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

Project(s): 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)

Abstract: These data include the quantification of specific microbial taxa within the sediments collected during Integrated Ocean Drilling Program (IODP) Expedition 347: Baltic Sea. DNA was extracted from the interior of frozen whole round cores sampled from Little Belt, Anholt Loch, Landsort Deep, and Bornholm Basin at The University of Tennessee. For a more detailed description of drill sites, access the data set, "IODP-347 drill site locations". Primers specifically targeting the 16S rRNA gene of bacteria, archaea, anaerobic methane oxidizers (ANME-1), and Miscellaneous Crenarchaeota Group (MCG; taxonomically reassigned as the Bathyarchaeota phylum of Archaea) were used to assess abundance of these microbial groups. Abundance data was generated using quantitative-PCR (qPCR) and a non-specific, intercalating DNA stain, SYBR Green. Values were compared against a standard curve to generate copies/uL. These data were collected by Alex Shumaker as part of Dr. Karen Lloyd and Dr. Andrew Steen's project funded by the National Science Foundation entitled, "Quantifying the contribution of the deep biosphere in the marine sediment carbon cycle using deep-sea sediment cores from the Baltic Sea". For a complete list of measurements, refer to the supplemental document 'Field_names.pdf', and a full dataset description is included in the supplemental file 'Dataset_description.pdf'. The most current version of this dataset is available at: http://www.bco-dmo.org/dataset/641358

Description: Quantitative PCR data from sediment samples

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.

Acquisition Description: **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

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

Deployment Information

Deployment description for MPSV GREATSHIP MANISHA IODP-347

http://iodp.pangaea.de/upload/images/Baltic_master3.jpg

Instrument Information

Instrument	BioRad iQ5
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 Name	PCR Thermal Cycler
Generic Instrument Description	General term for a laboratory apparatus commonly used for performing polymerase chain reaction (PCR). The device has a thermal block with holes where tubes with the PCR reaction mixtures can be inserted. The cycler then rises and lowers the temperature of the block in discrete, pre-programmed steps. (adapted from http://serc.carleton.edu/microbelife/research_methods/genomics/pcr.html)

Instrument	FastDNA® Spin Kit for Soil
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 Name	PCR Thermal Cycler
Generic Instrument Description	General term for a laboratory apparatus commonly used for performing polymerase chain reaction (PCR). The device has a thermal block with holes where tubes with the PCR reaction mixtures can be inserted. The cycler then rises and lowers the temperature of the block in discrete, pre-programmed steps. (adapted from http://serc.carleton.edu/microbelife/research_methods/genomics/pcr.html)