

Organic carbon and nitrogen in near-vent sediment samples in Paleochori Bay, Milos Island, Greece during 2012 (Hydrothermal Autotrophic Carbon Fixation project)

Website: <https://www.bco-dmo.org/dataset/551070>

Version: 2015-02-17

Project

» [Autotrophic carbon fixation at a shallow-water hydrothermal system: Constraining microbial activity, isotopic and geochemical regimes](#) (Hydrothermal Autotrophic Carbon Fixation)

Program

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

Contributors	Affiliation	Role
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Dataset Description

This dataset describes the sediment push cores taken by Scuba diving at a shallow-water hydrothermal vent site in Paleochori Bay, Milos Island, Greece for subsequent analyses to determine the total organic carbon (TOC), total organic nitrogen (TON), the stable carbon isotopic composition of TOC (d13C), the stable nitrogen isotopic composition of TON (d15N), and for the extraction of DNA for subsequent microbial community analyses based on sequencing 16S rRNA amplicons using 454 technology.

Methods & Sampling

Samples were collected by push cores along a transect from the hottest part of the venting area towards the unaffected, colder sediments, i.e., at 0 cm, 50 cm, 100 cm, 150 cm, 200 cm, and 300 cm. On shore, the push cores were sliced in 2 cm intervals, frozen on dry ice, and subsequently stored at -80°C. DNA was extracted using a commercially available extraction kit. We were able to successfully sequence 16S rRNA amplicons for Bacteria and Archaea from a total of 26 samples. Sequence data are currently being analyzed and will be deposited in GenBank prior to publication and will be made available to the scientific community. For TOC and TON analyses, dried sediment samples were weighed into methanol rinsed silver boats (4 x 6 mm, Costech). 96 well glass plates (combusted 4 hrs @ 450C) holding these samples were placed in a vacuum desiccator that also contained an open dish with about 50 ml fresh, concentrated (12N) HCl. An inverted crystallization dish was placed over the samples to protect them from water that can condense and rain down from the desiccator top during heating. The desiccator was closed and pumped out with an air driven aspirator, to a reading of about ~0.5 atm and the desiccator is placed in an oven kept between 60 and 65 C. Acidification was allowed to run for 60 to 72 hours. The samples were then transferred to another vacuum desiccator, this time charged with indicating silica gel (Fisher S162-500, activated by heating to 450°C overnight, and pumped down

again and dried for about 24 hours before use. Immediately before analysis, samples are wrapped in tin boats (Costech, 4x6, methanol rinsed).

Data Processing Description

We have used a commercially available extraction kit for extracting the DNA. 16S rRNA amplicons for Bacteria and Archaea were generated using 454 sequence technology. Obtained sequences are currently being analyzed using the QIIME pipeline. The reads are being dereplicated, denoised, screened for chimeric sequences and taxonomically classified using the RDP and GreenGenes databases. Multivariate ordination techniques are being used to discriminate among samples with similar community structures. For TOC and TON analyses, samples are analyzed in triplicate using a Fisons 1108 Elemental Analyzer equipped with a Costech "Zero Blank" sample carousel. Effluent gases from the EA flow into a Finnigan-MAT Conflo-II interface attached to a DeltaPlus Isotope Ratio Mass Spectrometer. Standard materials (USGS-40 glutamic acid, IAEA-N1 ammonium sulfate, NBS-19 limestone, as well as known glycine and calcites) are used to determine the area response and calibrate the isotopic reference gas.

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Data Files

File
orgC_N.csv (Comma Separated Values (.csv), 4.25 KB) MD5:ab7ec709fb4d64ffc4009d04b0b34400 Primary data file for dataset ID 551070

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Parameters

Parameter	Description	Units
location	sampling location	unitless
lat	latitude; north is positive	decimal degrees
lon	longitude; east is positive	decimal degrees
site	sampling site number	unitless
date	sampling date	yyyy-mm-dd
distance	distance from transect origin	cm
comment	comment	unitless
sediment_horizon	sediment horizon	cm
TOC	total organic carbon mean percent	percent
TOC_sd	total organic carbon standard deviation	percent
TOC_n	number of samples for total organic carbon	samples
TON	total organic nitrogen mean percent	percent
TON_st	total organic nitrogen standard deviation	percent
TON_n	number of samples for total organic nitrogen	samples
C_N	carbon:nitrogen ratio	unitless
d13C_org	organic d13C concentration	parts per thousand
d13C_org_sd	organic d13C standard deviation	parts per thousand
d13C_org_n	number of samples for organic d13C	samples
d15N_org	organic d15N concentration	parts per thousand
d15N_org_sd	organic d15N standard deviation	parts per thousand
d15N_org_n	number of samples for organic d15N	samples
DNA	whether DNA was extracted from the samples for subsequent microbial community analyses	unitless
rRNA	whether 16S rRNA Sequence Data for Bacteria and Archaea was collected	unitless

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Instruments

Dataset-specific Instrument Name	Elemental Analyzer
Generic Instrument Name	CHN Elemental Analyzer
Dataset-specific Description	Fisons 1108 Elemental Analyzer equipped with a Costech "Zero Blank" sample carousel
Generic Instrument Description	A CHN Elemental Analyzer is used for the determination of carbon, hydrogen, and nitrogen content in organic and other types of materials, including solids, liquids, volatile, and viscous samples.

Dataset-specific Instrument Name	Spectrometer
Generic Instrument Name	Spectrometer
Dataset-specific Description	Finnigan-MAT Conflo-II interface attached to a DeltaPlus Isotope Ratio Mass Spectrometer
Generic Instrument Description	A spectrometer is an optical instrument used to measure properties of light over a specific portion of the electromagnetic spectrum.

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Deployments

Milos_vents_2012

Website	https://www.bco-dmo.org/deployment/474391
Platform	shoreside Milos_vents
Start Date	2012-05-21
End Date	2012-05-30
Description	Vent fluids and sediment cores were collected at shallow-water hydrothermal vent sites at Paleochori Bay, Milos Island, Greece, and analyzed for their chemical composition (anion, cation, trace elements/metals).

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Project Information

Autotrophic carbon fixation at a shallow-water hydrothermal system: Constraining microbial activity, isotopic and geochemical regimes (Hydrothermal Autotrophic Carbon Fixation)

Coverage: Shallow-water hydrothermal vents, Paleochori Bay, Milos Island, Greece

In this project we studied the shallow-water hydrothermal vent sites at Milos Island (Greece) to better understand the extent of autotrophic carbon fixation and its chemical and isotopic signature along environmental (redox/thermal) gradients. This was a 12-day long expedition (May 18 to 30, 2012) to sample vent fluids, gases and retrieve sediment cores at Paleochori Bay by using SCUBA diving at 8-10 m depth. In addition to the submarine vent sites, two subaerial locations of venting were identified at 36° 40' 28" N - 24° 31' 14" E and 36° 40' 25" N - 24° 30' 44" E. Both the subaerial and submarine sites are located on the same

fracture zone that likely controls the hydrothermal circulation of evolved meteoritic water and seawater within the magmatic zone of Milos Island. To this end, the geochemistry of the fluids and gases emitted from subaerial sites provide important information towards identifying the linkage between the subaerial and submarine magmatic activity and provide insights on the metabolic functions (e.g. H₂ oxidation, Fe(III) reduction, C and S cycling) of the subsurface microbial community.

Abstract:

Currently, there is only limited information on the identity and activity of the microorganisms carrying out CO₂-fixation in situ, despite the fact that these organisms form the basis of their respective ecosystems. Representatives that are able to grow autotrophically are known to exist in almost all major groups of prokaryotes, and these organisms play essential roles in ecosystems by providing a continuous supply of organic carbon for heterotrophs. Microorganisms present in extreme environments utilize CO₂-fixation pathways other than the Calvin-Benson-Bassham (CBB) cycle. At present, five alternative autotrophic CO₂ fixation pathways are known. Different carbon fixation pathways result in distinct isotopic signatures of the produced biomass due to the isotopic discrimination between light (¹²C) and heavy (¹³C) carbon by the carboxylating enzymes. Thus, inferences about the carbon fixation pathway predominantly utilized by the microbial community can also be made based on the stable carbon isotopic composition of the organic matter, in extant systems as well as in the geological record. However, at present little is known about the systematics and extents of fractionation during carbon fixation by prokaryotic organisms, and to our knowledge no studies exist that have systematically studied the relationship between the operation of different carbon fixation pathways and how this is reflected in the stable carbon isotopic composition in a natural system. This is a 2-year interdisciplinary, international research program that employs a powerful combination of cutting-edge research tools aiming to improve our understanding of autotrophic carbon fixation and its chemical and isotopic signature along environmental gradients in a natural hydrothermal system. The following hypotheses are addressed:

1. The diversity of microorganisms present along a thermal and redox gradient, and rates of CO₂ fixation, will reflect adaptation to in situ temperatures and geochemical conditions
2. Microorganisms utilizing the CBB cycle for autotrophic CO₂-fixation will represent a smaller percentage of the chemolithoautotrophic community at higher temperatures, where microorganisms utilizing alternative CO₂-fixation pathways dominate
3. Isotopic values of biomass and specific biomarker molecules will vary along a thermal and redox gradient from zones characterized by a higher hydrothermal fluid flux and thus higher temperatures to the surrounding, cooler areas, corresponding to the physiology of the microorganisms utilizing different pathways for carbon fixation

The PIs will use a multidisciplinary approach to delineate the relative contribution of the different carbon fixation pathways along an environmental gradient by combining metagenomic analyses coupled with: 1) an assessment of the frequency and the expression of specific key genes involved in carbon fixation, and 2) with the measurement of carbon fixation rates. These data will be integrated with the determination of stable C isotopic composition of biomass, DIC, and specific hydrocarbons/lipids. Due to its easy accessibility, well-established environmental gradients, and extensive background information, the shallow-water vents off Milos (Greece) will be used as a natural laboratory to perform these studies.

Intellectual Merit. The data generated in this study will allow constraints on the relationship between autotrophic carbon fixation and the resulting isotopic signatures of biomass and specific biomarker molecules (e.g. CH₄, C₂+ alkanes, lipids) in a natural system. This has implications for assessing the importance of carbon fixation in extant ecosystems, and it will also provide a tool to improve the interpretation of isotopic values in the geological record.

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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-1124272

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