, focusing on the *Nitrosomonadaceae* and *Gallionellaceae* families. Tree was constructed using 139 concatenated single-copy core genes (10). Colored squares indicate the environment of origin; colored circles denote the lake of origin for genomes from the Laurentian Great Lakes; yellow stars indicate clades containing strains that lack nitrosocyanin.

Figure S4. Shared synteny surrounding the proteorhodopsin module in *Nitrospira* draft genomes from deep lakes. Light blue, shared genes on reverse strand; dark blue, shared genes on forward strand; pink, tRNA genes; orange, PR module in NspGL1 MAG; gray, genes unique to one MAG.

Figure S5. Phylogenomic tree of *Nitrosopumilaceae* and *Cenarchaeum*. Tree was constructed using concatenated single-copy core genes as defined in (11). This tree represents a zoomed in view of *Nitrosopumilaceae* and *Cenarchaeum* from larger tree of all *Thaumarchaeota* and *Aigarchaeota*. Colored boxes correspond to the environment of origin; genomes from the Laurentian Great Lakes are indicated by colored circles. Names in bold indicate cultured isolates or enrichments.

Figure S6. Phylogenomic tree of *Thaumarchaeota* and *Aigarchaeota*. Tree was constructed using concatenated single-copy core genes from (11), and all available genomes in Genbank as of February 6 2020. Colors denote clades described in (46). Star indicates placement of NarchGL.

Figure S8. Gene content distinguishing NtogaGL1a and NtogaGL1b. Genes colored in blue are shared between both genomes; orange colored genes are unique to either genome.

Figure S9. Phylogenomic tree of Lineage I and II *Nitrospira*. Tree was constructed using concatenated single-copy core genes described in (10). Colored boxes correspond to the environment of origin; genomes from the Laurentian Great Lakes are indicated by circles color coded by lake of origin.

Figure S10. Gene neighborhood surrounding photolyase genes in NspiraGL, compared to reference *N. moscoviensis*. Genes shared between the two genomes are colored light blue (protein coding) or magenta (rRNA, tRNA); non-shared genes are gray; photolyase genes are colored to match the tree in Figure 5.

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