# Denitrification and DNRA data from Little Lagoon, Alabama collected from 2012-2013

Website: https://www.bco-dmo.org/dataset/723966 Data Type: Other Field Results Version: 1 Version Date: 2018-01-16

#### Project

» <u>Groundwater Discharge, Benthic Coupling and Microalgal Community Structure in a Shallow Coastal Lagoon</u> (LittleLagoonGroundwater)

Contributors	Affiliation	Role
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#### Abstract

Denitrification and DNRA data from Little Lagoon, Alabama collected from 2012-2013

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# Coverage

Spatial Extent: Lat:30.241929 Lon:-87.773756 Temporal Extent: 2012 - 2013

# **Dataset Description**

Denitrification and DNRA data from Little Lagoon, Alabama.

#### Methods & Sampling

Little Lagoon is a shallow coastal lagoon that is tidally connected to the Gulf of Mexico but has no riverine inputs. The water in the lagoon is replenished solely from precipitation and groundwater inputs primarily on the East end (Su et al. 2012). Because of the rapid development in Baldwin County, a large amount of NO3- enters the Little Lagoon system through SGD (Murgulet & Tick 2008). In this region, there can be rapid changes in the depth to groundwater (Fig. 4.1 inset) and episodic SGD inputs to the lagoon (Su et al.2013). Within the lagoon, three sites were selected (East, Mouth, and West) to represent the gradient that exists across the lagoon from the input of groundwater. Sites were sampled on a near-monthly basis from February 2012 to February 2013.

#### DNRA

Approximately 1 L of outflow water was collected from the inflow water and each core forDNRA analysis.

Appropriate sample volume was determined after NH4 + nutrient analysis and expected atom % enrichment.  $\delta$ 15N-NH4 + was measured in samples, constructed blanks, and standards that bracketed the NH4 + concentration of the samples following a modified ammonium diffusion procedure (Holmes et al. 1998) that collects NH4 + dissolved in water by converting NH4 + to NH3 under basic conditions and then traps the NH3 on an acidified glass fiber filter. Non diffused standards were prepared according to Stark and Hart (1996) to account for blank corrections. After 15N analysis on a Europa Scientific SL-2020 system (Stable Isotope Lab, Utah State University), DNRA was calculated from the production rate of 15NH4 + (p15NH4 +) during the incubation according to Christensen et. al (2000): (7) where is the production of 15N-NH4 + and D14 and D15 are the denitrification rates of 14N-NO3 - and 15N-NO3 -, respectively. This assumes that DNRA takes place in the same sediment layers as denitrification and that the 15N labeling of NO3 - being reduced to NH4 + equals the 15N labeling of NO3 - being reduced to N2 (Christensen et al. 2000).

#### Denitrification and anammox from slurry assays

Volumetric rates of denitrification, anammox, and the relative contribution of anammox to gross N2 production were determined from sediment slurry incubations. Slurry rates for depth-integrated sediments (0-50 mm) were prepared in Exetainers (Thamdrup & Dalsgaard 2002) with artificial seawater (ASW) (70.2g NaCl, 3.0g KCl, 49.4 g MgSO4\*7H2O, 5.8g CaCl2\*2H2O L-1) constructed at a salinity of 52 and diluted with deionized water to match the salinity of each site. After dilution, homogenized sediment from 0 to 50 mm was added to the ASW and the incubation bottle was sparged with N2 and amended with 100  $\mu$ mol L-1 Na15NO3 - (99 atom %). Sediment slurry was dispensed to 12 ml Exetainers, yielding approximately 1 ml of sediment and 11 ml ASW with no headspace. For each site, 12 vials total were incubated with three vials stopped at time points 0 to 36 h. Incubations were stopped by adding 250  $\mu$ L of ZnCl2 and resealing the vials without headspace.

Excess 29N2 and 30N2 concentrations for intact core and slurry incubations were calculated from dissolved 29N2:28N2 and 30N2:28N2 measured using a MIMS. Rates of excess 29N2 (p29) and 30N2 (p30) production were calculated from the flux calculation described above. Rates of ambient 14N2 production (p14) in core incubations with 15NO3 - tracer addition were determined as (Nielsen 1992, Risgaard-Petersen et al. 2003):

(1)  $p14 = 2 \times r14 \pm [p29 + p30 \pm (1 - r16)]$ 

The 14N:15N ratio of NO3 - undergoing reduction to N2 (r14) was determined as follows:

(2) 
$$r14 = [R29 \times (1 - ra) - ra] \times (2 - ra) ^{-1}$$

where R29 was the ratio of p29 to p30 determined for the cores and ra was the relative contribution of anammox to gross N2 production determined in vial slurry incubations. Gross denitrification and anammox rates within intact sediment cores with 15NO3 - tracer addition were calculated as follows:

(3) denitrification =  $p14 \pm (1 - ra)$ 

(4) anammox = 
$$p14 \pm ra$$

Denitrification stimulated by the added 15N-NO3 - (D15) was calculated from the classical IPT (Nielsen 1992) and these amended rates are a measure of the denitrification capacity under field conditions when NO3 - is not limiting.

Rates of denitrification and anammox in vial slurry incubations with 15NO3 - amendments were calculated from the equations of Thamdrup and Dalsgaard (2002): (5) (6) where FN was the fraction of 15N in NO3 -. For months when anammox slurry incubations were not performed (August and November 2012), p14 is calculated as D14 from the IPT (Nielsen 1992). Potential denitrification and anammox rates were converted to an areal basis using the wet weight of the sediment in the slurry. All rates and fluxes pertaining to N species in this study were normalized to one atom N.

#### Additional methodology can be found in:

Bernard, Rebecca & Mortazavi, Behzad & A. Kleinhuizen, Alice. (2015). Dissimilatory nitrate reduction to ammonium (DNRA) seasonally dominates NO3– reduction pathways in an anthropogenically impacted sub-tropical coastal lagoon. Biogeochemistry. 125. 47-64. <u>10.1007/s10533-015-0111-6</u>.

Data were flagged as below detection limits if no measurable rates were returned after calculations. See equations in methodology section of:

Bernard, Rebecca & Mortazavi, Behzad & A. Kleinhuizen, Alice. (2015). Dissimilatory nitrate reduction to ammonium (DNRA) seasonally dominates NO3– reduction pathways in an anthropogenically impacted sub-tropical coastal lagoon. Biogeochemistry. 125. 47-64. <u>10.1007/s10533-015-0111-6</u>.

#### **Statistical Analysis**

To test the seasonal flux variability between sites in Little Lagoon, two-way ANOVAs with site and date as independent variables were performed. When data could not be transformed to meet ANOVA assumptions, Wilcoxon/Kruskal-Wallis nonparametric tests were used. When significant differences occurred, Tukey HSD or Steel-Dwass post hoc tests were used to determine significant interactions. A Principal component analysis (PCA) was conducted on all biogeochemical parameters to identify underlying multivariate components that may be influencing N fluxes. Spearman's rho correlation analysis was used to examine the relationship between the principal components and fluxes. Statistical significance of the data set was determined at  $\alpha$ =0.05 and error is reported as standard error. All statistical analyses were performed in SAS JMP 10 (SAS Institute Inc.).

#### **BCO-DMO Data Processing Notes:**

- Data reorganized into one table under one set of column names from both original files

- Units removed from column names
- Column names reformatted to meet BCO-DMO standards

- Information captured in original columns entered under column "Value\_Description" where units are also described

- Created column Year to describe to capture the metadata in the file name

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#### **Data Files**

File denitrification.csv(Comma Separated Values (.csv), 2.73 KB) MD5:9a180eb2232fee824157597a4d4163de Primary data file for dataset ID 723966

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# **Related Publications**

Bernard, R. J., Mortazavi, B., & Kleinhuizen, A. A. (2015). Dissimilatory nitrate reduction to ammonium (DNRA) seasonally dominates NO3 – reduction pathways in an anthropogenically impacted sub-tropical coastal lagoon. Biogeochemistry, 125(1), 47–64. doi:<u>10.1007/s10533-015-0111-6</u> *Methods* 

Murgulet, D., & Tick, G. R. (2008). Assessing the extent and sources of nitrate contamination in the aquifer system of southern Baldwin County, Alabama. Environmental Geology, 58(5), 1051–1065. doi:<u>10.1007/s00254-008-1585-5</u> *Methods* 

Nielsen, L. P. (1992). Denitrification in sediment determined from nitrogen isotope pairing. FEMS Microbiology Letters, 86(4), 357–362. doi:<u>10.1111/j.1574-6968.1992.tb04828.x</u> *Methods* 

Stark, J. M., & Hart, S. C. (1996). Diffusion Technique for Preparing Salt Solutions, Kjeldahl Digests, and Persulfate Digests for Nitrogen-15 Analysis. Soil Science Society of America Journal, 60(6), 1846. doi:<u>10.2136/sssaj1996.03615995006000060033x</u> *Methods* 

Su, N., Burnett, W.C., Eller, K.T., MacIntyre, H.L., Mortazavi, B., Leifer, J., Novoveska, L. (2012). Radon and radium isotopes, groundwater discharge and harmful algal blooms in Little Lagoon, Alabama. Interdisciplinary

Studies on Environmental Chemistry, 6, 329–337. *Methods* 

Su, N., Burnett, W.C., MacIntyre, H.L., Liefer, J.D., Peterson, R.N., Viso, R. (2013). Natural radon and radium isotopes for assessing groundwater discharge into Little Lagoon, AL: implications for harmful algal blooms. Estuaries Coasts, 1–18 *Methods* 

Thamdrup, B., & Dalsgaard, T. (2002). Production of N2 through Anaerobic Ammonium Oxidation Coupled to Nitrate Reduction in Marine Sediments. Applied and Environmental Microbiology, 68(3), 1312–1318. doi:10.1128/aem.68.3.1312-1318.2002 <a href="https://doi.org/10.1128/AEM.68.3.1312-1318.2002">https://doi.org/10.1128/AEM.68.3.1312-1318.2002</a> <a href="https://doi.org/10.1128/AEM.68.3.1312-1318.2002">https://doi.org/10.1128/AEM.68.3.1312-1318.2002</a> <a href="https://doi.org/10.1128/AEM.68.3.1312-1318.2002">https://doi.org/10.1128/AEM.68.3.1312-1318.2002</a> <a href="https://doi.org/10.1128/AEM.68.3.1312-1318.2002">https://doi.org/10.1128/AEM.68.3.1312-1318.2002</a> <a href="https://doi.org/10.1128/AEM.68.3.1312-1318.2002">https://doi.org/10.1128/AEM.68.3.1312-1318.2002</a> <a href="https://doi.org/10.1128/AEM.68.3.1312-1318.2002">https://doi.org/10.1128/AEM.68.3.1312-1318.2002</a>

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### Parameters

Parameter	Description	Units
Year	Year ID that samples were taken	unitless
Value_Description	Description of the measurment taken; description includes relevant units for each sample taken; Descriptions include: DIN:DIP = ratio of dissolved inorganic nitrogen to dissolved inorganic phosphate; Denitrification = Denitrification; p14 ambient denitrification = ambient denitrification rates; DNRA = dissimilatory nitrate reduction to ammonium; D15 denitrification = denitrification from added heavy labeled isotope.	unitless
Date	Month and day that samples were taken; MMM-DD	unitless
East	Denitrification and DNRA values collected at the East site; location of site is 30.253347, -87.724729	umol N m-2 hr-1; umol N m-2 d-1; mmol NH4+ m- 2 d-1
East_SE	Standard error of denitrification and DNRA values collected at the East site	umol N m-2 hr-1; umol N m-2 d-1; mmol NH4+ m- 2 d-1
Mouth	Denitrification and DNRA values collected at the Mouth site; location of site is 30.243683, -87.738407	umol N m-2 hr-1; umol N m-2 d-1; mmol NH4+ m- 2 d-1
Mouth_SE	Standard error of denitrification and DNRA values collected at the Mouth site	umol N m-2 hr-1; umol N m-2 d-1; mmol NH4+ m- 2 d-1
West	Denitrification and DNRA values collected at the West site; location of site is 30.247181, -87.767856	umol N m-2 hr-1; umol N m-2 d-1; mmol NH4+ m- 2 d-1
West_SE	Standard error of denitrification and DNRA values collected at the West site	umol N m-2 hr-1; umol N m-2 d-1; mmol NH4+ m- 2 d-1

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Instruments

Dataset-specific Instrument Name	Continuous Flow Analyzer
Generic Instrument Name	Continuous Flow Analyzer
Dataset-specific Description	Used to measure continuous flow rate
Generic Instrument Description	A sample is injected into a flowing carrier solution passing rapidly through small-bore tubing.

Dataset- specific Instrument Name	Europa Scientific SL-2020 system
Generic Instrument Name	Isotope-ratio Mass Spectrometer
Dataset- specific Description	Used for 15N analysis
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	MIMS
Generic Instrument Name	Membrane Inlet Mass Spectrometer
Dataset-specific Description	Used to measure dissolved gas
Generic Instrument Description	Membrane-introduction mass spectrometry (MIMS) is a method of introducing analytes into the mass spectrometer's vacuum chamber via a semipermeable membrane.

Dataset- specific Instrument Name	Multichannel proportioning pump
Generic Instrument Name	Pump
Dataset- specific Description	Used to filter sediment
Generic Instrument Description	A pump is a device that moves fluids (liquids or gases), or sometimes slurries, by mechanical action. Pumps can be classified into three major groups according to the method they use to move the fluid: direct lift, displacement, and gravity pumps

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# Deployments

LittleLagoon

Website	https://www.bco-dmo.org/deployment/528089
Platform	SmallBoat_FSU
Start Date	2010-04-05
End Date	2013-08-17
Description	The sampling sites were all accessed from small boats, here amalgamated to one deployment called LittleLagoon.

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# **Project Information**

# Groundwater Discharge, Benthic Coupling and Microalgal Community Structure in a Shallow Coastal Lagoon (LittleLagoonGroundwater)

Coverage: southern Alabama, east of Mobile

This project investigated the link between submarine groundwater discharge (SGD) and microalgal dynamics in Little Lagoon, Alabama. In contrast to most near-shore environments, it is fully accessible; has no riverine inputs; and is large enough to display ecological diversity (c. 14x 0.75 km) yet small enough to be comprehensively sampled on appropriate temporal and spatial scales. The PIs have previously demonstrated that the lagoon is a hot-spot for toxic blooms of the diatom *Pseudo-nitzchia spp.* that are correlated with discharge from the surficial aquifer. This project assessed variability in SGD, the dependence of benthic nutrient fluxes on microphytobenthos (MPB) abundance and productivity, and the response of the phytoplankton to nutrient enrichment and dilution. The work integrated multiple temporal and spatial scales and demonstrated both the relative importance of SGD vs. benthic recycling as a source of nutrients, and the role of SGD in structuring the microalgal community. (*paraphrased from Award abstract*)

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# Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-0962008

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