Volume-dependent offsets in NO3- N and O isotope ratios of reference materials (Biological Nitrogen Isotope Fractionation project)

Website: https://www.bco-dmo.org/dataset/865031
Data Type: experimental
Version: 1
Version Date: 2021-11-16

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

Contributors	Affiliation	Role
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Abstract
NO3- reference materials (IAEA-NO3 and USGS-34) in seawater and freshwater were used to demonstrate volume-dependent offsets of NO3- N and O isotope ratio analyses with the denitrifier method.

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Coverage

Temporal Extent: 2019-02-20 - 2019-03-21

Methods & Sampling

Sampling and analytical procedures:

1. Analysis of NO3- N and O isotope ratios with the denitrifier method

The denitrifying bacteria strains *Pseudomonas chlororaphis* f. sp. *aureofaciens* (ATCC 13985, Manassas, VA, USA) and *Pseudomonas. chlororaphis* (ATCC 43928, Manassas, VA, USA) were used. Cultures were inoculated from cryo-preserved aliquots (Weigand et al., 2011) into sterile growth media prepared as originally described (Sigman et al., 2001; Casciotti et al., 2002) in 700 mL glass bottles containing 600 mL of medium, then sealed with gas-tight lids. Cells were cultured for 7-10 days at 20˚C on a rotary shaker table. Cultures were harvested by centrifugation and resuspended into 220 mL of fresh medium without potassium nitrate addition, achieving *ca.* 3-fold concentration of the bacteria. Two mL of the cell concentrates were added to respective 20-mL headspace glass vials, capped with pre-rinsed butyl rubber septa and crimp-seals (McIlvin and Casciotti, 2011). Vials were sparged with a water-scrubbed N2 gas stream for 6 hours to remove any N2O produced from the

residual NO3- in the medium. NO3- samples were then injected into each vial to achieve a final sample size of 10 nmoles of N. Vials were incubated inverted in order to prevent potential N2O leakage. Following overnight incubation in the dark, *ca.* 0.1 ml of 10 mol L-1 NaOH was injected into each vial to kill the cultures and sequester CO2 into carbonate species. The N2O gas in the vials was extracted, purified and analyzed with a Delta V Advantage continuous flow gas chromatograph isotope ratio mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) interfaced with a modified Thermo Fisher Scientific Gas Bench sample preparation device fronted by dual cold traps (Casciotti et al., 2002) and a GC Pal autosampler (CTC Analytics, Zwingen, Switzerland). Samples were referenced to pure N2O injections from a common reference gas cylinder.

2. Demonstration of volume effects in analyzes of NO3- reference materials

The reference solutions were prepared from salts into primary stocks at 200 µmol L-1 in deionized water (DIW) from a Milli-QTM water purification system (EMD Millipore, Burlington, MA, USA), and stored frozen. Primary stocks of NO3- reference materials (IAEA-NO3 and USGS-34) were diluted in NO3--deplete surface Sargasso seawater or in aged DIW to concentrations of 1, 5 and 20 µmol L-1, corresponding to respective injection volumes of 10, 2 and 0.5 mL, in order to aliquot 10 nmoles of N analyte. The NO3- aliquots were injected into the sparged bacterial concentrates of either *P. chlororaphis* or *P. aureofaciens*. Following bacterial conversion, the resulting N2O in the reaction vials was extracted, purified and analyzed on the isotope ratio mass spectrometer.

Data Processing Description

Processing notes from submitter:

- Data were processed using Microsoft Excel

BCO-DMO processing notes

- Date formats were changed from mm/dd/yy to yyyy-mm-dd
- Spaces and units removed from column headers

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Data Files

File
res1.csv (Comma Separated Values (.csv), 4.63 KB) MD5:7ee472a699d28ffd94d7fe5ce6a80711 Primary data file for dataset ID 865031

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Related Datasets

IsSourceOf

Zhou, M., Granger, J., Chang, B. X. (2022) **δ15N (δ18O) scale contraction was calculated as the percent deviation of the difference between measured δ15N (δ18O) values of IAEA-NO3 and USGS-34 from the true difference (Biological Nitrogen Isotope Fractionation project).** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2021-11-16 doi:10.26008/1912/bco-dmo.865043.1 [view at BCO-DMO]

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Parameters

Parameter	Description	Units
Strain	The two denitrifying bacteria strains used in the laboratory experiment: Pseudomonas chlororaphis f. sp. Aureofaciens (P. aureofaciens) and Pseudomonas. chlororaphis (P. chlororaphis)	unitless
Solution	Internationally recognized nitrate reference materials IAEA-NO3 (International Atomic Energy Agency, Vienna, Austria) and USGS-34 (National Institute of Standards and Technology, Gaithersburg, MD, USA)	unitless
Aliquot	Types of aliquot: freshwater or seawater. Reference solutions were prepared from salts into primary stocks at 200 µM in deionized water (DIW). Then working aliquots were diluted from primary stocks with DIW or nitrate-deplete seawater	unitless
Date	Date of the experiments; yyyy-mm-dd	unitless
Trial	Trial name	unitless
Concentration	Concentrations of nitrate reference solutions	umol L^-1
Sample_volume	Sample volume injected to aliquot 10 nmol of nitrate	mL
N2O_peak_area	Recoverd N2O peak area measured with a Thermo Delta V GC-IRMS with modified Gas Bench II and a PAL autosampler	Vs
stdev_of_N2O_peak_area	Standard deviation of N2O peak area replicates in each trial	unitless
delta_15N	N isotopic composition of nitrate measured with the denitrifier method using a Thermo Delta V GC-IRMS with modified Gas Bench II and a PAL autosampler	‰ vs. N2Otank
stdev_of_delta_15N	Standard deviation of delta 15N replicates in each trial	unitless
delta_18O	O isotopic composition of nitrate measured with the denitrifier method using a Thermo Delta V GC-IRMS with modified Gas Bench II and a PAL autosampler	‰ vs. N2Otank
stdev_of_delta_18O	Standard deviation of _18O replicates in each trial	unitless

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Instruments

Dataset-specific Instrument Name	Delta V Advantage continuous flow gas chromatograph isotope ratio mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA)
Generic Instrument Name	Gas Chromatograph Mass Spectrometer
Dataset-specific Description	Delta V Advantage continuous flow gas chromatograph isotope ratio mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) interfaced with a modified Thermo Fisher Scientific Gas Bench sample preparation device fronted by dual cold traps (Casciotti et al., 2002) and a GC Pal autosampler (CTC Analytics, Zwingen, Switzerland) - to measure N and O isotope ratio of nitrate using the denitrified method.
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.

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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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