

# 16S gene sequencing of microbial communities in South China Sea sediments from January to March 2014

**Website:** <https://www.bco-dmo.org/dataset/748024>

**Data Type:** Cruise Results

**Version:** 1

**Version Date:** 2018-09-05

## Project

» [Edginess in the subsurface: Microbial diversity of deep seafloor ecotones](#) (Edginess in the subsurface)

## Program

» [Center for Dark Energy Biosphere Investigations](#) (C-DEBI)

| Contributors                       | Affiliation   | Role                      |
|------------------------------------|---|---------------------------|
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## Abstract

16S gene sequencing of microbial communities in South China Sea sediments from January to March 2014.

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## Coverage

**Spatial Extent:** N:18.3514 E:117 S:12.919 W:115.0472

**Temporal Extent:** 2014-01-30 - 2014-03-07

## Dataset Description

Three locations in the SCS were cored during IODP Expedition 349 at sites U1431 (15° 22.5379' N, 117° 00.0022' E, 4240 meters below sea level (mbsl)), U1432 (18° 21.0831' N, 116° 23.4504' E, 3829 mbsl), and U1433 (12° 55.1380' N, 115° 02.8345' E, 4379 mbsl). Cores were split lengthwise onboard to reveal geological interfaces and the exposed sediment was aseptically scraped away prior to sampling. Samples were collected only from the center of the cores, avoiding the outside edges of the cores that may have contacted the core barrel. Samples were stored at -80°C until analysis. Down-core measurements of sediment methane concentrations, alkalinity, and pore water sulfate, ammonium, and phosphate were made onboard. Sediment ages were calculated based on paleomagnetic analysis and nanofossil characterization for each site.

All samples described in this study were collected using an advanced piston core, a tool that minimizes core disturbance. In order to check whether contamination occurred, and consistent with other deep microbiology coring studies, we compared microbial communities present in fluids that drained from the core barrel (possibly from seawater intrusion during core retrieval) to those present in the center of the cores, a so-called

fluid community tracer (FCT) approach. Samples of the drilling fluid from core U1433 were collected immediately when cores were brought onto the deck and were frozen at -80°C until analysis. To compare microbial communities in the drilling fluids to those in the cores, DNA from 10 FCT samples (235-790 mbsf) and a whole-round sample (13.5 mbsf) was extracted using a FastDNA Spin Kit for Soil (MP Biomedical, OH, USA). Pyrosequencing of the archaeal 16S rRNA gene of the FCT samples and the whole-round sample was conducted with a Roche 454 GS FLX+ Titanium platform (Roche 454 Life Sciences, CT, USA) at the Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). Primers used for amplification were Arch\_344F (5'-ACGGGGCGCAGCAGGCGCGA-3') and Arch\_915R (5'-GTGCTCCCCCGCCAATTCCT-3').

Additional award numbers:

Consortium for Ocean Leadership; award number: IUSSP410  
C-DEBI; subaward number: 59209190  
DCO/DLC; subaward number: 53587

Data are publicly accessible at NCBI under accession number PRJNA362622.

## Methods & Sampling

DNA was extracted from 0.25 g of wet sediment using a MoBio PowerSoil DNA Isolation Kit. Extracted DNA was amplified in technical triplicate 25 µL reactions using universal 16S rRNA gene primers 515-fwd and 806-rev with Illumina sequencing adapters and barcodes. Triplicate PCR products were pooled, visualized on an agarose gel, and cleaned using a MoBio UltraClean PCR Clean-Up Kit. PCR products were quantified using a Qubit fluorometer and pooled prior to sequencing. Paired-end 250-bp sequencing was performed on an Illumina MiSeq at Oregon State University's Center for Genome Research and Biocomputing. A sediment-free extraction was amplified and sequenced alongside sediment samples.

## Data Processing Description

Sequences were processed using mothur (v 1.38.0; (38)). Reads were clustered at 97% and classified against the SILVA database (v. 119). Singleton OTUs were removed prior to analysis. OTUs that were present in greater than 1% relative abundance in the negative (sediment-free) control and classified as common human or kit contaminants (39) were removed from the dataset. To confirm their identity, phylogenetic trees were generated for all OTUs classified as ANME and for the 8 most abundant OTUs classified to known sulfate-reducing bacteria lineages with Fasttree (v. 2.1.10; 40)) using the Jukes-Cantor model (Supp. Figs. 1 and 2).

BCO-DMO Processing Notes:

- translated the "collection\_date" format from DD-Mon-YY (7-Mar-14) to YYYY-MM-DD (2014-03-14).
- added conventional header with dataset name, PI name, version date
- modified parameter names to conform with BCO-DMO naming conventions
- removed hemisphere declarations in the latitude and longitude fields.

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## Data Files

| File  |
|---|
| <b>scs16.csv</b> (Comma Separated Values (.csv), 4.86 KB)<br>MD5:06e03d357ea6882f2d65b14c962bf693 |
| Primary data file for dataset ID 748024   |

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## Related Publications

Graw, M. F., D'Angelo, G., Borchers, M., Thurber, A. R., Johnson, J. E., Zhang, C., ... Colwell, F. S. (2018). Energy Gradients Structure Microbial Communities Across Sediment Horizons in Deep Marine Sediments of the South China Sea. *Frontiers in Microbiology*, 9. doi:[10.3389/fmicb.2018.00729](https://doi.org/10.3389/fmicb.2018.00729)  
*Results*

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## Parameters

| Parameter       | Description                                     | Units           |
|-----------------|---|-----------------|
| Sample_Name     | sample name                                     | unitless        |
| collection_date | date the sediment was collected                 | unitless        |
| depth           | depth where samples were taken below sea floor. | meters (m)      |
| lat             | Latitude in decimal degrees north               | decimal degrees |
| lon             | Longitude in decimal degrees east               | decimal degrees |
| sedimentology   | description of sediment type                    | unitless        |
| elev            | water depth at the sampling site                | meters (m)      |

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## Instruments

|   |  |
|---|--|
| <b>Dataset-specific Instrument Name</b> | advanced piston core   |
| <b>Generic Instrument Name</b>          | Advanced Piston Corer  |
| <b>Dataset-specific Description</b>     | All samples described in this study were collected using an advanced piston core, a tool that minimizes core disturbance.  |
| <b>Generic Instrument Description</b>   | The JOIDES Resolution's Advanced Piston Corer (APC) is used in soft ooze and sediments. The APC is a hydraulically actuated piston corer designed to recover relatively undisturbed samples from very soft to firm sediments. More information is available from IODP (PDF). |

|   |  |
|---|--|
| <b>Dataset-specific Instrument Name</b> | Illumina MiSeq   |
| <b>Generic Instrument Name</b>          | Automated DNA Sequencer  |
| <b>Dataset-specific Description</b>     | Paired-end 250-bp sequencing was performed on an Illumina MiSeq at Oregon State University's Center for Genome Research and Biocomputing.  |
| <b>Generic Instrument Description</b>   | 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. |

|   |   |
|---|---|
| <b>Dataset-specific Instrument Name</b> | Qubit fluorometer   |
| <b>Generic Instrument Name</b>          | Fluorometer   |
| <b>Dataset-specific Description</b>     | PCR products were quantified using a Qubit fluorometer and pooled prior to sequencing.  |
| <b>Generic Instrument Description</b>   | 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. |

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## Deployments

### IODP-349

|                   |   |
|-------------------|---|
| <b>Website</b>    | <a href="https://www.bco-dmo.org/deployment/748037">https://www.bco-dmo.org/deployment/748037</a>                                   |
| <b>Platform</b>   | R/V JOIDES Resolution   |
| <b>Report</b>     | <a href="http://publications.iodp.org/proceedings/349/349title.html">http://publications.iodp.org/proceedings/349/349title.html</a> |
| <b>Start Date</b> | 2014-01-26  |
| <b>End Date</b>   | 2014-03-30  |

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## Project Information

**Edginess in the subsurface: Microbial diversity of deep seafloor ecotones (Edginess in the subsurface)**

## Coverage: South China Sea

### Project summary:

For seafloor microorganisms, defined geological and chemical gradients affect population sizes and community structure. We examined how distinct sediment types influence microbial diversity and community composition and the factors that drive deep-subsurface microbial community structure (e.g., depth, interstitial water chemistry, sample location). During IODP Expedition 349 (South China Sea Tectonics), either coupled ash/clay or turbidite/clay boundaries were sampled, DNA extracted, and the 16S rRNA gene analyzed on an Illumina MiSeq platform. Microbial communities in sediments were distinct from communities in drilling fluid, indicating that drilling-based contamination was unlikely. Illumina sequencing of the 16S rRNA gene yielded 5,453 OTUs (97% identity) representing 44 bacterial phyla and 3 archaeal phyla. Members of the Atribacteria dominated all microbial communities among all sites. Sulfate-reducing bacteria were relatively rare within sulfate-replete sediments in all cores. Ordination of the microbial communities using weighted UniFrac distances revealed significant differences in communities both between sites and between the sulfate reduction zone and methanogenic zone at two of the sites. The number of observed taxa followed an exponential decline with sediment age only in the sulfate reduction zone. Species evenness increased linearly with sediment age regardless of geochemical zonation in the sediment column. Our investigation helps to characterize the factors that drive microbial community structure of the seafloor and highlights the need to focus on habitat heterogeneity at a scale pertinent to bacteria and archaea in studies of microbial ecology.

This project is funded with:

Center for Dark Energy Biosphere Investigations (C-DEBI); subaward number: 59209190  
Deep Carbon Observatory (DCO) /Deep Life Community (DLC); subaward number: 53587  
Consortium for Ocean Leadership; award number: IUSSP410

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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 seafloor 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.

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#### **Funding**

| <b>Funding Source</b>                                    | <b>Award</b>                |
|--|-----------------------------|
| <a href="#">NSF Division of Ocean Sciences (NSF OCE)</a> | <a href="#">OCE-0939564</a> |

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