# Microbial diversity and geochemistry of marine sediment mesocosm, Cape Lookout Bight, North Carolina

**Website**: https://www.bco-dmo.org/dataset/649807
**Data Type**: Other Field Results
**Version**:
**Version Date**: 2016-07-27

**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)
» Discovering pathways for organic matter breakdown in deep subsurface sediments using detailed gene homologue analysis of metagenomes (Subsurface Organic Breakdown)

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

| Contributors | Affiliation | Role |
|---|---|---|
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## Dataset Description

This dataset resulted from a project 'Growth dynamics of methanogens and sulfate reducers in natural marine sediments'.

### Methods & Sampling

Samples were collected with SCUBA divers at Cape Lookout Bight, North Carolina (34.6205°N, 76.5500°W), October 2, 2013. Thirty 20 cm PVC push cores were collected, capped, refrigerated, and then returned to the lab on ice in Tennessee within 48 hrs. Bubbles of methane were released from the sediments as each core was taken, indicating the presence of methane at the surface of the core. Using a plunger inserted from the bottom the first three centimeters of sediment taken from each core was placed in a 2L Erlenmeyer flask by way of a funnel. About ten core tubes were needed to fill each of the three flasks to 1.5 liters of sediment. About 100 ml of slurried sediment were autoclaved and incubated alongside the experiments under anoxic conditions as a negative control. Cell counts, hydrogen, sulfate, and methane were measured for the negative control on day 260.  All measurements described below were performed for 18 weekly timepoints for all three incubations (0-122 days). An additional timepoint at 802 days was taken for 16S rRNA gene sequence analysis only.

**Geochemistry**

Each of the three flasks was fitted with a custom butyl rubber stopper with a hole drilled through the center to accommodate a wide bore (6 mm) glass and Teflon stopcock for the removal of samples. Two 18-gauge needles with stainless steel stopcocks were inserted into the stopper as well. Using the luer-lock fitting on the needles, ultra high purity nitrogen gas (99.999%) that had been scrubbed of oxygen using heated copper fillings was flowed through the bottles using the second needle for the outflow to make the headspace anoxic. Incubation 3 was then flushed with ≥ 99.0% methane gas (Sigma-Aldrich, St. Louis, MO). Then all the ports were closed and the flasks inverted so that sediment covered the gas ports, stopper, and stopcock, and placed in a ring stand at constant room temperature (21.4°C) in the dark.

The incubations were turned over once every seven days just before sampling. Prior to gas sampling, 2 ml of anoxic N2 gas (99.999%) was used to blow the needle clear of sediment. Hydrogen and methane gas samples were collected in glass gastight Hamilton syringes using the steel needle ports in the custom stopper. About 32 ml of sediment was removed through the glass and Teflon stopcock using a sterile 60 ml plastic catheter tip syringe. From this, two 15ml conical centrifuge tubes were filled and capped, one used for porewater analysis and the other frozen at -80°C for later molecular analysis. One ml of sediment was placed in a 2 ml screw cap tube to be fixed and used for cell counts as described below. After sampling, 30ml of oxygen- and hydrogen-scrubbed N2 was injected into the bottle to replace the lost volume. The 15 ml tube destined for porewater analysis was centrifuged at 5000 xG for 5 minutes. A syringe was used to remove the supernatant not in contact with the air. The porewater was then filtered using a 0.2 µm syringe filter into 100 µl of 10% HCl to a final volume of 1 ml. Porewater sulfate content was determined by ion chromatography (Dionex, Sunnyvale, CA), with the remaining porewater used for determining the pH.

500µl of headspace gas was injected into a Peak Performer 1 Reducing Compound Photometer (Peak Laboratories, Mountain View, CA). Premixed hydrogen ppm lab bottles (Sigma-Aldrich, St. Louis, MO) were used as standards. Hydrogen was assumed to be equilibrated between headspace and porewater, since the equilibration time for Cape Lookout Bight sediments is < 2 days (Hoehler et al. 1998), which is less than the 7 days between timepoints in our study. Therefore, gas phase partial pressures were converted to aqueous hydrogen concentrations using the solubility coefficient of hydrogen corrected for salinity of 35 ppt and temperature of 21.4°C (Crozier & Yamamoto 1980). Methane was determined by using injected 500 µl of gas from the headspace into an evacuated glass bottle to be later analyzed on a gas chromatograph with a flame ionized detector (Agilent, Santa Clara, CA). Methane concentrations were not assumed to be equilibrated with the aqueous phase, therefore concentrations are presented as partial pressures.

**Cell quantification**

Total cell counts were determined by direct epifluorescence microscopy SYBRGold DNA stain (Invitrogen, Carlsbad, CA). Sediments were sonicated at 20% power for 40 seconds to disaggregates cells from sediments and diluted 40-fold into PBS prior to filtration onto a 0.2 µm polycarbonate filter (Fisher Scientific, Waltham, MA) and mounted onto a slide. An autoclaved sediment sample was used as a negative control.

**Quantitative PCR**

DNA was extracted from sediment samples frozen at -80°C using the Fast DNA kit for Soil (MP Bio, Santa Ana, CA). Negative controls of sterilized sediment and a blank water extract were used as well. Quantitative PCR was used to determine 16S rRNA gene copy numbers of several taxa with Quantifast SYBRGreen kit (Qiagen) on a BioRad IQ5 machine. DNA standards were prepared from either existing stocks (Lloyd et al., 2011) or from TOPO plasmids (Invitrogen, Carlsbad, CA) containing PCR amplified 16S rRNA gene products of closely related relatives in the clades Methanomicrobiales and Methanosarcinales synthesized by Invitrogen (accession # AB236118 and AB679168 respectively). Standards were quantified using Hoechst dye in a flourimeter (Hoefer, Holliston, MA). 16s rRNA specific primers Methanomicrobiales and Methanosarcinales primers were selected to have good coverage of the taxa (Narihiro and Sekiguchi, 2011).

**16S Ribosomal RNA Gene Amplicons**

Extracted DNA was used for 16S rRNA gene amplicon analysis. The V4 region of each DNA extraction was amplified using primers 806r and 515f (Caporaso et al., 2012), as a universal primer pair for Bacteria and Archaea. Library preparations via Nexterra kit and sequencing using an Illumina MiSeq were performed at the Center for Environmental Biotechnology at the University of Tennessee in Knoxville. The Mothur MiSeq Standard Operating Procedure was used to make contigs of bidirectional sequences, cluster operational taxonomic units (OTUs) at 97% similarity, and classify them with the Silva reference set 119 (Schloss et al., 2009, Pruesse et al., 2007). 26.4% of unique sequences were removed as chimeric and then approximately 5% of total sequences were removed for failing to classify at the domain level. Reads were normalized against

the sum of reads classified as bacteria and archaea.

Analyses of time course patterns of various microbial taxa were considered only for those with more than 20 reads when summed from the 18 timepoints from each of the three incubations, leaving between 593 and 669 genus-level clades of bacteria and archaea across the three incubations. Total reads ranged from 20,922 to 329,380 for the 54 libraries. 16S rRNA sequences were normalized to total classifiable bacterial and archaeal reads. ]

**Data Processing Description**

Raw data has been converted to environmentally relevant units as calculated from appropriate standards. Data that is below detection limits is flagged as no data (nd).

**BCO-DMO Processing:**

- added conventional header with dataset name, PI name, version date
- renamed parameters to BCO-DMO and BODC standards
- reformatted date from m/d/yyyy to yyyy-mm-dd
- changed Incbuation to Incubation
- added lat and lon

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## Data Files

| File |
|---|
| **incubation.csv** (Comma Separated Values (.csv), 6.43 KB)<br>MD5:f15375c46483dd246743af0109225d5c<br>Primary data file for dataset ID 649807 |

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## Parameters

| Parameter | Description | Units |
|---|---|---|
| sample | sample identification | unitless |
| date | date sampled; formatted as YYYY-MM-DD | year-month-day |
| days_elapsed | duration of incubation | days |
| Hydrogen_nM | aqueous hydrogen concentration in the porewater of the sediment in the sample | mM |
| Sulfate_mM | aqueous sulfate concentration in the porewater of the sediment in the sample | mM |
| Methane_mM | aqueous methane concentration in the porewater of the sediment in the sample | mM |
| total_cells | Total cell counts determined by direct epifluorescence microscopy SYBRGold DNA stain | cells/gram |
| sediment_mass | mass of sediment | grams |
| pH_porewater | pH of the porewater | pH scale |
| Archaea | Archaea 16S rRNA gene abundance | copies/gram |
| Bacteria | Bacteria 16S rRNA gene abundance | copies/gram |
| Methanomicrobiales | Methanomicrobiales 16S rRNA gene abundance | copies/gram |
| Methanosarcinales | Methanosarcinales 16S rRNA gene abundance | copies/gram |

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## Instruments

| | |
|---|---|
| **Dataset-specific Instrument Name** | |
| **Generic Instrument Name** | Automated DNA Sequencer |
| **Dataset-specific Description** | Illumina MiSeq at the Center for Environmental Biotechnology at the University of Tennessee in Knoxville |
| **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** | Fluorescence Microscope |
| **Generic Instrument Description** | Instruments that generate enlarged images of samples using the phenomena of fluorescence and phosphorescence instead of, or in addition to, reflection and absorption of visible light. Includes conventional and inverted instruments. |

| | |
|---|---|
| **Dataset-specific Instrument Name** | |
| **Generic Instrument Name** | Fluorometer |
| **Dataset-specific Description** | flourimeter Hoefer, Holliston, MA |
| **Generic Instrument Description** | A fluorometer or fluorimeter is a device used to measure parameters of fluorescence: its intensity and wavelength distribution of emission spectrum after excitation by a certain spectrum of light. The instrument is designed to measure the amount of stimulated electromagnetic radiation produced by pulses of electromagnetic radiation emitted into a water sample or in situ. |

| | |
|---|---|
| **Dataset-specific Instrument Name** | |
| **Generic Instrument Name** | Gas Chromatograph |
| **Dataset-specific Description** | gas chromatograph with a flame ionized detector (Agilent, Santa Clara, CA) |
| **Generic Instrument Description** | Instrument separating gases, volatile substances, or substances dissolved in a volatile solvent by transporting an inert gas through a column packed with a sorbent to a detector for assay. (from SeaDataNet, BODC) |

| | |
|---|---|
| **Dataset-specific Instrument Name** | |
| **Generic Instrument Name** | Ion Chromatograph |
| **Dataset-specific Description** | Dionex, Sunnyvale, CA |
| **Generic Instrument Description** | Ion chromatography is a form of liquid chromatography that measures concentrations of ionic species by separating them based on their interaction with a resin. Ionic species separate differently depending on species type and size. Ion chromatographs are able to measure concentrations of major anions, such as fluoride, chloride, nitrate, nitrite, and sulfate, as well as major cations such as lithium, sodium, ammonium, potassium, calcium, and magnesium in the parts-per-billion (ppb) range. (from http://serc.carleton.edu/microbelife/research_methods/biogeochemical/ic....) |

| | |
|---|---|
| **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

**Cape_Lookout_Bight_NC**

| | |
|---|---|
| **Website** | https://www.bco-dmo.org/deployment/649811 |
| **Platform** | Unknown Platform |
| **Start Date** | 2013-10-03 |
| **End Date** | 2014-01-28 |
| **Description** | Sediment sampling |

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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.

**Discovering pathways for organic matter breakdown in deep subsurface sediments using detailed gene homologue analysis of metagenomes (Subsurface Organic Breakdown)**

**Coverage**: Peru Margin deep biosphere

Heterotrophy supports much of the energy-starved life in the deep biosphere, yet we know almost nothing about the pathways by which organic matter (OM) is remineralized in the deep subseafloor. The microbes responsible for OM breakdown in the subsurface are taxonomically diverse, but their lack of resemblance to well-characterized lab cultures makes it impossible to use taxonomy alone to predict the nature of their interactions with OM. Even when metagenomes or metatranscriptomes are available to provide functional information, common analysis tools only categorize annotated genes into general classes that do not distinguish between degradative, synthetic, or housekeeping processes. We propose to develop detailed gene homologue analyses to unlock hidden details about OM degradation pathways in subsurface metagenomes from a range of OM types and amounts in the Peru Margin deep biosphere. This will allow us to describe the intricate landscape of biological remineralization of OM in the marine subsurface. Finally, we will develop an open-source software tool that replicates our analysis methods (freely available to the community via web-interface or as source code from a public repository such as GitHub) to allow other researchers to perform this analysis automatically.

Reference:

Lloyd, K.G., Schreiber, L., Petersen, D.G., Kjeldsen, K.U., Lever, M.A., Steen, A.D., Stepanauskas, R., Richter, M., Kleindienst, S., Lenk, S., Schramm, A., Jørgensen, B.B. 2013. Predominant archaea in marine sediments degrade detrital proteins. Nature 496, 215–218. *C-DEBI Contribution 177*. Publisher's Link

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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.

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## Funding


| Funding Source | Award |
|---|---|
| NSF Division of Ocean Sciences (NSF OCE) | OCE-0939564 |
| NSF Division of Ocean Sciences (NSF OCE) | OCE-1431598 |


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