Amplicon sequencing of ammonia oxidizing archaea amoA gene from R/V Kilo Moana KM1314 in the North Pacific Ocean, Aug-Sept 2013 (Nitrification and Planktonic Biodiversity project)

Website: https://www.bco-dmo.org/dataset/672336
Data Type: Cruise Results
Version:
Version Date: 2017-01-04

Project
» Significance of nitrification in shaping planktonic biodiversity in the ocean (Nitrification and Marine Planktonic Biodiversity)

Program
» Dimensions of Biodiversity (Dimensions of Biodiversity)

Contributors	Affiliation	Role
Stahl, David A.	University of Washington (UW)	Principal Investigator
Armbrust, E. Virginia	University of Washington (UW)	Co-Principal Investigator
Devol, Allan	University of Washington (UW)	Co-Principal Investigator
Ingalls, Anitra E.	University of Washington (UW)	Co-Principal Investigator
Moffett, James W.	University of Southern California (USC)	Co-Principal Investigator
Copley, Nancy	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

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Dataset Description

Amplicon sequencing of ammonia oxidizing archaea amoA gene data from R/V Kilo Moana KM1314 in the North Pacific Ocean (Seattle to Honolulu, including Line P, Station ALOHA).

Related Datasets:
Ammonia oxidation rate profiles
Particulate vitamin B12 profiles

Methods & Sampling

We determined the community diversity for nitrite max samples at stations 1, 3, 5, 10, 11, 13, 14, 15, and 16 and chlorophyll max samples at station 8 (55 m). Marine AOA amoA genes were amplified from template DNA (~20 ng/uL) using a two-step enrichment procedure for target amplification and sample barcoding on a

Fluidigm Access Array (Fluidigm Inc., San Francisco CA). Gene targets were amplified using primer pairs ArchAmoA_1F and ArchAmoA1R and sample specific Golay primers (Francis et al. 2005). Amplified products were analyzed by gel electrophoresis to ensure the correct amplicon size, quantified using pico-green, pooled to an equal molar concentration and sequenced in pair-end 300 bp mode using the Illumina HiSeq Platform. Resulting sequences were assessed for quality using fastq_eestats and trimmed at the 5' (5bp) and 3' (225bp) ends (Edgar 2010). Trimmed reads were then quality filtered in QIIME using the recommendations described by Bokulich et al. (2013). The resulting reads were then analyzed using the UPARSE pipeline with an OTU clustering identity of 95% (Edgar 2013). Representative OTU sequences were analyzed against a reference database of AOA amoA genes in the ARB software using the parsimony tool for insertion (Ludwig et al. 2004; Pester et al. 2012). Sample-OTU matrices from UPARSE were converted to biom format and rarified to 1000 reads per sample using the single_rarfy.py script in QIIME. The resulting output file was then used for calculation of Bray-Curtis similarity values between samples and hierarchical clustering using the package vegan in R.

Data Processing Description

BCO-DMO Data Processing Notes:

- added conventional header with dataset name, PI name, version date, reference information
- renamed parameters to BCO-DMO standard
- converted lat and lon to decimal degrees

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Data Files

File	
amoA_reads.csv	(Comma Separated Values (.csv), 735 bytes) MD5:372d3a6796bbaf838920317c18eeaadc
Primary data file for dataset ID 672336	

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Parameters

Parameter	Description	Units
cruise_id	cruise identifier	unitless
sta	station number	unitless
depth	sampling depth	meters
lat	latitude; north is positive	decimal degrees
lon	longitude; east is positive	decimal degrees
Arch_OTU95_1_reads	amplicon sequencing reads of AOA amoA gene (encoding alpha unit of ammonia monooxygenase) for OTU (operational taxonomic unit) 1	amplicon reads
Arch_OTU95_2_reads	amplicon sequencing reads of AOA amoA gene (encoding alpha unit of ammonia monooxygenase) for OTU 2	amplicon reads
Arch_OTU95_5_reads	amplicon sequencing reads of AOA amoA gene (encoding alpha unit of ammonia monooxygenase) for OTU 5	amplicon reads
Arch_OTU95_29_reads	amplicon sequencing reads of AOA amoA gene (encoding alpha unit of ammonia monooxygenase) for OTU 29	amplicon reads
Arch_OTU95_34_reads	amplicon sequencing reads of AOA amoA gene (encoding alpha unit of ammonia monooxygenase) for OTU 34	amplicon reads
Arch_OTU95_38_reads	amplicon sequencing reads of AOA amoA gene (encoding alpha unit of ammonia monooxygenase) for OUT 38	amplicon reads

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Instruments

Dataset-specific Instrument Name	Illumina HiSeq Platform
Generic Instrument Name	Automated DNA Sequencer
Generic Instrument Description	General term for a laboratory instrument used for deciphering the order of bases in a strand of DNA. Sanger sequencers detect fluorescence from different dyes that are used to identify the A, C, G, and T extension reactions. Contemporary or Pyrosequencer methods are based on detecting the activity of DNA polymerase (a DNA synthesizing enzyme) with another chemoluminescent enzyme. Essentially, the method allows sequencing of a single strand of DNA by synthesizing the complementary strand along it, one base pair at a time, and detecting which base was actually added at each step.

Dataset-specific Instrument Name	
Generic Instrument Name	Thermal Cycler
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

KM1314

Website	https://www.bco-dmo.org/deployment/536050
Platform	R/V Kilo Moana
Start Date	2013-08-07
End Date	2013-09-05

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Project Information

Significance of nitrification in shaping planktonic biodiversity in the ocean (Nitrification and Marine Planktonic Biodiversity)

Microorganisms sustain the biogeochemical cycling of nitrogen, one of the most important nutrient cycles on earth. A key step in this cycle, the oxidation of ammonia to nitrite by autotrophic microorganisms, was for a century thought mediated by a few restricted bacterial genera. Significant ammonia oxidation, perhaps most, is now attributed to a previously enigmatic group of Archaea - the ammonia-oxidizing archaea (AOA) - of high abundance in both marine and terrestrial environments. The investigators prior physiological and environmental analyses, the foundation for this proposal, have shown that AOA are active within the marine photic zone and that their competitive fitness in the marine environment is at least in part attributable to an extremely high affinity for ammonia, growing at near maximum growth rates at concentrations of ammonia that would not sustain known bacterial ammonia oxidizers, and an unusual copper-based respiratory system that may render them more competitive in iron limited environments. The compelling inference from these prior analyses is that AOA alter and possibly control the forms of fixed nitrogen available to other microbial assemblages within the photic zone by converting ammonia, a nearly universally available form of nitrogen, into nitrite, a form only available to nitrite oxidizing bacteria and some phytoplankton. If correct, this has a significant impact on biodiversity.

The PIs will use the most recent technological advances in protein and high throughput sequencing to evaluate the significance of nitrification in shaping biodiversity (genomic and metagenomics), activity (transcriptome, proteome and stable isotope probing), and in controlling availability of an important trace element (copper). In turn, by resolving the environmental and biotic variables that influence the diversity, distribution and activity of AOA, they will advance general understanding of their taxonomy. More directly, functional knowledge of the contribution of AOA to regenerated nitrate will improve estimates of new ocean production ("biological pump")

based on nitrate assimilation, which in the past has mostly neglected the importance of nitrification as a major source of nitrate. Together these studies will transform understanding of the marine nitrogen cycle, estimates of new production, and will ultimately provide a better understanding of the impact of human activity on this critical nutrient cycle.

The nitrogen cycle has been profoundly affected by anthropogenic inputs of reactive nitrogen into terrestrial, marine, and atmospheric systems having, or predicted to have, major impacts on marine biological production, increased N20 emissions, nitrogen pollution, and eutrophication. Likewise, there is a poor understanding of the relationship between nitrogen cycling and productivity in marine ecosystems. Marine systems are increasingly affected by ocean acidification and by atmospheric inputs of reactive nitrogen. Since both changes greatly alter nitrogen available to microorganisms, the characterization of the response of these environmentally relevant AOA is of tremendous relevance to understanding the affect of acidification and anthropogenic nitrogen inputs on major ocean processes.

The proposed project encompasses and integrates the three dimensions (functional genetic, and taxonomic) of biodiversity. First, the project is framed by function: microbial control of one of the most important nutrient cycles on earth, the nitrogen-cycle. Second, it is motivated by recent genetic analyses that associate activities of a novel clade of Archaea (provisionally assigned to a new kingdom within the Archaea, the Thaumarchaeota) with control of ammonia oxidation in the ocean. Third, it is built upon a compelling synthesis of physiological and environmental data that lead to its central hypothesis that by altering and possibly controlling the form of nitrogen, the AOA also alter biodiversity and ecological function in one of the most productive environments on earth. It identifies a specific taxonomic imperative. The tremendous genetic diversity among the globally abundant AOA catalogued almost exclusively by gene sequencing surveys and therefore lacking formal description makes it essential to resolve membership into ecologically relevant groups or clades as a prelude to developing a formal taxonomy. The investigators have assembled a group of researchers with specific expertise in each of dimension and uniquely qualified to address the research objectives outlined in an integrative way.

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Program Information

Dimensions of Biodiversity (Dimensions of Biodiversity)

Website: http://www.nsf.gov/funding/pgm_summ.jsp?pims_id=503446

Coverage: global

(adapted from the NSF Synopsis of Program)
Dimensions of Biodiversity is a program solicitation from the NSF Directorate for Biological Sciences. FY 2010 was year one of the program. [MORE from NSF]

The NSF Dimensions of Biodiversity program seeks to characterize biodiversity on Earth by using integrative, innovative approaches to fill rapidly the most substantial gaps in our understanding. The program will take a broad view of biodiversity, and in its initial phase will focus on the integration of genetic, taxonomic, and functional dimensions of biodiversity. Project investigators are encouraged to integrate these three dimensions to understand the interactions and feedbacks among them. While this focus complements several core NSF programs, it differs by requiring that multiple dimensions of biodiversity be addressed simultaneously, to understand the roles of biodiversity in critical ecological and evolutionary processes.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1046017

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