Sediment NO3 reduction rates, associated genes, and environmental data from bimonthly samples collected along the York River Estuary from June 2018 to April 2019

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Project
» Alteration of carbon fluxes by intense phytoplankton blooms in a microtidal estuary (LYRE)

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Abstract
Sediment cores were collected from bimonthly samples taken along the York River Estuary from June 2018 to April 2019. Potential rates of NO3 reduction processes, environmental data, and associated gene abundances were measured.

Table of Contents

Coverage

Spatial Extent: **N**:37.4801 **E**:-76.4429 **S**:37.2518 **W**:-76.7595
Temporal Extent: 2018-06-13 - 2019-04-05

Methods & Sampling

Sampling:
Sampling took place at 5 stations along the length of the York River Estuary in June, August, and October of 2018, and February and April of 2019 using 24ft Carolina skiffs. Two sediment cores (5.6 cm diameter, 10 cm deep) were collected at each station; the top 2 cm of sediment were separated from the rest of the core and the top 2cm from both replicate cores were composited.

Slurry incubations:
One gram of sediment from each sample was weighed into 5 exetainer tubes. After flushing for 5 minutes with helium (He) gas to create anoxic conditions, the tubes were incubated at *in situ* temperatures overnight to remove all background nitrate and re-flushed with He gas. Denitrification and anammox potential rate measurements were performed following established protocols (Semedo and Song, 2020; Song and Tobias,

2011); DNRA potential rate measurements followed a modified protocol from Yin et al. (2014). Each gram of sediment was spiked with 100 nmol of $^{15}NO_3^-$ (99 atom %, Cambridge Isotopes) and incubated at *in situ* temperatures. The addition of 50% zinc chloride (0.5 ml) was used to stop all microbial activity immediately after spiking with $^{15}NO_3^-$, for T0, or after a 1-hour incubation, for TF. The amount of accumulated $^{30}N_2$ and $^{29}N_2$ was then measured in the gas fraction using an isotope ratio mass spectrometer (IRMS, Model Delta V, ThermoScientific). Immediately following IRMS analysis, the exetainers were frozen (-80 degC) until analyzed for DNRA.

Ammonium was extracted from the sediment incubation samples using 5 mL of 2 M potassium chloride (KCl). For each sample, 4mL of the KCl extract was diluted with 22 mL of autoclaved, Mili-Q filtered water and poured into two new exetainer tubes. One tube was left as a control and run on a membrane inlet mass spectrometer (MIMS, Pfeiffer Balzers Prisma) without any further additions; the second tube was spiked with 200 µL of a hypobromite solution that converts all NH_4^+ to N_2 (Yin et al., 2014), inverted, and incubated for at least 15 minutes before being run on the MIMS. The concentration of excess $^{29}N_2$ and $^{30}N_2$ produced by the addition of the hypobromite solution was calculated for each sample based on the method of Risgaard-Petersen and Rysgaard (1995) with the exception that a single air equilibrated DI water standard, held at the same temperature as the samples, was used. The concentrations of excess $^{29}N_2$ and $^{30}N_2$ were used to calculate the concentration of $^{15}NH_4^+$ present in the samples.

qPCR Gene Abundance Measurements:
DNA was extracted from each sample using 0.5g of sediment and the DNeasy PowerSoil Kit (Qiagen) following manufacturer protocols. The abundance of specific genes was measured using SYBR Green qPCR. The DNRA marker gene *nrfA* was measured using the primers nrfA2F/nrfA1R (Mohan et al., 2004; Welsh et al., 2014) and the following qPCR reaction: 6µL of GoTaq qPCR Master Mix (Promega), 0.03µL of CXR Reference Dye (Promega), 0.6µL of each primer, 0.25µL of MgCl2, and 4µL of sample DNA (at 1ng/ µL) with the remainder of the 12µL reaction volume made up with water. The *nrfA* qPCR protocol included an initial 10 minute step at 95°C followed by 50 cycles of: 95°C for 15s, 2°C for 45s, 72°C for 1 minute, and 80°C for 35s (Song et al., 2014). The qPCR reaction for *nirS*, the denitrification marker gene, included: 6µL of GoTaq qPCR Master Mix, 0.03µL of CXR Reference Dye, 0.6µL of the forward primer nirScdaF (Kandeler et al., 2006), 0.6µL of the reverse primer nirSR3cd (Kandeler et al., 2006), 0.12µL of BSA, and 4µL of sample DNA (at 1ng/ µL) with the remainder of the 12µL reaction volume made up with water and the protocol was: 95°C for 10 minutes, followed by 45 cycles of 95°C for 15s, 57°C for 1 minute, 72°C for 1 minute, and 80°C for 35s. The 16S rRNA qPCR reactions were set up in the same way as the nirS reactions, with the exception that the primers 515F-Y (Parada et al., 2016) and 806R (Caporaso et al., 2011) were used. The 16S qPCR protocol is as follows: 95°C for 10 minutes with 40 cycles of 95°C for 15s, 55°C for 30s, 70°C for 30s, with a melting curve analysis at the end. All qPCR samples were run in triplicate, with two no-template negative controls for each run. Gene abundance was calculated based on a standard curve produced with known quantities of the target gene.

Instruments:
Nutrient analyses (NO_3, NO_2, NH_4) were performed with a Lachat QuikChem 8000 automated ion analyzer (Lachat Instruments,Milwaukee, WI, USA; detection limits for NO_3, and NH_4, are 0.20 and 0.36 µM, respectively). Extracted chlorophyll-a was analyzed on a Beckman Coulter DU800 spectrophotometer. $^{29}N_2$ and $^{30}N_2$ was measured in the gaseous form by an isotope ratio mass spectrometer (Model Delta V, ThermoScientific) and in the liquid form by a membrane inlet mass spectrometer (MIMS, Balzers Prisma). C:N ratio was measured with a Costech elemental analyzer (Model 1040).

Data Processing Description

BCO-DMO Processing:
- changed date format to YYYY-MM-DD;
- renamed fields.

[table of contents | back to top]

Data Files

File
sediment_NO3.csv(Comma Separated Values (.csv), 3.90 KB) MD5:29c1be12e74a0980d44f721509979b62 Primary data file for dataset ID 854433

[table of contents | back to top]

Related Publications

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J., … Knight, R. (2010). Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the National Academy of Sciences, 108(Supplement_1), 4516–4522. doi:10.1073/pnas.1000080107
Methods

Kandeler, E., Deiglmayr, K., Tscherko, D., Bru, D., & Philippot, L. (2006). Abundance of narG, nirS, nirK, and nosZ Genes of Denitrifying Bacteria during Primary Successions of a Glacier Foreland. Applied and Environmental Microbiology, 72(9), 5957–5962. doi:10.1128/aem.00439-06
Methods

Mohan, S. B., Schmid, M., Jetten, M., & Cole, J. (2004). Detection and widespread distribution of the nrfA gene encoding nitrite reduction to ammonia, a short circuit in the biological nitrogen cycle that competes with denitrification. FEMS Microbiology Ecology, 49(3), 433–443. doi:10.1016/j.femsec.2004.04.012
Methods

Parada, A. E., Needham, D. M., & Fuhrman, J. A. (2015). Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environmental Microbiology, 18(5), 1403–1414. doi:10.1111/1462-2920.13023
Methods

Risgaard-Petersen, N., Rysgaard, S., 1995. Nitrate reduction in sediments and waterlogged soil measured by 15N techniques. Methods Appl. Soil Microbiol. Biogeochem. 287–295.
Methods

Semedo, M., & Song, B. (2019). From Genes to Nitrogen Removal: Determining the Impacts of Poultry Industry Wastewater on Tidal Creek Denitrification. Environmental Science & Technology. doi:10.1021/acs.est.9b03560
Methods

Song, B., & Tobias, C. R. (2011). Molecular and Stable Isotope Methods to Detect and Measure Anaerobic Ammonium Oxidation (Anammox) in Aquatic Ecosystems. Research on Nitrification and Related Processes, Part B, 63–89. https://doi.org/10.1016/b978-0-12-386489-5.00003-8 https://doi.org/10.1016/B978-0-12-386489-5.00003-8
Methods

Song, B., Lisa, J. A., & Tobias, C. R. (2014). Linking DNRA community structure and activity in a shallow lagoonal estuarine system. Frontiers in Microbiology, 5. doi:10.3389/fmicb.2014.00460
Methods

Welsh, A., Chee-Sanford, J. C., Connor, L. M., Löffler, F. E., & Sanford, R. A. (2014). Refined NrfA Phylogeny Improves PCR-BasednrfAGene Detection. Applied and Environmental Microbiology, 80(7), 2110–2119. doi:10.1128/aem.03443-13 https://doi.org/10.1128/AEM.03443-13
Methods

Yin, G., Hou, L., Liu, M., Liu, Z., & Gardner, W. S. (2014). A Novel Membrane Inlet Mass Spectrometer Method to Measure 15NH4+ for Isotope-Enrichment Experiments in Aquatic Ecosystems. Environmental Science & Technology, 48(16), 9555–9562. doi:10.1021/es501261s
Methods

[table of contents | back to top]

Parameters

Parameter	Description	Units

Date	date when the survey took place; format: YYYY-MM-DD	unitless
Station	numerical station number to differentiate sample locations	unitless
Lat	latitude of sample location	decimal degrees North
Long	longitude of sample location	decimal degrees East
Depth	depth of sample	meters (m)
DNF	potential rate of denitrification	nanomoles nitrogen per gram wet sediment per hour (nmole N/g/hr)
AMX	potential rate of anaerobic ammonium oxidation	nanomoles nitrogen per gram wet sediment per hour (nmole N/g/hr)
DNRA	potential rate of dissimilatory nitrate reduction	nanomoles nitrogen per gram wet sediment per hour (nmole N/g/hr)
NOx	concentration of nitrate+nitrite	micromolar (uM)
NO2	concentration of nitrite	micromolar (uM)
NO3	concentration of nitrate	micromolar (uM)
NH4	concentration of ammonium	micromolar (uM)
OP	concentration of organic phosphate	micromolar (uM)
Temp	temperature measured in situ	degrees Celsius
Salinity	salinity measured in situ	unitless
Chl_a	concentration of chlorophyll a	micrograms per liter (ug/L)
DO	concentration of dissolved oxygen measured in situ	milligrams per liter (mg/L)
organics	percent sediment organic matter	unitless (percent)

num_16S	number of 16S genes in sample	number of gene copies per gram sediment (copies/g)
num_nirS	number of nirS genes in sample	number of gene copies per gram sediment (copies/g)
num_nrfA	number of nrfA genes in sample	number of gene copies per gram sediment (copies/g)
C_N	sediment C:N ratio	unitless
PW_NH4	concentration of ammonium in pore water	micromolar (uM)
PW_NOx	concentration of nitrate+nitrite in pore water	micromolar (uM)

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	Costech elemental analyzer (Model 1040)
Generic Instrument Name	Costech International Elemental Combustion System (ECS) 4010
Dataset-specific Description	C:N ratio was measured with a Costech elemental analyzer (Model 1040).
Generic Instrument Description	The ECS 4010 Nitrogen / Protein Analyzer is an elemental combustion analyser for CHNSO elemental analysis and Nitrogen / Protein determination. The GC oven and separation column have a temperature range of 30-110 degC, with control of +/- 0.1 degC.

Dataset-specific Instrument Name	Lachat QuikChem 8000
Generic Instrument Name	Flow Injection Analyzer
Dataset-specific Description	Nutrient analyses (NO_3, NO_2, NH_4) were performed with a Lachat QuikChem 8000 automated ion analyzer (Lachat Instruments, Milwaukee, WI, USA).
Generic Instrument Description	An instrument that performs flow injection analysis. Flow injection analysis (FIA) is an approach to chemical analysis that is accomplished by injecting a plug of sample into a flowing carrier stream. FIA is an automated method in which a sample is injected into a continuous flow of a carrier solution that mixes with other continuously flowing solutions before reaching a detector. Precision is dramatically increased when FIA is used instead of manual injections and as a result very specific FIA systems have been developed for a wide array of analytical techniques.

Dataset-specific Instrument Name	isotope ratio mass spectrometer (Model Delta V, ThermoScientific)
Generic Instrument Name	Isotope-ratio Mass Spectrometer
Dataset-specific Description	$^{29}N_2$ and $^{30}N_2$ were measured in the gaseous form by an isotope ratio mass spectrometer (Model Delta V, ThermoScientific).
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	membrane inlet mass spectrometer (MIMS, Balzers Prisma)
Generic Instrument Name	Membrane Inlet Mass Spectrometer
Dataset-specific Description	$^{29}N_2$ and $^{30}N_2$ were measured in the liquid form by a membrane inlet mass spectrometer (MIMS, Balzers Prisma).
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	Beckman Coulter DU800 Spectrophotometer
Generic Instrument Name	Spectrophotometer
Dataset-specific Description	Extracted chlorophyll-a was analyzed on a Beckman Coulter DU800 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.

[table of contents | back to top]

Project Information

Alteration of carbon fluxes by intense phytoplankton blooms in a microtidal estuary (LYRE)

Coverage: York River Estuary, Virginia

NSF Award Abstract:
Estuaries, coastal water bodies where rivers mix with ocean water, are hotspots for the processing of carbon and nutrients moving from land to the coastal ocean. Within estuaries land-based nutrient inputs can cause intense blooms of single-celled algae called phytoplankton, which can have significant impacts on the ecosystem. As blooms move down-estuary some of the phytoplankton material is buried on the bottom, and some is decomposed, resulting in low oxygen conditions (hypoxia), harmful to marine life, and production of carbon dioxide (CO2), the major greenhouse gas, which can exchange with the atmosphere. The remaining phytoplankton material can be exported to the ocean. The type and amount of carbon exported from the estuary depend both on its biological activity and physical factors such as fresh water discharge, temperature, and light availability. If phytoplankton production is greater than decomposition, the estuary will take up

atmospheric CO2 and export phytoplankton carbon to the coastal ocean. On the other hand, if decomposition is greater than production the estuary will be a source of CO2 to the atmosphere and dissolved CO2 to the coastal ocean. The investigators expect that intense phytoplankton blooms will greatly amplify carbon exchanges with the atmosphere, coastal ocean, and bottom sediments. As intense phytoplankton blooms increase in the future due to increased nutrient inputs and temperature, low oxygen events may become more frequent with potential negative impacts on fisheries and increased export of carbon to the coastal ocean and atmosphere. This study will fill critical gaps identified by the Coastal Carbon Synthesis Program in knowledge of how microtidal estuaries transform and export C to the atmosphere, benthos, and coastal ocean. In addition, there will be a strong teaching and training component to this project, with support for graduate and undergraduate students. The graduate student will be partnered with secondary teachers to gain teaching experience and enrich the middle school educational programs. Summer undergraduate interns will be recruited for a summer program from Hampton University, a historically Black college. There will be public outreach through participation in existing programs at VIMS.

Estuaries serve as critical hotspots for the processing of carbon (C) as it transits from land to the coastal ocean. Recent attempts to synthesize what is known about sources and fates of C in estuaries have noted large data gaps; thus, the role of estuaries, especially those that are microtidal, as important sources of carbon dioxide (CO2) to the atmosphere and total organic carbon (TOC) and dissolved inorganic carbon (DIC) to the coastal ocean, or as a C sink in bottom sediments, remains uncertain. Intensive phytoplankton blooms are becoming increasingly frequent in many estuaries and are likely to have important and yet unknown impacts on the C cycle. The trophic status of an estuary will determine in large part the species of C exported to the atmosphere, bottom sediments, and coastal ocean. The overarching objective of this project is to identify the impacts of intense phytoplankton blooms on C speciation, net C fluxes and exchanges in the Lower York River Estuary (LYRE), a representative mesotrophic, microtidal mid-Atlantic estuary. Metabolic processes are hypothesized to be spatially and temporally dynamic, driving the speciation, abundance, and fates of C in the LYRE. High spatiotemporal resolution sampling in the LYRE will capture rates of C cycling under both baseline conditions throughout most of the year, and during periods when the estuary is perturbed by widespread and intense, but patchy, late summer phytoplankton blooms. The short-term effects of physical drivers (wind, temperature, salinity, fresh water discharge, nutrient and organic carbon loads) and biological drivers (metabolic rates, bacterial and phytoplankton abundances and composition) on C transformations, speciation, and exchanges will be assessed. Expected longer term variations in the C cycle due to anthropogenic and natural disturbances will be predicted through use of modeling. In addition, laboratory manipulations will examine the impacts of specific organisms dominating intensive phytoplankton blooms on benthic metabolism, processing of organic C by the microbial community, and C fluxes to the water column.

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1737258

[table of contents | back to top]