

# Dissolved Organic Nitrogen oxidation collected on 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/767048>

**Data Type:** Cruise Results

**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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| <a href="#">Popp, Brian N.</a>       | University of Hawai'i (UH)                          | Co-Principal Investigator |
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## Abstract

This dataset contains the results of analyses related to ammonia oxidation rates, including oxidation rates of  $15\text{N}$  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  $\leq 500$  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  $\leq 1$  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 ( $K_d$ ) was calculated from plots of  $\ln(\text{PAR})$  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 qPCR\_Parameters at <https://www.bco-dmo.org/dataset/767141>).

## Methods & Sampling

### Nutrient analysis

Nutrient samples were filtered through 0.22  $\mu\text{m}$  pore size Durapore GVWP filters (Millipore Sigma) and frozen at  $-20^\circ\text{C}$  immediately after collection, then stored at  $-80^\circ\text{C}$  until analysis. Dissolved nitrate ( $\text{NO}_3^-$ ), nitrite ( $\text{NO}_2^-$ ), phosphate ( $\text{PO}_4^{3-}$ ), and silicate ( $\text{SiO}_4^{4-}$ ) 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  $^{15}\text{N}$ -labeled substrates (98–99%  $^{15}\text{N}$ , Cambridge Isotope Laboratories) to measure the oxidation of N supplied as  $\text{NH}_4^+$ , urea, 1,2-diaminoethane (DAE), 1,3-diaminopropane (DAP), 1,4-diaminobutane (putrescine, PUT), L-glutamic acid (GLU), and L-arginine (ARG).  $^{15}\text{N}$  oxidation from  $\text{NH}_4^+$ , urea, PUT, and GLU were measured extensively, whereas  $^{15}\text{N}$  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  $\text{NH}_4^+$  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,  $^{15}\text{N}$ -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  $\text{pK}_a$ ) 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  $^{15}\text{N}$ -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  $18^\circ\text{C}$  in the dark. Incubation bottles were sampled for  $^{15}\text{N}$  analysis immediately after substrate addition and again after a period of  $\sim 24$  h.  $^{15}\text{N}$  samples were subsampled into 50 mL polypropylene centrifuge tubes, frozen at  $-20^\circ\text{C}$ , and stored at  $-80^\circ\text{C}$  until analysis. The  $^{15}\text{N}/^{14}\text{N}$  ratios of the  $\text{NO}_3^-$  plus  $\text{NO}_2^-$  ( $\text{NO}_x$ ) pools ( $\delta^{15}\text{NNO}_x$ ) in the samples were measured using the bacterial denitrifier method to convert  $\text{NO}_x$  to nitrous oxide ( $\text{N}_2\text{O}$ ; Sigman et al. 2001). The  $\delta^{15}\text{N}$  values of the  $\text{N}_2\text{O}$  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  $^{15}\text{N}$ , the amount of the N added as the  $^{15}\text{N}$  spike (in  $\mu\text{M}$ ) was multiplied by the number of moles of  $^{15}\text{N}$  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  $\alpha$ -amino position and three in the guanidine structure of its R-group). Abiotic oxidation of organic N was assessed by measuring  $^{15}\text{NOX}$  production following  $^{15}\text{N}$  amendment and incubation of 0.22  $\mu\text{m}$  filtered seawater (as described above), and potential metabolism of DON by the denitrifying bacteria used to convert NOX to  $\text{N}_2\text{O}$  was checked by adding  $^{15}\text{N}$ -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  $^{15}\text{N}$  activity was calculated using isotope mass balance using the known concentration and  $^{15}\text{N}$  activity of the labeled substrates added and assuming the concentrations described above and natural abundance  $^{15}\text{N}$  activity (i.e., 0.3663 atom%  $^{15}\text{N}$ ).

## 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
- converted latitude and longitude values from degrees minutes seconds to degrees and appended a new column (lat, lon).
- converted Date column to ISO convention and appended ISO\_Date column for those values.
- replaced "x 10" with "e" to signify power of 10.
- split columns containing " $\pm$ " into two columns, one for value and one for  $\pm$  value. Appended "\_sd" to the  $\pm$  value column.
- converted "nan" and blank cells to "nd".

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## Data Files

| File   |
|--|
| <b>DON.csv</b> (Comma Separated Values (.csv), 8.79 KB)<br>MD5:20a78c4d254911d58341260d712afb36<br>Primary data file for dataset ID 767048 |

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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](https://doi.org/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](https://doi.org/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](https://doi.org/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](https://doi.org/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                         |
|-----------|--|-------------------------------|
| Cruise_ID | R2R catalog identifier for this cruise   | unitless                      |
| Sta       | Station Identifier. Marsh Landing is on the Duplin River a tidal channel adjacent to Sapelo Island GA.   | unitless                      |
| Region    | Arbitrary assignment of stations to zones in the study area identified by location and water properties: I = Inshore; M = midshelf; S = Shelf-break; O = Oceanic | unitless                      |
| depth     | Depth sampled in meters  | meters                        |
| lon       | degrees longitude with positive values eastward  | decimal degrees               |
| lat       | degrees latitude with positive values northward  | decimal degrees               |
| Latitude  | Latitude in degrees and decimal minutes N  | degrees and decimal minutes N |
| Longitude | Longitude in degrees and decimal minutes W   | degrees and decimal minutes W |
| Date      | Sampling date: MM/DD/YYYY  | unitless                      |
| ISO_Date  | Date following the ISO convention of YYYY-MM-DD  | unitless                      |

|                        |  |                           |
|------------------------|--|---------------------------|
| Bacterial_16S_rRNA     | Concentration of genes for Bacteria 16S rRNA determined by qPCR (see qPCR parameter table) in units of copies L-1; Blank Cells = no data; either not sampled or the sample was lost; BLD = Below Limit of Detection. See qPCR parameter table. qPCR data are from single biological replicates; means of triplicate qPCR reactions. Limit of detection is given as copies per mL of template DNA; the limit thus varies by sample depending on volume filtered; extract volume and template volume per per reaction. The limits given are from this sample set assuming the minimum detectable concentration in the template used and 1 uL of template per reaction. They are thus conservative as some reactions were run with 10 uL of template.   | copies per liter filtered |
| Thaumarchaeal_16S_rRNA | Concentration of genes for Marine Group 1 Archaea (Thaumarchaeota) 16S rRNA determined by qPCR (see qPCR parameter table) in units of copies L-1 Blank Cells = no data either not sampled or the sample was lost; BLD = Below Limit of Detection. See qPCR parameter table. qPCR data are from single biological replicates; means of triplicate qPCR reactions. Limit of detection is given as copies per mL of template DNA; the limit thus varies by sample depending on volume filtered extract volume and template volume per per reaction. The limits given are from this sample set assuming the minimum detectable concentration in the template used and 1 uL of template per reaction. They are thus conservative as some reactions were run with 10 uL of template.                           | copies per liter filtered |
| WCA_amoA               | Concentration of genes for Archaeal ammonia monooxygenase subunit A from the Water Column A clade; determined by qPCR (see qPCR parameter table) in units of copies L-1; Blank Cells = no data; either not sampled or the sample was lost; BLD = Below Limit of Detection. See qPCR parameter table. qPCR data are from single biological replicates; means of triplicate qPCR reactions. Limit of detection is given as copies per mL of template DNA; the limit thus varies by sample depending on volume filtered; extract volume and template volume per per reaction. The limits given are from this sample set assuming the minimum detectable concentration in the template used and 1 uL of template per reaction. They are thus conservative as some reactions were run with 10 uL of template. | copies per liter filtered |
| WCB_amoA               | Concentration of genes for Archaeal ammonia monooxygenase subunit A from the Water Column A clade; determined by qPCR (see qPCR parameter table) in units of copies L-1; "Blank Cells = no data either not sampled or the sample was lost; BLD = Below Limit of Detection. See qPCR parameter table. qPCR data are from single biological replicates means of triplicate qPCR reactions. Limit of detection is given as copies per mL of template DNA; the limit thus varies by sample depending on volume filtered extract volume and template volume per per reaction. The limits given are from this sample set assuming the minimum detectable concentration in the template used and 1 uL of template per reaction. They are thus conservative as some reactions were run with 10 uL of template.   | copies per liter filtered |
|                        |  |                           |

|                      |  |                             |
|----------------------|--|-----------------------------|
| Nitrospina_16S_rRNA  | Concentration of genes for Nitrospina 16S rRNA determined by qPCR (see qPCR parameter table) in units of copies L-1; Blank Cells = no data; either not sampled or the sample was lost; BLD = Below Limit of Detection. See qPCR parameter table. qPCR data are from single biological replicates; means of triplicate qPCR reactions. Limit of detection is given as copies per mL of template DNA; the limit thus varies by sample depending on volume filtered; extract volume and template volume per per reaction. The limits given are from this sample set assuming the minimum detectable concentration in the template used and 1 uL of template per reaction. They are thus conservative as some reactions were run with 10 uL of template. | copies per liter filtered   |
| N15_added            | Final concentration of uniformly 15N labeled test substrates   | nanomoles per liter         |
| Ammonia_Oxidation    | Ammonia oxidation rate determined from conversion of 15N-labeled ammonium to 15N-labeled nitrite plus nitrate; BLD = Below Limit of Detection. 15N data are mean $\pm$ S.E.M. of duplicate biological replicates (see Damashek et al. 2018 for details) . Based on a propagation of error calculation; our conservative estimate of the precision of 15N measurements $\pm 4\%$ for samples at natural abundance and $\pm 5.2\%$ for samples artificially enriched with carrier 15N. Our limit of detection was similar to that reported by Santoro et al. (2013) and Beman et al. (2011).   | nanomoles per liter per day |
| Ammonia_Oxidation_sd | Deviation of ammonia oxidation rate determined from conversion of 15N-labeled ammonium to 15N-labeled nitrite plus nitrate; BLD = Below Limit of Detection. 15N data are mean $\pm$ S.E.M. of duplicate biological replicates (see Damashek et al. 2018 for details) . Based on a propagation of error calculation; our conservative estimate of the precision of 15N measurements $\pm 4\%$ for samples at natural abundance and $\pm 5.2\%$ for samples artificially enriched with carrier 15N. Our limit of detection was similar to that reported by Santoro et al. (2013) and Beman et al. (2011).  | nanomoles per liter per day |
| N15_ox_from_PUT      | Oxidation rate of 15N from putrescine (1;4 diamino butane) determined from conversion of the 15N label to 15N-labeled nitrite plus nitrate; BLD = Below Limit of Detection. 15N data are mean $\pm$ S.E.M. of duplicate biological replicates (see Damashek et al. 2018 for details) . Based on a propagation of error calculation our conservative estimate of the precision of 15N measurements $\pm 4\%$ for samples at natural abundance and $\pm 5.2\%$ for samples artificially enriched with carrier 15N. Our limit of detection was similar to that reported by Santoro et al. (2013) and Beman et al. (2011).   | nanomoles per liter per day |
|                      |  |                             |

|                     |   |                             |
|---------------------|---|-----------------------------|
| N15_ox_from_PUT_sd  | Deviation of oxidation rate of 15N from putrescine (1;4 diamino butane) determined from conversion of the 15N label to 15N-labeled nitrite plus nitrate; BLD = Below Limit of Detection. 15N data are mean $\pm$ S.E.M. of duplicate biological replicates (see Damashek et al. 2018 for details) . Based on a propagation of error calculation our conservative estimate of the precision of 15N measurements $\pm 4\%$ for samples at natural abundance and $\pm 5.2\%$ for samples artificially enriched with carrier 15N. Our limit of detection was similar to that reported by Santoro et al. (2013) and Beman et al. (2011). | nanomoles per liter per day |
| N15_ox_from_GLU     | Oxidation rate of 15N from L-glutamate determined from conversion of the 15N label to 15N-labeled nitrite plus nitrate; BLD = Below Limit of Detection. 15N data are mean $\pm$ S.E.M. of duplicate biological replicates (see Damashek et al. 2018 for details) . Based on a propagation of error calculation; our conservative estimate of the precision of 15N measurements $\pm 4\%$ for samples at natural abundance and $\pm 5.2\%$ for samples artificially enriched with carrier 15N. Our limit of detection was similar to that reported by Santoro et al. (2013) and Beman et al. (2011).                                 | nanomoles per liter per day |
| N15_ox_from_GLU_sd  | deviation of oxidation rate of 15N from L-glutamate determined from conversion of the 15N label to 15N-labeled nitrite plus nitrate; BLD = Below Limit of Detection. 15N data are mean $\pm$ S.E.M. of duplicate biological replicates (see Damashek et al. 2018 for details) . Based on a propagation of error calculation; our conservative estimate of the precision of 15N measurements $\pm 4\%$ for samples at natural abundance and $\pm 5.2\%$ for samples artificially enriched with carrier 15N. Our limit of detection was similar to that reported by Santoro et al. (2013) and Beman et al. (2011).                    | nanomoles per liter per day |
| N15_ox_from_UREA    | Oxidation rate of 15N from urea determined from conversion of the 15N label to 15N-labeled nitrite plus nitrate BLD = Below Limit of Detection. 15N data are mean $\pm$ S.E.M. of duplicate biological replicates (see Damashek et al. 2018 for details) . Based on a propagation of error calculation; our conservative estimate of the precision of 15N measurements $\pm 4\%$ for samples at natural abundance and $\pm 5.2\%$ for samples artificially enriched with carrier 15N. Our limit of detection was similar to that reported by Santoro et al. (2013) and Beman et al. (2011).   | nanomoles per liter per day |
| N15_ox_from_UREA_sd | Deviation of oxidation rate of 15N from urea determined from conversion of the 15N label to 15N-labeled nitrite plus nitrate BLD = Below Limit of Detection. 15N data are mean $\pm$ S.E.M. of duplicate biological replicates (see Damashek et al. 2018 for details) . Based on a propagation of error calculation; our conservative estimate of the precision of 15N measurements $\pm 4\%$ for samples at natural abundance and $\pm 5.2\%$ for samples artificially enriched with carrier 15N. Our limit of detection was similar to that reported by Santoro et al. (2013) and Beman et al. (2011).                            | nanomoles per liter per day |
|                     |   |                             |

|                    |  |                             |
|--------------------|--|-----------------------------|
| N15_ox_from_DAE    | Oxidation rate of 15N from 1;2 diamino ethane determined from conversion of the 15N label to 15N-labeled nitrite plus nitrate; BLD = Below Limit of Detection. 15N data are mean $\pm$ S.E.M. of duplicate biological replicates (see Damashek et al. 2018 for details) . Based on a propagation of error calculation; our conservative estimate of the precision of 15N measurements $\pm 4\%$ for samples at natural abundance and $\pm 5.2\%$ for samples artificially enriched with carrier 15N. Our limit of detection was similar to that reported by Santoro et al. (2013) and Beman et al. (2011).               | nanomoles per liter per day |
| N15_ox_from_DAE_sd | Deviation of oxidation rate of 15N from 1;2 diamino ethane determined from conversion of the 15N label to 15N-labeled nitrite plus nitrate; BLD = Below Limit of Detection. 15N data are mean $\pm$ S.E.M. of duplicate biological replicates (see Damashek et al. 2018 for details) . Based on a propagation of error calculation; our conservative estimate of the precision of 15N measurements $\pm 4\%$ for samples at natural abundance and $\pm 5.2\%$ for samples artificially enriched with carrier 15N. Our limit of detection was similar to that reported by Santoro et al. (2013) and Beman et al. (2011).  | nanomoles per liter per day |
| N15_ox_from_DAP    | Oxidation rate of 15N from 1;3 diamino propane determined from conversion of the 15N label to 15N-labeled nitrite plus nitrate; BLD = Below Limit of Detection. 15N data are mean $\pm$ S.E.M. of duplicate biological replicates (see Damashek et al. 2018 for details) . Based on a propagation of error calculation; our conservative estimate of the precision of 15N measurements $\pm 4\%$ for samples at natural abundance and $\pm 5.2\%$ for samples artificially enriched with carrier 15N. Our limit of detection was similar to that reported by Santoro et al. (2013) and Beman et al. (2011).              | nanomoles per liter per day |
| N15_ox_from_DAP_sd | Deviation of oxidation rate of 15N from 1;3 diamino propane determined from conversion of the 15N label to 15N-labeled nitrite plus nitrate; BLD = Below Limit of Detection. 15N data are mean $\pm$ S.E.M. of duplicate biological replicates (see Damashek et al. 2018 for details) . Based on a propagation of error calculation; our conservative estimate of the precision of 15N measurements $\pm 4\%$ for samples at natural abundance and $\pm 5.2\%$ for samples artificially enriched with carrier 15N. Our limit of detection was similar to that reported by Santoro et al. (2013) and Beman et al. (2011). | nanomoles per liter per day |
| N15_ox_from_ARG    | Oxidation rate of 15N from L-arginine determined from conversion of the 15N label to 15N-labeled nitrite plus nitrate; BLD = Below Limit of Detection. 15N data are mean $\pm$ S.E.M. of duplicate biological replicates (see Damashek et al. 2018 for details) . Based on a propagation of error calculation our conservative estimate of the precision of 15N measurements $\pm 4\%$ for samples at natural abundance and $\pm 5.2\%$ for samples artificially enriched with carrier 15N. Our limit of detection was similar to that reported by Santoro et al. (2013) and Beman et al. (2011).                        | nanomoles per liter per day |
|                    |  |                             |



|                    |  |                             |
|--------------------|--|-----------------------------|
| N15_ox_from_ARG_sd | Deviation of oxidation rate of 15N from L-arginine determined from conversion of the 15N label to 15N-labeled nitrite plus nitrate; BLD = Below Limit of Detection. 15N data are mean $\pm$ S.E.M. of duplicate biological replicates (see Damashek et al. 2018 for details) . Based on a propagation of error calculation our conservative estimate of the precision of 15N measurements $\pm 4\%$ for samples at natural abundance and $\pm 5.2\%$ for samples artificially enriched with carrier 15N. Our limit of detection was similar to that reported by Santoro et al. (2013) and Beman et al. (2011). | nanomoles per liter per day |
| Nitrate            | Concentration of nitrate determined by cadmium reduction to nitrite followed by subtraction nitrite already present in the sample; Samples run on an autoanalyzer by Francis Wilkerson's lab at San Francisco State University   | micromoles per liter        |
| Nitrite            | Concentration of nitrite determined by autoanalyzer; Samples run on an autoanalyzer by Francis Wilkerson's lab at San Francisco State University   | micromoles per liter        |
| Ammonium           | Concentration of ammonium determined by autoanalyzer; Samples run on an autoanalyzer by Francis Wilkerson's lab at San Francisco State University  | micromoles per liter        |
| Urea               | Concentration of urea determined by the carboxythiazole method; Samples run in Hollibaugh lab at UGA   | micromoles per liter        |
| Silicate           | Concentration of silicate determined by autoanalyzer; Samples run on an autoanalyzer by Francis Wilkerson's lab at San Francisco State University  | micromoles per liter        |
| Phosphate          | Concentration of phosphate determined by autoanalyzer; Samples run on an autoanalyzer by Francis Wilkerson's lab at San Francisco State University   | micromoles per liter        |
| Temperature        | Water temperature in centigrade degrees at the depth sampled measured by the environmental sensing package on the sampling rosette   | degrees centigrade          |
| Salinity           | Salinity at the depth sampled derived from temperature and conductivity measured by the environmental sensing package on the sampling rosette  | PSU                         |
| Diss_Oxygen        | Dissolved oxygen concentration at the depth sampled measured by the environmental sensing package on the sampling rosette  | milliliters per liter       |
|                    |  |                             |

|                |  |                       |
|----------------|--|-----------------------|
| Relative_Fluor | Relative Fluorescence measured by the fluorometer on the Niskin rosette sampler converted to mg Chl a L-1 using a regression equation based on extracted chlorophyll data: $\text{Chl a} = 1.7869\text{RF} - 2.2541$ ; $R^2 = 0.83$    | relative fluorescence |
| Atten_Coeff    | PAR attenuation coefficient $k_z$ in $\text{m}^{-1}$ calculated as the slope of $\log(\text{PAR})$ vs depth  | per meter             |
| comment        | identifier for the comment. comment value a signifies Environmental data, bacterial 16S, thaumarchaeal 16S, AOB amoA, ammonia oxidation, and urea oxidation data were reported previously in Liu et al. (2015) or Tolar et al. (2017); | unitless              |

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## Instruments

|   |  |
|---|--|
| <b>Dataset-specific Instrument Name</b> | Bran and Luebbe AA3 autoanalyzer   |
| <b>Generic Instrument Name</b>          | Bran Luebbe AA3 AutoAnalyzer   |
| <b>Dataset-specific Description</b>     | Dissolved nitrate ( $\text{NO}_3^-$ ), nitrite ( $\text{NO}_2^-$ ), phosphate ( $\text{PO}_4^{3-}$ ), and silicate ( $\text{SiO}_4^{4-}$ ) were measured using a Bran and Luebbe AA3 autoanalyzer as described previously (Wilkerson et al. 2015). |
| <b>Generic Instrument Description</b>   | Bran Luebbe AA3 AutoAnalyzer See the description from the manufacturer.  |

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|---|--|
| <b>Dataset-specific Instrument Name</b> | Finnigan MAT-252 isotope ratio mass spectrometer   |
| <b>Generic Instrument Name</b>          | Isotope-ratio Mass Spectrometer  |
| <b>Dataset-specific Description</b>     | The $\delta^{15}\text{N}$ values of the $\text{N}_2\text{O}$ produced were measured using a Finnigan MAT-252 isotope ratio mass spectrometer coupled with a modified GasBench II interface             |
| <b>Generic Instrument Description</b>   | 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). |

|   |   |
|---|---|
| <b>Dataset-specific Instrument Name</b> | C1000 Touch Thermal Cycler  |
| <b>Generic Instrument Name</b>          | qPCR Thermal Cycler   |
| <b>Dataset-specific Description</b>     | All reactions (25 $\mu$ L 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). |
| <b>Generic Instrument Description</b>   | An instrument for quantitative polymerase chain reaction (qPCR), also known as real-time polymerase chain reaction (Real-Time PCR).   |

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| <b>Dataset-specific Instrument Name</b> | Sea-Bird SBE25 CTD   |
| <b>Generic Instrument Name</b>          | Sea-Bird SBE 25 Sealogger CTD  |
| <b>Dataset-specific Description</b>     | 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).  |
| <b>Generic Instrument Description</b>   | The Sea-Bird SBE 25 SEALOGGER CTD is battery powered and is typically used to record data in memory, eliminating the need for a large vessel, electrical sea cable, and on-board computer. All SBE 25s can also operate in real-time, transmitting data via an opto-isolated RS-232 serial port. Temperature and conductivity are measured by the SBE 3F Temperature sensor and SBE 4 Conductivity sensor (same as those used on the premium SBE 9plus CTD). The SBE 25 also includes the SBE 5P (plastic) or 5T (titanium) Submersible Pump and TC Duct. The pump-controlled, TC-ducted flow configuration significantly reduces salinity spiking caused by ship heave, and in calm waters allows slower descent rates for improved resolution of water column features. Pressure is measured by the modular SBE 29 Temperature Compensated Strain-Gauge Pressure sensor (available in eight depth ranges to suit the operating depth requirement). The SBE 25's modular design makes it easy to configure in the field for a wide range of auxiliary sensors, including optional dissolved oxygen (SBE 43), pH (SBE 18 or SBE 27), fluorescence, transmissivity, PAR, and optical backscatter sensors. More information from Sea-Bird Electronics: <a href="http://www.seabird.com">http://www.seabird.com</a> . |

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## Deployments

### SAV-17-16

|                   |   |
|-------------------|---|
| <b>Website</b>    | <a href="https://www.bco-dmo.org/deployment/767055">https://www.bco-dmo.org/deployment/767055</a> |
| <b>Platform</b>   | R/V Savannah  |
| <b>Start Date</b> | 2017-08-16  |
| <b>End Date</b>   | 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 known 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                       |
|--|-----------------------------|
| <a href="#">NSF Division of Ocean Sciences (NSF OCE)</a> | <a href="#">OCE-1537995</a> |
| <a href="#">NSF Division of Ocean Sciences (NSF OCE)</a> | <a href="#">OCE-1538677</a> |

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