Results from experiment examining 15N-labeled contaminants in commercial 15N2 gas: Detected 15N-labeled nitrate (NO3) in commercial 15N2 gas (15N2 Contamination project)

Website: https://www.bco-dmo.org/dataset/542153
Version: 09 Dec 2014
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Project
» The Ocean Nitrogen Imbalance Paradox: Environmental Controls on the Denitrification Isotope Effect (15N2 Contamination)

Contributors	Affiliation	Role
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Dataset Description

delta 15N NO3 measurments 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 NH4
direct N2O
N2
particulate N

Methods & Sampling

Data was acquired from an isotope ratio mass spectrometer using Isodat 3.0 software.

Nitrate Calculations: 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.

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.

Nitrate and ammonium concentrations:

Nitrate concentrations in the experimental solutions were verified via reduction to nitric oxide in hot vanadium (III) solution followed by detection with a chemiluminescence NOx analyzer (model T200 Teledyne Advanced Pollution Instrumentation). Ammonium concentrations were measured by derivatization with orthophthaldialdehyde (OPA) and fluorometric detection on an AJN Scientific f-2500 Fluorescence Spectrophotometer.

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).

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 in some experiments (Sigma A3, Sigma B (40 mL and 10 mL solutions), Cambridge B (10 mL solution), Campro Scientific). In experiments where 46/44 was small relative 45/44, this was not necessary.

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
NO3.csv(Comma Separated Values (.csv), 15.08 KB) MD5:c25e14190a20f7f3b99f5a873d54e450 Primary data file for dataset ID 542153

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Related Publications

Dabundo, R., Lehmann, M. F., Treibergs, L., Tobias, C. R., Altabet, M. A., Moisander, P. H., & 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
Results

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Parameters

Parameter	Description	Units
lecture_bottle	Identifier of the lecture bottle of 15N-labeled N2 gas used in the experiment.	dimensionless
solution	Solution volume.	milliliters (mL)
headspace	Headspace volume.	milliliters (mL)
N2_injection	Quantity of 15N-labeled N2 gas supplemented in the headspace of the experimental samples. (Control solutions were NOT supplemented with the 15N2 gas).	milliliters (mL)
lot_number	Lot number of the 15N-labeled N2 gas; or 'Control' or 'Standard' for controls and standards respectively.	dimensionless
sample_ID	Sample identification number.	dimensionless
initial_NO3	Initial concentration of nitrate (NO3).	micromolar (uM)
uncorr_d45_44	Uncorrected d45/44 ratio.	dimensionless
uncorr_d46_44	Uncorrected d46/44 ratio.	dimensionless
delta_15N_NO3	delta 15N NO3.	per mille (o/oo)
apparent_delta_18O	Apparent delta 18O; experimental samples.	per mille (o/oo)
delta_18O	delta 18O; controls and standards.	per mille (o/oo)

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Instruments

Dataset-specific Instrument Name	T200 Teledyne Advanced Pollution Instrumentation
Generic Instrument Name	Chemiluminescence NOx Analyzer
Dataset-specific Description	Nitrate concentrations in the experimental solutions were verified via reduction to nitric oxide in hot vanadium (III) solution followed by detection with a chemiluminescence NOx analyzer (model T200 Teledyne Advanced Pollution Instrumentation).
Generic Instrument Description	The chemiluminescence method for gas analysis of oxides of nitrogen relies on the measurement of light produced by the gas-phase titration of nitric oxide and ozone. A chemiluminescence analyzer can measure the concentration of NO/NO2/NOX. One example is the Teledyne Model T200: https://www.teledyne-api.com/products/nitrogen-compound-instruments/t200

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).

Dataset-specific Instrument Name	AJN Scientific f-2500 Fluorescence Spectrophotometer
Generic Instrument Name	Spectrophotometer
Dataset-specific Description	Ammonium concentrations were measured by derivatization with orthophthaldialdehyde (OPA) and fluorometric detection on an AJN Scientific f-2500 Fluorescence Spectrophotometer.
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.

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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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