Incomplete assimilatory nitrate reduction by LLI Prochlorococcus in response to light and temperature stress.

Website: https://www.bco-dmo.org/dataset/969513
Data Type: experimental
Version: 1
Version Date: 2025-07-31

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

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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 light shock, and temperature shock.

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Coverage

Location: Laboratory cultures of picocyanobacteria
Temporal Extent: 2024-11-26 - 2024-12-19

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.

Methodology and sampling for light and temperature perturbations: Batch cultures were grown as triplicates in 35 mL of medium in borosilicate glass culture tubes at a temperature of 24^{o}C and a photon flux of 20 µmol photons m^{-2} s^{-1} of blue light. Cultures were acclimated for at least 10 generations prior to starting the experiment. Each biological replicate was diluted to 1 x 10^{8} cells mL^{1} in a total of 50 mL of nitrogen-free AMP1-Mo supplemented with 2.5 mM sodium bicarbonate in duplicate, one to serve as a control and one to be subjected to the perturbation. Shock experiments were conducted in light- and temperature-controlled water baths. In both experiments, the control box was maintained at 23–25^{o}C and 21–23 µmol photons m^{-2} s^{-1} of blue light. In the light shock experiment, experimental cultures were exposed to 220 µmol photons m^{-2} s^{-1} of broad spectrum light at 23-25^{o}C. In the cold shock experiment, the cultures were exposed to 21–23 µmol photons m^{-2} s^{-1} of blue light at 14–16^{o}C. Prior to initiating the cold shock, nitrogen-free AMP1-Mo medium was pre-chilled to 15^{o}C before inoculation with the harvested biomass. Prior to spiking with nitrate, an initial set of samples were collected for flow cytometry, nitrite concentration determination, and fluorescence induction and relaxation (FIRe) fluorometry. Nitrate was then added to each tube at a final concentration of 80 µM sodium nitrate. Samples for nitrite concentration determination were collected every 30 min for 150 min by syringe filtration of 4 mL of culture through a 0.2 µm PES membrane into a 14 mL polystyrene tube and stored at -20^{o}C. Endpoint flow cytometry and FIRe fluormetry samples were also collect at the final time point. Media-only control tubes were prepared using the same batch of nitrogen-free AMP1-Mo medium to facilitate assessment of background signal in nitrite concentration determination; the mean background signal was equivalent to 0.050 µM nitrite (+/- 0.004) for the light shock experiment and 0.076 µM nitrite (+/- 0.012) for the cold shock experiment.

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 (r^2 >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 induction and relaxation fluorometry: The efficiency for energy conversion at photosystem II (Fv/Fm) for cultures subjected to light or cold shocks was measured using a FIRe Fluorometer System (Satlantic, Halifax, NS, Canada). A sample of each culture was diluted 10 fold in nitrogen-free AMP1 medium, incubated in the dark for 10 minutes in order to allow photosystems to open, and then transferred to a cylindrical quartz cuvette. A total of 10 profiles were collected for each sample. Background profiles for cell-free AMP1-Mo medium at 21 different gain values were collected to facilitate data processing; these files are named in format "AMP1NON.000" where "000" is a 3 digit counter from 000 through 020.

Flow cytometry data were exported as FSC 3.0 formatted files and analyzed using FlowJo version 10.6.1 (BD Biosciences, Ashland, OR, USA)

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)

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Data Files

File
969513_v1_perturbations.csv (Comma Separated Values (.csv), 31.27 KB) MD5:c2a5fe8cd2ee0dec33baabb269448b49 Primary data file for dataset ID 969513, version 1

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Supplemental Files

File

FIRe_fluorometry_lightshock files for lightshock dataset [NEW--USE THIS}

filename: FIRe_fluorometry_lightshock.tar.gz

(GZIP (.gz), 124.44 KB)
MD5:524d59d76e0fb2da082af1572d8a0b59

File 1: FIRe_fluorometry_lightshock.tar.gz
Brief description of File 1: This file contains the raw fluorometry data collected using the Satlantic FIRe (Fluorescence Induction Relaxation) Fluorometer for the light perturbation experiment. Experimental data files use the “NO2_CA” prefix and a background sample (at different gains) for the artificial medium uses the “AMP1NON” prefix. Data can be reprocessed using fireworx using the included background data files.

[pmberube@orcd-login003 CA_CB_FIRe_Data]$ tar -cvzf FIRe_fluorometry_lightshock.tar.gz FIRe_fluorometry_lightshock/
FIRe_fluorometry_lightshock/
FIRe_fluorometry_lightshock/AMP1NON.001
FIRe_fluorometry_lightshock/NO2_CA.003
FIRe_fluorometry_lightshock/NO2_CA.009
FIRe_fluorometry_lightshock/AMP1NON.012
FIRe_fluorometry_lightshock/AMP1NON.018
FIRe_fluorometry_lightshock/NO2_CA.022
FIRe_fluorometry_lightshock/AMP1NON.020
FIRe_fluorometry_lightshock/NO2_CA.010
FIRe_fluorometry_lightshock/NO2_CA.017
FIRe_fluorometry_lightshock/AMP1NON.015
FIRe_fluorometry_lightshock/NO2_CA.004
FIRe_fluorometry_lightshock/AMP1NON.006
FIRe_fluorometry_lightshock/AMP1NON.002
FIRe_fluorometry_lightshock/AMP1NON.008
FIRe_fluorometry_lightshock/NO2_CA.000
FIRe_fluorometry_lightshock/NO2_CA.021
FIRe_fluorometry_lightshock/AMP1NON.011
FIRe_fluorometry_lightshock/NO2_CA.013
FIRe_fluorometry_lightshock/NO2_CA.019
FIRe_fluorometry_lightshock/NO2_CA.014
FIRe_fluorometry_lightshock/AMP1NON.016
FIRe_fluorometry_lightshock/NO2_CA.007
FIRe_fluorometry_lightshock/AMP1NON.005
FIRe_fluorometry_lightshock/NO2_CA.005
FIRe_fluorometry_lightshock/AMP1NON.007
FIRe_fluorometry_lightshock/NO2_CA.016
FIRe_fluorometry_lightshock/AMP1NON.014
FIRe_fluorometry_lightshock/AMP1NON.019
FIRe_fluorometry_lightshock/AMP1NON.013
FIRe_fluorometry_lightshock/NO2_CA.023
FIRe_fluorometry_lightshock/NO2_CA.011
FIRe_fluorometry_lightshock/AMP1NON.000
FIRe_fluorometry_lightshock/NO2_CA.008
FIRe_fluorometry_lightshock/NO2_CA.002
FIRe_fluorometry_lightshock/NO2_CA.006
FIRe_fluorometry_lightshock/AMP1NON.004
FIRe_fluorometry_lightshock/NO2_CA.015
FIRe_fluorometry_lightshock/AMP1NON.017
FIRe_fluorometry_lightshock/NO2_CA.020
FIRe_fluorometry_lightshock/AMP1NON.010
FIRe_fluorometry_lightshock/NO2_CA.018
FIRe_fluorometry_lightshock/NO2_CA.012
FIRe_fluorometry_lightshock/AMP1NON.009
FIRe_fluorometry_lightshock/AMP1NON.003
FIRe_fluorometry_lightshock/NO2_CA.001

File

FIRe_fluorometry_temperatureshock files for tempshock dataset [NEW--USE THIS]

filename: FIRe_fluorometry_temperatureshock.tar.gz (GZIP (.gz), 124.98 KB)
MD5:acae29649c2acd5269e26e5c6b9bcf92

File 2: FIRe_fluorometry_temperatureshock.tar.gz
Brief description of File 2: This file contains the raw fluorometry data collected using the Satlantic FIRe (Fluorescence Induction Relaxation) Fluorometer for the temperature perturbation experiment. Experimental data files use the "NO2_CB" prefix and a background sample (at different gains) for the artificial medium uses the "AMP1NON" prefix. Data can be reprocessed using fireworx using the included background data files.
[pmberube@orcd-login003 CA_CB_FIRe_Data]$ tar -cvzf FIRe_fluorometry_temperatureshock.tar.gz FIRe_fluorometry_temperatureshock/
FIRe_fluorometry_temperatureshock/
FIRe_fluorometry_temperatureshock/NO2_CB.007
FIRe_fluorometry_temperatureshock/AMP1NON.010
FIRe_fluorometry_temperatureshock/NO2_CB.014
FIRe_fluorometry_temperatureshock/AMP1NON.009
FIRe_fluorometry_temperatureshock/AMP1NON.003
FIRe_fluorometry_temperatureshock/NO2_CB.021
FIRe_fluorometry_temperatureshock/AMP1NON.004
FIRe_fluorometry_temperatureshock/NO2_CB.019
FIRe_fluorometry_temperatureshock/NO2_CB.013
FIRe_fluorometry_temperatureshock/AMP1NON.017
FIRe_fluorometry_temperatureshock/NO2_CB.000
FIRe_fluorometry_temperatureshock/AMP1NON.019
FIRe_fluorometry_temperatureshock/AMP1NON.013
FIRe_fluorometry_temperatureshock/NO2_CB.004
FIRe_fluorometry_temperatureshock/AMP1NON.000
FIRe_fluorometry_temperatureshock/NO2_CB.017
FIRe_fluorometry_temperatureshock/NO2_CB.022
FIRe_fluorometry_temperatureshock/NO2_CB.010
FIRe_fluorometry_temperatureshock/AMP1NON.007
FIRe_fluorometry_temperatureshock/NO2_CB.009
FIRe_fluorometry_temperatureshock/NO2_CB.003
FIRe_fluorometry_temperatureshock/AMP1NON.014
FIRe_fluorometry_temperatureshock/AMP1NON.016
FIRe_fluorometry_temperatureshock/NO2_CB.001
FIRe_fluorometry_temperatureshock/NO2_CB.020
FIRe_fluorometry_temperatureshock/AMP1NON.005
FIRe_fluorometry_temperatureshock/NO2_CB.012
FIRe_fluorometry_temperatureshock/NO2_CB.018
FIRe_fluorometry_temperatureshock/NO2_CB.015
FIRe_fluorometry_temperatureshock/AMP1NON.002
FIRe_fluorometry_temperatureshock/AMP1NON.008
FIRe_fluorometry_temperatureshock/NO2_CB.006
FIRe_fluorometry_temperatureshock/AMP1NON.011
FIRe_fluorometry_temperatureshock/NO2_CB.002
FIRe_fluorometry_temperatureshock/NO2_CB.008
FIRe_fluorometry_temperatureshock/AMP1NON.015
FIRe_fluorometry_temperatureshock/NO2_CB.023
FIRe_fluorometry_temperatureshock/NO2_CB.011
FIRe_fluorometry_temperatureshock/AMP1NON.006
FIRe_fluorometry_temperatureshock/AMP1NON.001
FIRe_fluorometry_temperatureshock/NO2_CB.016
FIRe_fluorometry_temperatureshock/AMP1NON.012
FIRe_fluorometry_temperatureshock/AMP1NON.018
FIRe_fluorometry_temperatureshock/NO2_CB.005
FIRe_fluorometry_temperatureshock/AMP1NON.020

flow_cytometry_lightshock.tar.gz

(GZIP (.gz), 9.88 MB)
MD5:182a4940a41a8b3e0d7e243c0fdf10e0

File 3: flow_cytometry_lightshock.tar.gz
Brief description of File 3: This file contains archived and compressed raw flow cytometry data files (FCS3 format) for the light shock experiment. File names match those provided in the primary data file.

flow_cytometry_temperatureshock.tar.gz

(GZIP (.gz), 10.83 MB)
MD5:988d4068bd8c01ad1048312a75fe3f75

File 4: flow_cytometry_temperatureshock.tar.gz
Brief description of File 4: This file contains archived and compressed raw flow cytometry data files (FCS3 format) for the temperature shock experiment. File names match those provided in the primary data file.

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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

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Related Datasets

IsRelatedTo

Berube, P., Chisholm, S. W., LeMaster, T. (2025) **Incomplete assimilatory nitrate reduction by LLI Prochlorococcus in response to nutrient stress.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2025-08-06 doi:10.26008/1912/bco-dmo.969427.1 [view at BCO-DMO]
Relationship Description: Nitrogen limitation shock dataset as part of same experiment.

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Parameters

Parameter	Description	Units
Experiment	Experiment type: Light Perturbations or Temperature Perturbations	unitless
ExpSampleID	Experiment Sample ID for nitrite analysis and flow cytometry	unitless
Strain	Strain name of pure culture used	unitless
Rep	Biological Replicate (A/B/C)	unitless
ExpCondition	Flag for experimental condition (Control/Shock)	unitless
N_source	Concentration and type of inorganic nitrogen provided as a nitrogen source	unitless
Light_Intensity	Photon flux that the cultures were cultivated under	micromoles photones per square meter per second (umol photons m-2 s-1)
Light_Color	Spectra of light that the cultures were cultivated under (W=White or B=Blue)	unitless

Temperature	Temperature of the waterbath the cultures were kept in over the course of sampling time period	Degrees Celsius (°C)
Culture_ID	Unique ID for culture tubes in format [A]-[B]-[C]-[DD]-[E]-[F]-[G]. Where A = strain B=ExpCondition; C =Replicate; DD=Light_Color & Light_Intensity; E = N_source;G = temperature	unitless
ExpTime	Time of sample collection measured from the of sodium nitrate addition; -1 min indicates sample collection immediate before the sodium nitrate addition	minutes
ISOTime	Date and time of sample collection in ISO 8601 format	unitless
Nitrite_Concentration	Extracellular concentration of dissolved nitrite in the culture determined from an AutoAnalyzer3; BDL = below detection limit of 0.010 micromole per liter (umol/L).	micromoles nitrite per liter (umol nitrite L-1)
Nitrite_Concentration_Notes	Says "BDL" when nitrite concentration is below detection limit of 0.010 micromole per liter (umol/L).	unitless
Cell_Concentration_FCM	Concentration of total cyanobacteria cells as determined by flow cytometry	cells per milliliter (cells mL-1)
FRRF_filename	Name of FRRF file corresponding to the sample	unitless
FCM_filename	Name of flowcytometry file corresponding to the sample	unitless
FCM_Beads_Filename	Name of flowcytometry file corresponding to the beads normalization well for that flow cytometry run	unitless
FCM_blank_filename	Name of flowcytometry file corresponding to the blank well for that flow cytometry run	unitless

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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	FIRe Fluorometer System - Satlantic, Halifax, NS, Canada
Generic Instrument Name	Fluorometer
Dataset-specific Description	FIRe fluorometry exposes cells to µsec scale flashlets of light while collecting data on cell fluorescence over the entire curve of photosystem excitation and relaxation. Data generated using FIRe can be processed to obtain parameters such as the minimum and maximum chlorophyll fluorescence, the maximum quantum yield of photochemistry at photosystem II, and the functional absorption cross section of photosystem II (see: https://datadocs.bco-dmo.org/docs/302/Upwelled_Phytoplankton_Dynamics/da...(FIRe).pdf).
Generic Instrument Description	A fluorometer or fluorimeter is a device used to measure parameters of fluorescence: its intensity and wavelength distribution of emission spectrum after excitation by a certain spectrum of light. The instrument is designed to measure the amount of stimulated electromagnetic radiation produced by pulses of electromagnetic radiation emitted into a water sample or in situ.

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.

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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.

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Funding

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

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