

# Sediment N isotope ratios from incubations amended with $^{15}\text{N}$ -labeled substrates from samples collected on cruise OC1703A aboard R/V Oceanus and cruise AT36 aboard R/V Atlantis

**Website:** <https://www.bco-dmo.org/dataset/862979>

**Data Type:** Cruise Results

**Version:** 1

**Version Date:** 2021-11-02

## Project

» [Nitrogen Fixation in Deep-Sea Sediments](#) (Deep Sediment N Fix)

Contributors	Affiliation	Role
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## Abstract

This dataset includes sediment N isotope ratios from incubations amended with  $^{15}\text{N}$ -labeled substrates. Sediments were collected on cruise OC1703A aboard R/V Oceanus in March 2017 and on cruise AT36 aboard R/V Atlantis in July-August 2016. Cores were collected using an MC-800 multicore and an MC-400 multicore. The generation of these data was completed on April 9, 2020.

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## Coverage

**Spatial Extent:** N:39.900193 E:-69.263835 S:35.68905 W:-124.92211

**Temporal Extent:** 2016-07-28 - 2017-03-21

## Acquisition Description

Sediment cores were collected in the northwest Pacific Ocean off the coast of San Francisco, CA on the R/V Oceanus during cruise OC1703A in March 2017 and in the northeast Atlantic off the coast of Woods Hole, MA on the R/V Atlantis during cruise AT36 in July 2016. Sediment cores in the northwest Pacific were 15–30 cm long and collected at five sites (100–4475 water depths) using an MC-800 multicore (Ocean Instruments). Sediment cores in the northeast Atlantic were 15 cm long and collected at two sites (1252–1496 water depth) using an MC-400 multicore (Ocean Instruments). Both multicores were modified with Go-Pro cameras in custom pressure housing to provide real-time video feeds and guide sampling (MISO Facility, Woods Hole Oceanographic Institute). Cores were stored at 4°C for  $\leq 24$  hours until they were sectioned on board in 2.5, 3, or 5 cm depth increments.

Sediment from two cores at each of the five Pacific sampling sites was sectioned in 5 cm intervals (3–5 sections per core) and mixed with 0.2  $\mu\text{m}$ -filtered, argon-sparged bottom water (1:1–1:2 ratio of sediment to seawater). About 60 mL of sediment slurry was aliquoted into each 120 mL glass serum bottle, sealed with NaOH pre-treated black butyl rubber stoppers (Geo-microbial technologies, Ochelata, OK, USA) and aluminum

crimp caps, and the headspace exchanged using argon. Incubations from each sediment depth were amended with  $^{15}\text{N}$ -ammonium (final concentration: 500  $\mu\text{M}$ ; Cambridge Isotopes, NLM-467-5, Lot I-19633L),  $^{13}\text{C}$ -glucose (50  $\mu\text{M}$ ; Cambridge Isotopes, CLM-1396-1, Lot PR-27921),  $^{13}\text{C}$ -bicarbonate (final concentration 1.15 mM; Cambridge Isotopes, CLM-441-5, Lot PR-27797), or 5 mL of  $^{15}\text{N}_2$  (Cambridge Isotopes, NLM-363-PK, Lot I-19197).  $^{15}\text{N}_2$  gas was passed through an acid trap before addition to incubation bottles to scavenge any undetected contaminants. Select  $^{15}\text{N}$ -ammonium and  $^{13}\text{C}$ -glucose incubations were additionally amended with non-isotope labeled glucose (50  $\mu\text{M}$ ) and ammonium (500  $\mu\text{M}$ ), respectively. Incubations were over-pressured to 30 psi using argon. All incubations were conducted in duplicate, with the exception of the  $^{15}\text{N}_2$  incubations, which were conducted in triplicate. All Pacific incubations were subsampled at 2, 4, 8, and 12 weeks for bulk C or N stable isotope analysis.

For the Atlantic samples, cores were sectioned into 3 cm sediment horizons and stored under anaerobic conditions at 4°C for 3 months. Incubation set up was the same as for the Pacific samples, except only 20 mL of slurry was aliquoted into each 60 mL serum bottle and they were sealed with blue butyl rubber stoppers (Bellco Glass, Vineland, NJ, USA). All incubations were amended with  $^{15}\text{N}$ -ammonium (final concentration: 1 mM; Cambridge Isotopes, NLM-467-5, Lot I-19633L) or 5 mL  $^{15}\text{N}_2$  (Cambridge Isotopes, NLM-363-PK, Lot I-19197) and incubations were subsampled at 0 and 30 days. A total of nearly 300 incubations of Pacific and Atlantic sediment were prepared.

Sediment slurries were subsampled with a needle and syringe and sampled slurry was immediately centrifuged at 4,000 x g for 30 seconds. The supernatant was removed and stored at -20°C, and the sediment pellet frozen at -20°C. For incubations amended with  $^{15}\text{N}$ -ammonium, sediment pellets were washed three times with PBS and KCl after defrosting and before drying as previously described (Dekas et al., 2014). Briefly, 1 x PBS was added to sediment pellets, vortexed, centrifuged at 15,100 x g for 15 min, the supernatant removed, and repeated with 2M KCl (incubated at 1 h at room temperature) and then 1 x PBS. For incubations amended with  $^{13}\text{C}$ -glucose and  $^{13}\text{C}$ -bicarbonate, sediment pellets were also washed, but we replaced the KCl incubation with another 1 x PBS wash step. Sediment pellets were dried overnight at 60°C and left to equilibrate to ambient humidity for at least three days. For N isotope analysis, samples were weighed into silver capsules and acid fumigated to remove carbonates as previously described (Harris et al., 2001). Acid fumigated samples were then encapsulated into tin capsules. All samples were analyzed using Elementar Vario EL Cube or Micro Cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced to either an Isoprime VISION IRMS (Elementar UK Ltd, Cheadle, UK) or a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at UC Davis Stable Isotope Facility. They report a long term deviation of 0.2‰ for  $^{13}\text{C}$  and 0.3‰ for  $^{15}\text{N}$ .

## Processing Description

### BCO-DMO Processing:

- renamed fields to conform with BCO-DMO naming conventions;
- replaced commas with semi-colons in the Core column;
- removed apostrophes from the Depth\_cm column;
- converted longitude negative values to indicate West direction;
- filled in "none" in "Amendment" column for samples AT36-5-t1, AT36-11-t1, AT36-17-t1, and AT36-23-t1.

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## Related Publications

Dekas, A. E., Chadwick, G. L., Bowles, M. W., Joye, S. B., & Orphan, V. J. (2014). Spatial distribution of nitrogen fixation in methane seep sediment and the role of the ANME archaea. *Environmental Microbiology*, 16(10), 3012–3029. doi:[10.1111/1462-2920.12247](https://doi.org/10.1111/1462-2920.12247)  
*Methods*

Harris, D., Horwáth, W. R., & van Kessel, C. (2001). Acid fumigation of soils to remove carbonates prior to total organic carbon or CARBON-13 isotopic analysis. *Soil Science Society of America Journal*, 65(6), 1853–1856. doi:[10.2136/sssaj2001.1853](https://doi.org/10.2136/sssaj2001.1853)  
*Methods*

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## Parameters

Parameter	Description	Units
Cruise	Cruise ID number	unitless
ISO_Date_Local	Sampling date (local) in format YYYY-MM-DD	unitless
Latitude	Sampling latitude	decimal degrees North
Longitude	Sampling longitude	decimal degrees East
Deployment	Deployment number	unitless
Water_Depth_m	Sampling water depth	meters (m)
Core	Multicore number	unitless
Depth_cm	Sampling sediment depth	centimeters (cm)
Sample_ID	Sample ID number	unitless
d15N	Delta 15N isotope ratio	permil
N_amount_micrograms	Amount of nitrogen	micrograms (ug)
Sediment_weight_mg	Sediment weight	milligrams (mg)
Time_point_weeks	Subsample time point	weeks
Amendment	Label amendment	unitless

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## Instruments

<b>Dataset-specific Instrument Name</b>	Isoprime VISION IRMS
<b>Generic Instrument Name</b>	Isotope-ratio Mass Spectrometer
<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>	DZ Europa 20-20 isotope ratio mass spectrometer
<b>Generic Instrument Name</b>	Isotope-ratio Mass Spectrometer
<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>	Go-Pro cameras in custom pressure housing
<b>Generic Instrument Name</b>	Camera
<b>Generic Instrument Description</b>	All types of photographic equipment including stills, video, film and digital systems.

<b>Dataset-specific Instrument Name</b>	MC-800 multicore (Ocean Instruments)
<b>Generic Instrument Name</b>	Multi Corer
<b>Generic Instrument Description</b>	The Multi Corer is a benthic coring device used to collect multiple, simultaneous, undisturbed sediment/water samples from the seafloor. Multiple coring tubes with varying sampling capacity depending on tube dimensions are mounted in a frame designed to sample the deep ocean seafloor. For more information, see Barnett et al. (1984) in Oceanologica Acta, 7, pp. 399-408.

<b>Dataset-specific Instrument Name</b>	MC-400 multicore (Ocean Instruments)
<b>Generic Instrument Name</b>	Multi Corer
<b>Generic Instrument Description</b>	The Multi Corer is a benthic coring device used to collect multiple, simultaneous, undisturbed sediment/water samples from the seafloor. Multiple coring tubes with varying sampling capacity depending on tube dimensions are mounted in a frame designed to sample the deep ocean seafloor. For more information, see Barnett et al. (1984) in Oceanologica Acta, 7, pp. 399-408.

<b>Dataset-specific Instrument Name</b>	Elementar Vario EL Cube or Micro Cube elemental analyzer
<b>Generic Instrument Name</b>	Elemental Analyzer
<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

### OC1703A

<b>Website</b>	<a href="https://www.bco-dmo.org/deployment/717423">https://www.bco-dmo.org/deployment/717423</a>
<b>Platform</b>	R/V Oceanus
<b>Start Date</b>	2017-03-14
<b>End Date</b>	2017-03-23
<b>Description</b>	See additional cruise information from the Rolling Deck to Repository (R2R): <a href="https://www.rvdata.us/search/cruise/OC1703A">https://www.rvdata.us/search/cruise/OC1703A</a>

### AT36

<b>Website</b>	<a href="https://www.bco-dmo.org/deployment/862503">https://www.bco-dmo.org/deployment/862503</a>
<b>Platform</b>	R/V Atlantis
<b>Start Date</b>	2016-07-28
<b>End Date</b>	2016-08-07
<b>Description</b>	See additional cruise information from the Rolling Deck to Repository (R2R): <a href="https://www.rvdata.us/search/cruise/AT36">https://www.rvdata.us/search/cruise/AT36</a>

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## Project Information

### Nitrogen Fixation in Deep-Sea Sediments (Deep Sediment N Fix)

**Coverage:** California Shelf (36,-123)

#### *NSF Award Abstract:*

Life requires nitrogen for growth. Atmospheric nitrogen (N<sub>2</sub>) is the most abundant form of nitrogen on the surface of the planet, but most organisms cannot assimilate N<sub>2</sub> directly. Habitats can therefore be nitrogen limited, meaning the demand for "bioavailable" nitrogen exceeds the supply, and its availability controls the overall growth and productivity of the community. A small subset of microorganisms, termed diazotrophs, convert N<sub>2</sub> to bioavailable forms of nitrogen, including ammonium and nitrogenous organic matter, in a process known as N<sub>2</sub> fixation. Diazotrophs are the largest natural source of bioavailable nitrogen on the planet, and the rate at which they fix N<sub>2</sub> can control the rates at which other important microbial processes occur, such as the production and consumption of greenhouse gases. Understanding diazotrophs in the environment - their identity, distribution, activity levels, and biogeochemical controls - is therefore essential to understanding overall microbial community activity and biogeochemical cycling. The goal of this project is to characterize N<sub>2</sub> fixation in deep-sea sediments, a generally understudied but expansive habitat, covering nearly two thirds of our planet. The project will have broader impacts via educational outreach, support and training of early career scientists, and scientific impact: since rates of marine methane, carbon dioxide, and nitrous oxide cycling are affected by nitrogen availability, the results will inform our understanding of greenhouse gas cycling in the marine environment, and therefore climate stability, a topic central to global security.

N<sub>2</sub> fixation is a critical and intensely studied metabolism in the marine photic zone. Much less is known about N<sub>2</sub> fixation in deep-sea sediments, but it could be an important factor in both benthic productivity and ocean-scale elemental cycling. Several observations have suggested or directly detected N<sub>2</sub> fixation at localized areas of enhanced productivity on the seafloor (e.g., methane seeps and hydrothermal vents), raising the possibility that deep-sea N<sub>2</sub> fixation is widespread. However, few measurements of N<sub>2</sub> fixation have been made outside of these anomalous areas, and thus little is known about N<sub>2</sub> fixation in the vast majority of the deep ocean floor. Preliminary data suggest N<sub>2</sub> fixation does occur in typical deep marine sediment, and is mediated by a diverse set of yet unidentified microorganisms. This project will combine techniques from molecular biology and geochemistry to systematically investigate N<sub>2</sub> fixation in representative deep-sea sediments collected along a depth profile (500 to 4500 m water depth) offshore California. The project will determine the (1) rates and distribution of N<sub>2</sub> fixation (2) abundance, diversity, and distribution of genes and transcripts associated with N<sub>2</sub> fixation (nif) (3) phylogenetic identity of the biological mediators (diazotrophs) and (4) physiochemical controls on diazotrophic community structure and activity. For context, the activity of the non-diazotrophic bacterial community will also be characterized. The results may lead to upward revisions of the estimates of new nitrogen production in the seafloor, and therefore change our understanding of the current balance of the marine nitrogen cycle. Together, this hypothesis-driven characterization of N<sub>2</sub> fixation in deep-sea sediments will shed light on an expansive, climatically important, and traditionally understudied habitat, and facilitate more accurate extrapolation of the rates and distribution of N<sub>2</sub> fixation on the whole seafloor as well as the metabolic response of the seafloor community to environmental change.

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## Funding

Funding Source	Award
<a href="#">NSF Division of Ocean Sciences (NSF OCE)</a>	<a href="#">OCE-1634297</a>

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