N and O isotope effects of nitrate reduction by 3 forms of nitrate reductase (15N2 Contamination project)

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Project
» The Ocean Nitrogen Imbalance Paradox: Environmental Controls on the Denitrification Isotope Effect (15N2 Contamination)

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Dataset Description

Enzyme assays were conducted to determine the N and O isotope effects of nitrate reduction by 3 forms of nitrate reductase: respiratory nitrate reductase (Nar), periplasmic nitrate reductase (Nap), and eukaryotic assimilatory nitrate reductase (EukNR).

Methods & Sampling

Sources of Nitrate Reductase:
Enzymatic assays were conducted on (a) cell homogenates from the denitrifying bacterial strain Paracoccus denitrificans (American Type Culture Collection [ATCC] 19367) cultured under anaerobic vs. aerobic conditions, on (b) cell homogenates from the photo-heterotrophic bacterial strain Rhodobacter sphaeroides (Deutsche Sammlung von Mikroorganismen [DSM] 158) cultured aerobically, and on (c) purified extracts of recombinant eukaryotic assimilatory nitrate reductases (EukNR) from the flowering plant Arabidopsis thaliana (AtNaR: E.C. 1.7.1.1) and from the yeast Pichia angusta (YNaR1: E.C. 1.7.1.2), both purchased from NECi (nitrate.com).

Enzymatic assay preparations:
Assays contained 0.5 or 1 mL of cell suspension or of commercially purified EukNR buffered solution, 1 mL of 200 umol L-1 reducing agent – either membrane-permeant benzyl viologen dichloride [Sigma-Aldrich, CAS: 1102-19-8], methyl viologen dichloride hydrate [Sigma-Aldrich, CAS: 75365-73-0], or hydroquinone (for dissimilatory reductases only; [MP Organics]) – 0.2 or 1 mL 10 mmol L-1 KNO3 to a final concentration of 200 or 1000 umol L-1, and the remaining volume of 100 mmol L-1 phosphate buffer [pH 7.9] containing 100 umol L-1 to a final assay volume of 10 mL. After drawing an initial 1 mL sample for quantitation of [NO3-] and [NO2-], the denitrification reaction was commenced by the addition of 1 mL of 57 mmol L-1 sodium dithionite in 29 mmol L-1 sodium bicarbonate, which reduces the electron donor. Initial [NO3-] and [NO2-] values are corrected for this dilution. Serial 1 mL samples were drawn approximately every 90 seconds during room temperature assays and every 3 minutes during assays conducted at 4 degrees C. Samples were mixed vigorously on a vortex mixer for 30 s immediately upon collection to halt the reaction through oxidation of the

methyl or benzyl viologen or hydroquinone. In selected assays, additional ~ 50 uL samples were also drawn throughout the assay reactions for determination of [NO2-] and were measured immediately. In order to ensure complete cessation of enzyme activity, samples placed in an 80 degrees C water bath for 2 to 10 minutes. NO2- was then removed from the samples via the addition of 55 uL 4% (wt/vol) sulfamic acid in 10% vol/vol HCl.

Determination of [NO3-]:
[NO3-] was measured in samples by chemiluminescence detection on a NOx analyzer (model T200 Teledyne Advanced Pollution Instrumentation) following reduction to NO in a heated vanadium solution (Braman and Hendrix 1989). Nitrite had been previously removed from these samples, as vanadium reduces both nitrate and nitrite to NO.

Nitrate N and O isotope ratio analyses:
NO3- δ15N and δ18O were determined with the denitrifier method (Sigman et al. 2001; Casciotti et al. 2002), wherein denitrifying bacteria lacking terminal nitrous oxide reductase (P. chlororaphis f. sp. aureofaciens ATCC 1398) quantitatively convert sample NO3- to N2O gas, which is then extracted, purified and analyzed through a modified Thermo-Scientific Gas Bench II and Delta V Advantage gas chromatograph isotope ratio mass spectrometer. Samples were standardized through comparison to reference standards IAEA-N3, USGS-34, and USGS-32, which have δ15N (vs air N2) and δ18O (vs V-SMOW) of 4.7‰ and 25.6‰, -1.8‰ and -27.9‰, and 180‰ and 25.6‰ respectively after individually being referenced to pure N2O injections from a common reference gas cylinder.

Data Processing Description

Nitrate Calculations: delta 15N and delta 18O NO3 were calculated from uncorr_d45_44 and uncorr_d46_44 using equations outlined in the "Nitrate isotope corrections" supplementary file (PDF), followed by reference to IAEA N-3, US34, US32 standards.

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Data Files

File
nitrate_reductases.csv (Comma Separated Values (.csv), 22.69 KB) MD5:336df3b22392eab9d3b73d02288dc54a Primary data file for dataset ID 564509

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Parameters

Parameter	Description	Units
assay	Assay identification number.	dimensionless
sample	Sample identification number.	dimensionless
date	Month, day, and year.	mmddyyyy
reductant	Chemical used to donate electrons for assay reductions (methyl viologen OR benzyl viologen OR hydroquinone).	dimensionless
enzyme_form	Whether cell suspensions or pure enzyme extracts were used in experimental assays (cell suspensions OR pure extract).	dimensionless
bacteria	Bacteria from which nitrate reductase enzymes used in assays were procured.	dimensionless
cells_lysed	Whether cells were lysed before incorporation in experimental assays (nd for pure extracts).	dimensionless
growth_conditions	Whether cells were grown aerobically or anaerobically before preparation for experimental assays (nd for pure extracts).	dimensionless
temp	Temperature at which assay was conducted.	degrees Celsius (C)
NO3_corr	Concentration of nitrate remaining in samples.	micromolar (uM NO3-)
d15N	delta-N15 of nitrate, reported as an average of replicate measurements.	permille (‰)
d15N_sd	Standard deviation of replicate delta-N15 measurements.	permille (‰)
d18O	delta-O18 of nitrate, reported as an average of replicate measurements.	permille (‰)
d18O_sd	Standard deviation of replicate delta-O18 measurements.	permille (‰)

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Instruments

Dataset-specific Instrument Name	gas chromatograph
Generic Instrument Name	Gas Chromatograph
Dataset-specific Description	NO3- δ15N and δ18O were determined with the denitrifier method (Sigman et al. 2001; Casciotti et al. 2002), wherein denitrifying bacteria lacking terminal nitrous oxide reductase (P. chlororaphis f. sp. aureofaciens ATCC 1398) quantitatively convert sample NO3- to N2O gas, which is then extracted, purified and analyzed through a modified Thermo-Scientific Gas Bench II and Delta V Advantage gas chromatograph isotope ratio mass spectrometer.
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	isotope ratio mass spectrometer
Generic Instrument Name	Isotope-ratio Mass Spectrometer
Dataset-specific Description	NO3- δ15N and δ18O were determined with the denitrifier method (Sigman et al. 2001; Casciotti et al. 2002), wherein denitrifying bacteria lacking terminal nitrous oxide reductase (P. chlororaphis f. sp. aureofaciens ATCC 1398) quantitatively convert sample NO3- to N2O gas, which is then extracted, purified and analyzed through a modified Thermo-Scientific Gas Bench II and Delta V Advantage gas chromatograph isotope ratio mass spectrometer.
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).

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Project Information

The Ocean Nitrogen Imbalance Paradox: Environmental Controls on the Denitrification Isotope Effect (15N2 Contamination)

Description from NSF award abstract:
This study will test the sensitivity of the amplitude of the denitrification isotope effect to culture conditions pertinent to the ocean environment. The isotope effect amplitude will be explored with respect to electron donor, trace oxygenation, and temperature, in both batch and continuous culture experiments of denitrifiers. The proposed work will also involve measurements of the enzymatic isotope effect of the respiratory nitrate reductase of denitrifiers, measurements of its enzymatic activity among cultures, and examination of cellular nitrate transport kinetics of denitrifying strains. The experiments are designed to reveal the physiological basis of the modulation of the isotope effect amplitude, which will further resolve this manifestation in the environment.

In regards to the broader significance and importance of this study, these new experimental data will provide a basis for integration of nitrogen isotope dynamics in ocean models to test how key environmental parameters can affect the global ocean distribution of nitrogen isotopes.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1233897

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