Metagenomic, metatranscriptomics and 16S rRNA gene sequence data from diel sampling at Groves Creek Marsh, Skidaway Island, GA during July 2014

Website: https://www.bco-dmo.org/dataset/762443
Data Type: Other Field Results
Version: 1
Version Date: 2019-03-19

Project
» Collaborative Research: Marine priming effect - molecular mechanisms for the biomineralization of terrigenous dissolved organic matter in the ocean (Marine priming effect)

Contributors	Affiliation	Role
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Abstract
Groves Creek Marsh (31.972° N, 81.028° W), a temperate salt marsh fringing Skidaway Island, GA served as the field site for this study. During July 16-17, 2014, samples were collected every two hours and four minutes to evenly sample across two tidal cycles and one diurnal cycle.

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Coverage

Spatial Extent: **Lat**:31.972 **Lon**:-81.028
Temporal Extent: 2014-07-16 - 2014-07-17

Dataset Description

Groves Creek Marsh (31.972° N, 81.028° W), a temperate salt marsh fringing Skidaway Island, GA served as the field site for this study. During July 16-17, 2014, samples were collected every two hours and four minutes to evenly sample across two tidal cycles and one diurnal cycle.

This dataset contains the environmental observations related to the genetic data found at the Joint Genome Institute (JGI) at the following
url: https://genome.jgi.doe.gov/portal/Geometdatstreams/Geometdatstreams.info... (last visited: 2019-03-18)

Methods & Sampling

Sample collection
Surface water samples were collected from approximately 1 m depth using a hand deployed Niskin bottle. Samples for dissolved constituents (dissolved organic carbon, colored dissolved organic matter, and nutrient

analyses) were filtered on site through 0.2 □m Polycap filters within minutes of collection and then transported to the laboratory for further processing. For cell counts by flow cytometry, samples were transported to the laboratory and fixed using 25% glutaraldehyde. For additional microbial analyses (microbial biomass collection for DNA and RNA extractions and bacterial production) were returned to the laboratory, which was within 10 minutes' drive of the field site. Salinity was measured for discrete samples collected in the field using a handheld multiparameter probe (YSI, Pro2030). Depth was recorded using a YSI 600OMS V2 Optical Monitoring Sonde deployed on the creek bed.

Microbial community analysis sample collection
Planktonic microbial cells from surface water samples were collected by filtration. Water was pre-filtered through a GF/D glass fiber filter (~2.7 µM pore size, Whatman, GE Healthcare Life Sciences, Marlborough, MA); 500 mL of the filtrate was passed through a 0.22 µM pore size, 47 mm diameter filter (Millipore, Burlington, MA). Filtration was completed within 30 min of sample collection. After filtration all filters were placed in cryovials and flash frozen in liquid nitrogen. The samples were stored at -80 C until processing.

Microbial community analysis sample processing
Nucleic acids were extracted from samples following standard methodology. Briefly, for DNA the filters were thawed and placed in a 2 mL tube with 0.3 g glass and zirconia beads (0.2 g glass and 0.1 g zirconia), 0.75 mL CTAB extraction buffer, 0.75 mL phenol:chloroform:isoamyl alcohol (25:24:1, pH 8), internal standards, proteinase K, 10% SDS, and lysozyme for DNA extractions. Samples were vortexed for 10 min to lyse the cells. For RNA extraction, sample tubes were centrifuged for 10 min at 10,000 rpm and 4° C. The lysates were transferred to a sterile 1.5 mL microcentrifuge tube and mixed with 0.75 mL chloroform:isoamyl alcohol (24:1). The aqueous phase was added to a sterile 1.5 mL microcentrifuge with $MgCl_2$, sodium acetate, and isopropanol. This solution was incubated at -80° C for 1.5 hours and then centrifuged at 4° C for 45 min at 10,000 rpm. The supernatant was discarded, and the RNA was washed with 70% EtOH twice. Following RNA extraction Turbo DNase was used to remove residual DNA. For metagenomic samples the lysate was centrifuged at 5,000 rpm for 5 min and washed twice with 0.5 mL of chloroform:isoamyl alcohol by centrifugation at 15,000 rpm for 5 min. The upper aqueous phase was incubated with isopropanol at room temperature for 2 hrs. The DNA was precipitated by centrifugation at 10,000 rpm for an hour and washed with 70% EtOH twice.

All sequencing, assembly, and annotation was performed by the DOE Joint Genome Institute (JGI). JGI generated 16S rRNA libraries, metagenomes, and metatranscriptomes. Plate-based DNA library preparation for Illumina sequencing was performed on the PerkinElmer Sciclone NGS robotic liquid handling system using Kapa Biosystems library preparation kit. DNA was sheared to 300 base pairs (bp) using the Covaris LE220 focused-ultrasonicator and size selected using SPRI beads (Beckman Coulter). The fragments were treated with end-repair, A-tailing, and ligation of Illumina compatible adapters (IDT, Inc) containing a unique molecular index barcode for each sample library. qPCR was used to determine the concentration of the libraries and were sequenced on the Illumina HiSeq-2500 to yield 150 bp paired-end reads at the DOE Joint Genome Institute. Quality filtered metagenomic sequences for each sample were assembled with metaSPAdes (version 3.10.1; and all contigs >200 bp were uploaded and annotated by the Integrated Microbial Genomes (IMG) pipeline. For metatranscriptomes, a plate-based RNA sample preparation was performed on the PerkinElmer Sciclone NGS robotic liquid handling system using the Illumina Ribo-Zero rRNA Removal Kit (bacteria) and the TruSeq Stranded Total RNA HT sample prep kit following the protocol outlined by Illumina. Total RNA starting material consisted of 100 ng per sample and included 10 cycles of PCR for library amplification. Illumina sequencing was performed as described for metagenome samples.

Quality filtered metatranscriptomic sequences for each sample were assembled with Megahit (version 1.10.6), and all contigs > 200 bp were annotated as described for the metagenome samples. Datasets which had assemblies for which the N50 was greater than three standard deviations from mean were not included in further analyses (Supplemental Tables 1 and 2) Resultant assemblies were combined with coding sequences (CDS) using bedtools2 (version 2.27.0) in order to generate an assembly with CDS embedded. Quality controlled raw reads were mapped to the assembly with gene features using bowtie2 (version 2.2.9). Coverage information on the number of reads mapping to each contig was generated using pileup in the BBmap suite of tools. The coverage information was used to normalize read counts to account for the length of reads and the length of CDS. Read counts within KEGG ortholog groups (KO) were summed and normalized as read counts per million mapped to KO-annotated contigs (genes per million [GPM], transcripts per million [TPM]). GPM and TPM were also used in taxonomic analyses.

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
- added lat and lon information

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Data Files

File
sample_info.csv (Comma Separated Values (.csv), 1.38 KB) MD5:77914a98f25d094a39a538f0c94f7158 Primary data file for dataset ID 762443

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Parameters

Parameter	Description	Units
Time_point	time point	unitless
Time	Time of observation	unitless
Temperature	temperature	degrees Celsius
Depth	depth	meters (m)
Salinity	salinity	parts per thousand (ppt)
Cell_Density	cell density	cells per mililiter (cells/mL)
Bacterial_Production	bacterial production	milimole per hour (mmol/h)
DOC	Dissolved Organic Carbon (DOC)	mm
TDN	TDN	mm
DOC_TDN	DOC/TDN	unitless
a254_DOC	a254/DOC	unitless
Lignin	Lignin	mg 1/ mg OC
lat	latitude; North is positive; negative denotes South	decimal degrees
lon	longitude; East is positive; negative denotes West	decimal degrees

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Instruments

Dataset-specific Instrument Name	liquid scintillation counter (Beckman LS-6500)
Generic Instrument Name	Liquid Scintillation Counter
Dataset-specific Description	Tubes were then placed in to a liquid scintillation counter (Beckman LS-6500) overnight and measured disintegrations per minute (DPM) for live samples were corrected using DPM recorded for killed controls.
Generic Instrument Description	Liquid scintillation counting is an analytical technique which is defined by the incorporation of the radiolabeled analyte into uniform distribution with a liquid chemical medium capable of converting the kinetic energy of nuclear emissions into light energy. Although the liquid scintillation counter is a sophisticated laboratory counting system used the quantify the activity of particulate emitting (ß and a) radioactive samples, it can also detect the auger electrons emitted from 51Cr and 125I samples. Liquid scintillation counters are instruments assaying alpha and beta radiation by quantitative detection of visible light produced by the passage of rays or particles through a suitable scintillant incorporated into the sample.

Dataset-specific Instrument Name	Niskin bottle
Generic Instrument Name	Niskin bottle
Dataset-specific Description	Surface water samples were collected from approximately 1 m depth using a hand deployed Niskin bottle.
Generic Instrument Description	A Niskin bottle (a next generation water sampler based on the Nansen bottle) is a cylindrical, non-metallic water collection device with stoppers at both ends. The bottles can be attached individually on a hydrowire or deployed in 12, 24, or 36 bottle Rosette systems mounted on a frame and combined with a CTD. Niskin bottles are used to collect discrete water samples for a range of measurements including pigments, nutrients, plankton, etc.

Dataset-specific Instrument Name	Lachat Quickchem FIA+ 8000 nutrient analyzer
Generic Instrument Name	Nutrient Autoanalyzer
Dataset-specific Description	Samples were analyzed for NOx, NH4, PO4 and SiO2 using a Lachat Quickchem FIA+ 8000 nutrient analyzer, following established colorimetric protocols.
Generic Instrument Description	Nutrient Autoanalyzer is a generic term used when specific type, make and model were not specified. In general, a Nutrient Autoanalyzer is an automated flow-thru system for doing nutrient analysis (nitrate, ammonium, orthophosphate, and silicate) on seawater samples.

Dataset-specific Instrument Name	Shimadzu TOC-VCPH analyzer
Generic Instrument Name	Shimadzu TOC-V Analyzer
Dataset-specific Description	Following filtration, sample aliquots were transferred to pre-combusted 40 mL glass vials, acidified to pH 2 (hydrochloric acid), and analyzed for non-purgable organic carbon using a Shimadzu TOC-VCPH analyzer fitted with a Shimadzu ASI-V autosampler.
Generic Instrument Description	A Shimadzu TOC-V Analyzer measures DOC by high temperature combustion method.

Dataset-specific Instrument Name	Agilent 8453 ultraviolet-visible spectrophotometer
Generic Instrument Name	Spectrophotometer
Dataset-specific Description	Filtered samples (non-acidified) were placed in a 1 cm quartz absorbance cell situated in the light path of an Agilent 8453 ultraviolet-visible spectrophotometer and CDOM absorbance spectra were recorded from 190 to 800 nm.
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.

Dataset-specific Instrument Name	YSI, Pro2030
Generic Instrument Name	YSI Professional Plus Multi-Parameter Probe
Dataset-specific Description	Salinity was measured for discrete samples collected in the field using a handheld multiparameter probe (YSI, Pro2030).
Generic Instrument Description	The YSI Professional Plus handheld multiparameter meter provides for the measurement of a variety of combinations for dissolved oxygen, conductivity, specific conductance, salinity, resistivity, total dissolved solids (TDS), pH, ORP, pH/ORP combination, ammonium (ammonia), nitrate, chloride and temperature. More information from the manufacturer.

Dataset-specific Instrument Name	YSI 600OMS V2 Optical Monitoring Sonde
Generic Instrument Name	YSI Sonde 6-Series
Dataset-specific Description	Depth was recorded using a YSI 600OMS V2 Optical Monitoring Sonde deployed on the creek bed.
Generic Instrument Description	YSI 6-Series water quality sondes and sensors are instruments for environmental monitoring and long-term deployments. YSI datasondes accept multiple water quality sensors (i.e., they are multiparameter sondes). Sondes can measure temperature, conductivity, dissolved oxygen, depth, turbidity, and other water quality parameters. The 6-Series includes several models. More from YSI.

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Deployments

Groves_Creek_2013-2015

Website	https://www.bco-dmo.org/deployment/682763
Platform	Groves Creek - SkIO
Start Date	2013-07-26
End Date	2015-03-11
Description	Studies of temporal and compositional changes in exported material in a saltmarsh, both the quantity and quality of dissolved organic matter (DOM) and particulate organic matter (POM) exported from Groves Creek.

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Project Information

Collaborative Research: Marine priming effect - molecular mechanisms for the biomineralization of terrigenous dissolved organic matter in the ocean (Marine priming effect)

Description from NSF award abstract:
Large fluxes of apparently refractory terrigenous dissolved organic matter (t-DOM) are transported through rivers to the coast each year, yet there are vanishingly low traces of t-DOM in the oceans. The removal of t-DOM is central to the global carbon cycle, yet the mechanisms that drive removal remain poorly understood. In soils, the presence of labile organic compounds is known to enhance the remineralization of recalcitrant compounds, a phenomenon known as the priming effect (PE). The PE is quantitatively important in soil systems, but has received little attention in aquatic systems despite its potential to explain C mineralization patterns at the land-sea interface. This project investigates the magnitude of PE in the coastal ocean and the metabolic and ecological mechanisms that give rise to it. It focuses on the microbial communities of US Atlantic Ocean coastal marshes. In these systems, river-borne t-DOM provides a particularly valuable and tractable model for evaluating the magnitude of the PE. The study utilizes a well-characterized DOM standard collected from a Georgia river as the model t-DOM material in a series of laboratory experiments with natural coastal microbial communities and cultures of heterotrophic marine bacteria of the Roseobacter lineage. Roseobacters are particularly appropriate biological models for this work as they are abundant in southeastern US coastal zones and are known to catabolize lignin and other plant-derived aromatic compounds. Long-term (60 day) incubation experiments will track the PE resulting from addition of labile DOM of differing chemical complexity.

Changes in lignin phenols will be the primary measure of the influence of PE on t-DOM degradation, but the research also monitors a broader suite of aromatic compounds represented by optical properties and identified by high-resolution mass spectrometry. Measurements of the microbial response to added labile organic matter, via extracellular enzyme activities, bacterial production, community composition and gene transcript analysis, will reveal the biological mechanisms responsible for the PE. Experiments using Roseobacter strains will allow detailed investigation of the relationship between metabolic pathways, specific bacteria, and organic carbon mineralization in a well-defined experimental system. Data on gene expression, microbial activity, and DOM transformations from the lab experiments will be integrated to elucidate the specific metabolic pathways invoked as part of the PE and guide development of molecular tools to track genetic signatures along a river to coastal ocean transect in the final year of the project.

The role of heterotrophic microorganisms in remineralizing t-DOM at the land-sea interface is a central question in biological oceanography. Components of t-DOM, principally lignin, are refractory in the sense that degradation rates are typically slow relative to other biomolecules, and yet lignin is effectively removed somewhere between land and the open ocean. The project will determine whether priming plays a role in the rapid removal of t-DOM in the coastal ocean, provide evidence for the types of labile organic matter most effective as priming agents, and attemp to discover the metabolic pathways by which the PE is mediated. These studies have the potential to reveal conserved and predictable metabolic responses that may contribute to regulation of the transformation and turnover of naturally occurring semi-labile/refractory DOM in marine environments. As climate change is likely to affect fluxes of both terrigenous carbon and nutrients to the coastal ocean, understanding the magnitude and mechanisms of PE will be necessary to predict the geochemical consequences of these changing fluxes.

This project is related to the project "Tempo and mode of salt marsh exchange" found at https://www.bco-dmo.org/project/564747.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1357242

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