GenBank accession numbers for ammonia oxidizer genes collected on the R/V Endeavor (SQO-Delta) in the San Francisco Bay Delta during September and October 2007.

Website: https://www.bco-dmo.org/dataset/654295
Data Type: Cruise Results
Version: 1
Version Date: 2016-08-18

Project
» Spatial and Temporal Dynamics of Nitrogen-Cycling Microbial Communities Across Physicochemical Gradients in the San Francisco Bay Estuary (N-Cycling Microbial Communities)

Contributors	Affiliation	Role
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Ake, Hannah	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
GenBank accession numbers for ammonia oxidizer genes collected on the R/V Endeavor (SQO-Delta) in the San Francisco Bay Delta during September and October 2007.

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Coverage

Spatial Extent: **N**:38.167117 **E**:-121.597033 **S**:38.017617 **W**:-121.850917
Temporal Extent: 2007-09-17 - 2007-10-10

Dataset Description

Surface sediment samples were collected for potential nitrification rates, using sediment slurries with filtered site water. Ammonia oxidizer gene abundances (AOA and AOB *amoA*) were quantified using qPCR, and clone libraries for each gene were sequenced using Sanger sequencing.

Related Manuscript: Damashel *et al.*, 2015

Methods & Sampling

Surface sediment was retrieved using a modified Van Veen grab. Duplicate cores were taken from each grab sample using sterile, cut-off 5 mL syringes and immediately placed on dry ice prior to storage at –80 degrees celsius. Bottom water nutrient samples were collected in triplicate using a hand-held Niskin bottle, immediately

filtered (0.2 um pore size), and frozen on dry ice prior to storage at –20 degrees celsius. Nutrient (NH_4^+, NO_2^-, and NO_3^-) concentrations were measured using a QuikChem 8000 Flow Injection Analyzer (Lachat Instruments).

Sediment samples for potential nitrification rate measurements were collected in triplicate into the barrels of cut-off 60 mL syringes, which were sealed with parafilm and transported to the laboratory on ice. Potential rates were measured using amended sediment slurries. Slurries included 5 g of sediment (top 1 cm) homogenized in 100 mL of filtered bottom water augmented with NH_4^+ and phosphate to final additional concentrations of 500 and 100 uM, respectively. Amended slurries were shaken (200 rpm) in the dark for 24 hours at room temperature (about 22 degrees celsius). Aliquots for the determination of NO_3^- plus NO_2^- (NOX) were collected at evenly spaced intervals through the incubation period and stored at –20 degrees celsius. Prior to analysis, aliquots were thawed and passed through Whatman No. 42 filter paper, and the filtrate was analyzed for the accumulation of NOx over time, using a SmartChem 200 Discrete Analyzer (Unity Scientific). Rates were determined by linear regression of NOx concentrations over time.

DNA was extracted from approximately 0.5 g of surface sediments by extruding and cutting the top 0.5 cm from frozen cores with a sterile scalpel and immediately proceeding with the FastDNA SPIN Kit for Soil (MP Biomedicals), including a FastPrep bead beating step of 30 s at speed 5.5. AOA and AOB *amoA* genes were quantified using gene-specific SYBR qPCR assays on a StepOnePlus Real-Time PCR System (Life Technologies). AOA *amoA* reactions contained iTaq SYBR Green Supermix with ROX (Bio-Rad Laboratories), 0.4 uM primers Arch-amoAF/Arch-amoAR (Francis et al., 2005) and 1 uL template DNA. AOA qPCR program details were identical to previously published protocols (Mosier and Francis, 2008) but with a 10 s detection step at 78.5 degrees celsius. AOB *amoA* qPCR reactions used primers amoA1F/amoA2R (Rotthauwe et al., 1997), and were set up following Mosier and Francis (2008) but with a 10 s detection step at 83 degress celsius. Each plate included a standard curve (5 to 10^6 copies/reaction) made by serial dilution of linearized plasmids extracted from previously sequenced clones, and negative controls that substituted sterile water for DNA. The diversity of ammonia oxidizing communities was determined by cloning and sequencing of PCR-amplified *amoA* genes using primers Arch-amoAF/Arch-amoAR (Francis et al., 2005) and amoA1F*/amoA2R (Rotthauwe et al., 1997; Stephen et al., 1999) for AOA and AOB, respectively. Reaction conditions and PCR programs followed previously published protocols (Mosier and Francis, 2008). Triplicate reactions were qualitatively checked by gel electrophoresis, pooled, and purified using the MinElute PCR Purification Kit or MinElute Gel Extraction Kit (Qiagen), following the manufacturer's instructions. Purified products were cloned using the pGEM-T Vector System II (Promega), and sequenced by Elim Biopharmaceuticals on a 3730xl capillary sequencer (Life Technologies). Sequences were imported into Geneious (version 6.1.6 created by Biomatters, available from http://www.geneious.com) and manually cleaned prior to operational taxonomic unit (OTU) grouping (greater than or equal to 95% sequence similarity) using mothur (Schloss et al., 2009). Rarefaction curves and diversity/richness estimators (Chao1 and Shannon indices) were calculated using mothur. OTUs were aligned with reference sequences using the MUSCLE alignment package within Geneious, using a gap open score of –750. Alignments were manually checked and used to build neighbor-joining bootstrap trees (Jukes-Cantor distance model, 1000 neighbor joining bootstrap replicates) within Geneious. The *amoA* sequences generated in this study have been deposited into GenBank with accession numbers KM000240 to KM000508 (AOB) and KM000509 to KM000784 (AOA).

Two-tailed Spearman rank correlation coefficients (ρ) were calculated using R (R Core Team, 2014) to determine correlations between variables, using the suggested critical value of 0.786 for 5% significance with a sample size of 7 (Zar, 1972). Principal component and non-metric multidimensional scaling analyses were performed using the vegan package in R (Oksanen, 2013). Environmental variables were z-transformed to standardize across different scales and units by subtracting the population mean from each measurement and dividing by the standard deviation. OTU count data were Hellinger-transformed to standardize to relative abundances (Legendre and Legendre, 2012). Other than unweighted UniFrac distances, which were calculated using the online UniFrac portal (Lozupone et al., 2006), distance/dissimilarity indices were calculated using the vegan package in R. All principle component analyses are presented using scaling 1; therefore, the distance between sites on the biplot represents their Euclidean distance, and the right-angle projection of a site onto a descriptor vector shows the approximate position of that site on the vector (Legendre and Legendre, 2012).

Data Processing Description

DMO Notes:

-created a row for every accession number, they were originally presented by the PI as a range.
-added links for every accession number.

-removed all spaces and replaced with underscores.
-reformatted column names to comply with BCO-DMO standards.
-reorganized the data so that all station numbers were grouped together

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Data Files

File
GenBank_accessions.csv (Comma Separated Values (.csv), 103.37 KB) MD5:8a6ea46c5891bfcc1e4c06509bc2abd1
Primary data file for dataset ID 654295

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Related Publications

Damashek, J., Smith, J. M., Mosier, A. C., & Francis, C. A. (2015). Benthic ammonia oxidizers differ in community structure and biogeochemical potential across a riverine delta. Frontiers in Microbiology, 5. doi:10.3389/fmicb.2014.00743
General

Francis, C. A., Roberts, K. J., Beman, J. M., Santoro, A. E., & Oakley, B. B. (2005). Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. Proceedings of the National Academy of Sciences, 102(41), 14683–14688. doi:10.1073/pnas.0506625102
Methods

Legendre, P., and Legendre, L. (2012). Numerical Ecology, 3rd Edn. San Francisco, CA: Elsevier. https://isbnsearch.org/isbn/978-0-444-538680
Methods

Lozupone, C., Hamady, M., & Knight, R. (2006). UniFrac - An online tool for comparing microbial community diversity in a phylogenetic context. BMC Bioinformatics, 7(1), 371. doi:10.1186/1471-2105-7-371
Methods

Mosier, A. C., & Francis, C. A. (2008). Relative abundance and diversity of ammonia-oxidizing archaea and bacteria in the San Francisco Bay estuary. Environmental Microbiology, 10(11), 3002–3016. doi:10.1111/j.1462-2920.2008.01764.x
Methods

Oksanen, J. (2013). Multivariate Analysis of Ecological Communities in R: Vegan Tutorial. Available online at: http://cc.oulu.fi/~jarioksa/opetus/metodi/vegantutor.pdf
Methods

Rotthauwe, J. H., Witzel, K. P., and Liesack, W. (1997). The ammonia monooxygenase structural gene amoA as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations. Appl. Environ. Microbiol. 63, 4704–4712.
Methods

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., … Weber, C. F. (2009). Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. Applied and Environmental Microbiology, 75(23), 7537–7541. doi:10.1128/aem.01541-09 https://doi.org/10.1128/AEM.01541-09
Software

Stephen, J. R., Chang, Y. J., Macnaughton, S. J., Kowalchuk, G. A., Leung, K. T., Flemming, C. A., et al. (1999). Effect of toxic metals on indigenous soil ß-subgroup proteobacterium ammonia oxidizer community structure and protection against toxicity by inoculated metal-resistant bacteria. Appl. Environ. Microbiol. 65, 95–101.
Methods

Zar, J. H. (1972). Significance Testing of the Spearman Rank Correlation Coefficient. Journal of the American Statistical Association, 67(339), 578–580. doi:10.1080/01621459.1972.10481251 https://doi.org/10.2307/2284441

Methods

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Parameters

Parameter	Description	Units
station	station where sample was taken	unitless
lat	latitude	decimal degrees
lon	longitude	decimal degrees
organism	organism analyzed	unitless
gene	gene analyzed	unitless
accession_numbers	GenBank accession numbers	unitless

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Instruments

Dataset-specific Instrument Name	SmartChem 200 Discrete Analyzer
Generic Instrument Name	Discrete Analyzer
Dataset-specific Description	Filtrate analyzed from the accumulation of NOx over time using this discrete analyzer.
Generic Instrument Description	Discrete analyzers utilize discrete reaction wells to mix and develop the colorimetric reaction, allowing for a wide variety of assays to be performed from one sample. These instruments are ideal for drinking water, wastewater, soil testing, environmental and university or research applications where multiple assays and high throughput are required.

Dataset-specific Instrument Name	QuikChem 8000
Generic Instrument Name	Flow Injection Analyzer
Dataset-specific Description	Concentrations measured via QuikChem 8000 Flow Injection Analyzer
Generic Instrument Description	An instrument that performs flow injection analysis. Flow injection analysis (FIA) is an approach to chemical analysis that is accomplished by injecting a plug of sample into a flowing carrier stream. FIA is an automated method in which a sample is injected into a continuous flow of a carrier solution that mixes with other continuously flowing solutions before reaching a detector. Precision is dramatically increased when FIA is used instead of manual injections and as a result very specific FIA systems have been developed for a wide array of analytical techniques.

Dataset-specific Instrument Name	Niskin bottle
Generic Instrument Name	Niskin bottle
Dataset-specific Description	Hand-held Niskin bottle
Generic Instrument Description	A Niskin bottle (a next generation water sampler based on the Nansen bottle) is a cylindrical, non-metallic water collection device with stoppers at both ends. The bottles can be attached individually on a hydrowire or deployed in 12, 24, or 36 bottle Rosette systems mounted on a frame and combined with a CTD. Niskin bottles are used to collect discrete water samples for a range of measurements including pigments, nutrients, plankton, etc.

Dataset-specific Instrument Name	StepOnePlus Real-Time PCR System
Generic Instrument Name	Thermal Cycler
Dataset-specific Description	Genes quantified using gene-specific SYBR qPCR assays
Generic Instrument Description	A thermal cycler or "thermocycler" is a general term for a type of laboratory apparatus, commonly used for performing polymerase chain reaction (PCR), that is capable of repeatedly altering and maintaining specific temperatures for defined periods of time. The device has a thermal block with holes where tubes with the PCR reaction mixtures can be inserted. The cycler then raises and lowers the temperature of the block in discrete, pre-programmed steps. They can also be used to facilitate other temperature-sensitive reactions, including restriction enzyme digestion or rapid diagnostics. (adapted from http://serc.carleton.edu/microbelife/research_methods/genomics/pcr.html)

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Deployments

SQO-Delta

Website	https://www.bco-dmo.org/deployment/654306
Platform	R/V Endeavor
Report	http://dmoserv3.bco-dmo.org/data_docs/N_Cycling_Microbial_Communities/cruise_reports/2007-08-21-Sediment.pdf
Start Date	2007-09-17
End Date	2007-10-17
Description	2007 Regional Monitoring Program (RMP) Sediment Cruise SQO-Delta Cruise Plan

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Project Information

Spatial and Temporal Dynamics of Nitrogen-Cycling Microbial Communities Across Physicochemical Gradients in the San Francisco Bay Estuary (N-Cycling Microbial Communities)

Coverage: San Francisco Bay

Description from the NSF award abstract:

This award is funded under the American Recovery and Reinvestment Act of 2009 (Public Law 111-5).

Although nitrogen (N) acts as a limiting nutrient in many marine ecosystems, from estuaries to the open ocean, N in excess can be extremely detrimental. Eutrophication is of particular concern in estuaries, with over half of the estuaries in the United States experiencing its effects. Harmful levels of N in estuaries can be diminished through tightly coupled processes in the microbial nitrogen cycle, including nitrification (chemoautotrophic oxidation of ammonia to nitrite and nitrate) and denitrification (the dissimilatory reduction of nitrate to N2 gas). In fact, coupled nitrification-denitrification can remove up to 50% of external dissolved inorganic nitrogen inputs to estuaries, thereby reducing the risk of eutrophication. Despite the biogeochemical importance of both nitrification and denitrification in estuarine systems, surprisingly little is known regarding the underlying microbial communities responsible for these processes, or how they are influenced by key physical/chemical factors.

The investigators will work in San Francisco Bay - the largest estuary on the west coast of the United States - using molecular, biogeochemical and cultivation approaches to explore how the distribution, diversity, abundance, and activities of key N-cycling communities are influenced by environmental gradients over temporal and spatial scales. Denitrifying communities will be studied using functional genes (nirK and nirS) encoding the key denitrification enzyme nitrite reductase, while genes encoding ammonia monooxygenase subunit A (amoA) will be used to study both ammonia-oxidizing bacteria (AOB) and the recently-discovered ammonia-oxidizing archaea (AOA)- members of one of the most ubiquitous and abundant prokaryotic groups on the planet, the mesophilic Crenarchaeota. Analyzing sediments from sites spanning a range of physical and chemical conditions in the Bay, seasonally over the course of several years, will represent an unprecedented opportunity to examine spatial, physical/chemical, and temporal effects on both denitrifier and ammonia-oxidizer communities in this large, urban estuary. Concurrently, an intensive cultivation effort will also be undertaken, in order to compile a novel culture collection of estuarine denitrifiers and ammonia-oxidizers, for which virtually nothing is currently known. Taken together, these complimentary approaches will help reveal how complex physical/chemical gradients influence the diversity and functioning of key estuarine N-cycling communities over time and space.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-0847266

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