Nitrogen isotope ratios (δ15N) in amino acid standards and in four field-collected samples

Website: https://www.bco-dmo.org/dataset/884976
Data Type: Other Field Results, experimental
Version: 1
Version Date: 2022-12-09

Project
» Collaborative research: Using individual amino acids N isotopes in sinking particles and surficial sediments to reconstruct euphotic zone N sources and trophic structure (Amino Acids N Isotopes)

Contributors	Affiliation	Role
Zhang, Lin	Texas A&M, Corpus Christi (TAMU-CC)	Principal Investigator
Lee, Wing Man Charlotte	Texas A&M, Corpus Christi (TAMU-CC)	Student
Rauch, Shannon	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
These data include Nitrogen Isotope Ratios (δ15N) in amino acid standards and in four field-collected samples. Certified δ15N values are either EA-IRMS values (Glutamic acid [Glu], USGS) or produced by the persulfate oxidization method (Phenylalanine [Phe], Knapp et al., 2005) or provided by McCarthy Lab (M-std and Cyano). Mixtures of 16 amino acids were also evaluated. The newly-developed method used here will help promote the use of δ15N-AA in important studies of nitrogen cycling and trophic ecology in a wide range of research areas. The Phe isotopic standards are available to the community for inter-lab method comparisons. These data were collected by PhD student Wingman (Charlotte) Lee and Dr. Lin Zhang (PI) at the Texas A&M University-Corpus Christi.

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Coverage

Spatial Extent: **N**:40.96 **E**:-64.17 **S**:26 **W**:-111.75
Temporal Extent: 2020-01 - 2020-08

Methods & Sampling

Sample Descriptions:
See Supplemental File "Field Sampling Info" (PDF) for the dates and locations of collection of the four field samples. In short:
Cyano = a cyanobacteria sample provided by Matt McCarthy lab (UCSC, δ15N-Phe and δ15N-Glu are 7.41±0.83‰ and 7.64±0.62‰, respectively).
Zoop = a zooplankton sample collected near Bermuda.
LIS = a sinking particle sample collected by a sediment trap in Long Island Sound, NY.

GoCA = a surface sediment sample collected from the Gulf of California under the oxygen-deficient zone.
Std1-4 = mixtures of 16 amino acids with different δ15N-Phe and δ15N-Glu values (see Supplemental File "Std1-4 Info" (PDF))
M-std = a standard mixture of 15 amino acids with known δ15N values from the Matt McCarthy lab (UCSC, δ15N-Phe and δ15N-Glu are 9.17‰ and -4.13‰, respectively).

The methodology for nitrogen isotopic compositions (d15N) analysis in amino acids (AA) employed here is comprised of four main steps: (1) sample preparation and amino acid extraction, (2) amino acid separation and collection, (3) conversion of amino acid to nitrite, and (4) conversion of nitrite to nitrous oxide (N2O) for nitrogen (N) isotope analysis using purge-and-trap continuous-flow isotope ratio mass spectrometry (PT/CF/IRMS).

Four mixtures of 16 proteinaceous AAs were created to identify AA in natural samples, as well as to quantify their concentrations and d15N values. USGS L-glutamic acid (Glu): USGS40 (-4.52 ‰) and 41a (+47.55‰) were mixed by weight with proportions of 62% vs 38% and 34% vs 66%, respectively, to create four different d15N values for Glu (United States Geological Survey, Reston, VA, USA). A series of phenylalanine (Phe) standards were created to cover a broad natural-abundance range in d15N adapted from a previously published procedure (Zhang and Altabet 2008), which were made by mixing isotopically labeled L-Phe (15N>98%, Cambridge Isotope, Cambridge, MA, USA) with unenriched Phe (Alfa Aesar, Haverhill, MA, USA). The d15N of these Phe at four different isotopic levels were determined using an adapted persulfate oxidation method in the Altabet Lab (Bronk et al., 2001, Knapp et al., 2005). These Glu and Phe were mixed with L-alanine (Ala), L-arginine (Arg), D/L-aspartic acid (Asp), L-cysteine (Cys), glycine (Gly), L-histidine (His), L-isoleucine (Ile), L-leucine (Leu), L-lysine (Lys), D/L-methionine (Met), D/L-serine (Ser), D/L-threonine (Thr), L-tyrosine (Tyr), and D/L-valine (Val). The AA in the mixtures were made with a concentration of 2.5mM except for Glu and Asp, which were at 5mM, respectively. The detailed isotopic composition of Phe and Glu in each standard mixture can be found in the Supplemental File "Std1-4 Info" (PDF).

Sample analyses took place between January and August 2020.

(1) Sample preparation and amino acid extraction:
The extraction of AA from proteinaceous samples followed previously established procedures (e.g., Zhang et al., 2021). Various amounts (Table SI-2 of Zhang et al. (2021)) of cyanobacteria, zooplankton, sinking particles, and sediment samples were placed in 20 milliliter (mL) glass vials with 10mL 6 M hydrochloric acid (HCl) (Thermo Fischer Scientific, Waltham, MA, USA), were flushed with nitrogen gas, sealed, and hydrolyzed at 110 °Celsius for 20 hours.

(2) Amino acid separation and collection:
Separations and collection of AAs were conducted using an ICS-5000+ Ion-change chromatography system (Thermo Fischer Scientific, Waltham, MA, USA). Baseline separation of 9 AAs (Arg, Lys, Met, Ser, Phe, His, Glu, Asp, and Tyr) was achieved on a CarboPac PA 10 semi-preparative scale column (9×250 mm, 10 micrometer (µm) particle size, pore size of < 10 angstroms (Å); Thermo Fischer Scientific, Waltham, MA, USA). Each sample injection was set at 25 microliters (µL) and the column compartment was set at a constant 30°C. The mobile phases used were (A) MilliQ water, (B)1M NaOH in MilliQ water, and (C)100mM NaOH in MilliQ water, and programmed as follows for each run: 0 to 30 min (A: 80%, C: 20%), 35 to 52 min (A: 50%, C: 50%), 55 to 62 min (A: 85%, B: 15%), 65 to 75 min (A: 75%, B: 25%), 80 to 140 min (A: 70%, B: 30%).

At the end of each run, 1M sodium acetate (NaOAc) was used to flush the column for 20 minutes to clean out any residue. Before the next run, the column was conditioned with 70% A and 30% B for 15 minutes, and then 80% A and 20% C for another 15 minutes. See Figure 2 in Zhang et al. (2021) for chromatograms and sample collection-window time intervals. 10% of the flow coming out of the ion chromatography (IC) column was directed to a Pulse Amperometric Detector (PAD; Thermo Fischer Scientific, Waltham, MA, USA) by a flow splitter for peak detection and quantitation. The rest of the flow was sent to an automated fraction collector (AFC-3000; Thermo Fischer Scientific, Waltham, MA, USA), which was placed in a glove bag filled with N2 gas. Purified AA fractions were collected into 40mL flat-bottom glass vials (Thermo Fischer Scientific, Waltham, MA, USA) using retention-time based collection method. IC procedural blanks were collected close to each AA (Phe and Glu) fractions and had the same concentration of NaOH as the corresponding AA fractions.

(3) Conversion of amino acid to nitrite:
A previously developed protocol (Zhang and Altabet 2008) was employed for the conversion of collected AA to nitrite. This adapted protocol involves three types of reagents:
1) a catalytical reagent that was prepared by mixing 0.665 grams (g) of sodium bromide (NaBr; Thermo Fischer Scientific, Waltham, MA, USA) with 52.5mLof 50% w/w sodium hydroxide (NaOH; Thermo Fischer Scientific, Waltham, MA, USA), and then diluted to 100mL with MilliQ water;
2) an oxidizing reagent that was prepared daily by diluting 4.25mL of commercial bleach (5.25% NaClO;

Thermo Fischer Scientific, Waltham, MA, USA) to 100 mL using MilliQ water;
and 3) a quenching reagent that was made by adding 5.1g sodium arsenite (Na2AsO2) into 100ml MilliQ water. 0.1mL of the catalytical reagent was added into the 10mL AA fractions and mixed well, followed by the addition of 0.1mL oxidizing reagent.

(4) Conversion of nitrite to N2O:
The analytical procedure of converting nitrite to nitrous oxide (N2O), and subsequent N isotope analysis of N2O, have been documented in previous studies (McIlvin and Altabet, 2005, Zhang et al., 2007). A slightly modified procedure was performed at UMD-SMAST. About 15 nanomoles (nmol) of nitrite converted from the AA samples or reference materials were pipetted into 20mL crimp-top sealed headspace glass vials and diluted to a consistent volume (i.e. 4mL) using MilliQ water. Then, pre-conditioned sodium azide solution with acetate buffer (0.2 M NaN3 in 85.5% acetic acid (aq), prepared by adding 10ml of a solution of 2M NaN3 in 45% acetic acid (aq) to 90ml of a 90% acetic acid (aq) solution) was pipetted into the same vials at a 0.14/1 (v/v) reagent-to-sample ratio and immediately capped with a butyl rubber stopper and crimped with aluminum seal followed by gentle swirling to fully mix the reagent and sample. It took an hour for this reduction reaction to complete at room temperature. The produced N2O in these crimped vials were purged with Helium using a Trace Gas interface autosampler (PAL), which was subsequently cryogenically focused and separated from N2, O2, CO2, and H2O using a gas chromatography column before sending into the IRMS for isotope analysis.

Data Processing Description

Data Processing:
A statistical summary is presented in the Supplemental File "Statistical Summary" (PDF).

BCO-DMO Processing:
- modified parameter names to comply with BCO-DMO naming convetions;
- created Supplemental File "Field Sampling Info" (PDF) containing the sampling information.

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Data Files

File
N_isotope_ratios.csv (Comma Separated Values (.csv), 6.44 KB) MD5:3e4ac1f71b4763c8fc6aa08d1b56e22a Primary data file for dataset ID 884976

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Supplemental Files

File	
Field Sampling Info filename: dataset_884976_sampling_info.pdf	(Portable Document Format (.pdf), 357.62 KB) MD5:4e3b38f9e55312e9d14f52912caa7408
Latitudes, longitudes, dates, and notes describing the four field samples examined in dataset 884976. Latitude and longitude are provided in decimal degrees. Dates are provided in YYYY-MM-DD format or Month and Year.	
Statistical Summary filename: dataset_884976_statistical_summary.pdf	(Portable Document Format (.pdf), 338.66 KB) MD5:9cbe5ff2a21e050b0c6ce613ca65c9ed
Statistical summary table for dataset 884976.	
Std1-4 Info filename: dataset_884976_Std1-4_info.pdf	(Portable Document Format (.pdf), 259.46 KB) MD5:94428bdb8abc64e65056a9821b8fa968
Table showing the δ15N-Phe and δ15N-Glu values for each standard sample (1-4) in dataset 884976.	

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Related Publications

Altabet, Mark. A., & Small, L. F. (1990). Nitrogen isotopic ratios in fecal pellets produced by marine Zooplankton. Geochimica et Cosmochimica Acta, 54(1), 155–163. https://doi.org/10.1016/0016-7037(90)90203-w https://doi.org/10.1016/0016-7037(90)90203-W
Methods

Broek, T. A. B., & McCarthy, M. D. (2014). A new approach to δ15N compound-specific amino acid trophic position measurements: preparative high pressure liquid chromatography technique for purifying underivatized amino acids for stable isotope analysis. Limnology and Oceanography: Methods, 12(12), 840–852. doi:10.4319/lom.2014.12.840
Methods

Bronk, D. A., Lomas, M. W., Glibert, P. M., Schukert, K. J., & Sanderson, M. P. (2000). Total dissolved nitrogen analysis: comparisons between the persulfate, UV and high temperature oxidation methods. Marine Chemistry, 69(1–2), 163–178. https://doi.org/10.1016/s0304-4203(99)00103-6 https://doi.org/10.1016/S0304-4203(99)00103-6
Methods

Knapp, A. N., Sigman, D. M., & Lipschultz, F. (2005). N isotopic composition of dissolved organic nitrogen and nitrate at the Bermuda Atlantic Time-series Study site. Global Biogeochemical Cycles, 19(1). doi:10.1029/2004gb002320
Methods

McIlvin, M. R., & Altabet, M. A. (2005). Chemical Conversion of Nitrate and Nitrite to Nitrous Oxide for Nitrogen and Oxygen Isotopic Analysis in Freshwater and Seawater. Analytical Chemistry, 77(17), 5589–5595. doi:10.1021/ac050528s
Methods

Zhang, L., & Altabet, M. A. (2008). Amino-group-specific natural abundance nitrogen isotope ratio analysis in amino acids. Rapid Communications in Mass Spectrometry, 22(4), 559–566. https://doi.org/10.1002/rcm.3393
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

Zhang, L., Lee, W. (Charlotte), Kreider-Mueller, A., Kuhnel, E., Baca, J., Ji, C., & Altabet, M. (2021). High-

precision measurement of phenylalanine and glutamic acid δ15N by coupling ion-exchange chromatography and purge-and-trap continuous-flow isotope ratio mass spectrometry. Rapid Communications in Mass Spectrometry, 35(13). Portico. https://doi.org/10.1002/rcm.9085
Results

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Parameters

Parameter	Description	Units
Sample	Sample name	unitless
Fraction	Collected fractions of blanks and amino acids on Ion-exchange chromatography	unitless
Concentration	Concentrations of produced NO2	micromolar (uM)
d15N	raw data, blank-corrected (Std1-4), or blank-corrected and standard-calibrated (natural samples)	per mil (‰)

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Instruments

Dataset-specific Instrument Name	custom purge-trap sample preparation system
Generic Instrument Name	Automated Purge and Trap System
Dataset-specific Description	Isotope determinations were made at the University of Massachusetts Dartmouth using a GV IsoPrime IRMS, a custom purge-trap sample preparation system, and a CTC PAL autosampler. Reproducibility was better than ± 0.5‰.
Generic Instrument Description	This equipment removes dissolved gases from the water samples, traps the extracted compounds on a cold trap and then heats the trap and injects the trapped gases into the gas chromatograph. It is automated and controlled by a laptop computer.

Dataset-specific Instrument Name	ICS-5000+ Ion-change chromatography system
Generic Instrument Name	Ion Chromatograph
Dataset-specific Description	Separations and collection of AAs were conducted using an ICS-5000+ Ion-change chromatography system (Thermo Fischer Scientific, Waltham, MA, USA).
Generic Instrument Description	Ion chromatography is a form of liquid chromatography that measures concentrations of ionic species by separating them based on their interaction with a resin. Ionic species separate differently depending on species type and size. Ion chromatographs are able to measure concentrations of major anions, such as fluoride, chloride, nitrate, nitrite, and sulfate, as well as major cations such as lithium, sodium, ammonium, potassium, calcium, and magnesium in the parts-per-billion (ppb) range. (from http://serc.carleton.edu/microbelife/research_methods/biogeochemical/ic....)

Dataset-specific Instrument Name	GV IsoPrime IRMS
Generic Instrument Name	Isotope-ratio Mass Spectrometer
Dataset-specific Description	Isotope determinations were made at the University of Massachusetts Dartmouth using a GV IsoPrime IRMS, a custom purge-trap sample preparation system, and a CTC PAL autosampler. Reproducibility was better than ± 0.5‰.
Generic Instrument Description	The Isotope-ratio Mass Spectrometer is a particular type of mass spectrometer used to measure the relative abundance of isotopes in a given sample (e.g. VG Prism II Isotope Ratio Mass-Spectrometer).

Dataset-specific Instrument Name	CTC PAL autosampler
Generic Instrument Name	Laboratory Autosampler
Dataset-specific Description	Isotope determinations were made at the University of Massachusetts Dartmouth using a GV IsoPrime IRMS, a custom purge-trap sample preparation system, and a CTC PAL autosampler. Reproducibility was better than ± 0.5‰.
Generic Instrument Description	Laboratory apparatus that automatically introduces one or more samples with a predetermined volume or mass into an analytical instrument.

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Project Information

Collaborative research: Using individual amino acids N isotopes in sinking particles and surficial sediments to reconstruct euphotic zone N sources and trophic structure (Amino Acids N Isotopes)

Coverage: Gulf of California, Equatorial Pacific, Sargasso Sea

NSF Award Abstract:
Nitrogen is a limiting nutrient over most of the surface ocean. Fixed nitrogen (N) such as nitrate controls absorption of atmospheric carbon dioxide and production of organic matter by marine plants and algae. Nitrogen availability, use patterns, and biological community structure in the surface ocean help determine the amount of organic matter passed onto higher organisms. Nitrogen availability also controls how much organic matter sinks into deep waters. This project will reconstruct past sources of nitrogen, use patterns, and trophic structures in surface waters of the Gulf of California, equatorial Pacific, and Sargasso Sea. The tool employed by the principal investigators from Texas A&M University in Corpus Christi and University of Massachusetts Dartmouth is nitrogen isotope ratios of individual amino acids. The investigators will measure isotope ratios in sinking particle samples collected by sediment traps such as those used by the Ocean Flux Program in the Sargasso Sea. This study will train graduate students in stable isotope biogeochemistry and oceanography. This project will also provide research funds for students in the McNair program. McNair students come from underrepresented and economically challenged backgrounds to pursue degrees in STEM fields at Texas A&M University Corpus Christi, a Hispanic and Minority Serving Institution. Data from this project will be made available to the public through the Biological and Chemical Oceanography-Data Management Office (www.bco-dmo.org).

There is great interest in reconstructing past climate-forced variations in nitrogen sources, their patterns of

utilization, and euphotic zone community structure using compound specific N isotope ratios in amino acids liberated from preserved proteinaceous materials in sediments and coral skeletons. However, it has not yet been verified whether 1) the nitrogen isotope ratios of individual amino acids produced in the euphotic zone are transported with fidelity by sinking particles to deep-sea corals and sediments and 2) the nitrogen isotope ratios of individual amino acids liberated from sedimentary organic matter have been altered by diagenesis. Through analysis of sediment trap material collected over time, this project seeks to verify that nitrogen isotope ratios in individual amino acids reflect the 1) overall spatial contrast in N sources, utilization patterns, and trophic structures among the Gulf of California, equatorial Pacific, and Sargasso Sea and 2) temporal variations in nitrogen sources, utilization patterns, and trophic structures within both the Gulf of California and equatorial Pacific due to seasonal upwelling and/or El Nino-Southern Oscillation. This study will also test if the nitrogen isotope ratios of total hydrolysable amino acids in sedimentary organic matter from the three locations retain the unaltered nitrogen isotope patterns carried by sinking particles. This project will, for the first time, compare nitrogen stable isotope ratios in amino acids collected from sediment trap samples with surficial sediments from deep-sea oxic sites to verify whether total hydrolysable amino acids in deep-sea sediments preserve unaltered nitrogen isotope signals produced in overlying euphotic zone, which can provide insights on addressing diagenetic alteration of bulk N isotope ratios that have hindered paleo-nitrogen cycle reconstruction.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1829947
NSF Division of Ocean Sciences (NSF OCE)	OCE-1829834

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