SSU rRNA gene sequences from marine sediments, marine subseafloor, and deep seawater sampled from the Juan de Fuca Ridge Flank from various R/V Atlantis cruises from 2008-2011 (microJdFR project)

Website: https://www.bco-dmo.org/dataset/660489
Data Type: Cruise Results
Version:
Version Date: 2016-10-07

Project
» Microbiology and biogeochemistry of Juan de Fuca Ridge flank borehole fluids (microJdFR)

Program
» Center for Dark Energy Biosphere Investigations (C-DEBI)

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Dataset Description

This dataset includes SSU rRNA gene sequence accession identifiers from marine sediments, marine subseafloor, and deep seawater along with quantities of various elements and compounds including (oxygen, ammonium, methane, hydrogen, total dissolved nitrogen, calcium, nitrate, nitrate, and total iron). Also included in this dataset are microbial cell abundance, pH, latitude and longitude. The sampling area in Northeast Pacific Ocean waters include long-term borehole observatories (CORKs) in the Juan de Fuca (JdF) Ridge Flank region from R/V Atlantis cruises AT15-35, AT15-55, AT15-66 and AT18-07..

These data have been published in the following references:

Jungbluth, Sean P., et al. "Data report: microbial diversity in sediment near Grizzly Bare Seamount in Holes U1363B and U1363G." *Proc. IODP Volume*. Vol. 327. 2013. http://dx.doi.org/10.2204/iodp.proc.327.201.2013

Jungbluth, Sean P., et al. "Novel microbial assemblages inhabiting crustal fluids within mid-ocean ridge flank subsurface basalt." *The ISME journal* (2016). dx.doi.org/10.1038/ismej.2015.248

Lin, H-T, Hsieh, C-C, Cowen, JP, Rappe, MS (2015). Data report: dissolved and particulate organic carbon in the deep sediments of IODP Site U1363 near Grizzly Bare seamount. *Proceedings of the Integrated Ocean Drilling Program* 327: 1-16. dx.doi.org/10.2204/iodp.proc.327.202.2015

Methods & Sampling

Sampling Methodology:

Sediment coring was performed by the IODP (described in Expedition 327 Scientists, 2011; *Integrated Ocean Drilling Program*). SSU rRNA genes were obtained as described in Jungbluth et al., 2013; *Proceedings of the Integrated Ocean Drilling Program* (see Supplementary Information Document(.DOC) for this publication).

CORK borehole fluids were sampled using a custom-built water sampler (described in Cowen et al., 2012). For a detailed description of the pump system see Lin, et al. 2012.

Pore water dissolved organic carbon (from Lin et al., 2015 Materials and Methods).

Sedimentary pore water DOC concentrations were measured by high-temperature combustion using a Shimadzu TOC-VCSH analyzer. The combustion temperature was set at 720C to ensure complete oxidation of organic matter. Samples were acidified to pH <2 by the addition of 45 uL of 2 M HCl to 3 mL samples. No acid contamination was observed based on monitoring the DOC value of low-carbon deionized water. Samples were purged with nitrogen gas within the autosampler syringe for 2 min in order to remove inorganic carbon. An injection volume of 150 uL was used, with five or six injections per sample. The reproducibility between replicate injections was <1 uM. Analytical reference materials (ARM) supplied by Dr. Dennis Hansell (RSMAS, University of Miami) were measured before, between, and after analysis of environmental samples (Sharp et al., 2002; Dickson et al., 2007). At least one ARM was measured every five samples. The average measured concentration of the ARM was 42 plus or minus 2 uM (n = 44); the reported value was 41–43 uM. Our detection limit for DOC concentrations was ~2 uM.

Sediment organic carbon and nitrogen (relevant text extracted from Lin et al., 2015 Materials and Methods).

Whole sediment samples were analyzed for concentration of total carbon, organic carbon, and total nitrogen using an elemental combustion system (Costech ECS 4010) connected inline to an isotope-ratio mass spectrometer (Thermo Finnigan Delta XP). The amount of powdered sediment used for the analyses was optimized to provide sufficient carbon and nitrogen for isotopic composition analysis and varied between 26 and 425 mg. A subset of samples was acidified by fuming with concentrated HCl (Hedges and Stern, 1984) in order to remove inorganic carbon and quantify the particulate organic carbon (POC) content. Acid fuming did not remove inorganic nitrogen, resulting in insignificant differences between whole and acid-fumed total particulate nitrogen (PN) concentrations.

Analytical methods for geochemistry

Text below extracted from Supplementary Information (PDF) Junbluth et al., 2016. See reference for full description.

Major ions (Ca^{2+}, Mg^{2+}, K^+ , Na^+, Cl^- , SO_4^{2-} and Br^-) were analyzed by ion chromatography on a Dionex ICS-1100s (Sunnyvale, CA, USA). In addition, magnesium and calcium concentrations were also analyzed by EDTA (colorimetric) and EGTA (electrometric) titration (Grasshoff et al., 1999), or inductively coupled plasma optical emission spectroscopy (ICP-OES) (Lin et al., 2012).

Silicate, nitrate, nitrite, phosphate, dissolved sulfide and dissolved manganese concentrations were measured by colorimetry (Brewer and Spencer, 1971; Phillips et al., 1997; Grasshoff et al., 1999).

Ammonium concentrations were measured by a flow injection-fluorometric method (Jones, 1991). The detection limit was ~2 µM for ammonium in basement fluids and the analytical uncertainty is 0.5 µM.

Ferrous iron was measured directly by a Ferrozine colorimetry method (Stookey, 1970; Gibbs, 1976).

For total iron analysis, samples were first reduced with ascorbic acid and analyzed as ferrous iron. The detection limit for both ferrous iron and total iron was 0.1 µM.

Dissolved organic carbon (DOC) was measured by high-temperature combustion using a TOC-VCSH analyzer (Sharp et al., 2002a; Dickson et al., 2007) (Shimadzu Corp., Kyoto, Japan).

Total dissolved nitrogen (TDN) was measured with a chemiluminescence detector in-line with a Shimadzu TOC-VCSH analyzer (Sharp et al., 2002b).

Alkalinity was determined by acid titration. Acid (0.1N HCl) was standardized with CO_2 certified reference

materials (CRMs) purchased from the office of Andrew Dickson at Scripps Institution of Oceanography.

An Orion 911600 Semi-micro pH electrode (ThermoFisher Scientific, Waltham, MA, USA) was used to measure the pH and electrode potential during the titration process. The Gran function plot method was used to evaluate titration end-points and calculate sample alkalinity (Dickson et al., 2007). The analytical reproducibility for alkalinity measurements was <0.02 mM.

SSU rRNA gene cloning and sequencing (from Supplementary Information Document(DOC) for Junbluth et al., 2013).

Small subunit ribosomal RNA (SSU rRNA) gene fragments were amplified via the polymerase chain reaction (PCR) using the universal oligonucleotide forward and reverse primers 519F (5'-CAGCMGCCGCGGTAATWC-3') and 1406R (5'-ACGGGCGGTGTGTRC-3'), respectively. Each 20 ul PCR reaction contained 0.25 U of PicoMaxx high fidelity DNA polymerase (Stratagene, La Jolla, CA), 1x PicoMaxx reaction buffer, 200 uM of each of the four deoxynucleoside triphosphates (dNTPs), 200 nM of both forward and reverse primer, and ~3-4 ng of environmental DNA template. PCR cycling conditions consisted of an initial denaturation step at 95C for 4 minutes, followed by 35 to 38 cycles of 95C denaturation for 30 sec, 55C annealing for 1 min, 72C extension for 2 min, and a final extension step at 72C for 20 min. For the 2008 borehole fluid sample, a 3-cycle reconditioning PCR was performed in order to help eliminate heteroduplexes (Thompson et al., 2002). Amplification products of the anticipated length were excised from an agarose gel and subsequently purified using the QIAquick gel extraction kit (Qiagen, Valencia, CA). Products were cloned using either the pGEM-T Easy kit (Promega, Madison, WI) or the TOPO TA Cloning kit (Invitrogen, Carlsbad, CA) following the manufacturer's instructions. Clones were sequenced unidirectionally on an ABI 3730XL DNA Analyzer (Applied Biosystems, Carlsbad, CA).

Fluorescence Microscopy: microbial cell counts (from Supplementary Information Document(DOC) for Junbluth et al., 2016).

Sample preparation for microscopy and fluorescence microscopy Fluid samples for microscopy collected in 2011 were prepared in similar fashion to those collected in sampling years 2008-2010 and described previously (Jungbluth et al., 2013). Briefly, 40 to 120 ml sub-samples were fixed with a final concentration of 3% of 0.2 um-filtered formaldehyde for 2 to 4 hours at 4C, and subsequently filtered through 0.2 um pore-sized polycarbonate membranes (Whatman, Maidstone, United Kingdom). After air-drying, membranes were stored desiccated at -80ºC until microscopic analysis. Filter sections were prepared for fluorescence microscopy using a mix of Citifluor/VectaShield/PBS/DAPI as described previously (Jungbluth et al., 2013a). Stained filter sections were inspected with a Leica DM5000B epifluorescence microscope (Leica Microsystems, Wetzlar, Germany) (samples: SSF1-2, SSF4, MIX1-4, SW1-5, SW9-11, SW14-15) or an Eclipse 90i (Nikon Corp., Tokyo, Japan) epifluorescence microscope (all other samples). Both microscopes were equipped with 100x objectives and filter sets appropriate for DAPI fluorescence.

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Lin, H-T, Hsieh, C-C, Cowen, JP, Rappe, MS (2015). Data report: dissolved and particulate organic carbon in the deep sediments of IODP Site U1363 near Grizzly Bare seamount. *Proceedings of the Integrated Ocean Drilling Program* 327: 1-16. dx.doi.org/10.2204/iodp.proc.327.202.2015

Lin, Huei-Ting, et al. "Inorganic chemistry, gas compositions and dissolved organic carbon in fluids from sedimented young basaltic crust on the Juan de Fuca Ridge flanks." *Geochimica et Cosmochimica Acta* 85 (2012): 213-227. http://dx.doi.org/10.1016/j.gca.2012.02.017

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Data Processing Description

These data have been quality controlled as described in Jungbluth et al., 2013, and Jungbluth et al., 2016.

For seawater samples, elevation was set equal to sea-level (value: 0) and all sample depths are reported as positive values.
For sediment and borehole samples, elevation was set equal to the depth of the seafloor (all values negative) and depths into the seafloor are reported as positive values.

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Data Files

File
accessions.csv (Comma Separated Values (.csv), 403.06 KB) MD5:61989e0951451b60f637ac81b3132291 Primary data file for dataset ID 660489

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Parameters

Parameter	Description	Units

database	Database to which the accession_id belongs to	unitless
Accession_id	Identification number for GenBank; SRA; or IMG databases	unitless
Accession_Link	URL link to the accession for GenBank; SRA; or IMG	unitless
BioSample_ID	Identifier for BioSample at NCBI. A BioSample corresponds to descriptions of biological source materials used in experimental assays.	unitless
BioProjectID	Identifier for BioProject at NCBI. A BioProject is a collection of biological data related to a single initiative originating from a single organization or from a consortium.	unitless
description	Description of sample and source material origin	unitless
sample_title	Project-specific sample title	unitless
sample_name	Descriptive sample title	unitless
organism	Type of organism(s) sampled	unitless
collection_date	Date of sample collection in format dd-mmm-yy.	unitless
depth	Depth of sample. Sea-water sample depths are reported as positive values. For sediment and borehole samples, elevation was set equal to the depth of the seafloor (all values negative) and depths into the seafloor are reported as positive values.	meters
elev	Elevation of sample. Sea-water samples are set to sea-level (0) . For sediment and borehole samples, elevation was set equal to the depth of the seafloor (all values negative).	meters
env_biome	Biome of sample site	unitless
env_feature	Environmental features of sample site	unitless
env_material	Environmental material of sample site	unitless
geo_loc_name	Geolocation name of sample site	unitless
lat	latitutde	decimal degrees
lon	longitude; west is negative	decimal degrees
ph	pH	pH scale
oxygen	oxygen (O2)	micromoles per liter
calcium	calcium (Ca)	millimoles per liter
magnesium	magnesium (Mg)	millimoles per liter
potassium	potassium (K)	millimoles per liter
sodium	sodium (Na)	millimoles per liter
chloride	chloride (Cl-)	millimoles per liter
bromide	bromide (Br-)	millimoles per liter
silicate	silicon dioxide (SiO2)	micromoles per liter
ammonium	ammonium (NH4)	micromoles per liter

phosphate	phosphate (PO4)	micromoles per liter
nitrite	nitrite (NO2)	micromoles per liter
nitrate	nitrate (NO3)	micromoles per liter
nitrate_and_nitrate	combined nitrate and nitrate (NO3 and NO2)	micromoles per liter
sulfate	sulfate (SO4)	millimoles per liter
dissolved_iron	dissolved iron (dFe)	micromoles per liter
total_iron	total iron (Fe)	micromoles per liter
Mn2plus	Manganese ion (Mn2+)	micromoles per liter
dissolved_hydrogen_sulfide	dissolved hydrogen sulfide (dissolved H2S)	micromoles per liter
dissolved_organic_carbon	dissolved organic carbon (DOC)	micromoles per liter
TDN	total dissolved nitrogen (TDN)	micromoles per liter
alkalinity	alkalinity	milliequivalents per liter
methane	methane (CH4)	micromoles per liter
hydrogen	hydrogen (H)	micromoles per liter
microbial_cell_abundance	microbial cell abundance	cells per milliliter

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Instruments

Dataset-specific Instrument Name	ABI 3730XL DNA Analyzer
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	Clones were sequenced unidirectionally on an ABI 3730XL DNA Analyzer (Applied Biosystems, Carlsbad, CA).
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	Dionex ICS-1100s
Generic Instrument Name	Ion Chromatograph
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	Thermo Finnigan Delta XP
Generic Instrument Name	Mass Spectrometer
Dataset-specific Description	Whole sediment samples were analyzed for concentration and isotopic composition of total carbon, organic carbon, and total nitrogen using an elemental combustion system (Costech ECS 4010) connected inline to an isotope-ratio mass spectrometer (Thermo Finnigan Delta XP).
Generic Instrument Description	General term for instruments used to measure the mass-to-charge ratio of ions; generally used to find the composition of a sample by generating a mass spectrum representing the masses of sample components.

Dataset-specific Instrument Name	Orion 911600 Semi-micro pH electrode
Generic Instrument Name	pH Sensor
Dataset-specific Description	Orion 911600 Semi-micro pH electrode (ThermoFisher Scientific, Waltham, MA, USA)
Generic Instrument Description	An instrument that measures the hydrogen ion activity in solutions. The overall concentration of hydrogen ions is inversely related to its pH. The pH scale ranges from 0 to 14 and indicates whether acidic (more H+) or basic (less H+).

Dataset-specific Instrument Name	pump
Generic Instrument Name	Pump
Dataset-specific Description	A custom pump system was used to sample basement fluid. It connected to the CORK observatorys' fluid delivery lines. The design of this system changed over the course of the study. It included the pump components Pelagic Electronics 5010 series deep sea pump, and Sea-Bird SBE-5T submersible titanium pump. For a detailed description see: Lin, Huei-Ting, et al. "Inorganic chemistry, gas compositions and dissolved organic carbon in fluids from sedimented young basaltic crust on the Juan de Fuca Ridge flanks." Geochimica et Cosmochimica Acta 85 (2012): 213-227. http://dx.doi.org/10.1016/j.gca.2012.02.017
Generic Instrument Description	A pump is a device that moves fluids (liquids or gases), or sometimes slurries, by mechanical action. Pumps can be classified into three major groups according to the method they use to move the fluid: direct lift, displacement, and gravity pumps

Dataset-specific Instrument Name	TOC-VCSH analyzer
Generic Instrument Name	Shimadzu TOC-V Analyzer
Generic Instrument Description	A Shimadzu TOC-V Analyzer measures DOC by high temperature combustion method.

Dataset-specific Instrument Name	NanoDrop ND-1000 spectrophotometer
Generic Instrument Name	Spectrophotometer
Dataset-specific Description	NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA) was used to quantify resulting genomic DNA.
Generic Instrument Description	An instrument used to measure the relative absorption of electromagnetic radiation of different wavelengths in the near infra-red, visible and ultraviolet wavebands by samples.

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Deployments

AT15-35

Website	https://www.bco-dmo.org/deployment/637087
Platform	R/V Atlantis
Start Date	2008-07-28
End Date	2008-08-13
Description	Science activities (according to WHOI's cruise synopsis): 1) Service instrumentation at up to seven subseafloor “CORK” hydrological observatories installed by ODP in 1996 and IODP in 2004; 2) make in situ, shipboard and shore-based measurements to characterize the microbial geochemistry of the subseafloor basement (basaltic crust) utilizing subset of above 7 CORK observatories; and 3) test underwater optical communication device associated with a temperature probe deployed within a thermal vent **Methods & Sampling** R/V Atlantis – AT15-35 – HOV Alvin II dive 4432

AT15-55

Website	https://www.bco-dmo.org/deployment/59044
Platform	R/V Atlantis
Start Date	2009-11-08
End Date	2009-11-18
Description	Cruise information and original data are available from the NSF R2R data catalog. **Methods & Sampling** R/V Atlantis - AT15-51 - HOV Alvin II dives 4532, 4533, 4434, 4536, & 4537

AT15-66

Website	https://www.bco-dmo.org/deployment/660524
Platform	R/V Atlantis
Start Date	2010-06-15
End Date	2010-07-01
Description	**Methods & Sampling** R/V Atlantis - AT15-66 - ROV Jason II dives J2-497, J2-498, J2-499, J2-502, J2-503, & J2-505, J2-500

AT18-07

Website	https://www.bco-dmo.org/deployment/660555
Platform	R/V Atlantis
Start Date	2011-06-29
End Date	2011-07-14
Description	**Methods & Sampling** R/V Atlantis - AT18-07 - ROV Jason II dives J2-566, J2-569, J2-571, & J2-573

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Project Information

Microbiology and biogeochemistry of Juan de Fuca Ridge flank borehole fluids (microJdFR)

Website: http://www.darkenergybiosphere.org/research/juandefuca.html

Coverage: Boreholes along the eastern flank of Juan de Fuca Ridge (47N, 128 W)

Extracted from the NSF award abstract:

Hydrothermally heated fluids circulate everywhere within the permeable basement rock of the upper ocean crust, providing warm temperatures and chemical gradients that support a deep subsurface marine biosphere. The volume of oceanic lithosphere habitable by microbial life is thought to be a substantial portion of the Earth's crust - extending thousands of meters below the seafloor. During expeditions from 2008 to 2014 we repeatedly sampled basalt-hosted, deep subseafloor crustal fluids from four different boreholes drilled along the Juan de Fuca Ridge flank in the Northeast Pacific Ocean using pumps and samplers capable of collecting whole water and filtered particulates in situ. The instrumented boreholes, sitting at 2600 m depth, penetrate ~100 to 260 m of bottom sediments and another ~48 to 300 m of igneous basement where they tap into hot (up to 65 degrees C), anoxic fluid within Earth's largest deep subsurface aquifer. Nearby bottom seawater and sediments were sampled as controls. Associated data sets include small subunit ribosomal RNA and functional gene amplicon DNA sequences, metagenome sequences, single cell genome sequences, direct counts of microbial cells and viruses, and a wide range of associated biogeochemical measurements including dissolved gases, particulate and dissolved organic carbon, sulfate, nitrate, and others.

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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.

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Funding

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

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