Incomplete assimilatory nitrate reduction by LLI Prochlorococcus in response to nutrient stress.

Website: https://www.bco-dmo.org/dataset/969427
Data Type: experimental
Version: 1
Version Date: 2025-08-06

Project
» Features and implications of nitrogen assimilation trait variability in populations of Prochlorococcus (Prochlorococcus N assimilation)

Contributors	Affiliation	Role
Berube, Paul	Massachusetts Institute of Technology (MIT)	Principal Investigator, Contact
Chisholm, Sallie W.	Massachusetts Institute of Technology (MIT)	Co-Principal Investigator
LeMaster, Trent	Massachusetts Institute of Technology (MIT)	Technician
Soenen, Karen	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
These data include Prochlorococcus cell density, nitrate (NO3-) and nitrite (NO2-) concentration, and fluorometry data for experiments that examined nitrite production by LLI Prochlorococcus following a pulse of nitrate under stress conditions. The conditions evaluated included nitrogen limitation.

Table of Contents

Coverage

Location: Laboratory cultures of picocyanobacteria
Temporal Extent: 2023-12-14 - 2024-05-24

Dataset Description

This dataset is part of a larger study on the impact of different physiological states/responses of Prochlorococcus on nitrite production potential in the presence of nitrate. The different perturbations yielding the different physiological states/responses and the respective analyses are listed below. Links to data for each perturbation can be found in the Related Datasets section.

The analyses include:

1. Cell concentration (Nitrogen limitation dataset, Light perturbation dataset, Temperature perturbation dataset)
2. Nitrite concentration (Nitrogen limitation dataset, Light perturbation dataset, Temperature perturbation dataset)

3. Nitrate + nitrite concentration (Nitrogen limitation dataset)
4. Bead standard normalized forward scatter (Nitrogen limitation dataset)
5. Bead standard normalized red (chlorophyll) fluorescence (Nitrogen limitation dataset)
6. FIRe fluorometry (Light perturbation dataset, Temperature perturbation dataset)

See Related Datasets section below for links to above mentioned datasets.

Methods & Sampling

Cultures: The strains used in this study included *Prochlorococcus* MIT0915 and *Prochlorococcus* MIT0917. Before each experiment, all strains were assayed for heterotrophic contaminants by staining cells with SYBR green and assessing the fluorescence and light scattering properties of both stained and unstained cells using a Guava easyCyte 12HT Flow Cytometer (MilliporeSigma, Burlington, MA, USA) – cultures that did not exhibit the presence of non-photosynthetic cells in the stained samples and had a single cyanobacteria population were presumed axenic and unialgal. Before each experiment, all presumed axenic cultures were assessed for purity by confirming a lack of turbidity after inoculation into a panel of purity test broths as described previously (Berube et al., 2015).

Culture medium: The cultures were grown using AMP1-Mo-NO3 medium which is a variant of AMP1 artificial medium (Moore et al., 2007) with the following modifications: 400 µM ammonium sulfate was replaced with 800 µM sodium nitrate; the sodium molybdate concentration was increased from 0.3 nM to 100 nM; 1 mM HEPES pH=7.5 was replaced with 5 mM TAPS pH=8.2; and the concentration of sodium bicarbonate was decreased from 6 mM to 2.5 mM. The medium was filter sterilized using a 0.2 µm polyethersulfone (PES) membrane filter instead of using steam sterilization in an autoclave. A nitrogen-free variant (AMP1-Mo) was also prepared by omitting all inorganic nitrogen sources. For chemostat experiments, the N and P concentrations in the AMP1-Mo-NO3 were adjusted to a 5:1 ratio at final concentrations of 40 µM sodium nitrate and 8 µM sodium phosphate.

Methodology and sampling for nitrogen limitation perturbations: Continuous cultures were grown in duplicate chemostats with nitrate as the limiting nutrient in AMP1-Mo-NO3 artificial medium adjusted to have a 5:1 nitrogen to phosphorus ratio. Chemostat cultures were grown at 24oC and 26 µmol photons m-2 s-1 of blue light. The volume of the chemostats was controlled through positive pressure coupled with an overflow tube placed at an empirically determined level to maintain a volume of 200 mL. Mixing, positive pressure, and aeration was provided by bubbling humidified air through a 0.2 µm PTFE venting filter. Cell concentrations were monitored three times per week using flow cytometry. The response of nitrate-limited cells to a pulse of nitrate was assessed at an imposed growth rate of 0.5 d-1. Four mL of 40 mM sodium nitrate was aseptically added to each chemostat to yield a final concentration of 800 µM (the same as standard AMP1-Mo-NO3 medium) which was predicted to decline to 50 µM over the course of 4 days at a dilution rte of 0.5 d-1. Samples for nitrite analysis were collected daily following the nitrate pulse by pumping culture into an acid-washed vessel, centrifuged in 35 mL acid-washed oakridge tubes at 12,500 RPM in a JA-25.50 rotor for 25 min to pellet biomass; the supernatant was stored at -20oC for no longer than 30 days before nitrite and/or nitrate analysis. Samples for nitrite and nitrate + nitrite analysis were additionally collected before the nitrate pulse to confirm undetectable levels of residual nitrate in stead state cultures.

Data Processing Description

Nitrate and nitrate + nitrite concentration assay: Nitrite was analyzed using an AA3 HR Autoanalyzer (Seal Analytical, Milwaukee, WI, USA). In this continuous segmented flow analyzer, nitrite is reacted with sulfanilamide and N-(1-naphthyl)ethylenediamine (NED) to produce a pink diazo dye. For determination of total nitrate and nitrite, any nitrate in the sample was first reduced to nitrite using a copper-cadmium coil. Absorbance was measured in a 1 cm flow cell using a 550 nm bandpass filter (SEAL Analytical method number G-384-08 for a multitest MT19B manifold). For each analysis, sodium nitrite and sodium nitrate standards were freshly prepared and serially diluted in nitrogen-free AMP1 medium to create a calibration curve at the following concentrations: 1.25, 0.5, 0.25, 0.08, 0.04, 0.03, and 0.015 µM. A linear calibration curve was applied to the data and the instrument's response factor, represented by the slope of the calibration curve, remained consistent and reproducible throughout the nitrite analysis period (r2 >0.99 in all runs). For samples in nitrogen-free AMP1 medium (AMP1-Mo) as a background matrix, the limit of detection (LOD) for nitrite was 0.010 µM and the limit of blank was 0.003 µM.

Cell concentration determination: Cell concentrations were obtained by flow cytometry using a Guava easyCyte 12HT Flow Cytometer (MilliporeSigma, Burlington, MA, USA). *Prochlorococcus* cells were detected based on the fluorescence of cellular pigments excited by a 488 nm laser. Cultures were first diluted to between 50 and 200 cells/µL and data were acquired for up to 6 min at a flow rate of 0.024 µL/s. Bead standards (Guava easyCheck beads; MilliporeSigma, Burlington, MA, USA), were run daily to confirm that the instrument was operating within normal parameters and within predefined tolerances for concentrations, scatter, and emission intensity of the beads. For each flow cytometry run, a diluted sample of Guava easyCheck beads was additionally run using the same parameters as the cultures to serve as a standard for the normalization of forward scatter and fluorescence data.

Fluorescence data were processed using fireworx version 0.9.1 (https://sourceforge.net/projects/fireworx/)

Continuous segmented flow nutrient autoanalyzer data were analyzed using AACE version 7.12 software (Seal Analytical, Mequon, WI, USA)

[table of contents | back to top]

Data Files

File
969427_v1_chemostat.csv (Comma Separated Values (.csv), 72.21 KB) MD5:3d114401b2a42101bb2bc6a1e065c4c9 Primary data file for dataset ID 969427, version 1

[table of contents | back to top]

Supplemental Files

File
flow_cytometry_chemostat_cultures.tar.gz (GZIP (.gz), 104.80 MB) MD5:6d6febb927c79d3d3de349da7ecf9ae4 This file contains archived and compressed raw flow cytometry data files (FCS3 format) for the chemostat cultures experiment. File names match those provided in the primary data file.

[table of contents | back to top]

Related Publications

Berube, P. M., Biller, S. J., Kent, A. G., Berta-Thompson, J. W., Roggensack, S. E., Roache-Johnson, K. H., Ackerman, M., Moore, L. R., Meisel, J. D., Sher, D., Thompson, L. R., Campbell, L., Martiny, A. C., & Chisholm, S. W. (2014). Physiology and evolution of nitrate acquisition in Prochlorococcus. The ISME Journal, 9(5), 1195–1207. https://doi.org/10.1038/ismej.2014.211
Methods

Berube, P. M., LeMaster, T., & Chisholm, S. W. (2025). Priming the pump: Enhanced nitrite release in response to a nitrate pulse by nitrogen-limited Prochlorococcus. https://doi.org/10.1101/2025.05.13.653865
Results

Moore, L. R., Coe, A., Zinser, E. R., Saito, M. A., Sullivan, M. B., Lindell, D., Frois-Moniz, K., Waterbury, J., & Chisholm, S. W. (2007). Culturing the marine cyanobacterium Prochlorococcus. Limnology and Oceanography: Methods, 5(10), 353–362. Portico. https://doi.org/10.4319/lom.2007.5.353
Methods

[table of contents | back to top]

Related Datasets

IsRelatedTo

Berube, P., Chisholm, S. W. (2025) **Incomplete assimilatory nitrate reduction by LLI Prochlorococcus in response to light and temperature stress.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2025-07-31 doi:10.26008/1912/bco-dmo.969513.1 [view at BCO-DMO]
Relationship Description: Light and temperature shock dataset as part of same experiment.

Massachusetts Institute of Technology. Continuous cultures of nitrogen-limited Prochlorococcus. 2025/05. In: BioProject [Internet]. Bethesda, MD: National Library of Medicine (US), National Center for Biotechnology Information; 2011-. Available from: http://www.ncbi.nlm.nih.gov/bioproject/PRJNA1262978. NCBI:BioProject: PRJNA1262978

[table of contents | back to top]

Parameters

Parameter	Description	Units
Strain	Strain name of pure culture used	unitless
Rep	Biological Replicate (A/B/C)	unitless
Collection_Time	Date and time of sample collection in ISO 8601 format	unitless
DeltaDays	Time since cultures were started in days	days
DilutionFactor_FCM	Dilution factor flow cytometry analysis	unitless
Cell_Concentration_FCM	Concentration of total cyanobacteria cells as determined by flow cytometry	cells per milliliter (cells mL-1)
FSC_Beads	Forward scatter values relative to beads as determined by flow cytometry	unitless
RedB_Beads	Red fluorescence values relative to beads as determined by flow cytometry	unitless
AutoAnalyzer3_dilutionFactor	Dilution factor for nitrite and/or nitrate analysis	unitless
Nitrate_NitriteConcentration	Extracellular concentration of dissolved nitrate+nitrite; BDL = below the detection limit of 0.010 micromole per liter as determined for nitrite	micromoles Nitrate+Nitrite per liter (umol L-1)
NitriteConcentration	Extracellular concentration of dissolved nitrite; BDL = below nitrite detection limit of 0.010 micromole per liter (umol/L)	micromoles Nitrite per liter (umol L-1)

FCM_filename	Name of the flow cytometry file corresponding to the sample	unitless
fcm_wellID	Well ID for the corresponding sample	unitless
FCM_Beads_Filename	Name of flow cytometry file corresponding to the beads normalization well for the respective flow cytometry run	unitless
FCM_blank_filename	Name of flow cytometry file corresponding to the blank well for the respective flow cytometry run	unitless
NCBI_SRA_Accession	Accession number for DNA sequencing data in NCBI's Short Read Archive under NCBI BioProject PRJNA1262978	unitless
GeneralNotes	General notes made regarding sample issues and imposed perturbations	unitless

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	Guava easyCyte 12HT Flow Cytometer - MilliporeSigma, Burlington, MA, USA
Generic Instrument Name	Flow Cytometer
Generic Instrument Description	Flow cytometers (FC or FCM) are automated instruments that quantitate properties of single cells, one cell at a time. They can measure cell size, cell granularity, the amounts of cell components such as total DNA, newly synthesized DNA, gene expression as the amount messenger RNA for a particular gene, amounts of specific surface receptors, amounts of intracellular proteins, or transient signalling events in living cells. (from: http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm)

Dataset-specific Instrument Name	AA3 HR AutoAnalyzer - Seal Analytical Inc, Mequon, WI, USA
Generic Instrument Name	Nutrient Autoanalyzer
Dataset-specific Description	Nutrient samples were taken according to best practices and were filtered using a 0.2 µm filter and immediately frozen until analysis. Samples were analyzed using methods provided by seal No. G-384-08 Rev 6 (multitest MT19B) which are based off Environmental Protection Agency standard methods for nitrate+nitrite, and nitrite. AA3 HR AutoAnalyzer is a fully automated Segmented Flow Analysis (SFA) system, ideal for water and seawater analysis. It comprises a modular system which integrates an autosampler, peristaltic pump, chemistry manifold and detector. The sample and reagents are pumped continuously through the chemistry manifold, and air bubbles are introduced at regular intervals forming reaction segments which are mixed using glass coils. The AA3 uses segmented flow analysis principles to reduce inter-sample dispersion, and can analyse up to 100 samples per hour using stable LED light sources. Please see http://seal-analytical.com/Products/AA3SFAAnalyzer/tabid/59/Default.aspx for a complete instrument description
Generic Instrument Description	Nutrient Autoanalyzer is a generic term used when specific type, make and model were not specified. In general, a Nutrient Autoanalyzer is an automated flow-thru system for doing nutrient analysis (nitrate, ammonium, orthophosphate, and silicate) on seawater samples.

[table of contents | back to top]

Project Information

Features and implications of nitrogen assimilation trait variability in populations of Prochlorococcus (Prochlorococcus N assimilation)

Coverage: Laboratory studies (MIT)

NSF Award Abstract:
The marine bacterium, *Prochlorococcus*, is a central part of the food web in the subtropical open ocean, one of the largest biomes on the planet. Like plants on land, *Prochlorococcus* and other phytoplankton are capable of photosynthesis, harnessing light to convert carbon dioxide into sugars and other organic matter. This matter feeds all the life in the sea. Akin to terrestrial plants, *Prochlorococcus* requires additional nutrients or "fertilizer" to grow and photosynthesize. Among these nutrients, nitrogen is often scarce across much of the sunlit ocean. Thus, understanding the means through which nitrogen is obtained and used by *Prochlorococcus* has important consequences for understanding how the nitrogen and carbon cycles are coupled in the ocean. Not all *Prochlorococcus* cells have the genetic capacity to use all sources of inorganic nitrogen, i.e. nitrate, nitrite, and ammonium. Some can use all three, some the last two, and some only ammonium. Cells that can use nitrate must sequentially transform it to nitrite and then to ammonium before they can make the building blocks for proteins. The genesis of this project derives from the observation that some *Prochlorococcus* cells release nitrite into the seawater during growth on nitrate. This project examines this feature of the physiology of these cell lines, and asks whether cells that release nitrite can support the growth of other cells than cannot use nitrate, in effect creating a cross-feeding situation that could make the system more robust. Understanding the drivers behind the coexistence of cells with different ways of obtaining nitrogen, a key currency in the ocean, will provide important insights on the flow of nitrogen in marine ecosystems. This project also sheds light on the structure of interactions between microbes and provides the broader scientific community (for instance, those studying diverse microbiomes related to human health and disease or agriculture) a new perspective on how microbes form beneficial partnerships. This project supports immersive laboratory-based research experiences for undergraduate students, who design and execute experiments directly related to the overall project goals. The project further supports the work of the investigators to engage with the general public on topics related to phytoplankton, photosynthesis, and the ecosystem services provided by these marine organisms.

In the low-light adapted LLI clade of *Prochlorococcus*, the focus of this project, nearly all cells possess the downstream half of the nitrate assimilation pathway (for the assimilation of nitrite). Only a fraction of LLI cells, however, have the complete nitrate assimilation pathway. Incomplete assimilatory nitrate reduction, with concomitant nitrite release, has been observed for LLI cells during growth on nitrate as the sole nitrogen source. Further, the nitrite released by cells growing on nitrate can support the growth of *Prochlorococcus* that can use nitrite but not the more oxidized nitrate. Overall, within a group of closely-related *Prochlorococcus*, there is genotypic and phenotypic diversity related to the production and consumption of nitrite, a central intermediate in the nitrogen cycle. The investigators propose to further develop *Prochlorococcus*as a model system to explore nitrite cycling within populations and provide new insights on how trait variability and the selection of complementary functions facilitates robustness and/or resiliency in microbial populations. The overarching hypothesis is that the population-level assembly of distinct functional types of *Prochlorococcus* emerges through interactions that are mediated, in part, by cross-feeding of nitrite. To address this broad hypothesis, the investigators are focusing on the following objectives: 1) assessing the physiological underpinnings of incomplete assimilatory nitrate reduction and nitrite release through in-vitro biochemical characterization of nitrite reductase enzymes and transcription profiling of cells subjected to light and temperature stress, 2) examining the nitrite production and consumption rates of *Prochlorococcus* strains across environmental gradients such as light, temperature, and nutrient availability in order to constrain the environmental parameters that modulate nitrite cycling, and 3) determining the frequencies and activities of nitrogen assimilation genotypes within laboratory and field populations, under varying environmental conditions and perturbations. Outcomes from objectives 1 and 2 help to constrain the tradeoffs associated with incomplete nitrate reduction and the release of nitrite (a valuable commodity to nitrogen limited cells) to facilitate modelling and interpretation of how partnerships between nitrogen assimilation genotypes are structured. These insights help to direct experiments in Objective 3, which aims to examine controlled laboratory co-cultures and field populations in order to produce quantitative data on the emergent features of *Prochlorococcus* populations where interactions are mediated by the cross-feeding of nitrite. These data are being used to develop an improved understanding of how interactions mediated by a common public good might give rise to emergent properties of populations, including resilience to perturbation and greater population-wide efficiency in nitrogen assimilation.

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.

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-2048470

[table of contents | back to top]