Phytoplankton Nitrogen isotope fractionation

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Project
» CAREER: The biological nitrogen isotope systematics of ammonium consumption and production (Biological Nitrogen Isotope Fractionation)

Contributors	Affiliation	Role
Granger, Julie	University of Connecticut (UConn)	Principal Investigator
Mathuri, Michael	University of Connecticut (UConn)	Contact
Newman, Sawyer	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
Laboratory experiments, both batch cultures and short-term ammonium (NH4+) uptake experiments, were conducted using marine phytoplankton to verify the concentration dependence of nitrogen (N) isotope fractionation for NH4+ assimilation

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Coverage

Spatial Extent: **Lat**:0 **Lon**:0

Methods & Sampling

Phytoplankton cultures

Two strains of marine algae, the diatom *Thalassiosira weissflogii* (actin) and the prasinophyte *Tetraselmis* sp. were grown in batch cultures in sterile, acid-washed borosilicate glass or polycarbonate bottles using artificial seawater medium in an environmental chamber at 180C, illuminated with fluorescent light (40 μmol photons m-2 s-1 photosynthetically available radiation) on a 12-hour light and 12-hour dark cycle. The artificial seawater medium was prepared from low nutrient Instant Ocean™ salt dissolved in Milli-Q water and filtered through a 47 mm Whatman GF/F glass microfiber filter (0.7 µm nominal pore-size) and sterilized by autoclaving at 1000C via Pasteurization cycle in PRIMUS steam sterilizer, then supplemented with filter-sterilized 100 – 250 μM NH4+, 10 μM phosphate, 100 μM silicic acid (only for *T. weissflogii* cultures), and f/2 trace metals and vitamins (Guillard and Ryther 1962). The batch cultures were initiated from inocula of approximately 1,000 cells mL-1, and cell densities monitored daily using Multisizer 4 Beckman Coulter counter. Media subsamples were collected during exponential growth for analyses of NH4+ concentration and N isotope ratios. Subsamples were filtered through a 0.45 µm pore-size polyether-sulfone (PES) syringe filters and collected into acid-washed High Density Poly-Ethylene (HDPE) bottles, solution pH adjusted to ca. 4.5 with dilute hydrochloric acid in order to minimize ammonia volatilization during storage, and samples stored at –200C pending analysis. Particulate organic nitrogen (PON) was also sampled during the exponential growth phase by filtering aqueous subsamples onto a pre-combusted 25 mm Whatman GF/F glass microfiber filters (0.7 µm pore-size) and

stored in pre-combusted aluminum foils at –200C pending analysis of N isotope ratio analysis. To capture lower NH4+ concentrations, short-term NH4+ uptake experiments with *T. weissflogii* and *Tetraselmis* sp. were conducted. A first set of experiments was conducted with *T. weissflogii* and *Tetraselmis* sp. cells in early stationary phase, wherein NH4+ was exhausted from the medium. The cells were collected by gentle filtration onto a 5 µm pore-size 47 mm IsoporeTM polycarbonate membrane filter and resuspended into fresh medium containing ~60 μM NH4+ for *T. weissflogii* and 20 μM NH4+ for *Tetraselmis* sp. Aqueous subsamples were collected at regular time intervals until NH4+ in the medium was exhausted. A second set of experiments was conducted with N-replete (cells in exponential growth phase) and N-starved cells (cells two days into stationary phase) of *T. weissflogii* and *Tetraselmis* sp. Cell cultures were either diluted into fresh medium containing ~20 µM NH4+, or gently filtered onto a polycarbonate membrane filter and resuspended into said medium. Short-term incubations occurred largely under constant illumination, although some *Tetraselmis* sp. uptake experiments were inadvertently subject to light-dark conditions.

Determination of NH4+ concentration

Ammonium concentrations at or above 50 μM were measured fluorometrically following derivatization with o-phthalaldehyde (OPA; Holmes et al. 1999) while concentrations < 50 µM were analyzed with the indophenol method (Solórzano 1969).

Analyses of N isotopes of NH4+ and PON

Ammonium samples were diluted to 5 µM or 1 µM with deep Atlantic seawater and N isotope ratios determined using the hypobromite-azide method (Zhang et al. 2007), wherein NH4+ is first oxidized to nitrite by hypobromite, after which nitrite is converted to a nitrous oxide gas analyte by reacting with azide. The N isotopic composition of the nitrous oxide product was analyzed using a continuous flow purge and dual cryogenic trap system coupled to a custom-modified Gas Bench II device interfaced with a Thermo Delta V gas chromatograph isotope ratio mass spectrometer (GC-IRMS; see Casciotti et al. 2002; McIlvin and Casciotti 2011). Calibration to reference (dinitrogen gas in air) was achieved from parallel reactions of NH4+ reference materials IAEA-N1 and IAEA-N2 diluted in deep Atlantic seawater (5 µM or 1 µM solutions), with respective assigned δ15N values of 0.43‰ and 20.3‰ vs. air (Böhlke et al. 1993). To analyze N isotopes of PON, frozen glass microfiber filters were lyophilized for 24 hours using an Edwards Super Modulyo freeze-dryer. The filters were packed into tin capsules and analyzed by combustion to dinitrogen gas on a Costech ECS 4010 Elemental Analyzer followed by N isotope ratio analysis of the resulting dinitrogen gas on a Thermo Delta V isotope ratio mass spectrometer. Samples were calibrated with corresponding aliquots of L-glutamic acid reference materials USGS-40 and USGS-41, with δ15N values of –4.52 and 47.57‰ vs. air, respectively (Qi et al. 2003).

Data Processing Description

No data values are represented by blank cells in the data table. These values indicate that no sample was collected at the given time point.

Data were generated from the laboratory experiments were processed using Microsoft Excel.

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Related Publications

BOHLKE, J. K., GWINN, C. J., & COPLEN, T. B. (1993). NEW REFERENCE MATERIALS FOR NITROGEN-ISOTOPE-RATIO MEASUREMENTS. Geostandards and Geoanalytical Research, 17(1), 159–164. doi:10.1111/j.1751-908x.1993.tb00131.x https://doi.org/10.1111/j.1751-908X.1993.tb00131.x
Methods

Casciotti, K. L., Sigman, D. M., Hastings, M. G., Böhlke, J. K., & Hilkert, A. (2002). Measurement of the Oxygen Isotopic Composition of Nitrate in Seawater and Freshwater Using the Denitrifier Method. Analytical Chemistry, 74(19), 4905–4912. doi:10.1021/ac020113w
Methods

Guillard, R. R. L., & Ryther, J. H. (1962). STUDIES OF MARINE PLANKTONIC DIATOMS: I. CYCLOTELLA NANA HUSTEDT, AND DETONULA CONFERVACEA (CLEVE) GRAN. Canadian Journal of Microbiology, 8(2), 229–239. doi:10.1139/m62-029
Methods

Holmes, R. M., Aminot, A., Kerouel, R., Hooker, B. A., & Peterson, B. J. (1999). A simple and precise method for measuring ammonium in marine and freshwater ecosystems. Canadian Journal of Fisheries and Aquatic Sciences, 56(10), 1801-1808. doi:10.1139/f99-128 https://doi.org/10.1139/cjfas-56-10-1801
Methods

McIlvin, M. R., & Casciotti, K. L. (2011). Technical Updates to the Bacterial Method for Nitrate Isotopic Analyses. Analytical Chemistry, 83(5), 1850–1856. doi:10.1021/ac1028984
Methods

Qi, H., Coplen, T. B., Geilmann, H., Brand, W. A., & Böhlke, J. K. (2003). Two new organic reference materials for δ13C and δ15N measurements and a new value for the δ13C of NBS 22 oil. Rapid Communications in Mass Spectrometry, 17(22), 2483–2487. doi:10.1002/rcm.1219
Methods

SOLÓRZANO, L. (1969). DETERMINATION OF AMMONIA IN NATURAL WATERS BY THE PHENOLHYPOCHLORITE METHOD 1 1 This research was fully supported by U.S. Atomic Energy Commission Contract No. ATS (11-1) GEN 10, P.A. 20. Limnology and Oceanography, 14(5), 799–801. doi:10.4319/lo.1969.14.5.0799
Methods

Zhang, L., Altabet, M. A., Wu, T., & Hadas, O. (2007). Sensitive Measurement of NH4+15N/14N (δ15NH4+) at Natural Abundance Levels in Fresh and Saltwaters. Analytical Chemistry, 79(14), 5297–5303. doi:10.1021/ac070106d
Methods

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Parameters

Parameters for this dataset have not yet been identified

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Instruments

Dataset-specific Instrument Name	Multisizer 4 Beckman Coulter Counter
Generic Instrument Name	Coulter Counter
Dataset-specific Description	A Multisizer 4 Beckman Coulter counter was used to determine phytoplankton cell density.
Generic Instrument Description	An apparatus for counting and sizing particles suspended in electrolytes. It is used for cells, bacteria, prokaryotic cells and virus particles. A typical Coulter counter has one or more microchannels that separate two chambers containing electrolyte solutions. from https://en.wikipedia.org/wiki/Coulter_counter

Dataset-specific Instrument Name	Costech ECS 4010 Elemental Analyzer
Generic Instrument Name	Elemental Analyzer
Dataset-specific Description	A Costech ECS 4010 Elemental Analyzer coupled with a Thermo Delta V isotope ratio mass spectrometer was used for the combustion of PON samples to produce dinitrogen gas for N isotope analyses, with calibration achieved using L-glutamic acid references USGS-40 and USGS-41.
Generic Instrument Description	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.

Dataset-specific Instrument Name	Turner Trilogy Fluorometer
Generic Instrument Name	Fluorometer
Dataset-specific Description	A Turner Trilogy Fluorometer was used to measure NH4+ concentration ≥ 50 µM.
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	Thermo Delta V gas chromatograph isotope ratio mass spectrometer (GC-IRMS)
Generic Instrument Name	Gas Chromatograph
Dataset-specific Description	A Thermo Delta V gas chromatograph isotope ratio mass spectrometer (GC-IRMS) - used to analyze N isotopes of nitrous oxide gas derived from the chemical oxidation of NH4+ using the hypobromite-azide method (Zhang et al. 2007), with calibrations achieved using NH4+ reference materials IAEA-N1 and IAEA-N2.
Generic Instrument Description	Instrument separating gases, volatile substances, or substances dissolved in a volatile solvent by transporting an inert gas through a column packed with a sorbent to a detector for assay. (from SeaDataNet, BODC)

Dataset-specific Instrument Name	U-3010 VIS Spectrophotometer
Generic Instrument Name	Spectrophotometer
Dataset-specific Description	A U-3010 VIS Spectrophotometer was used to measure NH4+ concentration < 50 µM.
Generic Instrument Description	An instrument used to measure the relative absorption of electromagnetic radiation of different wavelengths in the near infra-red, visible and ultraviolet wavebands by samples.

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Project Information

CAREER: The biological nitrogen isotope systematics of ammonium consumption and production (Biological Nitrogen Isotope Fractionation)

NSF Award Abstract:
The nitrogen (N) cycle in the marine environment is controlled by biological processes. Unfortunately, quantifying these processes and assessing their effect on the N cycle is difficult by direct measurements because of large spatial and temporal differences. Isotopic composition measurements of N provide a means to constrain these processes indirectly; however, there is still a great deal to be understood about isotope fractionation of recycled nitrogen through biological processes, which has made interpretation of novel nitrogen isotope data difficult. A researcher from the University of Connecticut plans to determine the influence of biological consumption and production on the isotope fractionation in ammonium. By helping to understand the processes surrounding fractionation of recycled ammonium at the organism level, this research will create a basis for which future researchers can better interpret isotope composition data to infer nitrogen cycle dynamics. A graduate student, a postdoctoral fellow, and two or more undergraduate students will be involved in the research. The researcher plans to integrate science with community-engaged learning by developing an undergraduate field and laboratory course that will require the students to present their research to stakeholders in the community. There will be a manual created for this course that will be disseminated in open-access forums for teachers hoping to develop similar courses.

Biological nitrogen isotope fractionation associated with nitrogen recycling remains poorly constrained despite the advent of a variety of new techniques to analyze nitrogen isotopes in recent years. The use of isotopic composition data can be incredibly useful to interpreting nitrogen cycle processes in the ocean that are difficult to measure directly, which makes it crucial to further understand the processes behind fractionation to catch up with the advancement of the datasets available to researchers. This research will characterize the isotope fractionation dynamics of ammonium during biological consumption and production. The researchers will investigate whether the characteristic low concentrations of ammonium in the surface ocean affect isotope fractionation when the ammonium is recycled and whether there is a trophic isotope effect associated with ammonium recycling by protozoan grazers. With this research, there will be a baseline from which researchers can interpret recycled nitrogen dynamics from ammonium isotope datasets. The methods of comparing nitrogen cycling studies will become significantly clearer with such a standard making interpretation uniform by removing significant uncertainties.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1554474

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