Results from experiment examining 15N-labeled contaminants in commercial 15N2 gas: 46-N2O contamination measured directly in commercial 15N2 gas (15N2 Contamination project)

Website: https://www.bco-dmo.org/dataset/546779
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Project
» The Ocean Nitrogen Imbalance Paradox: Environmental Controls on the Denitrification Isotope Effect (15N2 Contamination)

Contributors	Affiliation	Role
Granger, Julie	University of Connecticut (UConn)	Principal Investigator
Dabundo, Richard	University of Connecticut (UConn)	Contact
Rauch, Shannon	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

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Dataset Description

Direct measurments of N2O from experiments on 15N-labeled contaminants in commercial 15N2 gas; data used in Dabundo et al. 2014.

Refer to the following publication for more information:
Dabundo, R., Lehmann, M.F., Treibergs, L., Tobias, C.R., Altabet, M.A., Moisander, P.H., and Granger, J. 2014. The Contamination of Commercial 15N2 Gas Stocks with 15N–Labeled Nitrate and Ammonium and Consequences for Nitrogen Fixation Measurements. PLoS ONE, 9(10): e110335. doi:10.1371/journal.pone.0110335

See related datasets:
delta 15N NO3
delta 15N NH4
N2
particulate N

Methods & Sampling

Data was acquired from an isotope ratio mass spectrometer using Isodat 3.0 software. 15N2 was added to helium flushed exetainers containing 10 nmol tank N2O.

Direct Nitrous Oxide Calculations:
delta values calculated by the same method as nitrate (delta 15N NO3 and apparent delta 18O 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, and/or UBN-1 standards.)

moles 15N15N16O added equation:
moles added = [(46/44 sample)*(N2O final) - (46/44 control)*(N2O initial)]/(d value tracer)

"delta value tracer" is the expected 46/44 delta value of added N2O (‰) assuming it has the same enrichment as that reported for the tracer gas, at 98 atom %:
15N2 injected (moles) is calculated from the injection volume using the ideal gas law at T = 25 degrees C and P = 1 atm.

Refer to the following publication for more information:
Dabundo, R., Lehmann, M.F., Treibergs, L., Tobias, C.R., Altabet, M.A., Moisander, P.H., and Granger, J. 2014. The Contamination of Commercial 15N2 Gas Stocks with 15N–Labeled Nitrate and Ammonium and Consequences for Nitrogen Fixation Measurements. PLoS ONE, 9(10): e110335. doi:10.1371/journal.pone.0110335

Summary of methods from Dabundo et al. 2014:
Reagents:
Four lecture bottles of 98+ at% 15N-labeled N2 gas were purchased from Sigma-Aldrich, three from lot # SZ1670V, and one from lot # MBBB0968V. Two 1L lecture bottles of 98+ at% 15N2 were purchased from Cambridge Isotopes from lot #'s I1-11785A and I-16727. One 1L lecture bottle of 98+ at% 15N2 was purchased from Campro Scientific from lot # EB1169V. Ammonium and nitrate solutions were prepared with salts or with solutions obtained from different distributors: sodium nitrate (NaNO3), potassium nitrate (KNO3), and ammonium chloride (NH4Cl) from Fisher Scientific; analytical-grade potassium nitrate from Fluka Analytical and a gravimetric solution of ammonium chloride from SPEX CertiPrep.

Preparation of nitrate & ammonium solutions:
Aqueous solutions of natural abundance (unlabeled) ammonium and nitrate salts were equilibrated overnight with an air headspace supplemented with an injection of 15N2 gas (to determine whether the 15N2 gas stocks contained 15N-labeled ammonia (NH3) or nitrate and/or nitrite (NOx) contaminants). After equilibration, the 15N/14N ratio of ammonium and the 15N/14N and 18O/16O ratios of nitrate/nitrite in solution were measured, as well as the 15N/14N ratio of N2 gas in the headspace. The isotope ratios of nitrate and ammonium were compared to those in control solutions, which were not supplemented with 15N2 gas. Experiments with the Campro Scientific 15N2 stock were verified for 15N-nitrate/nitrite contaminants only (and not for 15N-ammonium).

Initial experiments consisted of 40 mL or 100 mL solutions of 10, 50, 100, 200, or 300 umol/L nitrate and 5 umol/L ammonium chloride in 60 mL or 120 mL serum vials that were sealed with stoppers. The 20 mL of air headspace in each of the treatment vials was supplemented with 0.1 mL of 15N2 gas from respective bottles from each of the three suppliers. The solutions were equilibrated overnight on a shaker, after which the 15N/14N and 18O/16O isotope ratios of nitrate were analyzed. The 15N/14N isotope ratio of ammonium was also analyzed in experimental solutions treated with the Sigma-Aldrich and Cambridge Isotopes stocks.

Additional experiments were carried out in which 2 mL 15N2 gas was equilibrated overnight in 20 mL serum vials containing 10 mL solutions of 10 umol/L sodium nitrate, after which the 15N/14N and 18O/16O ratios of nitrate were measured. Similarly, 10 mL solutions of 5 umol/L ammonium chloride were dispensed in 20 mL serum vials and equilibrated overnight with 2 mL 15N2 gas, after which the 15N/14N isotope ratios of ammonium were analyzed.

The measured 18O/16O ratios of nitrate/nitrite in solutions equilibrated with 15N2 gas from some stocks suggested the presence of 46-N2O contamination. As the analyte for isotope ratio analysis is N2O, and 46-N2O can be explained by both 15N15N16O and 14N14N18O, N2O that is doubly labeled with 15N is falsely detected as d18ONO3 enrichment. The presence of 46-N2O contamination in 15N2 gas was verified directly for one of the Sigma-Aldrich stocks (Lot # SZ1670V) by adding 0.0125, 0.020, or 0.025 mL of 15N2 stock to 20 mL serum vials containing 10 nmoles of reference N2O in helium. The N and O isotopic composition of the N2O was analyzed as described below, and compared to unamended N2O injections.

Nitrate N and O isotope ratio analyses:
Nitrate/nitrite nitrogen (15N/14N) and oxygen (18O/16O) isotope ratios were measured using the denitrifier method. Nitrate (and nitrite) in experimental samples was converted stoichiometrically to nitrous oxide (N2O) by a denitrifying bacterial strain (Pseudomonas chlororaphis f. sp. aureofaciens, ATCC 13985) that lacks nitrous oxide reductase. The N and O isotopic composition of N2O was then measured on a Delta V Advantage Isotope Ratio Mass Spectrometer (IRMS) interfaced with a modified Gas Bench II gas chromatograph (Thermo Fisher) purge and trap system. The isotope ratio measurements are reported in per mille (o/oo) units.

The 15N/14N reference is N2 in air, and the 18O/16O reference is Vienna Standard Mean Ocean water (V-

SMOW). Individual analyses on the GC-IRMS were referenced to injections of N2O from a pure N2O gas cylinder, and then standardized through comparison to the international nitrate standards USGS-34, USGS-32, and IAEA-NO-3, using standard bracketing techniques. Nitrate samples from experiments with Campro Scientific 15N2 were standardized with USGS-32 and IAEA-NO-3, and an additional internal lab nitrate standard (UBN-1).

Nitrous oxide N and O isotope ratio analyses:
N2O isotope ratios were measured directly on the GC-IRMS, and referenced against the N2O tank, which was standardized indirectly by comparison to the d15N and d18O of nitrate standards.

Headspace N2 isotope ratio analyses:
To measure the d15N of N2 gas in the headspace of experimental samples, 75 uL of headspace was injected into 12 mL Exetainer vials previously flushed with helium, then analyzed on a Gas Bench II GC-IRMS (Delta V Advantage Plus) operated in continuous flow mode. N2 and (O2+ Ar) were separated on a gas chromatography column. The analyses were standardized with parallel analyses of ambient N2 gas in air. These direct N2 gas measurements were carried out for experiments conducted using two of three lecture bottles from Sigma-Aldrich lot, and for experiments conducted using the lecture bottle from Cambridge Isotopes. The 15N2 concentration in the headspace of other experiments was estimated from the tracer injection volume rather than from direct measurements.

Data Processing Description

Since 46/44 enrichment is a function of 15N15N16O contamination rather than delta 18O enrichment of nitrate, as the delta 15N correction scheme presumes, uncorr delta 46/44 was replaced with the average control delta 46/44 value to calculate delta 15N.

Samples with the same ID are replicated measurements.

BCO-DMO Edits:
- Modified parameter names to conform with BCO-DMO naming conventions;
- Denoted 'Control' and 'Standard' in the lot_number column;
- Replaced spaces with underscores.

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Data Files

File
direct_N2O.csv (Comma Separated Values (.csv), 908 bytes) MD5:a1bd0522adc2c368912edafd5f4e069b Primary data file for dataset ID 546779

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Parameters

Parameter	Description	Units
sample_ID	Sample identification number.	dimensionless
lecture_bottle	Identifier of the lecture bottle of 15N-labeled N2 gas.	dimensionless
lot_number	Lot number of the 15N-labeled N2 gas; or 'Control' or 'Standard' for controls and standards respectively.	dimensionless
ratio_45_to_44	45/44	per mille (‰)
ratio_46_to_44	46/44	per mille (‰)
delta_15N	delta 15N.	per mille (‰)
delta_18O	delta 18O.	per mille (‰)
N2_injected_L	Amount of 15N2 injected.	liters (L)
N2O_initial	Initial N2O (moles).	moles
added_15N15N16O	Amount of 15N 15N 16O added (moles).	moles
N2_injected_moles	Amount of 15N2 injected (moles).	moles
ratio_15N15N16O_to_15N2	15N 15N 16O / 15N2 ratio.	mol/mol

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Instruments

Dataset-specific Instrument Name	Gas Chromatograph
Generic Instrument Name	Gas Chromatograph
Dataset-specific Description	The N and O isotopic composition of N2O was then measured on a Delta V Advantage Isotope Ratio Mass Spectrometer (IRMS) interfaced with a modified Gas Bench II gas chromatograph (Thermo Fisher) purge and trap system.
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	Delta V Advantage Isotope Ratio Mass Spectrometer (IRMS)
Generic Instrument Name	Isotope-ratio Mass Spectrometer
Dataset-specific Description	The N and O isotopic composition of N2O was then measured on a Delta V Advantage Isotope Ratio Mass Spectrometer (IRMS) interfaced with a modified Gas Bench II gas chromatograph (Thermo Fisher) purge and trap system. Data was acquired from an isotope ratio mass spectrometer using Isodat 3.0 software.
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
NSF Division of Ocean Sciences (NSF OCE)	OCE-1130495
Swiss National Science Foundation (SNSF)	R Equip 121258

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