Dissolved inorganic carbon (DIC) fixation and dissolved organic carbon (DOC) release measurements of marine nitrifier cultures grown under different culture conditions

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Data Type: experimental
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Project
» Collaborative Research: Underexplored Connections between Nitrogen and Trace Metal Cycling in Oxygen Minimum Zones Mediated by Metalloenzyme Inventories (CliOMZ)

Contributors	Affiliation	Role
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Abstract
This dataset contains dissolved inorganic carbon (DIC) fixation and dissolved organic carbon (DOC) release measurements of marine nitrifier cultures grown under different culture conditions. These data were generated through experiments conducted at the University of California, Santa Barbara over a period of time from December 2020 to August 2021.

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Coverage

Spatial Extent: **Lat**:34.413962 **Lon**:-119.84912
Temporal Extent: 2020-12-14 - 2021-08-13

Methods & Sampling

DIC fixation was measured via the incorporation of [14C]-bicarbonate as previously described (Herndl et al. 2005) with modifications. [14C]-bicarbonate (specific activity 56 mCi mmol-1/2.072 x 109 Bq mmol-1, Perkin Elmer) was added to 5 mL of culture (between 6 and 65 µCi were added depending on the activity of the culture). For every culture condition, at least three replicate live samples and one formaldehyde-fixed blank (3% v/v) were incubated in temperature-controlled incubators in the dark. Parallel incubations without [14C]-tracer additions were used to determine cell abundance and nitrite concentration. Incubations were terminated by adding formaldehyde (3% v/v) to 5 mL of sample. After 30-60 min, every sample was individually filtered onto 25 mm, 0.2 µm pore size polycarbonate filters (Millipore) and rinsed with 0.5 mL of artificial seawater using a glass filtration set (Millipore). The individual filtrates (5.5 mL per sample) were collected and transferred to scintillation vials to determine the fraction of [14C]-dissolved organic carbon ([14C]-DOC). Excess [14C]-bicarbonate from the filters was removed by exposing them to fumes of concentrated HCl (37 %) for 24 h. The filters were transferred to scintillation vials and 10 mL of scintillation cocktail (Ultima Gold, Perkin Elmer)

was added. The filtrates were acidified to pH ~2 with HCl (25 %) as previously described (Marañón et al. 2004), and filtrates were kept for 24 h in open scintillation vials placed on an orbital shaker before 10 mL scintillation cocktail was added to each vial. Samples were shaken for ca. 30 sec and incubated in the dark for at least 24 hours prior to counting the disintegrations per minute (DPM) in a scintillation counter (Beckman Coulter LS6500) for 15 min. Total radioactivity measurements were performed to verify added [14C]-bicarbonate concentrations by pipetting 100µl of sample into scintillation vials containing 400µl beta-phenylethylamine (to prevent outgassing of 14CO2). Scintillation cocktail was added, vials were shaken for ca. 30 sec and immediately measured in the scintillation counter.

A detailed description of materials and methods can be found in Bayer et al. 2022.

Data Processing Description

Data Processing:
The mean DPM of the samples were corrected for the DPM of the blank, converted into organic carbon fixed over time and corrected for the DIC concentration in the culture media. A detailed description of data processing can be found in Bayer et al. 2022.

BCO-DMO Processing:
- renamed fields to conform with BCO-DMO naming conventions;
- replaced "NA" with "nd" (no data).

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Data Files

File	
nitrifier_c_fixation_release.csv	(Comma Separated Values (.csv), 28.55 KB) MD5:fa7f4a2ca203d1185638b6ec4b9e1223
Primary data file for dataset ID 870832	

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Related Publications

Bayer, B., McBeain, K., Carlson, C. A., & Santoro, A. E. (2022). Carbon content, carbon fixation yield and dissolved organic carbon release from diverse marine nitrifiers. https://doi.org/10.1101/2022.01.04.474793
Results

Herndl, G. J., Reinthaler, T., Teira, E., van Aken, H., Veth, C., Pernthaler, A., & Pernthaler, J. (2005). Contribution of Archaea to Total Prokaryotic Production in the Deep Atlantic Ocean. Applied and Environmental Microbiology, 71(5), 2303–2309. https://doi.org/10.1128/aem.71.5.2303-2309.2005
Methods

Marañón, E., Cermeño, P., Fernández, E., Rodríguez, J., & Zabala, L. (2004). Significance and mechanisms of photosynthetic production of dissolved organic carbon in a coastal eutrophic ecosystem. Limnology and Oceanography, 49(5), 1652–1666. Portico. https://doi.org/10.4319/lo.2004.49.5.1652
Methods

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Parameters

Parameter	Description	Units

Nitrifier_strain	Name of cultured nitrifier	unitless
Substrate_conc	Concentration of substrate (NH4+ or NO2-)	micromoles per liter (umol/L)
Temp	Incubation temperature	degrees Celsius
Culture_medium	Type of culture medium (ASW, artificial seawater; NSW, natural seawater; ASW_HEPES, artificial seawater buffered with HEPES)	unitless
Treatment	Additional amendments to culture medium (none; NH4+ addition; tryptone addition)	unitless
Growth_phase	Growth phase of the culture when the measurement was taken (EEXP, early exponential growth; LEXP, late exponential growth; STAT, stationary phase)	unitless
DPM_POC_Blank_corrected	Activity measured on filters (particulate fraction) following incubation with 14C-bicarbonate. Blank value was subtracted	disintegrations per minute (DPM)
DPM_DOC_Blank_corrected	Activity measured in filtrates (dissolved fraction) following incubation with 14C-bicarbonate. Blank value was subtracted	disintegrations per minute (DPM)
DOC_release_percent	Released DOC as a fraction of fixed DIC	percent (%)
Incubation_time	Length of incubation with 14C-bicarbonate	hours (h)
Activity_tracer	Activity of added 14C-bicarbonate	microcurie (uCi)
DPM_tracer	Activity of added 14C-bicarbonate	disintegrations per minute (DPM)
DIC_conc	Concentration of dissolved inorganic carbon	micromoles per liter (umol/L)
Fixed_DIC	Total fixed DIC (excluding the fraction released as DOC)	micromoles per liter (umol/L)
Fixed_DIC_DOC	Total fixed DIC (including the fraction released as DOC)	micromoles per liter (umol/L)
Cell_abundance	Cell numbers of cultured nitrifiers	cells per liter (cells/L)

DIC_fixation_rate	Cell-normalized DIC fixation rate	femtomoles per cell per day (fmol/cell/d)
Produced_cells	Number of cells newly produced during the incubation period	cells per liter (cells/L)
C_content	Cellular carbon content	femtogram per cell (fg/cell)
N_oxidized	Amount of NH4+ or NO2- oxidized by cultured nitrifiers during the incubation period	micromoles per liter (umol/L)
DIC_fixation_yield	Moles of C fixed per mole of N oxidized	moles of carbon per mole of nitrogen (mol C/mol N)
DOC_corrected_DIC_fixation_yield	Moles of C fixed per mole of N oxidized including the fraction of C released as DOC	moles of carbon per mole of nitrogen (mol C/mol N)

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Instruments

Dataset-specific Instrument Name	Beckman Coulter LS6500 Scintillation counter
Generic Instrument Name	Liquid Scintillation Counter
Generic Instrument Description	Liquid scintillation counting is an analytical technique which is defined by the incorporation of the radiolabeled analyte into uniform distribution with a liquid chemical medium capable of converting the kinetic energy of nuclear emissions into light energy. Although the liquid scintillation counter is a sophisticated laboratory counting system used the quantify the activity of particulate emitting (ß and a) radioactive samples, it can also detect the auger electrons emitted from 51Cr and 125I samples.

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Project Information

Collaborative Research: Underexplored Connections between Nitrogen and Trace Metal Cycling in Oxygen Minimum Zones Mediated by Metalloenzyme Inventories (CliOMZ)

Coverage: Eastern Tropical Pacific

NSF abstract:
Though scarce and largely insoluble, trace metals are key components of sophisticated enzymes (protein molecules that speed up biochemical reactions) involved in biogeochemical cycles in the dark ocean (below 1000m). For example, metalloenzymes are involved in nearly every reaction in the nitrogen cycle. Yet, despite

direct connections between trace metal and nitrogen cycles, the relationship between trace metal distributions and biological nitrogen cycling processes in the dark ocean have rarely been explored, likely due to the technical challenges associated with their study. Availability of the autonomous underwater vehicle (AUV) Clio, a sampling platform capable of collecting high-resolution vertical profile samples for biochemical and microbial measurements by large volume filtration of microbial particulate material, has overcome this challenge. Thus, this research project plans an interdisciplinary chemistry, biology, and engineering effort to test the hypothesis that certain chemical reactions, such as nitrite oxidation, could become limited by metal availability within the upper mesopelagic and that trace metal demands for nitrite-oxidizing bacteria may be increased under low oxygen conditions. Broader impacts of this study include the continued development and application of the Clio Biogeochemical AUV as a community resource by developing and testing its high-resolution and adaptive sampling capabilities. In addition, metaproteomic data will be deposited into the recently launched Ocean Protein Portal to allow oceanographers and the metals in biology community to examine the distribution of proteins and metalloenzymes in the ocean. Undergraduate students will be supported by this project at all three institutions, with an effort to recruit minority students. The proposed research will also be synergistic with the goals of early community-building efforts for a potential global scale microbial biogeochemistry program modeled after the success of the GEOTRACES program, provisionally called "Biogeoscapes: Ocean metabolism and nutrient cycles on a changing planet".

The proposed research project will test the following three hypotheses: (1) the microbial metalloenzyme distribution of the mesopelagic is spatially dynamic in response to environmental gradients in oxygen and trace metals, (2) nitrite oxidation in the Eastern Tropical Pacific Ocean can be limited by iron availability in the upper mesopelagic through an inability to complete biosynthesis of the microbial protein nitrite oxidoreductase, and (3) nitrite-oxidizing bacteria increase their metalloenzyme requirements at low oxygen, impacting the distribution of both dissolved and particulate metals within oxygen minimum zones. One of the challenges to characterizing the biogeochemistry of the mesopelagic ocean is an inability to effectively sample it. As a sampling platform, we will use the novel biogeochemical AUV Clio that enables high-resolution vertical profile samples for biochemical and microbial measurements by large volume filtration of microbial particulate material on a research expedition in the Eastern Tropical Pacific Ocean. Specific research activities will be orchestrated to test the hypotheses. Hypothesis 1 will be explored by comparison of hydrographic, microbial distributions, dissolved and particulate metal data, and metaproteomic results with profile samples collected by Clio. Hypothesis 2 will be tested by incubation experiments using 15NO2- oxidation rates on Clio-collected incubation samples. Hypothesis 3 will be tested by dividing targeted nitrite oxidoreductase protein copies by qPCR (quantitative polymerase chain reaction)-based nitrite oxidizing bacteria abundance (NOB) to determine if cellular copy number varies with oxygen distributions, and by metalloproteomic analyses of NOB cultures. The demonstration of trace metal limitation of remineralization processes, not just primary production, would transform our understanding of the role of metals in biogeochemical cycling and provide new ways with which to interpret sectional data of dissolved and particulate trace metal distributions in the ocean. The idea that oxygen may play a previously underappreciated role in controlling trace metals due not just to metals' physical chemistry, but also from changing biological demand, will improve our ability to predict trace metal distributions in the face of decreasing ocean oxygen content.

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-1924512

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