Limits of detection and qPCR efficiencies from cruise SAV 17-16 in the South Atlantic Bight aboard the R/V Savannah from 2011 to 2017

Website: https://www.bco-dmo.org/dataset/767141
Data Type: experimental
Version: 1
Version Date: 2019-05-08

Project
» Collaborative Research: Direct Oxidation of Organic Nitrogen by Marine Ammonia Oxidizing Organisms (DON Oxidation)

Contributors	Affiliation	Role
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Abstract

This dataset contains the results of analyses related to ammonia oxidation rates, including oxidation rates of 15N supplied as ammonia, urea, 1,2 diamino ethane, 1,3 diamino propane, 1,4 diamino butane (putrescine), arginine and glutamate. Ancillary data including nutrient concentrations and the abundance of ammonia- and nitrite-oxidizing microorganisms are also reported. The samples analyzed to produce the dataset were collected off the coast of Georgia, USA. Most data were collected on one cruise in August 2017, incidental data from 2011, 2013 and 2016 are also reported.

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Coverage

Spatial Extent: **N**:31.99 **E**:-78.765 **S**:30.3175 **W**:-81.356
Temporal Extent: 2011-10-04 - 2017-08-19

Dataset Description

Samples were collected from four regions (inshore, midshelf, shelf-break, and oceanic) of the SAB off the Georgia (U.S.A.) coast (Fig. 1; Supporting Information Table S1), with terminology modified from Liu et al. (2018) as follows. “Inshore” stations were within the barrier island complex. “Mid-shelf” stations were outside the barrier island complex to depths < 40 m; due to limited sampling in this zone, no demarcation between “mid-shelf” and “nearshore” stations (as in Liu et al. 2018) was made. “Shelf-break” stations were between 40 m and 500 m depth. While Liu et al. (2018) did not sample waters past the shelf-break, we included deeper stations further offshore (bottom depth > 500 m), which are designated “oceanic” stations. Note that the maximum depth sampled was ≤ 500 m due to equipment limitations.

Inshore samples were collected from a dock at Marsh Landing on the Duplin River (Sapelo Island) and the dock at the Skidaway Institute of Oceanography (Fig. 1). Both inshore sites are salt marsh-dominated estuaries. Water from both sites was sampled from a depth ≤ 1 m and was processed immediately at a nearby laboratory (the University of Georgia Marine Institute on Sapelo Island or onboard the R/V Savannah). Water quality data for Marsh Landing samples were collected as part of the Sapelo Island National Estuarine Research Reserve monitoring program. Relevant data from the Lower Duplin ("LD") sonde were downloaded from NOAA/CDMO (http://cdmo. baruch.sc.edu/aqs/, last accessed 22 May 2018).

Most SAB samples were collected in August 2017 on the R/V Savannah (cruise SAV-17-16) along transects across the continental shelf and the Gulf Stream and into the western Sargasso Sea, with sampling focused around the shelf-break (Fig. 1). Water from multiple depths was collected using 12-liter Niskin bottles mounted on a rosette equipped with a Sea-Bird SBE25 CTD. Profiles of salinity, temperature, dissolved oxygen, fluorescence, and photosynthetically active radiation (PAR) were collected using the CTD system as described previously (Liu et al. 2018). PAR attenuation (Kd) was calculated from plots of ln(PAR) vs. depth as in Liu et al. (2018). Two additional SAB stations were sampled in October 2011 (described previously by Liu et al. 2015 and Tolar et al. 2017) and are referred to as "2011-4" and "2011-12" (Fig. 1). Environmental data and some of the microbial and rate data from 2011 stations are available in other publications (Liu et al. 2015; Tolar et al. 2017; see BCO-DMO dataset DON_Oxidation https://www.bco-dmo.org/dataset/767048).

Methods & Sampling

Nutrient analysis

Nutrient samples were filtered through 0.22 **μ**m pore size Durapore GVWP filters (Millipore Sigma) and frozen at −20_C immediately after collection, then stored at −80_C until analysis. Dissolved nitrate (NO3 −), nitrite (NO2 −), phosphate (PO4 3−), and silicate (SiO4 4−) were measured using a Bran and Luebbe AA3 autoanalyzer as described previously (Wilkerson et al. 2015). Ammonium and urea were measured manually using the phenolhypochlorite method (Solórzano 1969) and the diacetylmonoxime method (Rahmatullah and Boyde 1980; Mulvenna and Savidge 1992), respectively.

Oxidation rate measurements

We used 15N-labeled substrates (98–99% 15N, Cambridge Isotope Laboratories) to measure the oxidation of N supplied as NH4+, urea, 1,2-diaminoethane (DAE), 1,3-diaminopropane (DAP), 1,4-diaminobutane (putrescine, PUT), L-glutamic acid (GLU), and L-arginine (ARG). 15N oxidation from NH4+, urea, PUT, and GLU were measured extensively, whereas 15N oxidation from DAE, DAP, and ARG was only measured at a subset of stations (Supporting Information Table S1). GLU and ARG were included as a control for remineralization, as their central roles in microbial metabolism leads to rapid catabolism and NH4 + regeneration (Hollibaugh 1978; Goldman et al. 1987). PUT was used in routine assessments of the oxidation of polyamine-N because it is one of the most consistently detected polyamines in seawater (Nishibori et al. 2001a, 2003; Lu et al. 2014; Liu et al. 2015). Although spermine and spermidine are also common in seawater, 15N-labeled stocks of these polyamines were not commercially available. We measured the oxidation of N from DAE and DAP to investigate the effect of aliphatic chain length (which affects pK_a) on oxidation rate.

Duplicate seawater samples contained in 1-liter polycarbonate or 250 mL high density polyethylene (HDPE) bottles wrapped with aluminum foil (to exclude light) were amended with 10–50 nM 15N-labeled substrate. Marsh Landing samples were then placed in an incubator held at in situ temperature in the dark. Samples taken at the Skidaway dock were placed in a mesh bag and immersed at the sea surface at the sampling site. Samples collected at sea were incubated in a tank of flowing surface seawater or in an incubator held at 18 C in the dark. Incubation bottles were sampled for 15N analysis immediately after substrate addition and again after a period of ~ 24 h. 15N samples were subsampled into 50 mL polypropylene centrifuge tubes, frozen at −20_C, and stored at −80_C until analysis. The 15N/14N ratios of the NO3 − plus NO2 − (NOX) pools (**δ**15NNOx) in the samples were measured using the bacterial denitrifier method to convert NOX to nitrous oxide (N2O; Sigman et al. 2001). The **δ**15N values of the N2O produced were measured using a Finnigan MAT-252 isotope ratio mass spectrometer coupled with a modified GasBench II interface (Casciotti et al. 2002; Beman et al. 2011; McIlvin and Casciotti 2011). Oxidation rates were calculated using an endpoint model (Beman et al. 2011; Damashek et al. 2016). Since the substrates used were uniformly labeled with 15N, the amount of the N added as the 15N spike (in **μ**M) was multiplied by the number of moles of 15N per mole of substrate, which assumes that all of the N atoms have equal probability of being oxidized. This is likely true for urea, DAE, DAP, and PUT, which are symmetrical molecules, but not likely to be true for ARG, which contains 4 N atoms (one in the **α**-amino position and three in the guanidine structure of its R-group). Abiotic oxidation of organic N was assessed by measuring 15NOX production following 15N amendment and incubation of 0.22 **µ**m filtered seawater (as described above), and potential metabolism of DON by the denitrifying bacteria used to convert NOX to N2O was checked by adding 15N-labeled substrates into the bacterial cultures prior to mass spectrometry.

We were unable to measure the in situ concentrations of the individual components of DON used in oxidation experiments, other than urea. Based on previous measurements made in the SAB (Lu et al. 2014; Liu et al. 2015), we assumed concentrations of 1 nM and 0.25 nM for DAE, DAP and PUT, and 10 nM and 5 nM for GLU and ARG, at inshore and mid-shelf/shelf-break/oceanic stations, respectively. Rates of polyamine and amino acid oxidation reported below should therefore be considered potential rates, as amendments as low as 10–50 nM are likely to increase substrate concentrations substantially above in situ. Initial substrate 15N activity was calculated using isotope mass balance using the known concentration and 15N activity of the labeled substrates added and assuming the concentrations described above and natural abundance 15N activity (i.e., 0.3663 atom% 15N).

Data Processing Description

BCO-DMO Processing Notes:
- added conventional header with dataset name, PI name, version date
- modified parameter names to conform with BCO-DMO naming conventions
- tansposed the table
- combined continuous cells

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Data Files

File
qpcr.csv(Comma Separated Values (.csv), 1.25 KB) MD5:faa6013d744d2659229b6d93a374f83f Primary data file for dataset ID 767141

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Related Publications

Beman, J. M., Chow, C.-E., King, A. L., Feng, Y., Fuhrman, J. A., Andersson, A., … Hutchins, D. A. (2011). Global declines in oceanic nitrification rates as a consequence of ocean acidification. Proceedings of the National Academy of Sciences, 108(1), 208–213. doi:10.1073/pnas.1011053108
Methods

Beman, J. M., Popp, B. N., & Francis, C. A. (2008). Molecular and biogeochemical evidence for ammonia oxidation by marine Crenarchaeota in the Gulf of California. The ISME Journal, 2(4), 429–441. doi:10.1038/ismej.2007.118
Methods

Damashek, J., Tolar, B. B., Liu, Q., Okotie-Oyekan, A. O., Wallsgrove, N. J., Popp, B. N., & Hollibaugh, J. T. (2018). Microbial oxidation of nitrogen supplied as selected organic nitrogen compounds in the South Atlantic Bight. Limnology and Oceanography. doi:10.1002/lno.11089
Methods

Mincer, T. J., Jensen, P. R., Kauffman, C. A., & Fenical, W. (2002). Widespread and Persistent Populations of a Major New Marine Actinomycete Taxon in Ocean Sediments. Applied and Environmental Microbiology, 68(10), 5005–5011. doi:10.1128/aem.68.10.5005-5011.2002 https://doi.org/10.1128/AEM.68.10.5005-5011.2002
Methods

Mosier, A. C., & Francis, C. A. (2011). Determining the Distribution of Marine and Coastal Ammonia-Oxidizing Archaea and Bacteria Using a Quantitative Approach. Methods in Enzymology, 205–221. doi:10.1016/b978-0-12-381294-0.00009-2 https://doi.org/10.1016/B978-0-12-381294-0.00009-2

Methods

Santoro, A. E., Sakamoto, C. M., Smith, J. M., Plant, J. N., Gehman, A. L., Worden, A. Z., … Casciotti, K. L. (2013). Measurements of nitrite production in and around the primary nitrite maximum in the central California Current. Biogeosciences, 10(11), 7395–7410. doi:10.5194/bg-10-7395-2013
Methods

Suzuki, M. T., Taylor, L. T., & DeLong, E. F. (2000). Quantitative Analysis of Small-Subunit rRNA Genes in Mixed Microbial Populations via 5’-Nuclease Assays. Applied and Environmental Microbiology, 66(11), 4605–4614. doi:10.1128/aem.66.11.4605-4614.2000 https://doi.org/10.1128/AEM.66.11.4605-4614.2000
Methods

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Parameters

Parameter	Description	Units
Probe	Probe. All probes contained a 5' FAM tag and a 3' BHQ1 quencher.	unitless
Forward_primer	forward primer	unitless
Reverse_primer	reverse primer	unitless
Cycling_conditions	Cycling conditions	unitless
Efficiency	efficiency (%)	unitless
Limit_of_Detection_template	Limit of detection of template	copies per microliter of template
Limit_of_Detection_sample	Limit of detection of sample	copies per liter of sample
Number_plates_run	number of plates run	count
Reference	citation for values	unitless
qPCR_Parameters	qPCR parameters	unitless

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Instruments

Dataset-specific Instrument Name	Bran and Luebbe AA3 autoanalyzer
Generic Instrument Name	Bran Luebbe AA3 AutoAnalyzer
Dataset-specific Description	Dissolved nitrate (NO_3^-), nitrite (NO_2^-), phosphate (PO_4^{3-}), and silicate (SiO_4^{4-}) were measured using a Bran and Luebbe AA3 autoanalyzer as described previously (Wilkerson et al. 2015).
Generic Instrument Description	Bran Luebbe AA3 AutoAnalyzer See the description from the manufacturer.

Dataset-specific Instrument Name	Finnigan MAT-252 isotope ratio mass spectrometer
Generic Instrument Name	Isotope-ratio Mass Spectrometer
Dataset-specific Description	The δ15N values of the N2O produced were measured using a Finnigan MAT-252 isotope ratio mass spectrometer coupled with a modified GasBench II interface
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	C1000 Touch Thermal Cycler
Generic Instrument Name	qPCR Thermal Cycler
Dataset-specific Description	All reactions (25 µL total volume) were run in triplicate on a C1000 Touch Thermal Cycler equipped with a CFX96 Real-Time System (Bio- Rad), using either the iTaq Universal Green SYBR Mix (Bio-Rad) or the Platinum qPCR SuperMix-UDG (Thermo Fisher).
Generic Instrument Description	An instrument for quantitative polymerase chain reaction (qPCR), also known as real-time polymerase chain reaction (Real-Time PCR).

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Deployments

SAV-17-16

Website	https://www.bco-dmo.org/deployment/767055
Platform	R/V Savannah
Start Date	2017-08-16
End Date	2017-08-19

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Project Information

Collaborative Research: Direct Oxidation of Organic Nitrogen by Marine Ammonia Oxidizing Organisms (DON Oxidation)

Coverage: Coastal waters and the South Atlantic Bight continental shelf from Savannah GA out to the shelf break (SAV 17-16, UNOLS STR _104733, Marsden Grid 117, Navy Ops NA06), coastal waters around Sapelo Island, Georgia USA

NSF Abstract:

Nitrogen is an essential nutrient for phytoplankton that often limits primary production in the ocean, and its availability therefore plays a key role in global ocean productivity. The amounts and form in which nitrogen exist are controlled by microorganisms. One microorganism-mediated process is known as nitrification, which oxidizes ammonia or ammonium to nitrite and nitrite to nitrate, nitrate being the bioavailable form of nitrogen. While this is the well-accepted process of nitrification, preliminary results strongly suggest that a nitrogen-containing compound know as polyamine nitrogen may be directly converted by some microorganisms to nitrate. However, the importance of this process for global biogeochemical nitrogen cycling is unknown. The goal of this study is to evaluate the biogeochemical significance of direct oxidation of polyamine nitrogen, as a model organic nitrogen compound, to nitrification compared to canonical nitrification of ammonia. The project will result in training a postdoctoral researcher and provide opportunities for undergraduates to gain hands-on experience with research on microbial geochemistry and coastal ecosystem processes. Project personnel will also work with the Georgia Coastal Ecosystems Long-Term Ecological Research program to engage a K-12 science teacher in the project.

Ammonia oxidation is a key step in the process of converting fixed nitrogen to dinitrogen gas and thus is central to the global nitrogen cycle and to removing excess fixed nitrogen from coastal waters with high concentrations of nutrients. Recent research has shown that Thaumarchaeota play a major role in ammonia oxidation in the ocean. Experiments with enrichment cultures and coastal water samples where ammonia oxidizing archaea are the dominant ammonia oxidizers, show that some forms of organic nitrogen may be oxidized directly to nitrogen oxides without first being regenerated as ammonium. Of the substrates tested, polyamine and particularly putrescine nitrogen appear to be oxidized directly to nitrogen oxides, while amino acid and urea nitrogen is first regenerated as ammonium and then oxidized. The investigators will examine this process in detail over three years using enrichment cultures and experiments conducted with coastal bacterioplankton. Specifically, they will aim to better understand 1) the consequences of this novel process to ocean geochemistry, 2) the fate of the carbon present in polyamines, 3) what organisms are responsible for the direct oxidation, and 4) the chemical characteristics of the organic nitrogen compounds accessible to direct oxidation.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1537995
NSF Division of Ocean Sciences (NSF OCE)	OCE-1538677

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