Quantitative PCR data from sediment samples from MPSV GREATSHIP MANISHA IODP-347 cruise in the Baltic Sea in 2013 (IODP-347 Microbial Quantification project)

Website: https://www.bco-dmo.org/dataset/641358
Version: 25 March 2016
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Project
» Quantifying the contribution of the deep biosphere in the marine sediment carbon cycle using deep-sea sediment cores from the Baltic Sea (IODP-347 Microbial Quantification)

Programs
» Center for Dark Energy Biosphere Investigations (C-DEBI)
» International Ocean Discovery Program (IODP)

Contributors	Affiliation	Role
Lloyd, Karen G.	University of Tennessee Knoxville (UTK)	Principal Investigator, Contact
Steen, Andrew	University of Tennessee Knoxville (UTK)	Co-Principal Investigator
Gegg, Stephen R.	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

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Coverage

Spatial Extent: **N**:55.00483 **E**:58.622167 **S**:10.107828 **W**:18.254
Temporal Extent: 2013-09-13 - 2013-10-30

Dataset Description

Quantitative PCR data from sediment samples

Locations:
Site 59C (Little Belt); Site 60B (Anholt Loch); 63E (Landsort Deep); 65C (Bornholm Basin).
Subsurface samples as deep as 85 meters below the Baltic Sea floor.

Methods & Sampling

Sampling and Analytical Methodology:
Genomic DNA was extracted from Baltic Sea Basin sediments using FastDNA® Spin Kit for Soil (MP Biomedicals). 16S rRNA gene copy numbers of targets were quantified with qPCR using the primers in the table in datasheet. Results of qPCR were rejected if the R2 of the standard curve was

below 0.95, or if the melt curve showed evidence of primer dimers. SYBR green chemistry was used for all reactions, and Invitrogen mastermix was used for DNA copy number measurement on a BioRad iQ5 (Applied Biosystems, Foster City, California). Serial dilutions of full-length 16S rRNA gene PCR products from plasmids containing amplified partial 16S genes were used as standards.

Primers Used:

Primer name	Sequence (5' - 3')	Target	Reference
Bac340f	TCCTACGGGAGGCAGCAGT	Bacteria	Nadkarni et al., 2002
Bac 515r	CGTATTACCGCGGCTGCTGGCAC	Bacteria	Nadkarni et al., 2002
Arch915f	GTGCTCCCCCGCCAATTCCT	Archaea	Kubo et al., 2012
Arch1059r	GCCATGCACCWCCTCT	Archaea	Kubo et al., 2012
ANME1-628f	GCT TTC AGG GAA TAC TGC	ANME-1	Lloyd et al., 2011
ANME1-830r	TCG CAG TAA TGC CAA CAC	ANME-1	Lloyd et al., 2011
MCG528f	CGGTAATACCAGCTCTCCGAG		Kubo et al., 2012
MCG732r	CGCGTTCTAGCCGACAGC		Kubo et al., 2012

Primers used from the following publications:
Archaea of the Miscellaneous Crenarchaeotal Group are abundant, diverse and widespread in marine sediments

Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set

Environmental evidence for net methane production and oxidation in putative ANaerobic MEthanotrophic (ANME) archaea

Data Processing Description

Data Processing:
Absolute quantification was calculated by converting Ct values of samples into copy numbers per microliter of DNA with the linear equation produced by the standard curve with R2 greater than 0.95. The quantification limit was defined as having fluorescence threshold cycle numbers (Ct) well within those of the simultaneously-run standard curve and being at least 3 Ct below the non-template control Ct.

BCO-DMO Processing Notes
- Generated from original file "qPCR Exp IODP 347.xlsx, sheet: qPCR" contributed by Joy Buongiorno
- Parameter names edited to conform to BCO-DMO naming convention found at Choosing Parameter Name
- Latitude and Longitude for sample inserted from Sites data
- "nd" (no data) inserted into blank cells

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Data Files

File
qPCR.csv (Comma Separated Values (.csv), 2.91 KB) MD5:33bfe6bf077e8871a5e96512e2937026 Primary data file for dataset ID 641358

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Parameters

Parameter	Description	Units
Identifier	Sample Identifier	text
Latitude	Site Latitude (South is negative)	decimal degrees
Longitude	Site Longitude (West is negative)	decimal degrees
Depth	Depth of sample in core	meters
Archaea_replicate_1	Archaea replicate 1 (Units are copies of target DNA per microliter of DNA)	copies/uL
Archaea_replicate_2	Archaea replicate 2 (Units are copies of target DNA per microliter of DNA)	copies/uL
Bacteria_replicate_1	Bacteria replicate 1 (Units are copies of target DNA per microliter of DNA)	copies/uL
Bacteria_replicate_2	Bacteria replicate 2 (Units are copies of target DNA per microliter of DNA)	copies/uL
ANME1_replicate_1	ANME1 replicate 1 (Units are copies of target DNA per microliter of DNA)	copies/uL
ANME1_replicate_2	ANME1 replicate 2 (Units are copies of target DNA per microliter of DNA)	copies/uL
MCG_replicate_1	MCG replicate 1 (Units are copies of target DNA per microliter of DNA)	copies/uL
MCG_replicate_2	MCG replicate 2 (Units are copies of target DNA per microliter of DNA)	copies/uL

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Instruments

Dataset-specific Instrument Name	BioRad iQ5
Generic Instrument Name	Thermal Cycler
Dataset-specific Description	Genomic DNA was extracted from Baltic Sea Basin sediments using FastDNA® Spin Kit for Soil (MP Biomedicals). 16S rRNA gene copy numbers of targets were quantified with qPCR using the primers in the table in datasheet. Results of qPCR were rejected if the R2 of the standard curve was below 0.95, or if the melt curve showed evidence of primer dimers. SYBR green chemistry was used for all reactions, and Invitrogen mastermix was used for DNA copy number measurement on a BioRad iQ5 (Applied Biosystems, Foster City, California). Serial dilutions of full-length 16S rRNA gene PCR products from plasmids containing amplified partial 16S genes were used as standards. BioRad iQ5
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)

Dataset-specific Instrument Name	FastDNA® Spin Kit for Soil
Generic Instrument Name	Thermal Cycler
Dataset-specific Description	Genomic DNA was extracted from Baltic Sea Basin sediments using FastDNA® Spin Kit for Soil (MP Biomedicals). 16S rRNA gene copy numbers of targets were quantified with qPCR using the primers in the table in datasheet. Results of qPCR were rejected if the R2 of the standard curve was below 0.95, or if the melt curve showed evidence of primer dimers. SYBR green chemistry was used for all reactions, and Invitrogen mastermix was used for DNA copy number measurement on a BioRad iQ5 (Applied Biosystems, Foster City, California). Serial dilutions of full-length 16S rRNA gene PCR products from plasmids containing amplified partial 16S genes were used as standards. FastDNA® Spin Kit for Soil
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

IODP-347

Website	https://www.bco-dmo.org/deployment/641281
Platform	MPSV GREATSHIP MANISHA
Report	http://publications.iodp.org/preliminary_report/347/
Start Date	2013-09-12
End Date	2013-11-01
Description	IODP Expedition 347 Baltic Sea Basin Paleoenvironment During Integrated Ocean Drilling Program Expedition 347, sediments from different settings in the Baltic Sea Basin (BSB) spanning the last glacial–interglacial cycles will be cored to address four main research themes: - Climate and sea level dynamics of marine oxygen isotope Stage (MIS) 5, including onsets and terminations, - The complexities of the last glacial (MIS 4–MIS 2), - Glacial and Holocene climate forcing (MIS 2–MIS 1), and - Deep biosphere responses to glacial–interglacial cycles. Addressing these themes will be accomplished by drilling in six subbasins: one in the gateway of the BSB (Anholt Loch), focusing on sediments from MIS 6–MIS 5 and MIS 2–MIS 1; a subbasin in the southwesternmost part of the BSB (Little Belt) that possibly retains a unique MIS 5 record; two subbasins in the south (Bornholm Basin and Hanö Bay) to target long complete records from MIS 4–MIS 2; and one deep (450 m) subbasin in the central Baltic Sea (Landsort Deep) that contains a thick and continuous record of the last ~14,000 y. Finally, the subbasin in the very north (Ångermanälven River estuary) contains a unique varved (annually deposited) sediment record of the last >10,000 y. These six areas contain a combined suite of sediment sequences encompassing the last ~140,000 y, with paleoenvironmental information on a semicontinental scale, as the Baltic Sea drains an area four times as large as the basin itself. The location of the BSB in the heartland of the recurrently waning and waxing Scandinavian Ice Sheet (SIS) has resulted in a complex development history including repeated glaciations of different magnitude, sensitive responses to sea level and gateway threshold changes, large shifts in sedimentation patterns, and high sedimentation rates. Its position also makes it a unique link between Eurasian and northwest European terrestrial records. Therefore the sediments of this largest European intracontinental basin form a rare archive of climate evolution over the last glacial cycle. The high sedimentation rates provide an excellent opportunity to reconstruct climatic variability of global importance at a unique resolution from a marine-brackish setting in a location where comparable sequences from the surrounding onshore regions cannot be obtained. Furthermore, the large variability (salinity, climate, sedimentation pattern, and oxygenation) that the BSB has undergone during the last glacial cycle makes it optimal for new research on the deep biosphere, addressing questions such as its evolution, its biogeochemical processes, and how the postglacial diffusive penetration of conservative seawater ions may alter the chemical composition and microbial physiology in the subseafloor biosphere. IODP-347 General Information IODP Expedition Proceedings Note: Drill site locations used for deployment locations table

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Project Information

Quantifying the contribution of the deep biosphere in the marine sediment carbon cycle using deep-sea sediment cores from the Baltic Sea (IODP-347 Microbial Quantification)

Coverage: Baltic Sea

Marine sediments contain a microbial population large enough to rival that of Earth's oceans, but much about this vast community is unknown. Innovations in total cell counting methods have refined estimates of cell concentrations, but tell us nothing about specific taxa. Isotopic data provides evidence that a majority of subsurface microorganisms survive by breaking down organic matter, yet measurable links between specific microbial taxa and their organic matter substrates are untested. The proposed work overcomes these limitations, with a particular focus on the degradation of proteins and carbohydrates, which comprise the bulk of classifiable sedimentary organic matter. The project will link specific taxa to potential extracellular enzyme activity in the genomes of single microbial cells, apply newly-identified, optimal methods for counting viable cells

belonging to specific taxa using catalyzed reporter deposition fluorescent in situ hybridization (CARD-FISH), and measure the potential activity of their enzymes in situ. The resulting data will provide key evidence about the strategies subsurface life uses to overcome extreme energy limitation and contribute to the long-term carbon cycle.

The Principal Investigators are employing novel,improved methods to quantify cells of specific taxa in the marine subsurface and to determine the biogeochemical functions of those uncultured taxa, including:
1) Determine the pathway of organic carbon degradation in single cell genomes of uncultured, numerically dominant subsurface microorganisms.
2) Quantify viable bacteria and archaea in the deep subsurface using an improvement on the existing technology of CARD-FISH.
3)Measure the potential activities (Vmax values) of enzymes in deep Baltic Sea sediments, and use the abundances of enzyme-producing microorganisms to calculate depth profiles of cell-specific Vmax values.

The project combines these methods in order to identify and quantify the cells capable of degrading organic matter in deep sediments of the Baltic Sea, obtained from Integrated Ocean Drilling Program (IODP) expedition 347. These results will greatly expand our knowledge of the function and activity of uncultured microorganisms in the deep subsurface.

This project is associated with C-DEBI account number 157595.

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Program Information

Center for Dark Energy Biosphere Investigations (C-DEBI)

Website: http://www.darkenergybiosphere.org

Coverage: Global

The mission of the Center for Dark Energy Biosphere Investigations (C-DEBI) is to explore life beneath the seafloor and make transformative discoveries that advance science, benefit society, and inspire people of all ages and origins.

C-DEBI provides a framework for a large, multi-disciplinary group of scientists to pursue fundamental questions about life deep in the sub-surface environment of Earth. The fundamental science questions of C-DEBI involve exploration and discovery, uncovering the processes that constrain the sub-surface biosphere below the oceans, and implications to the Earth system. What type of life exists in this deep biosphere, how much, and how is it distributed and dispersed? What are the physical-chemical conditions that promote or limit life? What are the important oxidation-reduction processes and are they unique or important to humankind? How does this biosphere influence global energy and material cycles, particularly the carbon cycle? Finally, can we discern how such life evolved in geological settings beneath the ocean floor, and how this might relate to ideas about the origin of life on our planet?

C-DEBI's scientific goals are pursued with a combination of approaches:
(1) coordinate, integrate, support, and extend the research associated with four major programs—Juan de Fuca Ridge flank (JdF), South Pacific Gyre (SPG), North Pond (NP), and Dorado Outcrop (DO)—and other field sites;
(2) make substantial investments of resources to support field, laboratory, analytical, and modeling studies of the deep subseafloor ecosystems;
(3) facilitate and encourage synthesis and thematic understanding of submarine microbiological processes, through funding of scientific and technical activities, coordination and hosting of meetings and workshops, and support of (mostly junior) researchers and graduate students; and
(4) entrain, educate, inspire, and mentor an interdisciplinary community of researchers and educators, with an emphasis on undergraduate and graduate students and early-career scientists.

Note: Katrina Edwards was a former PI of C-DEBI; James Cowen is a former co-PI.

Data Management:
C-DEBI is committed to ensuring all the data generated are publically available and deposited in a data repository for long-term storage as stated in their Data Management Plan (PDF) and in compliance with the NSF Ocean Sciences Sample and Data Policy. The data types and products resulting from C-DEBI-supported research include a wide variety of geophysical, geological, geochemical, and biological information, in addition to education and outreach materials, technical documents, and samples. All data and information generated by C-DEBI-supported research projects are required to be made publically available either following publication of research results or within two (2) years of data generation.

To ensure preservation and dissemination of the diverse data-types generated, C-DEBI researchers are working with BCO-DMO Data Managers make data publicly available online. The partnership with BCO-DMO helps ensure that the C-DEBI data are discoverable and available for reuse. Some C-DEBI data is better served by specialized repositories (NCBI's GenBank for sequence data, for example) and, in those cases, BCO-DMO provides dataset documentation (metadata) that includes links to those external repositories.

International Ocean Discovery Program (IODP)

Website: http://www.iodp.org/index.php

Coverage: Global

The International Ocean Discovery Program (IODP) is an international marine research collaboration that explores Earth's history and dynamics using ocean-going research platforms to recover data recorded in seafloor sediments and rocks and to monitor subseafloor environments. IODP depends on facilities funded by three platform providers with financial contributions from five additional partner agencies. Together, these entities represent 26 nations whose scientists are selected to staff IODP research expeditions conducted throughout the world's oceans.

IODP expeditions are developed from hypothesis-driven science proposals aligned with the program's science plan *Illuminating Earth's Past, Present, and Future*. The science plan identifies 14 challenge questions in the four areas of climate change, deep life, planetary dynamics, and geohazards.

IODP's three platform providers include:

- The U.S. National Science Foundation (NSF)
- Japan's Ministry of Education, Culture, Sports, Science and Technology (MEXT)
- The European Consortium for Ocean Research Drilling (ECORD)

More information on IODP, including the Science Plan and Policies/Procedures, can be found on their website at http://www.iodp.org/program-documents.

A summary table with links to IODP datasets currently hosted on Zenodo (https://zenodo.org/communities/iodp) can be accessed using the following link: https://iodp.tamu.edu/database/zenodo.html

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1431598

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