Metaproteomics analyses of mixed-layer water from Hawaii Ocean Time-series Station ALOHA, collected on R/V Kilo Moana cruise KM2204 in March 2022, and incubated with 15N2 to track biosynthetic incorporation by diazotrophic microbes

Website: https://www.bco-dmo.org/dataset/958552
Data Type: Cruise Results, experimental
Version: 1
Version Date: 2025-04-10

Project
» EAGER: Tracking marine diazotrophy with isotope-labeling proteomics (15N2Fix Proteomics)

Contributors	Affiliation	Role
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Abstract
This project seeks to shed new light on outstanding questions in marine diazotroph ecology, including: 1) How is whole-community N2 fixation activity apportioned among different diazotroph taxa?; 2) How do diazotrophs and their symbiotic partners make biosynthetic use of the N they fix?; 3) How much diazotroph-derived N is redistributed to particular nondiazotroph taxa? To address these questions, we conducted shipboard field work at Hawaii Ocean Time-series Station ALOHA in the North Pacific, where we sampled mixed-layer ocean surface water microbial communities and performed 15N2-incubation experiments. We developed novel experimental approaches to highly enriching seawater with dissolved 15N2, which is essential for sensitive 15N-tracking proteomics. The protocols we developed were able to achieve >30 atom% 15N in dissolved N2 in shipboard incubations of natural seawater. We used metaproteomics analysis of the microbial biomass from these incubations to assess the N2-fixation activity of coexisting diazotrophic organisms, and to determine the biosynthetic allocation of the fixed nitrogen they produced. These data are available through MassIVE under accession number MSV000096410 (DOI: 10.25345/C5C24R05N).

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Coverage

Location: Hawaii Ocean Time-series Station ALOHA
Spatial Extent: **Lat**:22.75 **Lon**:-158
Temporal Extent: 2022-03-26 - 2022-03-30

Methods & Sampling

Mixed-layer water (25 meters (m) depth) was sampled at Station ALOHA using 12-liter (L) Niskin bottles on the HOT CTD rosette. To prepare $15N_2$-enriched seawater for incubations, water was filtered first through nitex mesh (250-micrometers (µm)) and then through 0.2 µm-pore filters (Millipak) into pre-cleaned 2L pyrex glass

bottles with gas-port caps (Chemglass). Water was then degassed under vacuum with vigorous stirring (Velp MST magnetic stir plates) for at least 30 minutes. The evacuated headspace was then filled with a slight overpressure of $15N_2$ gas (>98 atom% 15N; Cambridge Isotope), which was then allowed to equilibrate with continued vigorous stirring for at least 60 minutes. Incubations were initiated by mixing 2L of mesh-prefiltered mixed-later seawater (sampled from a hydrocast subsequent to that used for the $15N_2$-enriched water) in a 1:1 ratio with the 0.2µm-prefiltered $15N_2$-enriched water in 4L polycarbonate bottles. Parallel $14N_2$-incubations were prepared using 0.2µm-prefiltered, degassed seawater equilibrated with N_2 gas of natural isotopic abundance (0.37 atom% 15N). Bottles were then incubated in on-deck incubators that were temperature controlled with surface seawater from the ship's flow-through system and shaded to mimic the light field at 25m depth.

Samples for proteomic analysis were collected by filtering the contents of the 4L incubation bottles onto 0.2µm-pore PES membrane filters (MilliporeExpress) under gentle suction. Filters were frozen at -80 degrees Celsius (ºC) shipboard immediately upon collection and transported to UChicago in a LN_2-cooled dry shipper for analysis. Proteins were extracted from filters by agitation in a Beadbeater (40 seconds), sonication (QSonica Q500, 10 minutes in 10-second on/off pulses, 85% amplitude), and heating (95ºC, 25 minutes), all in a reducing and denaturing buffer (1% LDS, 200 millimolar (mM) Tris pH 8.0, 10 mM DTT) followed by alkylation of cysteine thiols with 40 mM iodoacetamide. Proteins were then precipitated in 4 volumes of acetone in glass centrifuge tubes overnight at -20ºC and pelleted by centrifugation for 60 minutes at 7000 x g. Protein pellets were dried, redissolved in denaturing buffer (8M urea, 0.2% deoxycholate, 1M ammonium bicarbonate pH 8), and purified using a modified eFASP (enhanced Filter-Aided Sample Preparation) protocol (Erde, et al. 2014) in passivated Sartorius Vivacon 500 concentrators (30 kDa nominal cutoff). Purified proteins were digested on-filter with MS-grade trypsin (37ºC, overnight, 2 micrograms (µg)). Peptides were eluted from the concentrators and dried by vacuum centrifugation.

For LC-MS analysis, peptide samples were reconstituted in 2% acetonitrile + 0.1% formic acid. All samples were separated on a monolithic capillary C18 column (GL Sciences Monocap Ultra, 100µm ID x 200-centimeter (cm) length) using a water-acetonitrile + 0.1% formic acid gradient (2-50% AcN over 180 minutes) at 360 nanoliters per minute (nL/min) using a Dionex Ultimate 3000 LC system with nanoelectrospray ionization (Proxeon Nanospray Flex source). Mass spectra were collected on an Orbitrap Elite mass spectrometer (Thermo Fisher Scientific) operating in a data-dependent acquisition mode, with one high-resolution (120,000 m/Δm) MS1 parent ion full scan triggering 15 Rapid mode MS2 CID fragment ion scans of selected precursors.

Data Processing Description

Peptide sequences were identified from mass spectral data using Sequest HT implemented in Proteome Discoverer v2.2 (Thermo Scientific). diDO-IPTL quantitation (from $14N_2$ incubations) was performed using MorpheusFromAnotherPlace (MFAP; Waldbauer et al., 2017; https://github.com/waldbauerlab). Precursor and product ion mass tolerances for MFAP searches were set to 20ppm and 0.6Da, respectively. Static cysteine carbamidomethylation and variable methionine oxidation, N-terminal (d4)-dimethylation, and C-terminal $18O_2$ were included as modifications. False discovery rate for peptide-spectrum matches was controlled by target-decoy searching to <1%. Protein-level relative abundances and standard errors were calculated in R using the Arm postprocessing scripts for diDO-IPTL data (https://github.com/waldbauerlab). Peptide atom %15N values (from $14N_2$ incubations) were determined using the cPIE (classified peptide isotope enrichment) pipeline (Zimmerman, et al. 2023; https://github.com/waldbauerlab).

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Related Publications

Erde, J., Loo, R. R. O., & Loo, J. A. (2014). Enhanced FASP (eFASP) to Increase Proteome Coverage and Sample Recovery for Quantitative Proteomic Experiments. Journal of Proteome Research, 13(4), 1885–1895. https://doi.org/10.1021/pr4010019
Methods

Waldbauer, J., Zhang, L., Rizzo, A., & Muratore, D. (2017). diDO-IPTL: A Peptide-Labeling Strategy for Precision Quantitative Proteomics. Analytical Chemistry, 89(21), 11498–11504. https://doi.org/10.1021/acs.analchem.7b02752
Methods

Zimmerman, A. E., Podowski, J. C., Gallagher, G. E., Coleman, M. L., & Waldbauer, J. R. (2023). Tracking nitrogen allocation to proteome biosynthesis in a marine microbial community. Nature Microbiology, 8(3), 498–509. https://doi.org/10.1038/s41564-022-01303-9
Methods

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Related Datasets

Results

Waldbauer, J. (2024). MassIVE MSV000096410 - HOT335 15N2-tracking Metaproteomics [Data set]. MassIVE. https://doi.org/10.25345/C5C24R05N

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Parameters

Parameters for this dataset have not yet been identified

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Instruments

Dataset-specific Instrument Name	Eppendorf 5430, 5424
Generic Instrument Name	Centrifuge
Generic Instrument Description	A machine with a rapidly rotating container that applies centrifugal force to its contents, typically to separate fluids of different densities (e.g., cream from milk) or liquids from solids.

Dataset-specific Instrument Name	Sartorius Vivacon 500 concentrators
Generic Instrument Name	Concentrator Device
Generic Instrument Description	A concentrator is a device designed to increase the weight per unit volume of a substance. This category includes vacuum centrifuge concentrator, which include a vacuum chamber within which a centrifuge rotor is mounted for spinning a plurality of vials containing a solution at high speed while subjecting the solution to a vacuum condition for concentration and evaporation. Alternative names: sample concentrator; speed vacuum; speed vac.

Dataset-specific Instrument Name	Dionex Ultimate 3000 LC system
Generic Instrument Name	High-Performance Liquid Chromatograph
Dataset-specific Description	Dionex UltiMate 3000 RSLCnano w/ GL Sciences Monocap Ultra monolithic capillary C18 column with nanoelectrospray ionization (Proxeon Nanospray Flex source)
Generic Instrument Description	A High-performance liquid chromatograph (HPLC) is a type of liquid chromatography used to separate compounds that are dissolved in solution. HPLC instruments consist of a reservoir of the mobile phase, a pump, an injector, a separation column, and a detector. Compounds are separated by high pressure pumping of the sample mixture onto a column packed with microspheres coated with the stationary phase. The different components in the mixture pass through the column at different rates due to differences in their partitioning behavior between the mobile liquid phase and the stationary phase.

Dataset-specific Instrument Name	Orbitrap Elite mass spectrometer (Thermo Fisher Scientific)
Generic Instrument Name	Mass Spectrometer
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	12L Niskin bottles
Generic Instrument Name	Niskin bottle
Generic Instrument Description	A Niskin bottle (a next generation water sampler based on the Nansen bottle) is a cylindrical, non-metallic water collection device with stoppers at both ends. The bottles can be attached individually on a hydrowire or deployed in 12, 24, or 36 bottle Rosette systems mounted on a frame and combined with a CTD. Niskin bottles are used to collect discrete water samples for a range of measurements including pigments, nutrients, plankton, etc.

Dataset-specific Instrument Name	QSonica Q500
Generic Instrument Name	ultrasonic cell disrupter (sonicator)
Generic Instrument Description	Instrument that applies sound energy to agitate particles in a sample.

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Deployments

KM2204

Website	https://www.bco-dmo.org/deployment/958555
Platform	R/V Kilo Moana
Start Date	2022-03-25
End Date	2022-03-30
Description	See more information at R2R: https://www.rvdata.us/search/cruise/KM2204

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Project Information

EAGER: Tracking marine diazotrophy with isotope-labeling proteomics (15N2Fix Proteomics)

Coverage: Station ALOHA

Throughout much of the global ocean, nitrogen fixation – the conversion of abundant atmospheric nitrogen gas into chemical forms usable by life – is a crucial source of nutrients for biological activity. In particular, life needs nitrogen to make proteins, the essential biochemical machines that power all cells. Nitrogen fixation in the oceans is carried out by a variety of microorganisms, whose surprising diversity and ecology oceanographers are working to uncover. This project uses a novel mass spectrometry technique to directly track the flow of nitrogen from nitrogen gas, via nitrogen fixation, into proteins. These measurements reveal which nitrogen-fixing organisms are active in a given part of the ocean, what proteins they make with the nitrogen they fix, and how that nitrogen ultimately flows to feed the entire marine biological community. This information will better inform models and predictions of how marine microbial ecosystems, and the essential biogeochemistry they perform, responds to changing conditions in the ocean. This project engages an undergraduate student researcher, recruited from groups underrepresented in science, in both the field and laboratory work.

Diazotrophy (biological N2 fixation) is the largest input of fixed nitrogen to the ocean and is carried out by a wide diversity of marine microbes, but we have limited ability to quantify the N2-fixation activity of the full diversity of marine diazotrophs, or to resolve how the N they fix ultimately flows to supply the broader microbial community. Since the primary biosynthetic fate of fixed N2 is protein production, proteomics is poised to make substantial contributions to our understanding of the marine nitrogen cycle, and to the molecular physiology and ecological roles of diazotrophs in particular. This project brings a novel 15N-tracking proteomics methodology to bear on three outstanding questions in marine diazotroph ecology:

1) How is whole-community N2 fixation activity apportioned among different diazotroph taxa?
2) How do diazotrophs and their symbiotic partners make biosynthetic use of the N they fix?
3) How much diazotroph-derived N is redistributed to particular non-diazotroph taxa?

The investigators are conducting 15N2-tracking proteomics experiments at the Hawaii Ocean Time-series (HOT) Station ALOHA, assaying N incorporation from 15N-labeled dinitrogen into proteins of both diazotrophs and the broader microbial community. This is the first use of proteomics to track diazotrophic 15N2 incorporation, representing a novel, molecular-level approach for investigating marine nitrogen fixation that encompasses the entire microbial community with high taxonomic resolution. 15N-tracking proteomics data provides unique insight into the flow of nitrogen currency through its key biological accounts in cellular proteomes, and into the ecophysiology and biogeochemical roles of marine diazotrophs.

This award reflects NSF's statutory mission and has been deemed worthy of support through evaluation using the Foundation's intellectual merit and broader impacts review criteria.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-2050685

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