Nitrogen isotope fractionation for ammonium assimilation by marine phytoplankton (Biological Nitrogen Isotope Fractionation project)

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

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

Table of Contents

Coverage

Temporal Extent: 2018-09-09 - 2020-01-03

Dataset Description

Model code description

To query the physiological mechanism of nitrogen (N) isotope fractionation during ammonium (NH_4^+) assimilation, we constructed a time-dependent finite-differencing box model that tracks discrete ^{14}N and ^{15}N in the different N pools during the growth of marine algae with NH_4^+ as the sole N source. The model entails three N reservoirs: external N (medium), intracellular reservoir, and the phytoplankton nitrogen (biomass). We prescribe a non-fractionating active NH_4^+ uptake by the cells via specialized NH_4^+ transport proteins (AMTs), that follows Michaelis-Menten kinetics with half-saturation constant of 50 nM. Inside the cell, NH_4^+ is condensed with glutamate by glutamine synthetase (GS) to biomass – the rate-determining and isotope-fractionating step for NH_4^+ assimilation. The isotope fractionation imparted internally is then communicated to

the external medium largely by passive diffusion of ammonia (NH_3). A full description of the physiological model and the different scenarios tested can be found in the article. Below is an outline of the various constants and variables used in the model.

Constants

0.00367 – initial ratio of $^{15}N/^{14}N$ for all the N pools other than external NH_4^+

0.003695 – initial ratio of $^{15}N/^{14}N$ for external NH_4^+

1.76×10^{-5} – equilibrium constant for protonation of NH_3

1.58×10^{-6} – concentration of OH^- in the external medium at pH 8.2 (mol L^{-1})

1.0×10^{-7} – concentration of OH^- in the cytoplasm at pH 7.0 (mol L^{-1})

1.0×10^{-9} – concentration of OH^- in the vacuole at pH 5.0 (mol L^{-1})

µmax – maximum specific growth rate (hr^{-1})

VGSmax – maximum GS rate for NH_4^+ condensation with glutamate (hr^{-1})

alphaGS – N isotope fractionation factor for GS

alphaCat – N isotope fractionation factor for catabolic NH_4^+ production

MultLA – multiplier of the maximum specific growth rate for the low-affinity uptake

MultHA – multiplier of the maximum specific growth rate for the high-affinity uptake

PC_NH3 – permeability coefficient for NH_3 (cm hr^{-1})

PC_NH4 – permeability coefficient for NH_4^+ (cm hr^{-1})

BetaNH4 – a term in the constant field equation for NH_4^+ diffusion across the cellular membrane

expBetaNH4 – exponent of BetaNH4

BetaVac – a term in the constant field equation for NH_4^+ diffusion across the vacuolar membrane

expBetaVac – exponent of BetaVac

SA – surface area of the phytoplankton cell (cm^{-2})

SAVac – surface area of the cell vacuole (cm^{-2})

Cellular N quota – 1.66×10^{-6} µmol N $cell^{-1}$

Phytoplankton cell volume – 1.15×10^{-12} L

Vacuole volume – 3.45×10^{-13} L

NH_4^+-NH_3 equilibrium isotope effect – 15‰

Variables

CellN14 – ^{14}N in the phytoplankton nitrogen in the culture (µmol L^{-1})

CellN15 – ^{15}N in the phytoplankton nitrogen in the culture (µmol L^{-1})

Gln14 – ^{14}N in the glutamine pool in the culture (µmol L^{-1})

Gln15 – ^{15}N in the glutamine pool in the culture (µmol L^{-1})

NH4cyt14 – ^{14}N in the cytoplasmic NH_4^+ in the culture (µmol L^{-1})

NH4cyt15 – ^{15}N in the cytoplasmic NH_4^+ in the culture (µmol L^{-1})

NH4out14 – ^{14}N in the external NH_4^+ pool (µmol L^{-1})

NH4out15 – ^{15}N in the external NH_4^+ pool (µmol L^{-1})

Vacuole14 – ^{14}N in the vacuolar NH_4^+ in the culture (µmol L^{-1})

Vacuole15 – ^{15}N in the vacuolar NH_4^+ in the culture (µmol L^{-1})

MMLAU – Michaelis-Menten term for low-affinity NH_4^+ uptake with half-saturation constant of 30 µmol L^{-1}

MMHAU – Michaelis-Menten term for high-affinity NH_4^+ uptake with half-saturation constant of 50 nmol L^{-1}

Cell_N_total – phytoplankton nitrogen in the culture (µmol L^{-1})

"Cells_L-1" – cell density per liter of the culture medium (cells L^{-1})

CytoplasmNH4conc – cellular concentration of NH_4^+ in the cytoplasm (µmol L^{-1})

CytoplasmNH3conc – cellular concentration of NH_3 in the cytoplasm (µmol L^{-1})

NH4concOut – external NH_4^+ pool (µmol L^{-1})

F15NH4out – fraction of ^{15}N in the external NH_4^+

NH3concOut – external NH_3 pool (µmol L^{-1})

Delta_NH3in – difference in NH_3 concentration between the cytoplasm and external medium (µmol L^{-1})

EffluxNH3 – rate of cellular NH_3 efflux from the cytoplasm to the external medium (µmol $cell^{-1}$ hr^{-1})

d15NH4cyt – N isotope composition of NH_4^+ in the cytoplasm (‰ vs. air)

d15NH3cyt – N isotope composition of NH_3 in the cytoplasm (‰ vs. air)

R15NH3cyt – ratio of ^{15}N in the cytoplasmic NH_3

F15NH3cyt – fraction of ^{15}N in the cytoplasmic NH_3

VENH314 – rate of ^{14}N NH_3 efflux from the cytoplasm to the external medium in the culture (µmol hr^{-1})

Delta_NH4in – difference in NH_4^+ concentration between the cytoplasm and external medium (µmol L^{-1})

EffluxNH4 – rate of cellular NH_4^+ efflux from the cytoplasm to the external medium (µmol $cell^{-1}$ hr^{-1})

F15NH4cyt – fraction of ^{15}N in the cytoplasmic NH_4^+

VENH414 – rate of ^{14}N NH_4^+ efflux from the cytoplasm to the external medium in the culture (µmol hr^{-1})

VINH3Vac14 – rate of ^{14}N NH_3 influx from the cytoplasm to the vacuole in the culture (µmol hr^{-1})

MMGS – Michaelis-Menten term for GS with half-saturation constant of 10 µmol L^{-1}

GS14 – rate of ^{14}N NH_4^+ condensation with glutamate by GS in the culture (µmol hr^{-1})

GlnConc – cellular concentration of glutamine (µmol L^{-1})

MMGln – Michaelis-Menten term for glutamate synthase (GOGAT) with half-saturation constant of 700 µmol L^{-1}

F15Gln – fraction of ^{15}N in the glutamine pool

GOGAT14 – rate of assimilation of ^{14}N glutamine into phytoplankton nitrogen by GOGAT in the culture (µmol hr^{-1})

VENH315 – rate of ^{15}N NH_3 efflux from the cytoplasm to the external medium in the culture (µmol hr^{-1})

VENH415 – rate of ^{15}N NH_4^+ efflux from the cytoplasm to the external medium in the culture (µmol hr^{-1})

VINH3Vac15 – rate of ^{15}N NH_3 influx from the cytoplasm to the vacuole in the culture (µmol hr^{-1})

GS15 – rate of ^{15}N NH_4^+ condensation with glutamate by GS in the culture (µmol hr^{-1})

GOGAT15 – rate of assimilation of ^{15}N glutamine into phytoplankton nitrogen by GOGAT in the culture (µmol hr^{-1})

VENH4Vac14 – rate of ^{14}N NH_4^+ efflux from the vacuole to the cytoplasm in the culture (µmol hr^{-1})

VENH4Vac15 – rate of ^{15}N NH_4^+ efflux from the vacuole to the cytoplasm in the culture (µmol hr^{-1})

VU14 – rate of ^{14}N NH_4^+ uptake into the cytoplasm via AMTs in the culture (µmol hr^{-1})

alphaTR – sigmoidal parametrization of N isotope fractionation factor for NH_4^+ transport via AMTs

VU15 – rate of ^{15}N NH_4^+ uptake into the cytoplasm via AMTs in the culture (µmol hr^{-1})

d15NCell – N isotope composition of cellular N (‰ vs. air)

d15NH4Vac – N isotope composition of NH_4^+ in the vacuole (‰ vs. air)

d15NH3Vac – N isotope composition of NH_3 in the vacuole (‰ vs. air)

d15NH4out – N isotope composition of NH_4^+ in the external pool (‰ vs. air)

NH4concVac – vacuolar concentration of NH_4^+ (µmol L^{-1})

NH3concVac – vacuolar concentration of NH_3 (µmol L^{-1})

Delta_NH3Vac – difference in NH_3 concentration between the cytoplasm and vacuole (µmol L^{-1})

Delta_NH4Vac – difference in NH_4^+ concentration between the cytoplasm and vacuole (µmol L^{-1})

EffluxNH4Vac – rate of cellular NH_4^+ efflux from the vacuole to the cytoplasm (µmol $cell^{-1}$ hr^{-1})

F15NH4Vac – fraction of ^{15}N in the vacuolar NH_4^+

InfluxNH3Vac – rate of cellular NH_3 influx from the cytoplasm to the vacuole (µmol $cell^{-1}$ hr^{-1})

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

Processing notes from data submitter:

- Data table generated from laboratory experiments were processed using Microsoft Excel
- Model code written in MatLab

BCO-DMO Processing Description

- Dates converted from mm-dd-yy format to yyyy-mm-dd format
- UTC date time column added
- Column names changed to meet FAIR standards (spaces were replaced with underscores and special characters were removed).

[table of contents | back to top]

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
Related Research

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
Related Research

Holmes, R. M., Aminot, A., Kérouel, 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
Methods

Mathuri, M. et. al., Concentration dependence of nitrogen isotope fractionation during ammonium assimilation by marine phytoplankton
Results

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

[table of contents | back to top]

Parameters

Parameter	Description	Units
Strain	The two marine algal strains used in the laboratory experiments; a diatom Thalassiosira weissflogii and a prasinophyte Tetraselmis sp.	unitless
Experiment_type	The algal strains were incubated either under batch cultures or short-term ammonium uptake experiments	unitless
Experiment_number	Experiment identifier; a and b represent replicate trials for batch cultures	unitless
Date_and_time_EST	Date and time of sampling in EST; %Y-%m-%d %H:%M	unitless
Date_and_time_UTC	Date and time of sampling in UTC; %Y-%m-%dT%H:%MZ	unitless
Incubation_time_in_days	Number of days from the start of the experiment	days
Cell_density	Number of cells per one milliliter of the culture media at different times during the incubation period as determined using Beckman Coulter counter	cells mL-1
Initial_cell_density	Number of cells per one milliliter of the culture media at start of short-term uptake experiments	cells mL-1
NH4_plus	Ammonium concentration. Concentrations ≥ 50 µM measured fluorometrically on Turner Trilogy Fluorometer with the o-phthalaldehyde (OPA) method (Holmes et al. 1999). Concentrations < 50 _M analyzed on U-3010 VIS Specrophotometer with the indophenol method (Solorzano 1969)	umol L-1
stdev_sigma_NH4_plus	Standard deviation for [NH4+] derived from analytical replicates	unitless
negative_natural_logarithm_of_NH4_plus	Negative natural logarithm of ammonium concentration as used in the Rayleigh substrate (NH4+) equation (Mariotti et al. 1981)	unitless
delta_15NNH4_plus	N isotope composition of NH4+ measured using hypobronite-azide method (Zhang et al. 2007) on Thermo Delta V GC-IRMS with modified Gas Bench II and a PAL autosampler	‰ vs. air
stdev_sigma_delta_15NNH4_plus	Standard deviation for _15NNH4+ derived from analytical replicates using monte carlo error propagation	units
f_lnf_1_f	Used in the Rayleigh product (PON) equation (Mariotti et al. 1981), where f represents the fraction of ammonium in the media at every sampling time relative to the initial concentration	units
delta_15NPON	N isotope composition of PON measured by combustion on a Thermo Delta V IRMS linked through a Costech ECS 4010 Elementral Analyzer	‰ vs. air
Light_conditions	Experiments conducted under 12-hour light and 12-hour dark conditions. Most of the short-term experiments were conducted in light but a few with Tetraselmis sp. extended into the dark period	unitless
Growth_stage	Short-term uptake experiments were conducted with cells harvested in their exponential growth phase (N-replete), early stationary phase (stationary), and in late stationary phase (N-starved).	unitless
Notes	Notes	unitless

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	Multisizer 4 Beckman Coulter Counter
Generic Instrument Name	Coulter Counter
Dataset-specific Description	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 coupled with Thermo Delta V isotope ratio mass spectrometer
Generic Instrument Name	Elemental Analyzer
Dataset-specific Description	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	Thermo Delta V gas chromatograph isotope ratio mass spectrometer (GC-IRMS)
Generic Instrument Name	Gas Chromatograph Mass Spectrometer
Dataset-specific Description	Used to analyze N isotopes of nitrous oxide gas derived from 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	Instruments 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 by a mass spectrometer.

Dataset-specific Instrument Name	U-3010 VIS Spectrophotometer
Generic Instrument Name	Spectrophotometer
Dataset-specific Description	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.

Dataset-specific Instrument Name	Turner Trilogy Fluorometer
Generic Instrument Name	Turner Designs Trilogy fluorometer
Dataset-specific Description	Used to measure NH4+ concentration ≥ 50 µM.
Generic Instrument Description	The Trilogy Laboratory Fluorometer is a compact laboratory instrument for making fluorescence, absorbance, and turbidity measurements using the appropriate snap-in application module. Fluorescence modules are available for discrete sample measurements of various fluorescent materials including chlorophyll (in vivo and extracted), rhodamine, fluorescein, cyanobacteria pigments, ammonium, CDOM, optical brighteners, and other fluorescent compounds.

[table of contents | back to top]

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.

[table of contents | back to top]

Funding

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

[table of contents | back to top]