

# The acetate and inorganic carbon uptake rates as determined via stable isotopic tracers from Maria S. Merian cruise MSM20-5 in 2012; data generated using the formation fluids recovered from CORKs installed at North Pond

**Website:** <https://www.bco-dmo.org/dataset/637804>

**Data Type:** experimental, Cruise Results

**Version:** 1

**Version Date:** 2016-02-03

## Project

» [Collaborative Research: Characterization of Microbial Transformations in Basement Fluids, from Genes to Geochemical Cycling](#) (North Pond Microbes)

## Programs

» [International Ocean Discovery Program](#) (IODP)

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

Contributors	Affiliation	Role
<a href="#">Girguis, Peter</a>	Harvard University	Principal Investigator
<a href="#">Glazer, Brian</a>	University of Hawaii at Manoa (SOEST)	Co-Principal Investigator
<a href="#">Huber, Julie</a>	Marine Biological Laboratory (MBL)	Co-Principal Investigator
<a href="#">Kraft, Beate</a>	Harvard University	Contact
<a href="#">Rauch, Shannon</a>	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

## Abstract

This dataset includes the acetate and inorganic carbon uptake rates as determined via stable isotopic tracers from Maria S. Merian cruise MSM20-5 in 2012; data were generated using the formation fluids recovered from CORKs installed at North Pond.

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## Coverage

**Spatial Extent:** Lat:22.75 Lon:-46.08333

**Temporal Extent:** 2012-04-11 - 2012-05-10

## Dataset Description

The acetate and inorganic carbon uptake rates as determined via stable isotopic tracers; data generated using the formation fluids recovered from the CORKs installed at the North Pond in 2012.

The North Pond is an isolated, northeast-trending, ~8 km × 15 km sediment pond located on the western flank of the Mid-Atlantic Ridge (MAR) at 22°45' N and 46°05' W.

Details of these CORKS and their positions, construction and depth can be found in the Proceedings of the IODP expedition 336. See: [http://publications.iodp.org/scientific\\_prospectus/336/336sp\\_6.htm](http://publications.iodp.org/scientific_prospectus/336/336sp_6.htm)

In addition to NSF OCE-1061934 (to Girguis), this dataset was funded by C-DEBI (OCE-0939564) sub-award number 41940192 granted to Beate Kraft.

## Acquisition Description

To determine potential rates of autotrophic and heterotrophic metabolism within crustal aquifer fluids and deep Atlantic bottom water, fluids were incubated with either  $^{13}\text{C}$ -labeled bicarbonate (autotrophy) or  $^{13}\text{C}$ -labeled acetate (heterotrophy). For this, 20 mL of freshly sampled fluids were injected into sterile, butyl stoppered Balch tubes using a 60 mL syringe and hypodermic needle using sterile technique. Overpressure was released by insertion of a second hypodermic needle. The tubes were pre-amended with a mix of either unlabeled bicarbonate and 10%  $^{13}\text{C}$ -labeled bicarbonate (to a final concentration of 1.8 mM  $\text{NaHCO}_3$  and 0.2 mM  $\text{Na}^{13}\text{CO}_3$ ) or unlabeled acetate and 10%  $^{13}\text{C}$ -labeled acetate (to a final concentration of 13.5  $\mu\text{M}$   $\text{C}_2\text{H}_3\text{NaO}_2$  and 1.5  $\mu\text{M}$   $^{13}\text{C}_2\text{H}_3\text{NaO}_2$ ). All tubes were additionally amended with resazurin (20  $\mu\text{M}$  final concentration) in order to follow the change in redox potential as a result of oxygen consumption throughout the incubation period. Sterile controls were set up as described above but with an additional filter (0.2  $\mu\text{m}$  pore size) inserted between the syringe outlet and the hypodermic needle. Tubes were incubated in the dark at either 5 or 25 degrees C. Incubations were stopped at distinct time intervals by addition of either 0.5 mL of a 1 M NaOH solution (for incubations with bicarbonate) or 5 mL of a 20% zinc acetate solution (for incubations with acetate). Tubes were stored at -20 degrees C until further processing.

For analysis of  $^{13}\text{C}$ -labeled biomass, tubes were thawed and the residual pool of bicarbonate or acetate was removed by acidification to a pH of 2 by adding 25% HCl (molecular grade) while stirring and sparging with  $\text{N}_2$  for at least 30 minutes. The entire volume was then filtered through a pre-combusted glass fiber filter (25mm diameter, 0.7  $\mu\text{m}$  particle retention, Whatman, UK). The filters were dried in a desiccator overnight and stored at 5 degrees C until further processing. Filters were weighed into tin capsules and analyzed for  $^{13}\text{C}/^{12}\text{C}$  ratios with an automated Isotope Cube elemental analyzer (Elementar, Germany) interfaced to a Delta Advantage isotope ratio mass spectrometer (Thermo, Germany). Rates of potential autotrophic metabolism (carbon fixation from  $^{13}\text{C}$ -labeled bicarbonate) and heterotrophic metabolism (degradation of  $^{13}\text{C}$ -labeled acetate) were calculated from  $\delta^{13}\text{C}$  of the carbon pool on the filters at the start and the end of the incubations (time intervals of not more than 13 days). For this, the transfer of  $^{13}\text{C}$  between pools was calculated, according to the following equation:

$$\delta^{\text{Biomass-Final}} \cdot \text{CBiomass-Final} \cdot \text{V}_{\text{Final}} = (\text{CBiomass-Initial} \cdot \text{V} \cdot \delta^{\text{Biomass-Initial}}) + (\text{C}_{\text{Transferred}} \cdot \text{V} \cdot \delta^{\text{Label}})$$

where  $\delta$  is the isotopic ratio  $((R_{\text{sample}}/R_{\text{standard}} - 1) \cdot 1000)$ , V is the volume of the incubation (20mL) and C is the concentration of carbon pool. For incubations with  $^{13}\text{C}$ -labeled bicarbonate we assumed a concentration of 2.3 mM endogenous dissolved inorganic carbon (DIC) in addition to the added mix of  $^{13}\text{C}$ -labeled bicarbonate, thus decreasing the amount of label in the substrate pool. For incubations with  $^{13}\text{C}$ -labeled acetate we assumed a concentration of 150  $\mu\text{M}$  endogenous dissolved organic carbon (DOC) in addition to the added  $^{13}\text{C}$ -labeled acetate.

## Processing Description

### Comments from Middlestead lab in re: C-contet:

- 1) Due to the nature of the samples (on ggf), and the small quantities being analysed, the standard deviation is higher than normal.
- 2) Nitrogen data is included where the peak size is at about the minimum. This data should not actually be used.
- 3) The quantities of N and C are not as accurate as on an EA-only run.
- 4) Even the standards start to have homogeneity issues at this level.

Blind std C-55: C13: -28.3; n = 2; std dev = 0.90. Expected value to date = -28.50

### BCO-DMO Processing:

- Modified parameter names to conform with BCO-DMO naming conventions;
- Replaced spaces with underscores;
- Moved incubation\_duration from comment to a column;

- Created notes column for additional comments;
- Replaced blanks with 'nd' (ie. in the 'Background control' row).

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## Parameters

Parameter	Description	Units
study	Excel sheet.	dimensionless
incubation	Fluids were incubated with either 13C-labeled bicarbonate (autotrophy) or 13C-labeled acetate (heterotrophy).	dimensionless
sample	Sample identifier/location.	dimensionless
temp	Temperature. RT = room temperature	degrees Celsius
time_point	Time point.	dimensionless
delta_13Cvpdb_DOC	delta 13Cvpdb DOC (VPDB = Vienna Pee Dee Belemnite)	per mil
incubation_duration	How long the incubation lasted.	days
DOC	Dissolved organic carbon (DOC). C-content only.	parts per million (ppm C)
filter_one_punch	Filter (one punch)	ppm C
notes	Notes/comments.	dimensionless

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## Instruments

<b>Dataset-specific Instrument Name</b>	Delta Advantage isotope ratio mass spectrometer
<b>Generic Instrument Name</b>	Isotope-ratio Mass Spectrometer
<b>Dataset-specific Description</b>	Filters were weighed into tin capsules and analyzed for 13C/ 12C ratios with an automated Isotope Cube elemental analyzer (Elementar, Germany) interfaced to a Delta Advantage isotope ratio mass spectrometer (Thermo, Germany).
<b>Generic Instrument Description</b>	The Isotope-ratio Mass Spectrometer is a particular type of mass spectrometer used to measure the relative abundance of isotopes in a given sample (e.g. VG Prism II Isotope Ratio Mass-Spectrometer).

<b>Dataset-specific Instrument Name</b>	Isotope Cube elemental analyzer
<b>Generic Instrument Name</b>	Elemental Analyzer
<b>Dataset-specific Description</b>	Filters were weighed into tin capsules and analyzed for <sup>13</sup> C/ <sup>12</sup> C ratios with an automated Isotope Cube elemental analyzer (Elementar, Germany) interfaced to a Delta Advantage isotope ratio mass spectrometer (Thermo, Germany).
<b>Generic Instrument Description</b>	Instruments that quantify carbon, nitrogen and sometimes other elements by combusting the sample at very high temperature and assaying the resulting gaseous oxides. Usually used for samples including organic material.

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## Deployments

### MSM20-5

<b>Website</b>	<a href="https://www.bco-dmo.org/deployment/555399">https://www.bco-dmo.org/deployment/555399</a>
<b>Platform</b>	R/V Maria S. Merian
<b>Report</b>	<a href="http://dmoserv3.whoi.edu/data_docs/Huber/Fahrtbericht_MSM20_5_02.pdf">http://dmoserv3.whoi.edu/data_docs/Huber/Fahrtbericht_MSM20_5_02.pdf</a>
<b>Start Date</b>	2012-04-11
<b>End Date</b>	2012-05-10

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## Project Information

### **Collaborative Research: Characterization of Microbial Transformations in Basement Fluids, from Genes to Geochemical Cycling (North Pond Microbes)**

**Coverage:** North Pond, mid-Atlantic Ridge

#### *Description from NSF award abstract:*

Current estimates suggest that the volume of ocean crust capable of sustaining life is comparable in magnitude to that of the oceans. To date, there is little understanding of the composition or functional capacity of microbial communities in the sub-seafloor, or their influence on the chemistry of the oceans and subsequent consequences for global biogeochemical cycles. This project focuses on understanding the relationship between microbial communities and fluid chemistry in young crustal fluids that are responsible for the transport of energy, nutrients, and organisms in the crust. Specifically, the PIs will couple microbial activity measurements, including autotrophic carbon, nitrogen and sulfur metabolisms as well as mineral oxide reduction, with quantitative assessments of functional gene expression and geochemical transformations in basement fluids. Through a comprehensive suite of in situ and shipboard analyses, this research will yield cross-disciplinary advances in our understanding of the microbial ecology and geochemistry of the sub-seafloor biosphere. The focus of the effort is at North Pond, an isolated sediment pond located on ridge flank oceanic crust 7-8 million years old on the western side of the Mid-Atlantic Ridge. North Pond is currently the target for drilling on IODP expedition 336, during which it will be instrumented with three sub-seafloor basement observatories.

The project will leverage this opportunity for targeted and distinct sampling at North Pond on two German-US research cruises to accomplish three main objectives:

1. to determine if different basement fluid horizons across North Pond host distinct microbial communities and chemical milieus and the degree to which they change over a two-year post-drilling period.
2. to quantify the extent of autotrophic metabolism via microbially-mediated transformations in carbon, nitrogen, and sulfur species in basement fluids at North Pond.
3. to determine the extent of suspended particulate mineral oxides in basement fluids at North Pond and to characterize their role as oxidants for fluid-hosted microbial communities.

Specific outcomes include quantitative assessments of microbial activity and gene expression as well as geochemical transformations. The program builds on the integrative research goals for North Pond and will provide important data for guiding the development of that and future deep biosphere research programs. Results will increase understanding of microbial life and chemistry in young oceanic crust as well as provide new insights into controls on the distribution and activity of marine microbial communities throughout the world's oceans.

There are no data about microbial communities in ubiquitous cold, oceanic crust, the emphasis of the proposed work. This is an interdisciplinary project at the interface of microbial ecology, chemistry, and deep-sea oceanography with direct links to international and national research and educational organizations.

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## Program Information

### 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>.

### 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**

<b>Funding Source</b>	<b>Award</b>
<a href="#">NSF Division of Ocean Sciences (NSF OCE)</a>	<a href="#">OCE-1061934</a>
<a href="#">NSF Division of Ocean Sciences (NSF OCE)</a>	<a href="#">OCE-0939564</a>

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