Results from experiment examining 15N-labeled contaminants in commercial 15N2 gas: The measured quantity of 15N2 added to nitrate and ammonium solutions equilibrated with 15N2 gas

Website: https://www.bco-dmo.org/dataset/549112
Version: 04 Feb 2015
Version Date: 2015-02-04

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

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Dataset Description

The measured quantity of 15N2 added to nitrate and ammonium solutions equilibrated with 15N2 gas.

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
direct N2O
particulate N

Methods & Sampling

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

Dinitrogen (N2) calculations:
Moles of 15N2 added were calculated from 28, 29, and 30 areas compared to the atmosphere.

Calculation:
atm = atmosphere
variables labeled "A" correspond to measured area values

Constants:

15Natm mole fraction = 0.003663
14Natm mole fraction = 0.996337
Therefore the fraction of 30N2atm = 1.34176E-05; 29N2atm = 0.007299165; 28N2atm = 0.992687418
30N2atm/ 28N2atm = 1.35164E-05 = 30/28natural_abundance
28N2 total = 28N2 total in incubation was calculated from the ideal gas law at 25 degrees C and Henry's Law. Found to be 654 umol in incubations with 40 mL solution and 20 mL headspace, and 690 umol in incubations with 100 mL solution and 20 mL headspace.

The following equations were applied to air standards to correct for the blank 30/28:
30area from binomial = 28A * 30/28nat_abundance
30/28area ratio blank = (30A/28A) – 30area_from_binomial/28A
30/28area ratio blank is graphed (y axis) vs. 28A (x axis) to account for variation relating to the injection volume.
The slope (mratio blank) and intercept (iratio blank) of the corresponding linear regression are found.

The blank is then removed from the measured 30A/28A ratio of samples:
30A/28A real = 30A/28A – (mratio_blank * 28A + iratio_blank)

Added 30N2 is calculated:
30N2total = 30A/28A real * 28N2total
30N2added = 30N2total – (28N2total * 30/28natural_abundance)

Total 15N added = 2 * 30N2added + 29N2added. However, 29N2added was negligible, as expected from the 98+ atom % 15N2 gas. Therefore, the reported 15N2 gas added is equivalent to added 30N2.

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).

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.
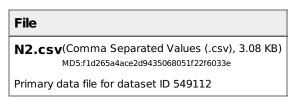
Data Processing Description

Samples with the same ID are replicated measurements.

BCO-DMO Edits:
- Modified parameter names to conform with BCO-DMO naming conventions;
- Replaced spaces with underscores.

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Data Files

File
N2.csv (Comma Separated Values (.csv), 3.08 KB) MD5:f1d265a4ace2d9435068051f22f6033e
Primary data file for dataset ID 549112

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Parameters

Parameter	Description	Units
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
sample_ID	Sample identification number.	dimensionless
Area_28_Vxs	28 Area (V *s) - Volt second measurements of mass 28 nitrogen gas output by the mass spectrometer.	volt seconds
Area_29_Vxs	29 Area (V *s) - Volt second measurements of mass 29 nitrogen gas output by the mass spectrometer.	volt seconds
Area_30_Vxs	30 Area (V *s) - Volt second measurements of mass 30 nitrogen gas output by the mass spectrometer.	volt seconds
added_15N2	Amount of added 15N2.	moles
V_injection_air_standard	V injection (in microliters) for the air standards.	microliters (uL)

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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. 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.
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. 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.
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:

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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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