Microbial cell abundance,carbon fixation rates, and nitrate concentrations during shipboard incubations of vent fluids at the diffuse-flow vent Crab Spa, East Pacific Rise on RV/Atlantis cruise AT37-12, May 2017

Website: https://www.bco-dmo.org/dataset/788911
Data Type: Cruise Results
Version: 1
Version Date: 2020-01-30

Project
» Collaborative Research: Environmental Drivers of Chemoautotrophic Carbon Production at Deep-Sea Hydrothermal Vents - Comparative Roles of Oxygen and Nitrate (vent O2 NO3 roles)

Contributors	Affiliation	Role
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Abstract
Microbial cell abundance, carbon fixation rates, and nitrate concentrations during shipboard incubations of vent fluids at the diffuse-flow vent Crab Spa, East Pacific Rise in the Eastern Tropical North Pacific (ETNP) on RV/Atlantis cruise AT37-12, May 2017.

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Coverage

Spatial Extent: **N**:9.8398 **E**:-104.2912 **S**:9.8387 **W**:-104.2913
Temporal Extent: 2017-05-08

Dataset Description

This dataset includes microbial cell abundance, carbon fixation rates, and nitrate concentrations during shipboard incubations of vent fluids at the diffuse-flow vent Crab Spa site, East Pacific Rise in the Eastern Tropical North Pacific (ETNP) on RV/Atlantis cruise AT37-12, May 2017. Crab Spa is at 9.8398N, 104.2913W, and depth 2503 meters.

Methods & Sampling

During Alvin Dive 4905, May 8, 2017, 5 major samples were collected at the diffuse-flow vent Crab Spa. The fluid from these samplers was used in an on-deck Vent-SID incubation. The purpose of the incubation was to

simulate a sea-floor incubation of the Vent-SID. Upon arrival of majors on the ship, we transferred fluid from the majors into N2 flushed 1L Restek bags. The bags were stored at 4˚C and taken out as needed for setting up a new Vent-SID incubation. In total, six incubations were conducted, all at 25(+/-2)˚C.

Microbial cell counts: Samples for cell numbers were fixed with formaldehyde (1% final concentration) and then counted on the board the ship after staining with acridine orange by fluorescence microscopy as described in McNichol et al. (2016).

Carbon fixation rates: 13C-labeled bicarbonate was added to the incubation chambers to assess chemoautotrophic production. At the beginning and the end of the incubation, fluids were filtered onto pre combusted GFF filters, which were frozen until analysis back in the shore lab. Gas chromatography combustion (Fisons 1108 Elemental Analyzer equipped with a Costech "Zero Blank" sample carousel) coupled to an isotope ratio mass spectrometer (GC-IRMS) (Finnigan-MAT Conflo-II interface attached to a DeltaPlus Isotope Ratio Mass Spectrometer) was used to measure the incorporation of 13C-labelled bicarbonate into biomass during the incubations to determine chemoautotrophic production.

Nitrate concentrations: Vent fluid from the Crab Spa site was incubated on the deck of the ship in the Vent-SID reaction chamber, in the dark at a temperature of 25(+/- 2)°C. Incubations consisted of a vent fluid with added NO3- (10 µmol/L), NO2- (1 µmol/L) and H13CO3- (0.7 mmol/L) and time-point samples were taken for nitrate+nitrite measurements at 0, 3, and 6 hours. Six incubations were conducted that varied in combinations of 15NO3-/14NO3 or 15NO2-/14NO2-, keeping the total concentration of added NO3- and NO2- constant across incubations.

Nitrate concentrations were measured in time-point samples utilizing the vanadium(III) reduction of nitrate and nitrite method described by Braman and Hendrix, 1989. Samples were injected into a heated Vanadium acid solution whereby NO3- and NO2- were reduced to NOx gases. The NOx then passed into a Teledyne T200 NOx analyzer where a photodetector measured the light produced from the chemiluminescent reaction of NOx and instrument generated ozone.

After initial collection, vent fluid samples were stored in 15 mL conical tubes at -20C until analyzed on the Teledyne NOx analyzer. Once thawed, samples were injected using Hamilton 1700 series gastight syringes and Nitrate concentrations were calculated alongside standards generated from a Ricca Nitrate Nitrogen Standard (CAT#5459-16, 1000 ppm N, 4427 ppm NO3). The series of standards ranged from 1 uM to 50 uM and were made up by diluting the Ricca standard in Milli-Q water.

Peak Simple Version 4.49 was used to generate chromatographs from the photodetector and raw data was further processed in Microsoft Excel.

Data Processing Description

BCO-DMO Processing
- combined cell abundance, carbon fixation rates, and nitrate concentration excel tables into a single table

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Data Files

File
incubations.csv (Comma Separated Values (.csv), 402 bytes) MD5:b2c7fa8bb48590599854367e78a04555 Primary data file for dataset ID 788911

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Related Publications

McNichol, J., Sylva, S. P., Thomas, F., Taylor, C. D., Sievert, S. M., & Seewald, J. S. (2016). Assessing microbial processes in deep-sea hydrothermal systems by incubation at in situ temperature and pressure. Deep Sea

Research Part I: Oceanographic Research Papers, 115, 221–232. doi:10.1016/j.dsr.2016.06.011
Methods

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Parameters

Parameter	Description	Units
incubation	incubation run identifier	unitless
time_elapsed	time since start of incubation	hours
cell_abund	microbial cell abundance	cells/milliliter
carbon_fix	carbon fixation rate	microgram Carbon/liter/day
NOX	Nitrate+nitrite concentration during incubations	micromol/liter

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Instruments

Dataset-specific Instrument Name	• Fisons 1108 Elemental Analyzer equipped with a Costech "Zero Blank" sample carousel
Generic Instrument Name	Elemental Analyzer
Dataset-specific Description	Used for carbon fixation measurements
Generic Instrument Description	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.

Dataset-specific Instrument Name	Teledyne T200 NOx analyzer with a SRI Model 333 Peak Simple Chromatography Data System
Generic Instrument Name	Elemental Analyzer
Dataset-specific Description	Used to generate chromatographs from the photodetector and thus measure nitrate concentrations.
Generic Instrument Description	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.

Dataset-specific Instrument Name	
Generic Instrument Name	Fluorescence Microscope
Dataset-specific Description	Used for microbial cell counts.
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	DeltaPlus Isotope Ratio Mass Spectrometer with attached Finnigan-MAT Conflo-II interface
Generic Instrument Name	Isotope-ratio Mass Spectrometer
Dataset-specific Description	Used for carbon fixation measurements.
Generic Instrument Description	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).

Dataset-specific Instrument Name	Vent-SID
Generic Instrument Name	Shipboard Incubator
Dataset-specific Description	A Vent-SID, Vent-Submersible Incubation Device, was used for on-deck incubations. It draws seawater and microbes into incubation chambers and measures the biochemical business going on under natural conditions. For further description of this device, see https://www.whoi.edu/oceanus/feature/bringing-a-lab-to-the-seafloor/.
Generic Instrument Description	A device mounted on a ship that holds water samples under conditions of controlled temperature or controlled temperature and illumination.

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Deployments

AT37-12

Website	https://www.bco-dmo.org/deployment/734074
Platform	R/V Atlantis
Report	http://datadocs.bco-dmo.org/docs/Vent_O2_NO3_Roles/data_docs/AT37-12_Cruise_Report.pdf
Start Date	2017-04-24
End Date	2017-05-15

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Project Information

Collaborative Research: Environmental Drivers of Chemoautotrophic Carbon Production at Deep-Sea Hydrothermal Vents - Comparative Roles of Oxygen and Nitrate (vent O2 NO3 roles)

Coverage: Deep-Sea hydrothermal vent field at 9 deg N on the East Pacific Rise

NSF award abstract:

Deep-sea hydrothermal vents, first discovered in 1977, are exemplary ecosystems where microbial chemosynthesis rather than photosynthesis is the primary source of organic carbon. Chemosynthetic microorganisms use the energy generated by oxidizing reduced inorganic chemicals contained in the vent fluids, like hydrogen sulfide or hydrogen gas, to convert carbon dioxide (CO2) into cell material. By doing so, they effectively transfer the energy from a geothermal source to higher trophic levels, in the process supporting the unique and fascinating ecosystems that are characterized by high productivity - oases in the otherwise barren deep ocean landscape. While the general view of the functioning of these ecosystems is established, there are still major gaps in our understanding of the microbiology and biogeochemistry of these systems. Particularly lacking are studies measuring rates of microbial activity in situ, which is ultimately needed to understand production of these ecosystems and to assess their impact on global biogeochemical cycles. This project makes use of the Vent-Submersible Incubation Device (Vent-SID), a robotic micro-laboratory that was recently developed and tested in the field. This instrument makes it possible for the first time to determine rates of carbon fixation at both in situ pressures and temperatures, revolutionizing the way we conduct microbial biogeochemical investigations at deep-sea hydrothermal vents. This is an interdisciplinary and collaborative effort between two US and foreign institutions, creating unique opportunities for networking and to foster international collaborations. This will also benefit two graduate students working in the project, who will get exposed to a wide range of instrumentation and scientific fields, facilitating their interdisciplinary education. In collaboration with Dr. Nitzan Resnick, academic dean of The Sage School, an elementary school outreach program will be developed and a long-term partnership with the school established. Further, a cruise blog site to disseminate the research to schools and the broader public will be set up. The results will be the topic of media coverage as well as be integrated into coursework and webpages existing either in the PI's labs or at the institution.

This project is using a recently developed robotic micro-laboratory, the Vent-SID, to measure rates of chemoautotrophic production and to determine the relative importance of oxygen and nitrate in driving chemosynthesis at deep-sea hydrothermal vents at in situ pressures and temperatures and to tackle the following currently unresolved science objectives: 1) obtain in situ rates of chemoautotrophic carbon fixation, 2) obtain in situ nitrate reduction rate measurements, and 3) directly correlate the measurement of these processes with the expression of key genes involved in carbon and energy metabolism. Although recent data suggests that nitrate reduction either to N2 (denitrification) or to NH4+ (dissimilatory reduction of nitrate to ammonium) might be responsible for a significant fraction of chemoautotrophic production, NO3-reduction rates have never been measured in situ at hydrothermal vents. The researchers hypothesize that chemoautrophic growth is strongly coupled to nitrate respiration in vent microbial communities. During a cruise that will take place approximately 12 months into the project (~Feb 2017), the researchers will carry out a total of 4 deployments of the Vent-SID as well as ancillary sampling collection at the 9°46N to 9°53N segment of the East Pacific Rise. They will focus efforts on two diffuse-flow vent sites, "Crab Spa" and "Teddy Bear". "Crab Spa" is a diffuse flow vent site (T: 25°C) that has been used as a model system to gain insights into chemoautotrophic processes and has been frequently sampled over the last several years. This vent site has been very well characterized, both geochemically and microbiologically, providing excellent background data for the proposed process oriented studies. "Teddy Bear" is a diffuse-flow site that was discovered in Jan 2014, and it has a lower temperature (T: 12°C), making it a good comparative site. The researchers will perform a number of short duration time-course incubations to assess the role of different environmental parameters that have been identified as likely key variables (e.g., O2, temperature, NO3-), and to link these process rate measurements to the expression of functional genes using metatranscriptomic analyses. This study will be the first attempt to measure critical metabolic processes of hydrothermal vent microbial assemblages under critical in situ conditions and to assess the quantitative importance of electron donor and acceptor pathways in situ. In the future, it is envisioned that the Vent-SID will become a routine application by the oceanographic

community for measuring time series rates of relevant metabolic processes at hydrothermal vents under in situ pressures and vent fluid temperatures.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1559198
NSF Division of Ocean Sciences (NSF OCE)	OCE-1559042

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