Effect of freshwater mussels on the vertical distribution of anaerobic ammonia oxidizers and other nitrogen-transforming microorganisms in upper Mississippi river sediment

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ABSTRACT

Targeted qPCR and non-targeted amplicon sequencing of 16S rRNA genes within sediment layers identified the anaerobic ammonium oxidation (anammox) niche and characterized microbial community changes attributable to freshwater mussels. Anammox bacteria were normally distributed (Shapiro-Wilk normality test, W-statistic $= 0.954$, $p = 0.773$) between 1 and 15 cm depth and were increased by a factor of 2.2 ($p < 0.001$) at 3 cm below the water-sediment interface when mussels were present. Amplicon sequencing of sediment at depths relevant to mussel burrowing (3 and 5 cm) showed that mussel presence reduced observed species richness ($p = 0.005$), Chao1 diversity ($p = 0.005$), and Shannon diversity ($p < 0.001$), with more pronounced decreases at 5 cm depth. A non-metric, multidimensional scaling model showed that intersample microbial species diversity varied as a function of mussel presence, indicating that sediment below mussels harbored distinct microbial communities. Mussel presence corresponded with a 4-fold decrease in a majority of operational taxonomic units (OTUs) classified in the phyla Gemmatimonadetes, Actinobacteria, Acidobacteria, Plantomycetes, Chloroflexi, Firmicutes, Crenarcheota, and Verrucomicrobia. 38 OTUs in the phylum Nitrospirae were differentially abundant ($p < 0.001$) with mussels, resulting in an overall increase from 25% to 35%. Nitrogen (N)-cycle OTUs significantly impacted by mussels belonged to anammmox genus *Candidatus* Brocadia, ammonium oxidizing bacteria family Nitrosomonadaceae, ammonium oxidizing archaea genus *Candidatus* Nitrososphaera, nitrite oxidizing bacteria in genus *Nitrospira*, and nitrate- and nitrite-dependent anaerobic methane oxidizing organisms in the archaeal family "ANME-2d" and bacterial phylum "NC10", respectively. Nitrosomonadaceae (0.9-fold ($p < 0.001$)) increased with mussels, while NC10 (2.1-fold ($p < 0.001$)), ANME-2d (1.8-fold ($p < 0.001$)), and *Candidatus* Nitrososphaera (1.5-fold ($p < 0.001$)) decreased with mussels. Co-occurrence of 2-fold increases in *Candidatus* Brocadia and *Nitrospira* in shallow sediments suggests that mussels may enhance microbial niches at the interface of oxic–anoxic conditions, presumably through biodeposition and burrowing. Furthermore, it is likely that the niches of *Candidatus* Nitrososphaera and nitrite- and nitrate-dependent anaerobic methane oxidizers were suppressed by mussel biodeposition and sediment aeration, as these phylotypes require low ammonium

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concentrations and anoxic conditions, respectively. As far as we know, this is the first study to characterize freshwater mussel impacts on microbial diversity and the vertical distribution of N-cycle microorganisms in upper Mississippi river sediment. These findings advance our understanding of ecosystem services provided by mussels and their impact on aquatic biogeochemical N-cycling.

Subjects Aquaculture, Fisheries and Fish Science, Ecosystem Science, Marine Biology, Microbiology, Molecular Biology
Keywords Freshwater mussels, Anaerobic ammonia oxidizers, Anammox, Nitrogen cycle, Sediment microbiology

INTRODUCTION

Native freshwater mussels (Order Unionida) are ecosystem engineers that significantly alter benthic habitats through biodeposition of feces and pseudofeces, rich in ammonium (NH_4^+) and organic carbon (C), into sediment (*Thorp et al., 1998*; *Vaughn & Hakenkamp, 2001*; *Bril et al., 2014*). The estimated mussel filtration capacity in a 480 km, Upper Mississippi River (UMR) segment, as a percentage of river discharge, is up to 1.4% at high flows, up to 4.4% at moderate flows and up to 12.2% during low flows (*Newton et al., 2011*). The mussels in this river segment collectively filter over 14 billion gallons of water, remove tons of biomass from the overlying water, and deposit tons of reduced C and nitrogen (N) at the water-sediment interface each day (*Newell, 2004*). The pocketbook mussel (*Lampsilis cardium*) and threeridge mussel (*Amblema plicata*) comprise up to 38% and 56% of the mussel biomass in the UMR, respectively (*Newton et al., 2011*). A habitat near Buffalo, Iowa, in UMR Pool 16, had mean densities of 1.56 *L. cardium*-m^{-2} and 7.18 *A. plicata*-m^{-2} that correlated with fine sediment diameters ($d_{50} = 0.300 \pm 0.121$ mm) which were presumably influenced by mussel burrowing (*Young, 2006*). Mussels live primarily buried in sediment, with their posterior end often flush with the sediment surface (*Haag, 2012*), or slightly below the surface in soft sediments (*Allen & Vaughn, 2009*; *Allen, 1923*; *Matteson, 1955*). This positions adult freshwater mussels 6–10 cm into the sediment with tendencies toward more shallow burrowing during the spring and summer (*Schwalb & Pusch, 2007*). Extensive observations in the UMR concluded that *A. plicata* were often found with portions of their shell above the water-sediment interface, while *L. cardium* burrow a few cm into the sediment during the summer (*Newton, Zigler & Gray, 2015*). Additionally, *A. plicata* often burrowed up to 2.5 cm vertically (*Allen & Vaughn, 2009*) in response to stressors while *L. cardium* moved more horizontally when stressed (*Newton, Zigler & Gray, 2015*). Two common stressors, that happen to be created by the mussels themselves, are low dissolved oxygen (DO) and elevated ammonia (NH_3) and NH_4^+ (*Bril et al., 2017*; *Haag, 2012*). We hypothesize that this frequent vertical and horizontal movement by mussels, many times as an indirect and/or direct response to their own waste production, has a significant impact on porewater chemistry and microbiology in UMR sediments.

The evidence for freshwater mussel impacts on aquatic chemistry is compelling, especially for nutrients. A dense mussel population can sequester 2 g C $day^{-1}m^{-2}$, 200 mg N day

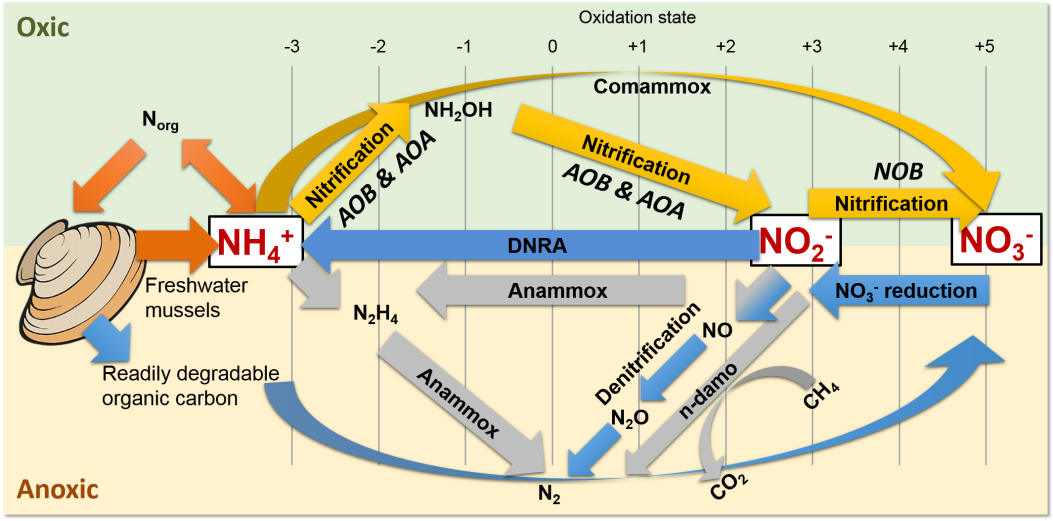

Figure 1 Freshwater mussels deposit feces and pseudofeces containing nitrogen and carbon at the water-sediment interface (i.e., oxic-anoxic transition). NH_4^+ resulting from mussel biodeposits may be oxidized via nitrification and comammox in oxic conditions (yellow arrows), and/or by anammox and n-damo near the oxic-anoxic interface (gray arrows). Oxidized nitrogen species (NO_2^- and NO_3^-) may be reduced by dissimilatory nitrate reduction to ammonium (DNRA) and denitrification pathways (blue).

$^{-1}m^{-2}$, and 50 mg phosphorus $day^{-1}m^{-2}$ from river water into sediment (*Strayer, 2014*). During the summer months, biodeposition-derived N from mussels was roughly 67% NH_4^+, 28% amino acids, and 5% urea (*Bayne, 1973*). Mussel biodeposition accounted for up to 40% of total N demand in freshwaters and up to 74% of N in the food web, but was sometimes dampened (*Atkinson, Kelly & Vaughn, 2014*) in high nutrient environments (*Atkinson, Kelly & Vaughn, 2014*). Our previous work showed mussel burrowing and biodeposition, just below the water-sediment interface, increased porewater NH_4^+, nitrate (NO_3^-), nitrite (NO_2^-), and total organic C concentrations by 160%, 38%, 40%, and 26%, respectively (*Bril et al., 2017*; *Bril et al., 2014*). However, the experimental design of our previous work limited our ability to assess the effects of mussels on the broad microbial community that was transforming N simultaneously and, quite likely, synergistically.

The UMR is an N-rich agro-ecosystem (*Hill et al., 2011*; *Hill et al., 2008*; *Houser & Richardson, 2010*; *Ikenberry et al., 2014*; *Schilling, Wolter & McLellan, 2015*) shown to foster high microbial N transformations (*Mason et al., 2016*; *Millar et al., 2015*) potentially making the effects of mussels on a variety of N-transforming bacteria and archaea more pronounced than in any other freshwater environment. The first step in transforming biologically active N is nitrification by aerobic ammonium oxidizing bacteria (AOB) (Fig. 1, yellow arrows), such as the genera *Nitrosomonas* and *Nitrosospira* in the Nitrosomonadaceae family (*Aakra et al., 2001*; *Burrell, Phalen & Hovanec, 2001*; *Hayatsu, Tago & Saito, 2008*; *Prosser, Head & Stein, 2014*), and aerobic ammonium oxidizing archaea (AOA) in multiple candidate genera. AOB and AOA are metabolically diverse (*Leininger et al., 2006*) and serve a functionally important role of catalyzing the rate limiting step of nitrification (*Martens-Habbena et al., 2009*) in various freshwater niches. For example, *Candidatus*

Nitrososphaera (phylum Thaumarchaetoa), a group of thermophilic AOA (*Hatzenpichler et al., 2008*), and AOB species in genera *Nitrosospira* and *Nitrosococcus* (*Koper et al., 2004*) can use urea as an alternative source of NH_4^+ (*Spang et al., 2012*). AOA often outnumber AOB (*Prosser & Nicol, 2008*) due to their ability to grow at NH_4^+ concentrations below 10 nM (*Martens-Habbena et al., 2009*), compared to 10 μM for some AOB species (*Bollmann, Bar-Gilissen & Laanbroek, 2002*). In the second step of nitrification, nitrite oxidizing bacteria (NOB), such as *Nitrospira* (phylum Nitrospirae), and *Nitrobacter*, *Nitrococcus*, and *Nitrospina* (phylum Proteobacteria) (*Prosser, Head & Stein, 2014*), aerobically oxidize NO_2^- to NO_3^- (Fig. 1, yellow arrows).

Nitrospira are the most abundant and diverse group of NOB and dominate numerous habitats, ranging from freshwater sediment to engineered wastewater treatment plants (*Daims et al., 2015*; *Koch et al., 2015*; *Lucker et al., 2010*). Furthermore, NOB species *Nitrospira moscoviensis* and *Nitrospira lenta* can derive NH_4^+ from urea hydrolysis, provide NH_4^+ to AOB, and subsequently may oxidize NO_2^- from AOB in a process deemed "reciprocal feeding of nitrifiers" (*Daims et al., 2015*; *Koch et al., 2015*). *Candidatus* Nitrospira inopinata, can also use urea as an alternative NH_4^+ source (*Daims et al., 2015*) and has all the genes necessary for complete ammonia oxidation (comammox) to NO_3^- (*Van Kessel et al., 2015*) (Fig. 1, yellow curved arrow). Denitrifiers complete the conventional N-cycle by sequentially reducing NO_3^- to nitric oxide (NO), nitrous oxide (N_2O), and nitrogen gas in anoxic and high C environments (Fig. 1, blue arrows) (*Hayatsu, Tago & Saito, 2008*).

A specialized group of bacteria in the phylum Planctomycetes, anaerobic ammonium oxidizing (anammox) bacteria, oxidize NH_4^+, utilize NO_2^- as a terminal electron acceptor, and produce N_2 gas (*Kartal et al., 2011*; *Kuenen, 2008*) (Fig. 1, gray arrows). Anammox bacteria thrive at the interface of oxic–anoxic conditions due to dependence on NO_2^- production by AOB or AOA (*Thamdrup, 2012*). Anammox and NOB compete for NO_2^- in low substrate environments, and this is especially true for *Nitrospira* NOB, which share a homologous form of the key enzyme catalyzing NO_2^- oxidation with anammox (*Lucker et al., 2010*). In another example, *N. moscoviensis* can adapt to a range of oxygen concentrations by coupling formate oxidation and NO_3^- reduction (*Koch et al., 2015*). Recently, an N-cycling enrichment culture revealed comammox bacteria co-occurring with anammox bacteria in the genus *Candidatus* Brocadia, presumably enhanced by the ability of commamox organisms to oxidize NH_4^+ in low oxygen conditions (<3.1 μM) (*Van Kessel et al., 2015*). *Nitrospira* species, *N. moscoviensis* and "*Ca.* Nitrospira inopinata" in particular, are examples of NOB which harbor a unique ability to assist or compete with anammox for N-substrate in a variety of niches (*Lucker et al., 2010*).

Shallow sediments also pose a competitive niche for anammox bacteria because of high NH_4^+ fluxes into oxic sediment and NO_2^- limitations from denitrification (*Thamdrup, 2012*; *Trimmer & Engstrom, 2011*). Another addition to the suite of known N-transformations includes prokaryotic coupling of anaerobic oxidation of methane with denitrification (*Raghoebarsing et al., 2006*). In nitrite- and nitrate-dependent anaerobic methane (CH_4) oxidation (n-damo) (*Raghoebarsing et al., 2006*; *Thamdrup, 2012*; *Welte et al., 2016*), NO_3^- reduction to NO_2^- and NO_2^- reduction to N_2 are coupled with

CH_4 oxidation to CO_2 (Fig. 1, gray line and curved arrows). Nitrate-damo biochemical processes have been linked to family "ANME-2d" (*Ding et al., 2016*; *Haroon et al., 2013*), while nitrite-damo was discovered for "*Candidatus* Methylomirabilis oxyfera"(*Ettwig et al., 2010*) in phylum "NC10" (*Ettwig et al., 2009*; *Luesken et al., 2011*; *Padilla et al., 2016*), and both are widespread in anoxic freshwater sediments (*Deutzmann & Schink, 2011*; *Ding et al., 2016*; *Ettwig et al., 2009*; *Hu et al., 2009*).

Mollusks have been shown to influence the diversity of microbial communities and abundance of N-transforming microorganisms. For example, metagenomic profiling revealed a marine California mussel (*Mytilus californianus*) shell provided a niche for N- and C-transforming microorganism populations (*Pfister, Meyer & Antonopoulos, 2010*), and a restored oyster reef enhanced nitrification and denitrification rates greater than 10-fold (*Kellogg et al., 2013*). Furthermore, an experimental microcosm study reported enhanced prokaryotic metabolic activity and diversity following a biodeposition rate of 10 g m^{-2} d^{-1} of mussel feces and pseudofeces (*Pollet et al., 2015*). Additionally, clusters of the zebra mussel (*Dreissena polymorpha*) in a lake increased heterotrophic bacteria density, activity, and diversity (*Lohner et al., 2007*). Since the impact of native freshwater mussels on prokaryotic diversity and abundance in the UMR is largely unknown, this study utilized targeted and non-targeted sequencing of the 16S rRNA gene to determine how N-transforming microorganisms and microbial community structure differs in sediments with mussels compared to sediments without mussels.

MATERIALS AND METHODS

UMR sediments were collected within a dense, well-characterized mussel assemblage in the Buffalo Habitat of UMR Pool 16 (*Young, 2006*) (41.452804, −90.763299), and from a slightly up-river location with no mussels (41.451540, −90.753275) using a 3-inch diameter, hammer-driven, acrylic tube (Batch 1 samples) or a 2-inch diameter, post-driver sediment sampler with a polypropylene liner (Multi-Stage Sediment Sampler, Batch 2 samples; Art's Manufacturing and Supply, Inc., American Falls, ID, USA). Batch 1 sediment was used to identify the vertical distribution of anammox bacteria below freshwater mussels. For Batch 1, the acrylic tube for each core ($n = 3$ with-mussels) was penetrated at 1, 3, 5, 7, 11, and 15 cm sediment depths with a 3/8th-inch diameter, ethanol flame-sterilized drill bit to enable sediment collection. In comparison, Batch 2 sediment was used to characterize anammox abundance, microbial diversity, and community structure in shallow sediments below mussels. For Batch 2, the polypropylene liner for each sediment core ($n = 5$ with-mussels, $n = 5$ no-mussels) was penetrated at depths of 3 cm and 5 cm. Sediment was sampled for DNA isolation (in quadruplicate) for a combined sample size of $n = 20$ for 3 cm depth with-mussels, $n = 20$ for 5 cm depth with-mussels, $n = 20$ for 3 cm depth without mussels, and $n = 20$ for 5 cm depth without mussels. Genomic DNA was isolated from 0.25 g of each sediment sample (PowerSoil® DNA Isolation Kit; MoBio Laboratories, Inc., Carlsbad, CA, USA) and stored at −20 °C. Batch 2 Genomic DNA was used for anammox-targeted qPCR ($n = 20$ for each treatment) and 16S rRNA gene amplicon sequencing ($n = 10$ for each treatment).

Anammox 16S rRNA gene quantification

Microbial culture from a sidestream deammonification process (Hampton Roads Sanitation District, Virginia Beach, VA, USA) served as a source of anammox genetic material for qPCR standard curve construction. PCR products (primers A483f (5′-GTCRGGAGTTADGAAATG-3′) and A684r (5′-ACCAGAAGTTCCACTCTC-3′) (*Sonthiphand & Neufeld, 2013*)) of the anammox 16S rRNA gene was purified with Qiaquick PCR purification Kit (Qiagen Inc.; Valencia, CA, USA), and cloned into the pCR 2.1-TOPO® vector using the TOPO® TA cloning Kit (Invitrogen Corp.; Carlsbad, CA, USA). Clones were Sanger sequenced at the University of Iowa Institute of Human Genetics with M13F (5′-TGTAAAACGACGGCCAGT-3′) and M13R (5′-CAGGAAACAGCTATGAC-3′) primers to ensure anammox 16S rRNA PCR products were inserted into the vector. Nucleotide sequences were aligned using the Standard Nucleotide Basic Local Alignment Search Tool (*Altschul et al., 1997*) (GenBank Accession: KU047953) and classified as *Candidatus* Brocadiales (of the Planctomycetes phylum) with a 95% confidence threshold using RDP Naïve Bayesian rRNA Classifier Version 2.10 (*Wang et al., 2007*). Plasmid DNA concentration was quantified with Qubit® Fluorometer 1.0 (Thermo Fisher Scientific, Inc.; Waltham, MA, USA), serially diluted, and used to construct qPCR calibration curves.

The anammox 16S rRNA gene from batches 1 and 2 was quantified (*Wang et al., 2015*) with qPCR using QuantStudio^{TM} 7 Flex Real-Time PCR System (Thermo Fisher Scientific, Inc.; Waltham, MA, USA) with primers A483f and A684r (*Sonthiphand & Neufeld, 2013*) and analyzed with QuantStudio^{TM} Real-Time PCR Software (Thermo Fisher Scientific, Inc.; Waltham, MA, USA). The threshold cycle (C_t) curves were satisfactory (slope $= -3.374$, Y-int $= 36.702$, $R^2 = 0.998$, and amplification efficiency $= 97.99\%$), and PCR product dissociation curves revealed singe peaks centered at a melting temperature of 83 °C. The statistical significance of 16S rRNA gene copies was determined via a one-way, repeated measures analysis of variance (ANOVA) (SigmaPlot 13.0, Systat Software, Inc., Chicago, IL, USA) between the 4 treatment groups ($n = 20$) following a passed normality test ($p = 0.826$, Shapiro–Wilk) and an equal variance test ($p = 0.073$, Brown-Forsythe). Pairwise multiple comparison procedures were completed via the Holm-Sidak method with a significance level of 0.050 and a power of 0.990.

Non-targeted amplicon sequencing of the 16S rRNA gene

Batch 2 genomic DNA (20 μL, 1–50 ng/μL) was analyzed by the Argonne National Laboratory, Environmental Sample Preparation and Sequencing Facility (ESPSF) utilizing the Earth Microbiome Project protocol (http://www.earthmicrobiome.org/emp-standard-protocols/16s/). All samples were analyzed together in one batch. The v4 region of prokaryotic 16S rRNA gene (515F-806R) was amplified using the following conditions: 3 min at 94 °C, 35 cycles of 94 °C for 45 s, 50 °C for 60 s, and 72 °C for 90 s, followed by 10 min at 72 °C (*Caporaso et al., 2012*). The PCR mixture consisted of 13.0 μL PCR grade water, 10.0 μL 5 PRIME HotMasterMix (Quanta Biosciences, Beverly, MA, USA), 1.0 μL genomic DNA, and 0.5 μL forward and reverse primers (10 μM). 16S rRNA gene amplicon libraries were sequenced by ESPSF using Illumina MiSeq paired end reads (2 × 151 bp)

(*Caporaso et al., 2012*) and uploaded to MG-RAST (ID's: 4705672.3–4705709.3) and NCBI (BioProject ID PRJNA374585).

Determining the operational taxonomic units ("OTUs") in each sample from the raw 16S rRNA gene amplicon reads was accomplished using the default Quantitative Insights into Microbial Ecology (QIIME) open-reference pipeline (*Navas-Molina et al., 2013*). Briefly, the QIIME open-reference pipeline takes paired-end reads as input, which are then joined, demultiplexed, filtered, and clustered into OTUs with uclust (*Edgar, 2010*). Representative sequences from each cluster were aligned (*Caporaso et al., 2010*) to GreenGenes 13.5 reference database (*DeSantis et al., 2006*) with a 97% similarity threshold. RDP classifier (*Wang et al., 2007*) was used for taxonomy assignment, PyNAST (*Caporaso et al., 2010*) was used for multiple sequence alignment. Phylogenetic trees were constructed using FastTree2.1.3 with default settings (*Price, Dehal & Arkin, 2010*). The OTU table from QIIME open reference picking ('otu_table_mc2_w_tax_no_pynast_failures_json.biom' in the standard QIIME workflow) was imported into R using the phyloseq package (*McMurdie & Holmes, 2013*) for downstream analysis, along with the corresponding phylogenetic tree ('rep_set.tre') and a metadata mapping file. These datasets were merged to create a single 'physeq' object representing the experiment. Alpha-diversity was calculated on the unfiltered OTU abundance data using the Observed species, Chao1 (*Chao & Chiu, 2001*), and Shannon (*Li et al., 2011*) metrics. Beta-diversity was calculated using a matrix of Bray-Curtis (*Bray & Curtis, 1957*) intersample distances and ordination plots calculated with non-metric multidimensional scaling (NMDS). Differential abundance analysis was carried out using the DESeq2 (*Love, Huber & Anders, 2014*) R package with default settings (test type was "Wald," fit type was "parametric"). Translating physeq objects into a compatible DESeq2 object was performed with the "phyloseq_to_deseq2" function. The complete data analysis R script can be downloaded from the public GitHub repository: https://github.com/mchimenti/black_chimenti_just_phyloseq/blob/master/phyloseq.r.

Analysis at the OTU level provided a fine scale resolution for significant differences in microbial ecology between mussel and no mussel treatments. To put these results into a biological context, the genus-level OTU file was used to compare relative abundances for N-cycle phylotypes. These groups include AOA genus *Candidatus* Nitrososphaera, nitrate-damo family "ANME-2d", NOB genus *Nitrospira*, anammox genus *Candidatus* Brocadia, AOB family Nitrosomonadaceae, and nitrite-damo phylum "NC10". Relative abundance counts for each N-cycle group was tested for statistical significance between treatments, using metadata groups "3 cm with-mussels" ($n = 10$), "5 cm with-mussels" ($n = 10$), "3 cm no-mussels" ($n = 10$), and "5 cm no-mussels" ($n = 10$). 1-way ANOVA's of each N-cycle group was performed using the Kruskal–Wallis test ($p < 0.05$) with Dunn's multiple correction test (Padj < 0.05) (GraphPad Prism 7.0; La Jolla, CA, USA). Similarly, multiple comparisons were made between all N-cycle phylotype groups and their respective treatments ($n = 10$); significant differences between relative abundances were tested using the Kruskal–Wallis test ($P < 0.0001$) and Dunn's multiple comparison test (Padj < 0.05).

RESULTS

Anammox-targeted 16S rRNA gene quantification

The targeted 16S rRNA gene data from Batch 1 ($n = 3$, with-mussels) indicated an anammox bacterial gene copy maximum ($\sim 3 \times 10^5$ copies g^{-1} sediment) between 3 cm and 7 cm sediment depth in the presence of mussels (Fig. 2A). The Batch 1 data was normally distributed between 1 cm and 15 cm (Shapiro–Wilk normality test, W-statistic = 0.954, $p = 0.773$). Only one sediment core went beyond 7 cm leaving anammox bacterial gene copy data at 11 cm and 15 cm without replicates. The Batch 2 data ($n = 20$ for 3 cm with-mussels, $n = 20$ for 5 cm with-mussels, $n = 20$ for 3 cm no-mussels, $n = 20$ for 5 cm no-mussels) showed that anammox bacteria experienced a 2.2-fold increase ($p < 0.001$) at 3 cm with-mussels compared to the no-mussels control (Fig. 2B). The anammox gene copies measured at 5 cm were statistically indistinguishable between the with-mussels and no-mussels treatments.

Non-targeted sequencing of the 16S rRNA gene

Summing across all samples, a total of 2,103,661 amplicon sequences were analyzed and about 76,000 unique OTUs were reported by QIIME. Of the unique OTUs, 18,777 had 10 or more reads and 3,916 OTUs had counts exceeding 100 reads. Mussel bed samples had read counts of 45,290 (±15,271) at 3 cm sediment depth and 52,451 (±7,044) at 5 cm sediment depth, while no-mussel samples had 48,920 (±7,517) read counts at 3 cm depth and 63,706 (±25,379) at 5 cm sediment depth (read depths depicted in Fig. S1). The top phyla in mussel bed sediments were Proteobacteria (40.7%), Nitrospirae (35.2%), Chloroflexi (5.9 %), Euryarchaeota (5.0%), Chlorobi (4.2%), and Bacteroidetes (2.3%). Proteobacteria decreased by about 6% with mussels while Nitrospirae increased by 10% with mussels. The most abundant taxonomic families in the Nitrospirae phylum were *Thermodesulfovibrionaceae* (55%), "FW" (33%), and Nitrospiraceae (13%), and were 5% less, 3% and 2% greater than in no-mussel samples, respectively. With mussels, Proteobacteria taxonomic classes consisted of the following proportions: 68% Deltaproteobacteria (8% less than without-mussels), 16% Gammaproteobacteria, and 15% Betaproteobacteria. A majority of these Deltaproteobacteria OTUs were from "BPC076", Desulfarculales, and Syntrophobacterales taxanomic orders, while orders Burkholderiales and Xanthomonodales made up a majority of Betaproteobacteria and Gammaproteobacteria taxons.

Species richness was analyzed using three common measures: Observed species, Chao1 and Shannon indices ($n = 20$ with-mussels and $n = 20$ without mussels). Together, the three measures indicated a decrease in microbial community richness and evenness in the presence of mussels as compared to sediments without mussels (Fig. 3A). The observed decrease in alpha-diversity reached significance for each of the three measures tested ($p = 0.0054$ or lower). A similar result was obtained when calculating alpha-diversity measures in samples exclusively from 3 cm ($n = 10$) or exclusively from 5 cm ($n = 10$)

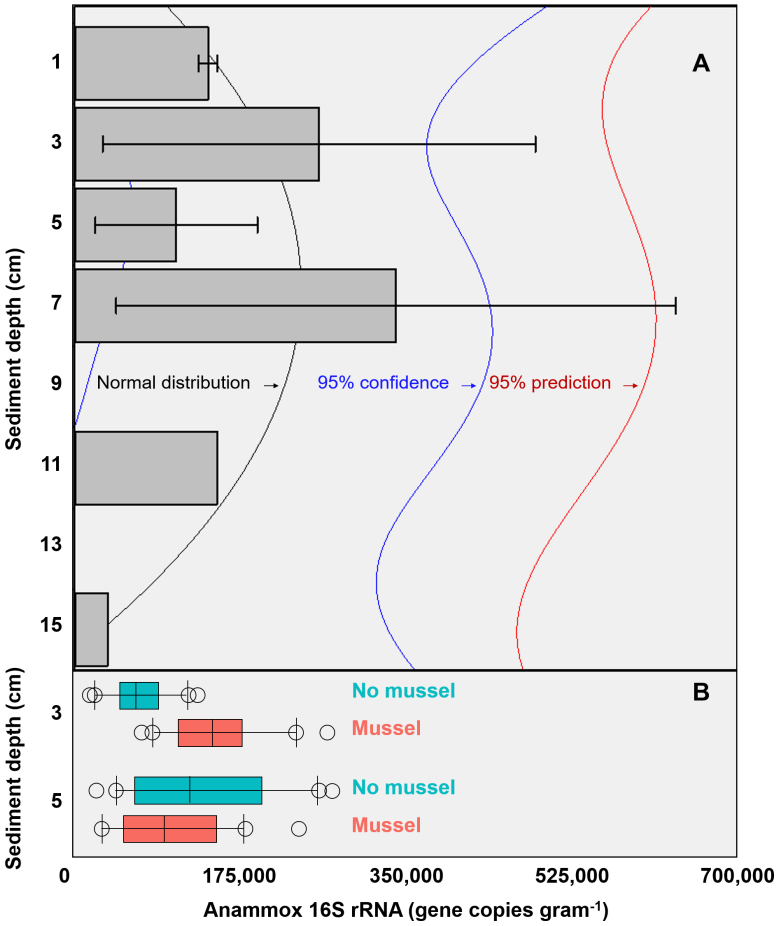

Figure 2 (A) The mean anammox 16S rRNA gene copies (per gram of sediment) in the presence of mussels were normally distributed (Shapiro–Wilk normality test, W-statistic = 0.954, $p = 0.773$) with depth (Batch 1 data). Error bars represent 1 standard deviation from the mean. (B) Mussels (salmon-colored data) significantly increased the anammox 16S rRNA gene copies at 3 cm depth ($p < 0.001$; Batch 2 data). The anammox gene copies were statistically indistinguishable with mussels as compared to the no mussels (turquoise-colored data) sediments at 5 cm (Batch 2 data). The outer most open circles in Fig. 2B represent data outliers, box boundaries represent the 25th and 75th percentile, the line within the box is the median, and error bars indicate 10th and 90th percentiles.

depths in the presence and absence of mussels. However, the decrease in richness was more pronounced at 5 cm than at 3 cm depth (Figs. S2 and S3).

To compare intersample diversity in species abundances and community composition ("beta diversity"), we employed NMDS scaling to accurately visualize, in 2D space, the higher-order community structure between with-mussels and no-mussels samples (Fig. 3B). The NMDS model produced an excellent representation of the bray–curtis distances for all samples (convergence in 20 iterations, stress ~ 0.06; shepard plot shown in Fig. S4). The beta diversity clearly differentiated as a function of mussel presence, but not sediment depth (Fig. 3B). Taken together, these data show that mussel presence had a pronounced influence on the microbial community evenness, richness, and composition within the sediment.

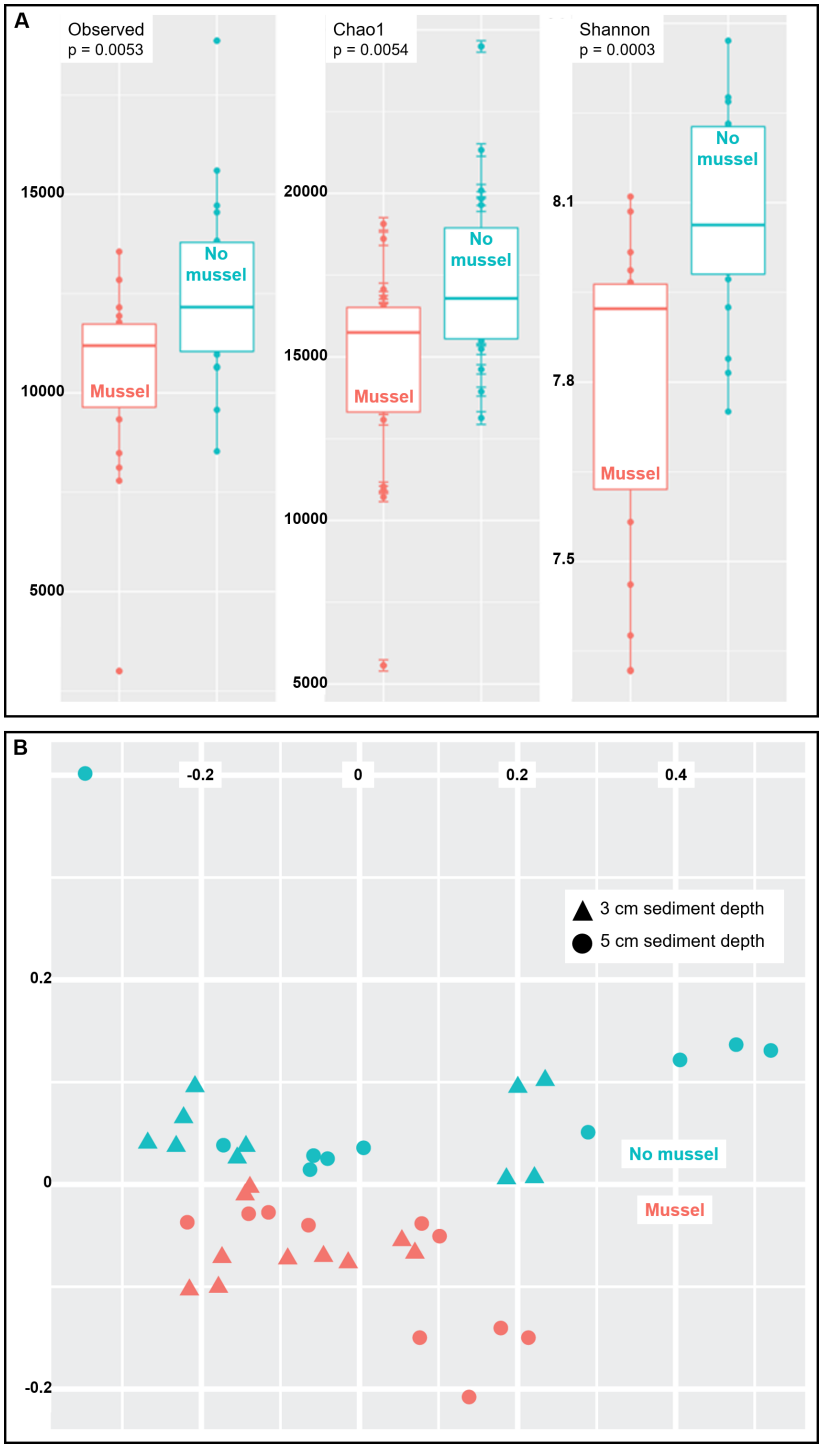

Figure 3 (A) Sediments with mussels have lower observed species richness ($p = 0.005$), Chao1 diversity ($p = 0.005$), and Shannon ($p = 0.0003$) diversity than no-mussel sediments. (B) NMDS analysis using Bray-Curtis distances revealed sample clustering as a function of mussel presence, but not sediment depth.

Differential abundances in OTUs did not reach significance for metadata values of sediment depth or comparisons between sediment cores. On the other hand, there were numerous differences in OTU abundances when comparing sediment with mussels and without mussels. We performed a differential abundance estimation with the DESeq2 R package using mussel presence status ($n = 20$ with-mussels, $n = 20$ no-mussels) as our covariate. 734 OTUs (or 0.94% of the 77,288 OTUs tested) reached significance with a false discovery rate of 0.01. The vast majority of OTUs belonging to the phyla Gemmatimonadetes, Actinobacteria, Acidobacteria, Plantomycetes, Chloroflexi, Firmicutes, Crenarcheota, and Verrucomicrobia decreased by at least 4-fold in the presence of mussels. In contrast, Proteobacteria showed a marked decrease in order Alphaproteobacteria, while showing mixed increasing and decreasing OTUs among Beta-, Delta-, and Gammaproteobacteria. Phylum Nitrospirae also had 38 OTUs which were differentially abundant with p-adj < 0.001. OTUs assigned to the GreenGenes taxonomic family of "0319-6A21" were the most abundant among those OTUs increasing without mussels, while families *Thermodesulfovibrionaceae* and "FW" were most abundant among those OTUs increasing with mussels.

Many of the Nitrospirae taxons that increased without mussels did so from a smaller average abundance (17 average counts for Nitrospira and up to 126 average counts for *Thermodesulfovibrionaceae*) relative to those that were increased with mussels (209 average counts for *Nitrospira* and up to 581 average counts for *Thermodesulfovibrionaceae*). This explains the 10% increase in Nitrospirae abundance when summing across all samples with mussels. Figure 4 shows the Log2FC categorized by phyla for OTUs with p-adj < 0.0001 (to enhance visual clarity). Significant differences within the Nitrospirae phylum were represented by increases of genus "HB118" in family *Thermodesulfovibrionaceae* (2.0Log2FC from a mean count of 52, $p < 0.001$) and unclassified *Nitrospira* species (0.8Log2FC from an average count of 209, $p < 0.001$) with mussels. No-mussel treatments showed increases in genus "LCP-6" from family *Thermodesulfovibrionaceae* (3.6Log2FC from an average count of 126, $p < 0.001$) and unclassified *Nitrospira* species (2.1Log2FC from an average count of 17, $p < 0.001$).

Despite seemingly even representation of phylum Thaumarchaeota between treatments, unclassified species from *Candidatus* Nitrososphaera were enhanced from an average abundance of 126 (1.73Log2FC, $p < 0.001$) without mussels, and AOA species, *Candidatus* Nitrososphaera gargensis increased from an average count of 16 (2.85Log2FC, $p < 0.001$) without mussels. One OTU classified in the anammox genus, *Candidatus* Brocadia, increased from an average count of 17 (3.72Log2FC, $p < 0.001$) without mussels, while another OTU classified as an unknown *Candidatus* Brocadia species increased from a mean count of 16 (1.2Log2FC, $p = 0.001$) with-mussels. Furthermore, OTUs belonging to the AOB family Nitrosomonadaceae increased from an average abundance of 6 (1.9Log2FC, $p < 0.001$) with mussels. Without mussels, taxonomic groups capable of nitrite-damo, phylum "NC10", increased from average abundances up to 130 (4.4 Log2FC, $p < 0.001$), and nitrate-damo family "ANME-2d" increased from average abundances up to 59 (3.4 Log2FC, $p < 0.001$). A summary of Log2FC values for OTUs relevant to N-transformations are listed in Table S1.

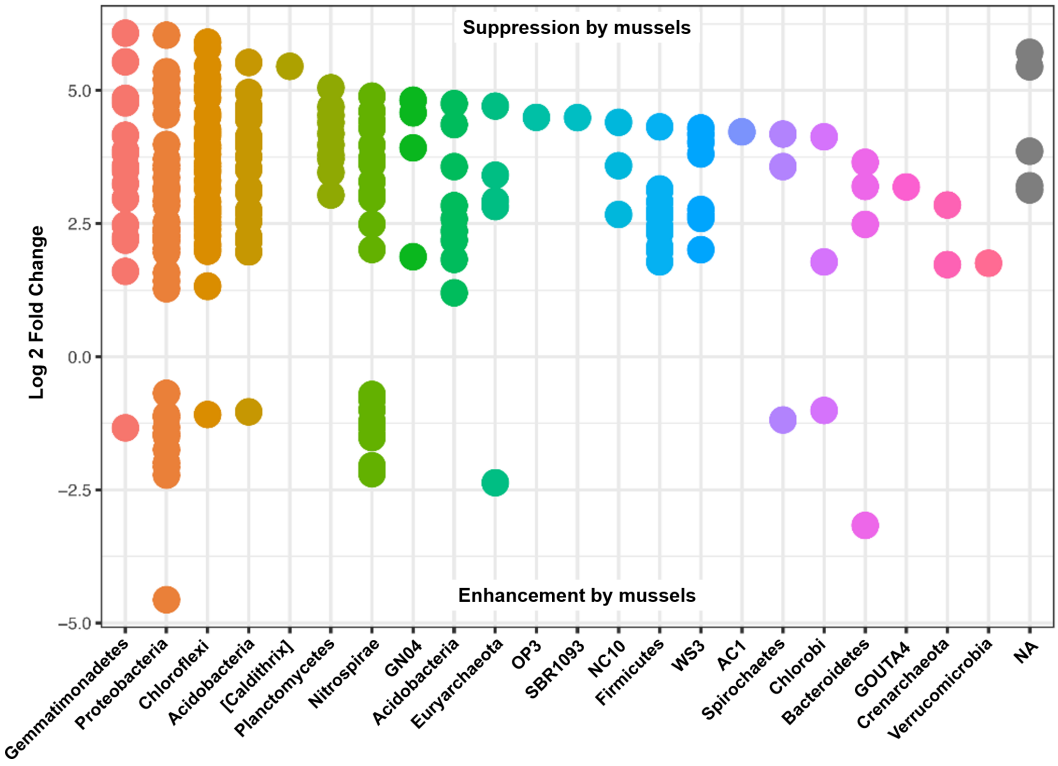

Figure 4 Results from a DESeq2 differential abundance analysis expressed as Log2FC comparison of with-mussels and no-mussels samples. Negative Log2FC represent phyla enhanced in the mussel bed and each point represents an individual OTU. To enhance clarity, only those OTUs with p-adj < 0.0001 are shown.

N-cycle phylotypes were examined for statistically significant relative abundances between treatments of mussel presence and sediment depth (Table 1). *Candidatus* Nitrososphaera experienced a 2.6-fold decrease ($p = 0.047$) with mussels at 5 cm sediment depth. ANME-2d was three times greater ($p = 0.049$) at 5 cm sediment depth without mussels, compared to 3 cm sediment depth without mussels. Within the mussel bed, *Nitrospira* were 1.7 times greater ($p = 0.0497$) at 3 cm depth, and experienced a 1.9-fold increase ($p = 0.025$) with mussels at 3 cm sediment depth versus control. *Candidatus* Brocadia was three times greater ($p = 0.013$) at 5 cm depth without mussels versus 3 cm without mussels, and the 3 cm sediment showed a 2-fold increase ($p = 0.002$) with mussels versus control. Nitrosomonadaceae was 2.7 times greater ($p = 0.015$) at 3 cm with mussels versus 5 cm depth with mussels.

Relative abundances of N-cycle phylotypes were compared within each treatment (Figs. 5A, 5B, 5D, 5E) and between treatments (Figs. 5C, 5F, 5G–5I). Within 3 cm sediment samples with mussels (Fig. 5A), *Nitrospira* was statistically greater in abundance than *Candidatus* Brocadia, and ANME-2d was less abundant than *Nitrospira*. Sediment without mussels at 3 cm depth (Fig. 5B) contained statistically greater abundances of *Candidatus* Nitrososphaera than *Candidatus* Brocadia, and greater *Nitrospira* abundances compared to *Candidatus* Brocadia, Nitrosomonadaceae, and ANME-2d.

Table 1 The percent relative abundance of N-cycle organisms for mussel and depth treatments.

Taxonomic classification	N-cycle classification	Mean percent relative abundance			
		3 cm with-mussels	3 cm no-mussels	5 cm with-mussels	5 cm no-mussels
Candidatus Nitrososphaera	AOA	0.26	0.44	0.22	0.58
ANME-2D	Nitrate-damo	0.12	0.21	0.11	0.63
NC10	Nitrite-damo	0.0039	0.02	0.0035	0.08
Nitrospira	NOB/comammox	1.92	1.00	1.11	0.85
Candidatus Brocadia	Anammox	0.10	0.05	0.07	0.15
Nitrosomonadaceae	AOB	0.27	0.13	0.10	0.08

Relative abundance comparisons between mussel and no-mussel treatments at 3 cm depth (Fig. 5C) showed that *Candidatus* Nitrososphaera was reduced in the mussel treatment, while *Nitrospira* and *Candidatus* Brocadia were enhanced with mussels. Within mussel sediment samples at 5 cm depth, *Nitrospira* was more abundant than *Candidatus* Brocadia, ANME-2d, and Nitrosomonadaceae. (Fig. 5D). On the other hand, *Candidatus* Nitrososphaera and *Nitrospira* were both more abundant than Nitrosomonadaceae without mussels at 5 cm sediment depth (Fig. 5E). Comparing microbial communities at 5 cm depth between mussel and no-mussel treatments (Fig. 5F) revealed that *Candidatus* Nitrososphaera was less abundant with mussels versus the no-mussel population. *Nitrospira* and Nitrosomonadaceae phylotypes were more prominent with mussels in shallow sediment depths (Fig. 5G).

Overall, *Nitrospira* made up larger proportions of microbial communities with and without mussels compared to many N-cycle organisms, especially *Candidatus* Brocadia and Nitrosomonadaceae (Figs. 5C, 5F, and 5G–5I). Without mussels at 3 cm sediment depth, *Candidatus* Brocadia made up a smaller proportion of the N-cycling microbial community, especially when compared to *Candidatus* Nitrososphaera, ANME-2d, NC10, and *Nitrospira* in deeper sediments (Fig. 5H).

DISCUSSION

Numerous studies have found Proteobacteria to be the most abundant phylum in freshwater sediments (*Bucci et al., 2014*; *Dai et al., 2016*; *Wakelin, Colloff & Kookana, 2008*; *Zeng et al., 2008*; *Zhang et al., 2015*), sediments with mollusks (*Fernandez et al., 2014*; *Lee et al., 2015*), and also mollusk microbiomes (*Frischer et al., 2000*; *Neta et al., 2015*; *Ngangbam et al., 2015*; *Trabal et al., 2012*). Although our results showed Proteobacteria were the most abundant phylum, we observed a decrease in Proteobacteria by 6% and an increase in Nitrospirae by 10% in the presence of mussels. Families *Thermodesulfovibrionaceae* and "FW" accounted for many of the Nitrospirae OTUs that increased with mussels and helps explain decreases in species richness for mussel bed sediment.

Sediments contain the most phylogenetically diverse microbial communities (*Lozupone & Knight, 2007*) and structure and diversity of soil microbial communities is often determined by soil biogeochemistry (*Fierer & Jackson, 2006*), further supporting the impact mussels have on biogeochemical cycling. In support of our hypothesis, our data indicated

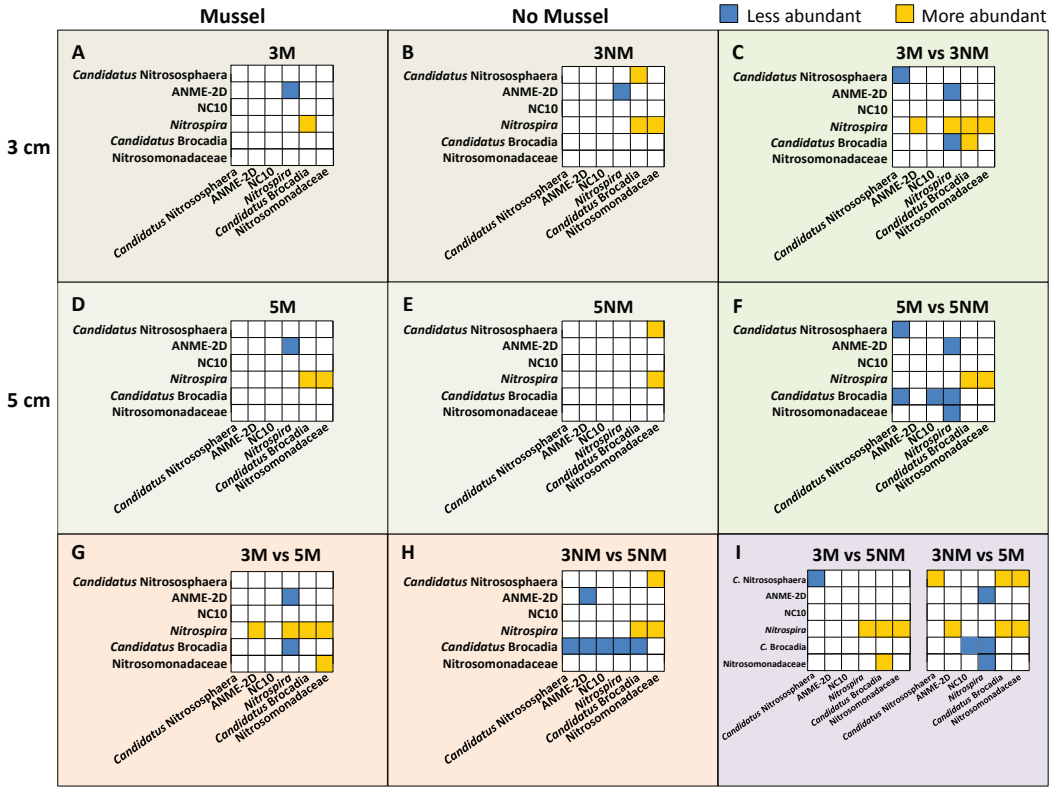

Figure 5 **Image of N-cycle phylotype comparisons between treatments.** Statistically significant differences in N-cycle organism abundances ($P < 0.05$). Statistical significance was determined by non-parametric ANOVA with Dunn's multiple correction test. All boxes show y-axes compared to the "baseline" x-axes, with no boxes representing comparisons not meeting significance. (A–B), (D–E), Comparisons within treatment conditions of mussel presence and depth. (C) Differentially abundant organisms between 3 cm Mussel and 3 cm No Mussel treatments. (F) Abundance comparisons between 5 cm Mussel and 5 cm No Mussel treatments. (G) Differential N-cycle organism abundance between 3 cm Mussel and 5 cm Mussel samples. (H) Comparisons between 3 cm No Mussel and 5 cm No Mussel treatments. (I) Abundance comparisons of 3 cm Mussel versus 5 cm No Mussel, and 3 No Mussel versus 5 Mussel samples.

that mussel presence in the UMR had a pronounced influence on the microbial community evenness, richness, and composition within the sediment. The observed changes in sediment microbial community structure and diversity showed mussels created a niche for specific microorganisms and may be attributable to the diverse chemical composition of mussel biodeposits, mixing of sediment from mussel burrowing, or the microbes living on mussels. Our findings of distinct microbial communities in mussel bed sediment are corroborated by a study of the California mussel (*Pfister, Gilbert & Gibbons, 2014*) where taxonomic richness increased and taxa evenness increased following the removal of mussels from a rocky shore habitat.

In contrast to our results of decreased microbial diversity with freshwater mussels, research has shown invasive zebra mussels (*Dreissena polymorpha*) increased bacterial community diversity and richness (*Lee et al., 2015*), and metabolic diversity and activity in freshwater sediments (*Lohner et al., 2007*). Increased microbial diversity and activity

has been attributed to the variety of C and N components in feces and pseudofeces, and also selects for the dominant microbial species (*Lohner et al., 2007*; *Pollet et al., 2015*). An experiment combining estuarine bivalve species (*N. virens, M. arenaria, and M. balthica*) implicated mussel-induced changes in O_2, NH_4^+ and NO_3^- fluxes for the alteration of microbial community composition (*Michaud et al., 2009*). On the other hand, investigation of microbiota in Thick-shelled River Mussel (*Unio crassus*) beds did not find any difference in microorganism diversity, abundance, and composition (*Richter et al., 2016*). This may be explained by the drastic differences in the study site, with high mussel densities (23–433 mussels/m^2) and control plots containing low microbial diversities with mean species richness of 48 OTUs/sample with high evenness (*Richter et al., 2016*). The contrasting findings of microbial community diversity and composition indicate that mussel density and/or mollusk species may produce different responses by microorganism communities.

Additionally, alterations in sediment microbial community structure may arise from exposure to the mussel shell, tissues, or fecal microbiome. Mussel tissue and fecal material has been shown to contain less diverse microbiomes than the surrounding water and sediment for the zebra mussel (*Frischer et al., 2000*), tropical oyster (*Crassostrea rhizophorae*) (*Neta et al., 2015*), and marine mussel, *Mytilus californianus* (*Frischer et al., 2000*; *Pfister, Gilbert & Gibbons, 2014*). Some studies have attributed immediate increased sediment microbial activity to the mussel intestinal microbiome (*Grenz et al., 1990*). Furthermore, mollusk biodeposition rates and biodeposit chemical compositions are highly dependent on mollusk species (*Hegaret, Wikfors & Shumway, 2007*; *Tenore & Dunstan, 1973*), and food availability (*Bril et al., 2017*; *Cranford et al., 2007*; *Vaughn & Hakenkamp, 2001*), so it makes sense that our results differ from studies with dissimilar mollusk species, densities, and study location.

Changes in mussel bed sediment microbial communities was also likely enhanced by mussel burrowing, because diffusion of substrates across the water-sediment interface is a relatively slow process (*Kristensen et al., 2012*) and is increased by mollusk burrowing (*Vaughn & Hakenkamp, 2001*), which ultimately affects microbial communities. For example, the burrow of shrimp species *Upogebia deltaura* and *Callianassa subterranean* contained distinct bacterial communities and a 3-fold increase in taxon richness (*Laverock et al., 2010*), and the estuarine bivalve, *C. fluminea*, stimulated microbial diversity via bioturbation (*Novais et al., 2016*). It is likely that UMR mussel bed sediments also experience the benefits from bioturbation, such as sediment mixing (*McCall, Tevesz & Schwelgien, 1979*) and aeration (*Vaughn & Hakenkamp, 2001*). Furthermore, bioturbation has been linked to increased NH_4^+ concentrations which alters the N-transforming microbial community (*Chen & Gu, 2017*), with greatest effects on bacteria growth found at 4–6 cm depth below the water-sediment interface (*McCall, Matisoff & Tevesz, 1986*).

N-cycle microbial community

Our research revealed an increase in anammox bacteria abundance 3 cm below the water-sediment interface when mussels were present, shown for the anammox community using anammox-targeted qPCR (2.2-fold increase) and for *Candidatus* Brocadia using non-targeted 16S rRNA gene amplicon sequencing (2-fold increase). The significance of

agreement between these techniques is finding that increases in the genus *Candidatus* Brocadia are representative for the anammox phylotype as a whole. *Candidatus* Brocadia may also make up a majority of the anammox community in UMR sediment, as amplicon sequencing did not detect anammox bacteria belonging to other genera. We are confident in these conclusions, as *Candidatus* Brocadia is often the dominant anammox genus in freshwater sediments (*Humbert et al., 2009*; *Shen et al., 2016*; *Sonthiphand, Hall & Neufeld, 2014*). One study showed that feeding of NH_4^+, NO_2^-, NO_3^-, and acetate led to an 80% enrichment of *Candidatus* 'Brocadia fulgida', signifying that B. *fulgida* could outcompete anammox species in genera *Candidatus* Anammoxoglobus and *Candidatus* Kuenenia, species *Candidatus* 'Brocadia anammoxidans', and even denitrifiers when acetate is present (*Kartal et al., 2008*). This indicates that *Candidatus* Brocadia has a distinct ecological niche and can utilize intermediates from anaerobic degradation of organic C to reduce NO_3^- (*Kartal et al., 2008*). Therefore, it is possible that a portion of our observed increases in *Candidatus* Brocadia with mussels was attributable to C biodeposition in the UMR.

Our research also revealed a vertical distribution of anammox bacteria with higher abundances near the sediment surface, which reflects the vertical distribution found in an agricultural field (*Shen et al., 2017*), oxygen minimum zone (*Galán et al., 2009*), flooded paddy fields (*Shen et al., 2017*; *Zhu et al., 2011*), and an urban wetland (*Shen et al., 2015*). A vertical anammox distribution has been shown to coincide with NH_4^+ presence and NO_2^- production (*Oshiki, Satoh & Okabe, 2016*; *Shen, Xu & He, 2014*; *Shen et al., 2015*; *Sun et al., 2014*) and anammox "hotspots" occur in zones of low, but not entirely absent, O_2 availability (*Zhu et al., 2013*). Anammox abundance in freshwater sediment can range between 7×10^4 and 8×10^6 gene copies g^{-1} sediment (*Shen et al., 2016*), or between 10^6 and 10^7 gene copies g^{-1} sediment in peak NO_2^- microniches at the oxic–anoxic interface (*Nie et al., 2015*; *Shen et al., 2015*; *Zheng et al., 2016*). Studies have shown anammox bacteria increase 1.5 to 2-fold within their niche (*Nie et al., 2015*; *Zheng et al., 2016*), similar to our findings of a 2.2-fold increase in anammox bacteria 3 cm below the water-sediment interfacewith mussels.

Co-occurrence of aerobic NH_4^+ oxidation and anammox niches are likely due to linked NO_2^- oxidation and reduction, respectively (*Shen, Xu & He, 2014*). Interestingly, we found that mussels also enhanced taxa from the AOB family Nitrosomonadaceae and the OTUs made up a greater proportion of mussel bed sediment populations near the water-sediment interface. To this point, the pacific oyster (*C. gigas*) was found to increase porewater NH_4^+ and elevate the concentration of NH_4^+ oxidizing microorganisms (*Green, Boots & Crowe, 2012*). Furthermore, our previous research (*Bril et al., 2014*; *Bril et al., 2017*) showed elevated NH_4^+ and NO_2^- in porewater of a similar depth below mussels. It makes sense that these groups of N-transforming bacteria co-occur where their substrate microniches overlap, and is likely enhanced by mussels periodically aerating the sediment (*Chen & Gu, 2017*). Intermittent aeration has shown to enrich microbial cultures in AOB and anammox bacteria in engineered partial nitritation-anammox processes (*Shannon et al., 2015*; *Yang et al., 2015*), and similar to our findings, enriches the anammox genus *Candidatus* Brocadia (*Shannon et al., 2015*).

On the other hand, we saw a decrease in *Candidatus* Nitrososphaera (AOA) with mussels at 3 cm (1.7-fold) and 5 cm (2.6-fold) sediment depths. It makes sense that mussels suppress abundance of AOA since these organisms typically dominate sediment niches with low NH_4^+ concentrations (*Hatzenpichler, 2012*; *Martens-Habbena et al., 2009*). Furthermore, a group of OTUs suppressed by mussels were classified at the species level as *Candidatus* 'Nitrososphaera gargensis', which are partially inhibited by NH_4^+ concentrations (3.08 mM) much lower than AOB (*Hatzenpichler, 2012*; *Hatzenpichler et al., 2008*; *Nakagawa & Takahashi, 2015*; *Pester, Schleper & Wagner, 2011*). Furthermore, nitrifier niche partitioning studies using agricultural soil showed that AOB increased in abundance and activity following the addition of urine-derived N, while AOA remained unchanged (*Di et al., 2009*; *Hatzenpichler, 2012*; *Jia & Conrad, 2009*). Therefore, it is possible that mussel biodeposits and an increased flux of agriculturally-fed water into sediment by mussel burrowing enhanced porewater NH_4^+ composition such that Nitrosomonadaceae out competed *Candidatus* Nitrososphaera. Our results agree with *Chen & Gu (2017)*, who found bioturbated sediment corresponded with a greater diversity of AOB and lower diversity of AOA microbial communities (*Chen & Gu, 2017*). On the other hand, our results of decreased abundance of *Candidatus* Nitrososphaera co-occurring with an increase in *Nitrospira* is in contrast to previous findings that these organisms may exhibit similar niche partitioning (*Pester, Schleper & Wagner, 2011*). For example, some species in *Candidatus* Nitrososphaera can adjust their metabolism for low oxygen availability (*Zhalnina et al., 2014*) and *Nitrospira* species are adapted to low oxygen concentrations (*Maixner et al., 2006*; *Nowka, Daims & Spieck, 2015*; *Zhalnina et al., 2014*) and microoxic environments (*Schramm et al., 2000*). Alternatively, our detected increased abundance of *Nitrospira* may include species with a variety of environmental niches.

Some *Nitrospira* species have shown to occupy a niche at oxic–anoxic interfaces, in opposition to NOB with higher O_2 tolerances such as those in genus *Nitrobacter* (*Schramm et al., 2000*). This supports our mussel-attributed increases in relative *Nitrospira* abundances (1.9-fold) at 3 cm sediment depths. Although we saw two different *Nitrospira* OTUs suppressed and enhanced by mussels, the mussel-enhanced OTUs had a larger mean abundance by about 12%. Different NOB OTUs enhanced with and without mussels further suggests that mussel bed sediments harbor specific NOB strains sensitive to microoxic niches. Despite OTU variability, we can conclude that mussels enhance the *Nitrospira* phylotype, especially near the water-sediment interface where *Nitrospira* were 1.7-times greater than deeper mussel bed depths.

On the other hand, we did not expect to see an increase in both NOB and anammox phylotypes due to competition of NO_2^- as a substrate. The co-occurrence of *Nitrospira* and anammox bacteria may be explained by the metabolic versatility of *Nitrospira* species, especially if mussel-derived urea provided an additional source of NH_3 and NO_2^- via reciprocal feeding between ammonia oxidizers and *Nitrospira*. Furthermore, these phylotypes have been shown to coexist in an oxygen minimum zone, where anammox bacteria obtained a majority of NO_2^- from NO_3^- reducers (*Lam et al., 2009*). The similar effect size of mussels on *Nitrospira* (1.9-fold) and *Candidatus* Brocadia (2-fold) at 3 cm depth suggests that mussels may exert similar influences on the niches of these phylotypes.

It is possible that these anammox and *Nitrospira* phylotypes were functionally linked in shallow mussel bed sediment, which has been shown for microoxic niches (*Van Kessel et al., 2015*). Furthermore, it is possible that the *Nitrospira* co-occurring with *Candidatus* Brocadia were *Nitrospira* species with the genetic potential for comammox, as a fluorescence in-situ hybridization study confirmed the extensive aggregation of the 2 phylotypes in hypoxic conditions (<3.1 μM O_2) (*Van Kessel et al., 2015*). Despite *Nitrospira* comammox being identified in numerous aquatic environments (*Chao et al., 2016*; *Daims et al., 2015*; *Pinto et al., 2016*), we cannot conclusively identify comammox without sequencing the ammonia monooxygenase gene (*Pinto et al., 2016*; *Van Kessel et al., 2015*).

In contrast to studies which found significant N-reduction on both a marine mussel (*Mytilus californianus*) (*Pfister, Meyer & Antonopoulos, 2010*) and a freshwater mussel (*Limnoperna fortunei*) (*Zhang, Cui & Huang, 2014*), our results showed that mussels suppressed n-damo OTUs in phylum "NC10" (2.1-fold) and family "ANME-2d" (1.8-fold). One study determined NO_3^--damo was responsible for NO_3^- reduction and anammox for NO_2^- reductions in a bioreactor supplied with NH_4^+, NO_2^-, NO_3^-, CH_4, and anoxic conditions, thus concluding anammox outcompeted NO_2^--damo (*Hu et al., 2015*). These findings make sense, because anammox bacteria have a higher affinity for NO_2^- (*Luesken et al., 2011*), anammox outperform n-damo in bioturbated sediments with higher NH_4^+ and lower NO_2^- and NO_3^- (*Chen & Gu, 2017*), and anammox and n-damo communities have a competitive relationship in burrowed mangrove sediment (*Chen & Gu, 2017*). Furthermore, NC10 bacteria in a peatland were most prevalent at depths with porewater CH_4 concentrations near 300 μM, where NO_3^- consumption exceeds production, and in completely anoxic conditions (*Zhu et al., 2012*). According to the literature, it makes sense that we found UMR mussels enhanced *Candidatus* Brocadia and suppressed NO_2^- reducing-NC10. Perhaps n-damo organisms did not have a favorable niche in mussel bed sediment because biodeposition products created an excess of NH_4^+ in sediment porewater (*Winkler et al., 2015*), or burrowing activity increased oxygen concentrations and made methane oxidation unfavorable (*Van Bodegom et al., 2001*). Our finding that no-mussel sediment contained three times more ANME-2d in deeper, and presumably anoxic sediment, further suggests that mussels broaden the oxic–anoxic interface niche (*Chen & Gu, 2017*; *Luesken et al., 2011*). However, we cannot extrapolate these findings to all denitrifying organisms, since denitrifying species are sporadically distributed among various taxonomic lineages, and are difficult to identify solely with16S rRNA amplicon sequencing (*Ishii et al., 2011*).

Although we observed greater relative abundances of *Nitrospira* than *Candidatus* Brocadia in a majority of treatments, both phylotypes increased by a factor of 2 with mussels at 3 cm depth. No-mussel samples contained a significantly smaller proportion of *Candidatus* Brocadia in shallow sediments compared to almost all N-transformers found in the deeper control sediments. Our phylotype-level analyses revealed similarities with the OTU-level differential abundance comparisons. For example, phylotype comparisons showed ANME-2d was less abundant than *Nitrospira* in 3 cm sediments with mussels, and *Candidatus* Nitrososphaera was more abundant than *Candidatus* Brocadia in 3 cm sediment samples without mussels. These results relate to DESeq2 OTU comparisons

which found *Candidatus* Brocadia and *Nitrospira* enhanced with mussels while ANME-2d and *Candidatus* Nitrososphaera were suppressed with mussels.

Extending our focus beyond N-cycling organisms, we demonstrated that mussels promoted a large effect size for OTUs classified as *Thermodesulfovibrionaceae* (Nitrospirales order). In contrast to *Nitrospira*, the Nitrospirales genus *Thermodesulfovibrio* contains multiple sulfate reducing species (*Kirchman, 2012*; *Sekiguchi et al., 2008*) and can outcompete other anaerobic organisms when sulfate is present (*He et al., 2015*). These findings are corroborated by discoveries of significantly greater C and sulfate concentrations from mussel biodeposits and 63% greater sulfate reduction in sediments with mussels (*McKindsey et al., 2011*). Biodeposition products often lead to increasingly anoxic sediment and greater activity of anoxic microorganisms (*Kellogg et al., 2013*; *McKindsey et al., 2011*), presumably due to consumption of excretion products by oxygen-consuming microorganisms (*Pollet et al., 2015*). Interestingly, *Fdz-Polanco et al. (2001)* observed simultaneous N and sulfate removal in an anaerobic fluidized-bed reactor and proposed simultaneous anammox and sulfate reduction. Coupled biological sulfate reduction and anammox reactions are metabolically feasible (*Schrum et al., 2009*; *Strous et al., 2002*) and have been of interest in the recent history (*Ali et al., 2013*; *Cai, Jiang & Zheng, 2010*; *Rikmann et al., 2016*; *Rios-Del Toro & Cervantes, 2016*), therefore warranting further research. Therefore, we showed that *Thermodesulfovibrionaceae* are significantly increased in the presence of mussels which may affect sulfate reduction (*Mahmoudi et al., 2015*) in tandem with anammox reactions in UMR sediments.

As a whole, mussels do have an impact on microbial niches and lower the overall community diversity. Mussel-influenced changes in microbiological diversity may have larger ecosystem implications, such as macrobiota richness and diversity (*Arribas et al., 2014*; *Borthagaray & Carranza, 2007*). Native freshwater mussels are capable of increasing macrobiota diversity as a result of being keystone species (*Hartmann et al., 2016*) and ecosystem engineers (*Chowdhury, Zieritz & Aldridge, 2016*; *Lopes-Lima et al., 2014*). Mussel biogeochemical hotspots can lead to a bottom-up trophic cascade by enhancing N substrates normally limiting primary productivity, ultimately leading to increased richness (*Atkinson et al., 2013*) and biodiversity (*Allen et al., 2012*) of higher trophic levels.

CONCLUSION

As far as we know, this is the first study to characterize freshwater mussel effects on microbial community diversity, composition, and the vertical distribution of N-cycle microorganisms in the UMR. qPCR of the anammox-specific 16S rRNA gene revealed an increase in anammox bacteria abundance 3 cm below the water-sediment interface when mussels were present, and confirmed anammox bacteria were normally distributed with depth. Non-targeted 16S rRNA gene amplicon sequencing revealed mussel presence suppressed AOA (*Candidatus* Nitrososphaera) and that the families *Thermodesulfovibrionaceae* and "FW" (Nitrospirales order) were overrepresented among the enhanced OTUs with-mussels. Mussel bed sediment contained microbial communities with 10% greater Nitrospirae and 6% fewer OTUs belonging to the phylum Proteobacteria, which ultimately had a

pronounced influence on microbial community evenness, richness, and composition. This was indicated by lower observed species richness, Chao1 diversity, Shannon diversity, and clustering of mussel samples in an NMDS analysis. We have shown that native freshwater mussels affect niche differentiation of N-cycle microorganisms, as evidenced by increased abundances of AOB family Nitrosomonadaceae, anammox genus *Candidatus* Brocadia, and NOB genus *Nitrospira*, while exhibiting a decrease in AOA genus *Candidatus* Nitrososphaera, and n-damo organisms in the phylum NC10 and family ANME-2d. Co-occurring 2-fold increases in *Candidatus* Brocadia and *Nitrospira* in shallow sediment suggests that mussels may enhance microbial niches at the interface of oxic–anoxic conditions, presumably through biodeposition and burrowing. Ultimately, this study demonstrates the large impact mussels have on biogeochemical N-cycling and ecosystem services in freshwater agroecosystems.

ADDITIONAL INFORMATION AND DECLARATIONS

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Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Ellen M. Black and Michael S. Chimenti conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.
- Craig L. Just conceived and designed the experiments, analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.

DNA Deposition

The following information was supplied regarding the deposition of DNA sequences:
MG-RAST (ID: 4705672.3-4705709.3).
Bioproject Accession: PRJNA374585.

Data Availability

The following information was supplied regarding data availability:
GitHub: https://github.com/mchimenti/black_chimenti_just_phyloseq/blob/master/phyloseq.r.

Project name: Mussel1_longlistminus2.
Static link: http://metagenomics.anl.gov/linkin.cgi?project=mgp18682.
Metagenomes: 4705672.3 through 4705709.3.
Project name: Mussel1_shortlist.
Static link: http://metagenomics.anl.gov/linkin.cgi?project=mgp18674.
Metagenomes: mgm4705417.3; mgm4705418.3.
Individual IDs:
Mussel IDs: 4705672.3, 4705673.3, 4705675.3, 4705676.3, 4705677.3, 4705680.3, 4705681.3, 4705683.3, 4705684.3, 4705690.3, 4705691.3, 4705693.3, 4705695.3, 4705696.3, 4705699.3, 4705705.3, 4705706.3, 4705708.3, 4705709.3, 4705417.3.
No-mussel IDs: 4705674.3, 4705678.3, 4705679.3, 4705682.3, 4705685.3, 4705686.3, 4705687.3, 4705688.3, 4705689.3, 4705692.3, 4705694.3, 4705697.3, 4705698.3, 4705700.3, 4705701.3, 4705702.3, 4705703.3, 4705704.3, 4705707.3, 4705418.3.

Supplemental Information

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REFERENCES

Aakra A, Utaker JB, Pommerening-Roser A, Koops HP, Nes IF. 2001. Detailed phylogeny of ammonia-oxidizing bacteria determined by rDNA sequences and DNA homology values. *International Journal of Systematic and Evolutionary Microbiology* **51**:2021–2030 DOI 10.1099/00207713-51-6-2021.

Ali M, Chai LY, Tang CJ, Zheng P, Min XB, Yang ZH, Xiong L, Song YX. 2013. The increasing interest of ANAMMOX research in China: bacteria, process development, and application. *BioMed Research International* **2013**:Article 134914 DOI 10.1155/2013/134914.

Allen WR. 1923. Studies of the biology of freshwater mussels. II, the nature and degree of response to certain physical and chemical stimuli. *Ohio Journal of Science* **23(2)**:57–82.

Allen DC, Vaughn CC. 2009. Burrowing behavior of freshwater mussels in experimentally manipulated communities. *Journal of the North American Benthological Society* **28**:93–100 DOI 10.1899/07-170.1.

Allen DC, Vaughn CC, Kelly JF, Cooper JT, Engel MH. 2012. Bottom-up biodiversity effects increase resource subsidy flux between ecosystems. *Ecology* **93**:2165–2174 DOI 10.1890/11-1541.1.

Altschul SF, Madden TL, Schaffer AA, Zhang JH, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* **25**:3389–3402 DOI 10.1093/nar/25.17.3389.

Arribas LP, Donnarumma L, Palomo MG, Scrosati RA. 2014. Intertidal mussels as ecosystem engineers: their associated invertebrate biodiversity under contrasting wave exposures. *Marine Biodiversity* **44**:203–211 DOI 10.1007/s12526-014-0201-z.

Atkinson CL, Kelly JF, Vaughn CC. 2014. Tracing consumer-derived nitrogen in riverine food webs. *Ecosystems* **17**:485–496 DOI 10.1007/s10021-013-9736-2.

Atkinson CL, Vaughn CC, Forshay KJ, Cooper JT. 2013. Aggregated filter-feeding consumers alter nutrient limitation: consequences for ecosystem and community dynamics. *Ecology* **94**:1359–1369 DOI 10.1890/12-1531.1.

Bayne BL. 1973. Physiological changes in mytilus-edulis-l induced by temperature and nutritive stress. *Journal of the Marine Biological Association of the United Kingdom* **53**:39–58 DOI 10.1017/S0025315400056629.

Bollmann A, Bar-Gilissen MJ, Laanbroek HJ. 2002. Growth at low ammonium concentrations and starvation response as potential factors involved in niche differentiation among ammonia-oxidizing bacteria. *Applied and Environmental Microbiology* **68**:4751–4757 DOI 10.1128/AEM.68.10.4751-4757.2002.

Borthagaray AI, Carranza A. 2007. Mussels as ecosystem engineers: their contribution to species richness in a rocky littoral community. *Acta Oecologica-International Journal of Ecology* **31**:243–250 DOI 10.1016/j.actao.2006.10.008.

Bray JR, Curtis JT. 1957. An ordination of the upland forest communities of Southern Wisconsin. *Ecological Monographs* **27**:326–349.

Bril JS, Durst JJ, Hurley BM, Just CL, Newton TJ. 2014. Sensor data as a measure of native freshwater mussel impact on nitrate formation and food digestion in continuous-flow mesocosms. *Freshwater Science* **33**:417–424 DOI 10.1086/675448.

Bril JS, Langenfeld K, Just CL, Spak SN, Newton TJ. 2017. Simulated mussel mortality thresholds as a function of mussel biomass and nutrient loading. *PeerJ* **5**:e2838 DOI 10.7717/peerj.2838.

Bucci J, Szempruch A, Caldwell J, Ellis J, Levine J. 2014. Seasonal changes in microbial community structure in freshwater stream sediment in a North Carolina river basin. *Diversity* **6**:18–32 DOI 10.3390/d6010018.

Burrell PC, Phalen CM, Hovanec TA. 2001. Identification of bacteria responsible for ammonia oxidation in freshwater aquaria. *Applied and Environmental Microbiology* **67**:5791–5800 DOI 10.1128/AEM.67.12.5791-5800.2001.

Cai J, Jiang JX, Zheng P. 2010. Isolation and identification of bacteria responsible for simultaneous anaerobic ammonium and sulfate removal. *Science China-Chemistry* **53**:645–650 DOI 10.1007/s11426-010-0053-8.

Caporaso JG, Bittinger K, Bushman FD, DeSantis TZ, Andersen GL, Knight R. 2010. PyNAST: a flexible tool for aligning sequences to a template alignment. *Bioinformatics* **26**:266–267 DOI 10.1093/bioinformatics/btp636.

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, Owens SM, Betley J, Fraser L, Bauer M, Gormley N, Gilbert JA, Smith G, Knight R. 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME Journal* **6**:1621–1624 DOI 10.1038/ismej.2012.8.

Chao A, Chiu C-H. 2001. *Nonparametric estimation and comparison of species richness. eLS*. Hoboken: John Wiley & Sons, Ltd.

Chao Y, Mao Y, Yu K, Zhang T. 2016. Novel nitrifiers and comammox in a full-scale hybrid biofilm and activated sludge reactor revealed by metagenomic approach. *Applied Microbiology and Biotechnology* **100**:8225–8237 DOI 10.1007/s00253-016-7655-9.

Chen J, Gu J-D. 2017. Faunal burrows alter the diversity, abundance, and structure of AOA, AOB, anammox and n-Damo communities in coastal mangrove sediments. *Microbial Ecology* **74**:140–156 DOI 10.1007/s00248-017-0939-5.

Chowdhury GW, Zieritz A, Aldridge DC. 2016. Ecosystem engineering by mussels supports biodiversity and water clarity in a heavily polluted lake in Dhaka, Bangladesh. *Freshwater Science* **35**:188–199 DOI 10.1086/684169.

Cranford PJ, Strain PM, Dowd M, Hargrave BT, Grant J, Archambault MC. 2007. Influence of mussel aquaculture on nitrogen dynamics in a nutrient enriched coastal embayment. *Marine Ecology Progress Series* **347**:61–78 DOI 10.3354/meps06997.

Dai Y, Yang YY, Wu Z, Feng QY, Xie SG, Liu Y. 2016. Spatiotemporal variation of planktonic and sediment bacterial assemblages in two plateau freshwater lakes at different trophic status. *Applied Microbiology and Biotechnology* **100**:4161–4175 DOI 10.1007/s00253-015-7253-2.

Daims H, Lebedeva EV, Pjevac P, Han P, Herbold C, Albertsen M, Jehmlich N, Palatinszky M, Vierheilig J, Bulaev A, Kirkegaard RH, Von Bergen M, Rattei T, Bendinger B, Nielsen PH, Wagner M. 2015. Complete nitrification by Nitrospira bacteria. *Nature* **528**:504–509 DOI 10.1038/nature16461.

DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P, Andersen GL. 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and Environmental Microbiology* **72**:5069–5072 DOI 10.1128/AEM.03006-05.

Deutzmann JS, Schink B. 2011. Anaerobic Oxidation of methane in sediments of lake constance, an oligotrophic freshwater lake. *Applied and Environmental Microbiology* **77**:4429–4436 DOI 10.1128/AEM.00340-11.

Di HJ, Cameron KC, Shen JP, Winefield CS, O/'Callaghan M, Bowatte S, He JZ. 2009. Nitrification driven by bacteria and not archaea in nitrogen-rich grassland soils. *Nature Geoscience* **2**:621–624 DOI 10.1038/ngeo613.

Ding J, Fu L, Ding ZW, Lu YZ, Cheng SH, Zeng RJ. 2016. Experimental evaluation of the metabolic reversibility of ANME-2d between anaerobic methane oxidation and methanogenesis. *Applied Microbiology and Biotechnology* **100**:6481–6490 DOI 10.1007/s00253-016-7475-y.

Edgar RC. 2010. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26**:2460–2461 DOI 10.1093/bioinformatics/btq461.

Ettwig KF, Butler MK, Le Paslier D, Pelletier E, Mangenot S, Kuypers MMM, Schreiber F, Dutilh BE, Zedelius J, De Beer D, Gloerich J, Wessels HJCT, Van Alen T, Luesken F, Wu ML, Van de Pas-Schoonen KT, Op den Camp HJM, Janssen-Megens EM, Francoijs K-J, Stunnenberg H, Weissenbach J, Jetten MSM, Strous M. 2010. Nitrite-driven anaerobic methane oxidation by oxygenic bacteria. *Nature* **464**:543–548 DOI 10.1038/nature08883.

Ettwig KF, Van Alen T, Van de Pas-Schoonen KT, Jetten MSM, Strous M. 2009. Enrichment and molecular detection of denitrifying methanotrophic bacteria of the NC10 phylum. *Applied and Environmental Microbiology* **75**:3656–3662 DOI 10.1128/AEM.00067-09.

Fdz-Polanco F, Fdz-Polanco M, Fernandez N, Urueña MA, Garcia PA, Villaverde S. 2001. New process for simultaneous removal of nitrogen and sulphur under anaerobic conditions. *Water Research* **35**:1111–1114 DOI 10.1016/S0043-1354(00)00474-7.

Fernandez NT, Mazon-Suastegui JM, Vazquez-Juarez R, Ascencio-Valle F, Romero J. 2014. Changes in the composition and diversity of the bacterial microbiota associated with oysters (Crassostrea corteziensis, Crassostrea gigas and Crassostrea sikamea) during commercial production. *FEMS Microbiology Ecology* **88**:69–83 DOI 10.1111/1574-6941.12270.

Fierer N, Jackson RB. 2006. The diversity and biogeography of soil bacterial communities. *Proceedings of the National Academy of Sciences of the United States of America* **103**:626–631 DOI 10.1073/pnas.0507535103.

Frischer ME, Nierzwicki-Bauer SA, Parsons RH, Vathanodorn K, Waitkus KR. 2000. Interactions between zebra mussels (Dreissena polymorpha) and microbial communities. *Canadian Journal of Fisheries and Aquatic Sciences* **57**:591–599 DOI 10.1139/f00-001.

Galán A, Molina V, Thamdrup B, Woebken D, Lavik G, Kuypers MMM, Ulloa O. 2009. Anammox bacteria and the anaerobic oxidation of ammonium in the oxygen minimum zone off northern Chile. *Deep Sea Research Part II: Topical Studies in Oceanography* **56**:1021–1031 DOI 10.1016/j.dsr2.2008.09.016.

Green DS, Boots B, Crowe TP. 2012. Effects of non-indigenous oysters on microbial diversity and ecosystem functioning. *PLOS ONE* **7(10)**:e48410 DOI 10.1371/journal.pone.0048410.

Grenz C, Hermin MN, Baudinet D, Daumas R. 1990. Insitu biochemical and bacterial variation of sediments enriched with mussel biodeposits. *Hydrobiologia* **207**:153–160 DOI 10.1007/BF00041452.

Haag WR. 2012. *North American freshwater mussels: natural history, ecology, and conservation*. New York: Cambridge University Press.

Haroon MF, Hu S, Shi Y, Imelfort M, Keller J, Hugenholtz P, Yuan Z, Tyson GW. 2013. Anaerobic oxidation of methane coupled to nitrate reduction in a novel archaeal lineage. *Nature* **500**:567–570 DOI 10.1038/nature12375.

Hartmann JT, Beggel S, Auerswald K, Stoeckle BC, Geist J. 2016. Establishing mussel behavior as a biomarker in ecotoxicology. *Aquatic Toxicology* **170**:279–288 DOI 10.1016/j.aquatox.2015.06.014.

Hatzenpichler R. 2012. Diversity, physiology, and niche differentiation of ammonia-oxidizing archaea. *Applied and Environmental Microbiology* **78**:7501–7510 DOI 10.1128/AEM.01960-12.

Hatzenpichler R, Lebedeva EV, Spieck E, Stoecker K, Richter A, Daims H, Wagner M. 2008. A moderately thermophilic ammonia-oxidizing crenarchaeote from a hot

spring. *Proceedings of the National Academy of Sciences of the United States of America* **105**:2134–2139 DOI 10.1073/pnas.0708857105.

Hayatsu M, Tago K, Saito M. 2008. Various players in the nitrogen cycle: diversity and functions of the microorganisms involved in nitrification and denitrification. *Soil Science and Plant Nutrition* **54**:33–45 DOI 10.1111/j.1747-0765.2007.00195.x.

He SM, Malfatti SA, McFarland JW, Anderson FE, Pati A, Huntemann M, Tremblay J, Del Rio TG, Waldrop MP, Windham-Myers L, Tringe SG. 2015. Patterns in wetland microbial community composition and functional gene repertoire associated with methane emissions. *Mbio* **6(3)**:e00066-15 DOI 10.1128/mBio.00066-15.

Hegaret H, Wikfors GH, Shumway SE. 2007. Diverse feeding responses of five species of bivalve mollusc when exposed to three species of harmful algae. *Journal of Shellfish Research* **26**:549–559 DOI 10.2983/0730-8000(2007)26[549:DFROFS]2.0.CO;2.

Hill BH, Bolgrien DW, Herlihy AT, Jicha TM, Angradi TR. 2011. A synoptic survey of nitrogen and phosphorus in tributary streams and great rivers of the Upper Mississippi, Missouri, and Ohio river basins. *Water Air and Soil Pollution* **216**:605–619 DOI 10.1007/s11270-010-0556-0.

Hill BH, Elonen CM, Jicha TM, Bolgrien DW. 2008. Nutrient chemistry and microbial activity in the Upper Mississippi river basin: stoichiometry and downstream patterns. In: *American water resources association annual meeting*. New Orleans: National Health and Environmental Effects Research Laboratory.

Houser JN, Richardson WB. 2010. Nitrogen and phosphorus in the Upper Mississippi river: transport, processing, and effects on the river ecosystem. *Hydrobiologia* **640**:71–88 DOI 10.1007/s10750-009-0067-4.

Hu SH, Zeng RJ, Burow LC, Lant P, Keller J, Yuan ZG. 2009. Enrichment of denitrifying anaerobic methane oxidizing microorganisms. *Environmental Microbiology Reports* **1**:377–384 DOI 10.1111/j.1758-2229.2009.00083.x.

Hu S, Zeng RJ, Haroon MF, Keller J, Lant PA, Tyson GW, Yuan Z. 2015. A laboratory investigation of interactions between denitrifying anaerobic methane oxidation (DAMO) and anammox processes in anoxic environments. *Scientific Reports* **5**:8706 DOI 10.1038/srep08706.

Humbert S, Tarnawski S, Fromin N, Mallet M-P, Aragno M, Zopfi J. 2009. Molecular detection of anammox bacteria in terrestrial ecosystems: distribution and diversity. *ISME Journal* **4**:450–454.

Ikenberry CD, Soupir ML, Schilling KE, Jones CS, Seeman A. 2014. Nitrate-nitrogen export: magnitude and patterns from drainage districts to downstream river basins. *Journal of Environmental Quality* **43**:2024–2033 DOI 10.2134/jeq2014.05.0242.

Ishii S, Ashida N, Otsuka S, Senoo K. 2011. Isolation of oligotrophic denitrifiers carrying previously uncharacterized functional gene sequences. *Applied and Environmental Microbiology* **77**:338–342 DOI 10.1128/AEM.02189-10.

Jia ZJ, Conrad R. 2009. Bacteria rather than Archaea dominate microbial ammonia oxidation in an agricultural soil. *Environmental Microbiology* **11**:1658–1671 DOI 10.1111/j.1462-2920.2009.01891.x.

Kartal B, Maalcke WJ, De Almeida NM, Cirpus I, Gloerich J, Geerts W, Den Camp H, Harhangi HR, Janssen-Megens EM, Francoijs KJ, Stunnenberg HG, Keltjens JT, Jetten MSM, Strous M. 2011. Molecular mechanism of anaerobic ammonium oxidation. *Nature* **479**:U127–U159 DOI 10.1038/nature10453.

Kartal B, Van Niftrik L, Rattray J, Van de Vossenberg JLCM, Schmid MC, Sinninghe Damsté J, Jetten MSM, Strous M. 2008. Candidatus 'Brocadia fulgida': an autofluorescent anaerobic ammonium oxidizing bacterium. *FEMS Microbiology Ecology* **63**:46–55 DOI 10.1111/j.1574-6941.2007.00408.x.

Kellogg ML, Cornwell JC, Owens MS, Paynter KT. 2013. Denitrification and nutrient assimilation on a restored oyster reef. *Marine Ecology Progress Series* **480**:1–19 DOI 10.3354/meps10331.

Kirchman DL. 2012. *Processes in microbial ecology*. Oxford: Oxford University Press.

Koch H, Lücker S, Albertsen M, Kitzinger K, Herbold C, Spieck E, Nielsen PH, Wagner M, Daims H. 2015. Expanded metabolic versatility of ubiquitous nitrite-oxidizing bacteria from the genus Nitrospira. *Proceedings of the National Academy of Sciences of the United States of America* **112**:11371–11376 DOI 10.1073/pnas.1506533112.

Koper TE, El-Sheikh AF, Norton JM, Klotz MG. 2004. Urease-encoding genes in ammonia-oxidizing bacteria. *Applied and Environmental Microbiology* **70**:2342–2348 DOI 10.1128/AEM.70.4.2342-2348.2004.

Kristensen E, Penha-Lopes G, Delefosse M, Valdemarsen T, Quintana CO, Banta GT. 2012. What is bioturbation? The need for a precise definition for fauna in aquatic sciences. *Marine Ecology Progress Series* **446**:285–302 DOI 10.3354/meps09506.

Kuenen JG. 2008. Anammox bacteria: from discovery to application. *Nature Reviews Microbiology* **6**:320–326 DOI 10.1038/nrmicro1857.

Lam P, Lavik G, Jensen MM, Van de Vossenberg J, Schmid M, Woebken D, Gutiérrez D, Amann R, Jetten MSM, Kuypers MMM. 2009. Revising the nitrogen cycle in the Peruvian oxygen minimum zone. *Proceedings of the National Academy of Sciences of the United States of America* **106**:4752–4757 DOI 10.1073/pnas.0812444106.

Laverock B, Smith CJ, Tait K, Osborn AM, Widdicombe S, Gilbert JA. 2010. Bioturbating shrimp alter the structure and diversity of bacterial communities in coastal marine sediments. *ISME Journal* **4**:1531–1544 DOI 10.1038/ismej.2010.86.

Lee PO, McLellan SL, Graham LE, Young EB. 2015. Invasive dreissenid mussels and benthic algae in Lake Michigan: characterizing effects on sediment bacterial communities. *FEMS Microbiology Ecology* **91**:1–12 DOI 10.1093/femsec/fiu001.

Leininger S, Urich T, Schloter M, Schwark L, Qi J, Nicol GW, Prosser JI, Schuster SC, Schleper C. 2006. Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature* **442**:806–809 DOI 10.1038/nature04983.

Li M, Cao HL, Hong YG, Gu JD. 2011. Seasonal dynamics of anammox bacteria in estuarial sediment of the mai po nature reserve revealed by analyzing the 16S rRNA and hydrazine oxidoreductase (hzo) genes. *Microbes and Environments* **26**:15–22 DOI 10.1264/jsme2.ME10131.

Lohner RN, Sigler V, Mayer CM, Balogh C. 2007. A comparison of the benthic bacterial communities within and surrounding Dreissena clusters in lakes. *Microbial Ecology* **54**:469–477 DOI 10.1007/s00248-007-9211-8.

Lopes-Lima M, Teixeira A, Froufe E, Lopes A, Varandas S, Sousa R. 2014. Biology and conservation of freshwater bivalves: past, present and future perspectives. *Hydrobiologia* **735**:1–13 DOI 10.1007/s10750-014-1902-9.

Love MI, Huber W, Anders S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology* DOI 10.1186/s13059-014-0550-8.

Lozupone CA, Knight R. 2007. Global patterns in bacterial diversity. *Proceedings of the National Academy of Sciences of the United States of America* **104**:11436–11440 DOI 10.1073/pnas.0611525104.

Lucker S, Wagner M, Maixner F, Pelletier E, Koch H, Vacherie B, Rattei T, Damste JSS, Spieck E, Le Paslier D, Daims H. 2010. A nitrospira metagenome illuminates the physiology and evolution of globally important nitrite-oxidizing bacteria. *Proceedings of the National Academy of Sciences of the United States of America* **107**:13479–13484 DOI 10.1073/pnas.1003860107.

Luesken FA, Sánchez J, Van Alen TA, Sanabria J, Op den Camp HJM, Jetten MSM, Kartal B. 2011. Simultaneous nitrite-dependent anaerobic methane and ammonium oxidation processes. *Applied and Environmental Microbiology* **77**:6802–6807 DOI 10.1128/AEM.05539-11.

Mahmoudi N, Robeson MS, Castro HF, Fortney JL, Techtmann SM, Joyner DC, Paradis CJ, Pfiffner SM, Hazen TC. 2015. Microbial community composition and diversity in Caspian Sea sediments. *FEMS Microbiology Ecology* DOI 10.1093/femsec/fiu013.

Maixner F, Noguera DR, Anneser B, Stoecker K, Wegl G, Wagner M, Daims H. 2006. Nitrite concentration influences the population structure of Nitrospira-like bacteria. *Environmental Microbiology* **8**:1487–1495 DOI 10.1111/j.1462-2920.2006.01033.x.

Martens-Habbena W, Berube PM, Urakawa H, De la Torre JR, Stahl DA. 2009. Ammonia oxidation kinetics determine niche separation of nitrifying Archaea and Bacteria. *Nature* **461**:976–U234 DOI 10.1038/nature08465.

Mason OU, Canter EJ, Gillies LE, Paisie TK, Roberts BJ. 2016. Mississippi river plume enriches microbial diversity in the Northern Gulf of Mexico. *Frontiers in Microbiology* DOI 10.3389/fmicb.2016.01048.

Matteson MR. 1955. Studies on the natural history of the Unionidae. *The American Midland Naturalist* **53**:126–145 DOI 10.2307/2422303.

McCall PL, Matisoff G, Tevesz MJS. 1986. The effects of a unionid bivalve on the physical, chemical, and microbial properties of cohesive sediments from Lake Erie. *American Journal of Science* **286**:127–159 DOI 10.2475/ajs.286.2.127.

McCall PL, Tevesz MJS, Schwelgien SF. 1979. Sediment mixing by lampsilis radiata siliquoidea (mollusca) from Western Lake Erie. *Journal of Great Lakes Research* **5**:105–111 DOI 10.1016/S0380-1330(79)72135-6.

McKindsey CW, Archambault P, Callier MD, Olivier F. 2011. Influence of suspended and off-bottom mussel culture on the sea bottom and benthic habitats: a review. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* **89**:622–646 DOI 10.1139/z11-037.

McMurdie PJ, Holmes S. 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLOS ONE* **8(4)**:e61217 DOI 10.1371/journal.pone.0061217.

Michaud E, Desrosiers G, Aller RC, Mermillod-Blondin F, Sundby B, Stora G. 2009. Spatial interactions in the Macoma balthica community control biogeochemical fluxes at the sediment-water interface and microbial abundances. *Journal of Marine Research* **67**:43–70 DOI 10.1357/002224009788597926.

Millar JJ, Payne JT, Ochs CA, Jackson CR. 2015. Particle-associated and cell-free extracellular enzyme activity in relation to nutrient status of large tributaries of the Lower Mississippi River. *Biogeochemistry* **124**:255–271 DOI 10.1007/s10533-015-0096-1.

Nakagawa T, Takahashi R. 2015. Nitrosomonas stercoris sp. nov. a chemoautotrophic ammonia-oxidizing bacterium tolerant of high ammonium isolated from composted cattle manure. *Microbes and Environments* **30**:221–227 DOI 10.1264/jsme2.ME15072.

Navas-Molina JA, Peralta-Sanchez JM, Gonzalez A, McMurdie PJ, Vazquez-Baeza Y, Xu ZJ, Ursell LK, Lauber C, Zhou HW, Song SJ, Huntley J, Ackermann GL, Berg-Lyons D, Holmes S, Caporaso JG, Knight R. 2013. Advancing our understanding of the human microbiome using QIIME. *Methods in Enzymology* **531**:371–444 DOI 10.1016/B978-0-12-407863-5.00019-8.

Neta MTS, Maciel BM, Lopes ATS, Marques ELS, Rezende RP, Boehs G. 2015. Microbiological quality and bacterial diversity of the tropical oyster Crassostrea rhizophorae in a monitored farming system and from natural stocks. *Genetics and Molecular Research* **14**:15754–15768 DOI 10.4238/2015.December.1.27.

Newell RIE. 2004. Ecosystem influences of natural and cultivated populations of suspension-feeding bivalve molluscs: a review. *Journal of Shellfish Research* **23**:51–61.

Newton TJ, Zigler SJ, Gray BR. 2015. Mortality, movement and behaviour of native mussels during a planned water-level drawdown in the Upper Mississippi River. *Freshwater Biology* **60**:1–15 DOI 10.1111/fwb.12461.

Newton TJ, Zigler SJ, Rogala JT, Gray BR, Davis M. 2011. Population assessment and potential functional roles of native mussels in the Upper Mississippi River. *Aquatic Conservation-Marine and Freshwater Ecosystems* **21**:122–131 DOI 10.1002/aqc.1170.

Ngangbam AK, Baten A, Waters DLE, Whalan S, Benkendorff K. 2015. Characterization of bacterial communities associated with the tyrian purple producing gland in a marine gastropod. *PLOS ONE* **10(10)**:e0140725 DOI 10.1371/journal.pone.0140725.

Nie SA, Li H, Yang XR, Zhang ZJ, Weng BS, Huang FY, Zhu GB, Zhu YG. 2015. Nitrogen loss by anaerobic oxidation of ammonium in rice rhizosphere. *ISME Journal* **9**:2059–2067 DOI 10.1038/ismej.2015.25.

Novais A, Souza AT, Ilarri M, Pascoal C, Sousa R. 2016. Effects of the invasive clam Corbicula fluminea (Muller, 1774) on an estuarine microbial community. *Science of the Total Environment* **566**:1168–1175 DOI 10.1016/j.scitotenv.2016.05.167.

Nowka B, Daims H, Spieck E. 2015. Comparison of oxidation kinetics of nitrite-oxidizing bacteria: nitrite availability as a key factor in niche differentiation. *Applied and Environmental Microbiology* **81**:745–753 DOI 10.1128/AEM.02734-14.

Oshiki M, Satoh H, Okabe S. 2016. Ecology and physiology of anaerobic ammonium oxidizing bacteria. *Environmental Microbiology* **18**:2784–2796 DOI 10.1111/1462-2920.13134.

Padilla CC, Bristow LA, Sarode N, Garcia-Robledo E, Gomez Ramirez E, Benson CR, Bourbonnais A, Altabet MA, Girguis PR, Thamdrup B, Stewart FJ. 2016. NC10 bacteria in marine oxygen minimum zones. *ISME Journal* **10**:2067–2071 DOI 10.1038/ismej.2015.262.

Pester M, Schleper C, Wagner M. 2011. The Thaumarchaeota: an emerging view of their phylogeny and ecophysiology. *Current Opinion in Microbiology* **14**:300–306 DOI 10.1016/j.mib.2011.04.007.

Pfister CA, Gilbert JA, Gibbons SM. 2014. The role of macrobiota in structuring microbial communities along rocky shores. *Peerj* **2**:e631 DOI 10.7717/peerj.631.

Pfister CA, Meyer F, Antonopoulos DA. 2010. Metagenomic profiling of a microbial assemblage associated with the California mussel: a node in networks of carbon and nitrogen cycling. *PLOS ONE* **5(5)**:e10518 DOI 10.1371/journal.pone.0010518.

Pinto AJ, Marcus DN, Ijaz UZ, Bautista-de lose Santos QM, Dick GJ, Raskin L. 2016. Metagenomic evidence for the presence of comammox nitrospira-like bacteria in a drinking water system. *Sphere* **1**:e00054–e00015 DOI 10.1128/mSphere.00054-15.

Pollet T, Cloutier O, Nozais C, McKindsey CW, Archambault P. 2015. Metabolic activity and functional diversity changes in sediment prokaryotic communities organically enriched with mussel biodeposits. *PLOS ONE* **10(4)**:e0123681 DOI 10.1371/journal.pone.0123681.

Price MN, Dehal PS, Arkin AP. 2010. FastTree 2-approximately maximum-likelihood trees for large alignments. *PLOS ONE* **5(3)**:e9490 DOI 10.1371/journal.pone.0009490.

Prosser JI, Head IM, Stein LY. 2014. The family nitrosomonadaceae. In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F, eds. *The prokaryotes: alphaproteobacteria and betaproteobacteria*. Berlin: Springer Berlin Heidelberg, 901–918.

Prosser JI, Nicol GW. 2008. Relative contributions of archaea and bacteria to aerobic ammonia oxidation in the environment. *Environmental Microbiology* **10**:2931–2941 DOI 10.1111/j.1462-2920.2008.01775.x.

Raghoebarsing AA, Pol A, Van de Pas-Schoonen KT, Smolders AJP, Ettwig KF, Rijpstra WIC, Schouten S, Damste JSS, Op den Camp HJM, Jetten MSM, Strous M. 2006. A microbial consortium couples anaerobic methane oxidation to denitrification. *Nature* **440**:918–921 DOI 10.1038/nature04617.

Richter A, Stoeckl K, Denic M, Geist J. 2016. Association between the occurrence of the Thick-shelled River Mussel (Unio crassus) and macroinvertebrate, microbial, and diatom communities. *Freshwater Science* **35**:922–933 DOI 10.1086/687811.

Rikmann E, Zekker I, Tomingas M, Tenno T, Loorits L, Vabamae P, Mandel A, Raudkivi M, Daija L, Kroon K, Tenno T. 2016. Sulfate-reducing anammox for

sulfate and nitrogen containing wastewaters. *Desalination and Water Treatment* **57**:3132–3141 DOI 10.1080/19443994.2014.984339.

Rios-Del Toro EE, Cervantes FJ. 2016. Coupling between anammox and autotrophic denitrification for simultaneous removal of ammonium and sulfide by enriched marine sediments. *Biodegradation* **27**:107–118 DOI 10.1007/s10532-016-9759-4.

Schilling KE, Wolter CF, McLellan E. 2015. Agro-hydrologic landscapes in the Upper Mississippi and Ohio River Basins. *Environmental Management* **55**:646–656 DOI 10.1007/s00267-014-0420-x.

Schramm A, De Beer D, Gieseke A, Amann R. 2000. Microenvironments and distribution of nitrifying bacteria in a membrane-bound biofilm. *Environmental Microbiology* **2**:680–686 DOI 10.1046/j.1462-2920.2000.00150.x.

Schrum HN, Spivack AJ, Kastner M, D'Hondt S. 2009. Sulfate-reducing ammonium oxidation: a thermodynamically feasible metabolic pathway in subseafloor sediment. *Geology* **37**:939–942 DOI 10.1130/G30238A.1.

Schwalb AN, Pusch MT. 2007. Horizontal and vertical movements of unionid mussels in a lowland river. *Journal of the North American Benthological Society* **26**:261–272 DOI 10.1899/0887-3593(2007)26[261:HAVMOU]2.0.CO;2.

Sekiguchi Y, Muramatsu M, Imachi H, Narihiro T, Ohashi A, Harada H, Hanada S, Kamagata Y. 2008. Thermodesulfovibrio aggregans sp nov and Thermodesulfovibrio thiophilus sp nov., anaerobic, thermophilic, sulfate-reducing bacteria isolated from thermophilic methanogenic sludge, and emended description of the genus Thermodesulfovibrio. *International Journal of Systematic and Evolutionary Microbiology* **58**:2541–2548 DOI 10.1099/ijs.0.2008/000893-0.

Shannon JM, Hauser LW, Liu X, Parkin GF, Mattes TE, Just CL. 2015. Partial nitritation ANAMMOX in submerged attached growth bioreactors with smart aeration at 20 °C. *Environmental Science: Processes & Impacts* **17**:81–89 DOI 10.1039/C4EM00481G.

Shen JP, Xu ZH, He JZ. 2014. Frontiers in the microbial processes of ammonia oxidation in soils and sediments. *Journal of Soils and Sediments* **14**:1023–1029 DOI 10.1007/s11368-014-0872-x.

Shen LD, Liu S, He ZF, Lian X, Huang Q, He YF, Lou LP, Xu XY, Zheng P, Hu BL. 2015. Depth-specific distribution and importance of nitrite-dependent anaerobic ammonium and methane-oxidising bacteria in an urban wetland. *Soil Biology & Biochemistry* **83**:43–51 DOI 10.1016/j.soilbio.2015.01.010.

Shen LD, Wu HS, Liu X, Li J. 2017. Vertical distribution and activity of anaerobic ammonium-oxidising bacteria in a vegetable field. *Geoderma* **288**:56–63 DOI 10.1016/j.geoderma.2016.11.007.

Shen L-D, Wu H-S, Gao Z-Q, Ruan Y-J, Xu X-H, Li J, Ma S-J, Zheng P-H. 2016. Evidence for anaerobic ammonium oxidation process in freshwater sediments of aquaculture ponds. *Environmental Science and Pollution Research* **23**:1344–1352 DOI 10.1007/s11356-015-5356-z.

Sonthiphand P, Hall MW, Neufeld JD. 2014. Biogeography of anaerobic ammonia-oxidizing (anammox) bacteria. *Frontiers in Microbiology* **5**:399 DOI 10.3389/fmicb.2014.00399.

Sonthiphand P, Neufeld JD. 2013. Evaluating primers for profiling anaerobic ammonia oxidizing bacteria within freshwater environments. *PLOS ONE* **8(3)**: DOI 10.1371/journal.pone.0057242.

Spang A, Poehlein A, Offre P, Zumbragel S, Haider S, Rychlik N, Nowka B, Schmeisser C, Lebedeva EV, Rattei T, Bohm C, Schmid M, Galushko A, Hatzenpichler R, Weinmaier T, Daniel R, Schleper C, Spieck E, Streit W, Wagner M. 2012. The genome of the ammonia-oxidizing Candidatus Nitrososphaera gargensis: insights into metabolic versatility and environmental adaptations. *Environmental Microbiology* **14**:3122–3145 DOI 10.1111/j.1462-2920.2012.02893.x.

Strayer DL. 2014. Understanding how nutrient cycles and freshwater mussels (Unionoida) affect one another. *Hydrobiologia* **735**:277–292 DOI 10.1007/s10750-013-1461-5.

Strous M, Kuenen JG, Fuerst JA, Wagner M, Jetten MSM. 2002. The anammox case—a new experimental manifesto for microbiological eco-physiology. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology* **81**:693–702 DOI 10.1023/A:1020590413079.

Sun W, Xu MY, Wu WM, Guo J, Xia CY, Sun GP, Wang AJ. 2014. Molecular diversity and distribution of anammox community in sediments of the Dongjiang River, a drinking water source of Hong Kong. *Journal of Applied Microbiology* **116**:464–476 DOI 10.1111/jam.12367.

Tenore KR, Dunstan WM. 1973. Comparison of feeding and biodeposition of three bivalves at different food levels. *Marine Biology* **21**:190–195 DOI 10.1007/BF00355249.

Thamdrup B. 2012. New pathways and processes in the global nitrogen cycle. *Annual Review of Ecology, Evolution, and Systematics* **43**:407–428.

Thorp JH, Delong MD, Greenwood KS, Casper AF. 1998. Isotopic analysis of three food web theories in constricted and floodplain regions of a large river. *Oecologia* **117(4)**:551–563 DOI 10.1007/s004420050692.

Trabal N, Mazon-Suastegui JM, Vazquez-Juarez R, Asencio-Valle F, Morales-Bojorquez E, Romero J. 2012. Molecular analysis of bacterial microbiota associated with oysters (Crassostrea gigas and Crassostrea corteziensis) in different growth phases at two cultivation sites. *Microbial Ecology* **64**:555–569 DOI 10.1007/s00248-012-0039-5.

Trimmer M, Engstrom P. 2011. Distribution, activity, and ecology of anammox bacteria in aquatic environments. *Nitrification* 201–235.

Van Bodegom P, Stams F, Mollema L, Boeke S, Leffelaar P. 2001. Methane oxidation and the competition for oxygen in the rice rhizosphere. *Applied and Environmental Microbiology* **67**:3586–3597 DOI 10.1128/AEM.67.8.3586-3597.2001.

Van Kessel M, Speth DR, Albertsen M, Nielsen PH, Op den Camp HJM, Kartal B, Jetten MSM, Lucker S. 2015. Complete nitrification by a single microorganism. *Nature* **528**:555–571 DOI 10.1038/nature16459.

Vaughn CC, Hakenkamp CC. 2001. The functional role of burrowing bivalves in freshwater ecosystems. *Freshwater Biology* **46**:1431–1446 DOI 10.1046/j.1365-2427.2001.00771.x.

Wakelin SA, Colloff MJ, Kookana RS. 2008. Effect of wastewater treatment plant effluent on microbial function and community structure in the sediment of a freshwater stream with variable seasonal flow. *Applied and Environmental Microbiology* **74**:2659–2668 DOI 10.1128/AEM.02348-07.

Wang HL, Ji GD, Bai XY, He CG. 2015. Assessing nitrogen transformation processes in a trickling filter under hydraulic loading rate constraints using nitrogen functional gene abundances. *Bioresource Technology* **177**:217–223 DOI 10.1016/j.biortech.2014.11.094.

Wang Q, Garrity GM, Tiedje JM, Cole JR. 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology* **73**:5261–5267 DOI 10.1128/AEM.00062-07.

Welte CU, Rasigraf O, Vaksmaa A, Versantvoort W, Arshad A, Op den Camp HJM, Jetten MSM, Lüke C, Reimann J. 2016. Nitrate- and nitrite-dependent anaerobic oxidation of methane. *Environmental Microbiology Reports* **8**:941–955 DOI 10.1111/1758-2229.12487.

Winkler MKH, Ettwig KF, Vannecke TPW, Stultiens K, Bogdan A, Kartal B, Volcke EIP. 2015. Modelling simultaneous anaerobic methane and ammonium removal in a granular sludge reactor. *Water Research* **73**:323–331 DOI 10.1016/j.watres.2015.01.039.

Yang J, Trela J, Zubrowska-Sudol M, Plaza E. 2015. Intermittent aeration in one-stage partial nitritation/anammox process. *Ecological Engineering* **75**:413–420 DOI 10.1016/j.ecoleng.2014.11.016.

Young NC. 2006. Physical characterization of freshwater mussel habitats in upper Mississippi River pool 16. PhD Thesis, University of Iowa, Iowa City, Iowa.

Zeng J, Yang LY, Liang Y, Li JY, Xiao L, Jiang LJ, Zhao DY. 2008. Spatial distribution of bacterial communities in sediment of a eutrophic lake revealed by denaturing gradient gel electrophoresis and multivariate analysis. *Canadian Journal of Microbiology* **54**:1053–1063 DOI 10.1139/W08-098.

Zhalnina KV, Dias R, Leonard MT, Dorr de Quadros P, Camargo FAO, Drew JC, Farmerie WG, Daroub SH, Triplett EW. 2014. Genome sequence of candidatus nitrososphaera evergladensis from group I.1b enriched from everglades soil reveals novel genomic features of the ammonia-oxidizing archaea. *PLOS ONE* **9**:e101648 DOI 10.1371/journal.pone.0101648.

Zhang JX, Yang YY, Zhao L, Li YZ, Xie SG, Liu Y. 2015. Distribution of sediment bacterial and archaeal communities in plateau freshwater lakes. *Applied Microbiology and Biotechnology* **99**:3291–3302 DOI 10.1007/s00253-014-6262-x.

Zhang RJ, Cui B, Huang SB. 2014. Algae consumption and nitrate removal in a raw water transport system by limnoperna fortunei and its associated microorganisms. *Water Environment Research* **86**:2301–2308 DOI 10.2175/106143014X13987223590209.

Zheng YL, Hou LJ, Liu M, Yin GY, Gao J, Jiang XF, Lin XB, Li XF, Yu CD, Wang R. 2016. Community composition and activity of anaerobic ammonium oxidation bacteria in the rhizosphere of salt-marsh grass Spartina alterniflora. *Applied Microbiology and Biotechnology* **100**:8203–8212 DOI 10.1007/s00253-016-7625-2.

Zhu B, Van Dijk G, Fritz C, Smolders AJP, Pol A, Jetten MSM, Ettwig KF. 2012. Anaerobic oxidization of methane in a minerotrophic peatland: enrichment of nitrite-dependent methane-oxidizing bacteria. *Applied and Environmental Microbiology* **78**:8657–8665 DOI 10.1128/AEM.02102-12.

Zhu G, Wang S, Wang Y, Wang C, Risgaard-Petersen N, Jetten MSM, Yin C. 2011. Anaerobic ammonia oxidation in a fertilized paddy soil. *The ISME Journal* **5**:1905–1912 DOI 10.1038/ismej.2011.63.

Zhu X, Burger M, Doane TA, Horwath WR. 2013. Ammonia oxidation pathways and nitrifier denitrification are significant sources of N2O and NO under low oxygen availability. *Proceedings of the National Academy of Sciences of the United States of America* **110**:6328–6333 DOI 10.1073/pnas.1219993110.