Illinois-Indiana Sea Grant (IISG) Research Project Annual Report for Year_2016-2017___

Title of Project: Impact of taxonomic and genetic diversity on dissolved organic carbon uptake by bacterial communities

Principal Investigator: Rachel Poretsky

1. Table 1. Key results for IISG specialists and communicators.

List all presentations given by project investigators and partners (include PDF copies of poster or slides)	1. International Symposium for Microbial Ecology 16 in Montreal, August 2016. Freshwater microbial community response to autochthonous and allochthonous dissolved organic carbon Adit Chaudhary, Sarah Turner, Rachel Macam, Rachel Poretsky 2. International Association for Great Lakes Research, Detroit, May 2017 Abstract accepted for a poster Metagenomics of Lake Michigan bacterioplankton and in response to allochthonous dissolved organic matter
List all publications in review or in press authored by project investigators and partners (include extension publications and popular articles as well as peer-reviewed articles)	None yet, but we are preparing our first paper for imminent submission.
List all students who have helped with this work (even if not directly supported with funding)	Adit Chaudhary, PhD student Sarah Turner, PhD student Rachel Macam, undergraduate student

2. Progress Toward Objectives:

We asked for a no cost extension last year to complete the RNA work and finish the initial analyses. We have now completed all analyses except for the metatranscriptomes, which were recently sent to our sequencing facility following extensive optimization for sample processing, manipulation, and library preparation. We anticipate receiving the sequences within the next few weeks.

We collected samples on the CSMI cruise in September, 2015. Due to logistical complications, we were unable to get ship time on the summer CSMI cruise, which delayed our intended sampling. We were also unable to coordinate with the R/V Lake Explorer to get samples from a northern transect in the Lake, as their sampling overlapped with that of the R/V Lake Guardian and we did not have personnel or space to be on both vessels. Nevertheless, we carried out a comprehensive experiment and sampling along the CSMI Saugatuck transect. Samples were

taken at the nearshore site (Sauga_18) and offshore (Sauga_110) surface and depth= mid-hypolimnion (~60 m).

Objective 1: Determine how Lake Michigan microbial communities compare between near- to offshore sites and sites experiencing different nutrient inputs.

Progress: Samples were collected from one nearshore surface (Sauga_18) and one offshore (Sauga_110) surface and depth (~60 m) stations. The free-living (<5.0 μm diameter) microbiome from these water samples were processed for analysis using metagenomics (Table 2). The DNA for metagenomics have been sent off for sequencing along with our metatranscriptomic samples (below).

Objective 2: Evaluate which organisms are breaking down and assimilating various DOM sources.

Progress: We conducted an experiment over 18 hours using samples collected from the three locations described above. In these experiments, microcosms were established in triplicate in which either no DOM was added (Ctrl, Table 2), or a final concentration of 120 μM were added of either terrestrially-derived DOM prepared from leaf litter (Ter, Table 2) or phytoplankton-derived DOM made from laboratory cultures of freshwater species of diatoms, dinoflagellates, and cyanobacteria commonly found in Lake Michigan (Phy, Table 2). The microcosms were sampled at 0, 2, and 18 hours post-DOM addition. From these samples, we will analyze 16S rRNA gene sequences to determine diversity and abundance of microorganisms throughout the incubations. DNA for 16S gene sequencing (Table 2 was completed in summer 2016. In order to compare the different DOM sources and their use by microorganisms, we are also conducting the most ambitious aspect of the study, which is sequencing mRNA transcripts from each (pooled) sample/timepoint/treatment. RNA extractions and library preparations were completed in Fall 2016 and recently sent for sequencing (Table 2) and we are hoping to have them completed in the next few weeks. *We had to do a significant amount of troubleshooting and optimization to prepare these samples for sequencing and it ended up taking substantially longer than expected.* To address objective 2, our main comparisons will be between the organisms and expressed genes in the ctrl vs. Ter vs. Phy treatments, as well as an evaluation of these differences with respect to nearshore vs. offshore and surface vs. depth, where major natural differences in DOM sources and abundances will be seen (metagenomes, Objective 1).

Objective 3: Determine how the response to a DOM pulse is transmitted through the community over time.

Progress: To address this objective, we will compare the community composition and transcriptomic response from the beginning of the experiment described above and over the course of the 18 hours for which it was carried out. Achieving this objective requires the receipt of our RNA sequence data, which should happen soon. We used the new NextSeq platform at the Genomics Core at UIC. We plan to conduct a thorough bioinformatics analysis of all sequence data in the context of our experiment, as well as using data collected along this transect by others, including our USGS partners; this data is still being processed by our EPA and USGS colleagues, but we received the first set of data in January 2017.

3. **Outputs and Outcomes Not Listed in Table 1:**
   Rachel Macam received a prestigious UIC Liberal Arts and Sciences Undergraduate Research Initiative (LASURI) grant ($3,500, “Study of Lake Michigan microbial community diversity and composition at nearshore and offshore sites and surface vs. depth”) in April 2016 and will be presenting her work at the Undergraduate Research Forum at UIC.

Reports are due January 31 of each calendar year, regardless of when the project started.
Graphs, figures and/or photos should be embedded in your text. We may wish to include these items in IISG publications with the appropriate credits.

Table 2. Samples collected in September 2015

S.No.	Timepoint (hr)	Site	Treatment	Replicate
1	0	OFS	No treatment (metagenome)	-
2	0	OFD	No treatment (metagenome)	-
3	0	NSF	No treatment (metagenome)	-
4	2	OFS	Ctrl	A
5	2	OFS	Ctrl	B
6	2	OFS	Ctrl	C
7	2	OFS	Ter	A
8	2	OFS	Ter	B
9	2	OFS	Ter	C
10	2	OFS	Phy	A
11	2	OFS	Phy	B
12	2	OFS	Phy	C
13	2	OFD	Ctrl	A
14	2	OFD	Ctrl	B
15	2	OFD	Ctrl	C
16	2	OFD	Ter	A
17	2	OFD	Ter	B
18	2	OFD	Ter	C
19	2	OFD	Phy	A
20	2	OFD	Phy	B
21	2	OFD	Phy	C
22	2	NSF	Ctrl	A
23	2	NSF	Ctrl	B

24	2	NSF	Ctrl	C
25	2	NSF	Ter	A
26	2	NSF	Ter	B
27	2	NSF	Ter	C
28	2	NSF	Phy	A
29	2	NSF	Phy	B
30	2	NSF	Phy	C
31	18	OFS	Ctrl	A
32	18	OFS	Ctrl	B
33	18	OFS	Ctrl	C
34	18	OFS	Ter	A
35	18	OFS	Ter	B
36	18	OFS	Ter	C
37	18	OFS	Phy	A
38	18	OFS	Phy	B
39	18	OFS	Phy	C
40	18	OFD	Ctrl	A
41	18	OFD	Ctrl	B
42	18	OFD	Ctrl	C
43	18	OFD	Ter	A
44	18	OFD	Ter	B
45	18	OFD	Ter	C
46	18	OFD	Phy	A
47	18	OFD	Phy	B
48	18	OFD	Phy	C
49	18	NSF	Ctrl	A
50	18	NSF	Ctrl	B
51	18	NSF	Ctrl	C

52	18	NSF	Ter	A
53	18	NSF	Ter	B
54	18	NSF	Ter	C
55	18	NSF	Phy	A
56	18	NSF	Phy	B
57	18	NSF	Phy	C

Site Code	
OFS	Offshore surface
OFD	Offshore depth
NSF	Nearshore surface

Treatment Codes	
Ctrl	Control
Ter	Terrigenous DOC addition (120 µM)
Phy	Phytoplankton DOC addition (120 µM)

Section A. Summary

• Title of Project

Impact of taxonomic and genetic diversity on dissolved organic carbon uptake by bacterial communities

• Completion Date

January 31, 2017

• Principal Investigator

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• Abstract

Freshwater bacterioplankton play important ecological and biogeochemical roles by production and assimilation of dissolved organic matter (DOM). They are highly sensitive to changes in nutrient regimes, which is critical as human activities shift carbon dynamics in freshwater ecosystems. Despite the importance of microorganisms in carbon flux, only a handful of studies have used modern –omics techniques to study freshwater microbial communities and *none* of these have been in Lake Michigan. Inputs from terrestrial environments, the invasive dreissenid mussels, primary producers including cyanobacteria, picoeukaryotes, and phytoplankton have all been known to affect lake food web dynamics. The microbial community is often the first to respond to pulses of nutrients and organic matter.

This project explored the microbial community composition (*i.e., What is the diversity, genetic potential, and taxonomic structure?*) and activity (*i.e., What genes are being used?*) in Lake Michigan as microbes break down and assimilate different organic matter sources. Water samples were collected along a coastal-to-offshore transect with a natural DOM gradient beginning at the mouth of Kalamazoo River. Analysis of the native bacterial community using metagenomics showed nearshore and offshore communities composed of known freshwater taxa, with certain taxa (*Pelagibacteria, Acidimicrobiales*) differing in relative abundance across the transect. The nearshore community exhibited a higher alpha diversity. Microcosms from these samples were amended with DOM and subsampled over time to analyze the bacterial response to DOM using 16S rRNA gene and mRNA sequencing. Results revealed differential responses of the nearshore and offshore bacteria, indicating different adaptations to processing this carbon.

• Keywords

Bacteria, DOM, carbon, metatranscriptomics, 16S rRNA gene

• Lay Summary

Millions of microbes (bacteria and archaea) inhabit each milliliter of water in Lake Michigan. These organisms are crucial players in the Earth's carbon cycle and are expected to be highly sensitive to climate change drivers. Among their many possible roles, arguably the most important one for bacteria in the carbon cycle is the use of dissolved organic carbon (DOC) and other components of the dissolved organic material (DOM) pool. DOC is one of the largest carbon pools in the biosphere. It is operationally defined as the organic matter that passes through a small pore size filter and serves as a primary food source for aquatic food webs. The flux of carbon through the DOM pool is also quite large. DOC is influenced by internal (e.g., photosynthesis) and allocthonous (e.g., runoff, terrestrial) sources. Carbon assimilation by microorganisms serves as the first step in the microbial food web; carbon flux through DOM is determined by assimilation and respiration by bacteria and ultimately by the rest of the microbial loop. The concentration of human activities along coastlines causes direct and indirect perturbations to these natural processes through pollution, eutrophication, water withdrawal, and wetland loss. These increased stresses on the coastal carbon cycle impact productivity, food web structure, atmospheric CO_2 exchange, and other key processes in ways that are very poorly understood. The purpose of this study was to examine how Lake Michigan bacteria respond to supplements of carbon and nutrients either from adjacent land drive or production by photosynthetic organisms in the lake. We accomplished this by looking at the microbial communities that live nearshore and comparing them to those that live farther offshore. We then conducted experiments where we provided carbon supplements of either terrestrial (leaf) or photosynthetic (phytoplankton) origin and looked at how different lake microbial communities respond to these different DOC sources.

Section B. Accomplishments

• Introduction

The millions of freshwater microbes that inhabit each milliliter of water are crucial players in the Earth's carbon cycle. Among many possible roles, arguably the most important one for bacteria in the carbon cycle is the use of dissolved organic carbon (DOC) and other components of dissolved organic material (DOM) pool. DOC is one of the largest carbon pools in the biosphere, equivalent in size to atmospheric carbon dioxide (1). The flux of carbon through the DOM pool is also quite large, equivalent to roughly half of primary production in many aquatic ecosystems (2). Carbon assimilation by microorganisms also serves as the first step in the microbial food web. In addition to DOC derived from primary production, terrigenous DOC can be a significant energy source, providing bacteria with a role similar to phytoplankton in freshwater lake food webs (3, 4) (Fig. 1). How phylogenetic and genetic diversity impact this flux is a major unsolved

problem in microbial ecology. Supplements of carbon and nutrients from adjacent land drive high rates of microbial activity, impacting productivity, food web structure, atmospheric CO_2 exchange, and other key processes in ways that are very poorly understood.

Despite the importance of DOM flux through bacterioplankton to the pelagic food web, only a handful of studies have used –omics techniques to study freshwater microbial communities (5-8) and *none* of these have been in Lake Michigan. DOC supporting bacterial production (BP) can originate from phytoplankton, grazers, viral lysis, and dissolution of particulate organic carbon (POC). In freshwater, another important source is terrestrially-derived DOC (9) (Fig. 1). Several decades ago, it was shown that BP in Lake Michigan is limited by DOM availability (10); carbon demands are partially met by phytoplankton production in the lake and partially by external loads of terrigenous DOM (11, 12). Furthermore, BP decreases with distance from the coast, implicating nearshore inputs in coastal community productivity (11). Although phytoplankton production in Lake Michigan was stable in the 1970's and 80's (13), there is evidence that phytoplankton blooms have been impacted since the introduction of non-native dreissenid mussels (14). Therefore, the importance of different carbon sources on bacterioplankton communities has likely shifted, perhaps along with their composition and diversity. This proposed project aims to explore the microbial community composition and activity in Lake Michigan as microbes break down and assimilate different organic matter sources, providing insights into the microbial response to nutrient pulses with implications to carbon flux throughout the lake food web.

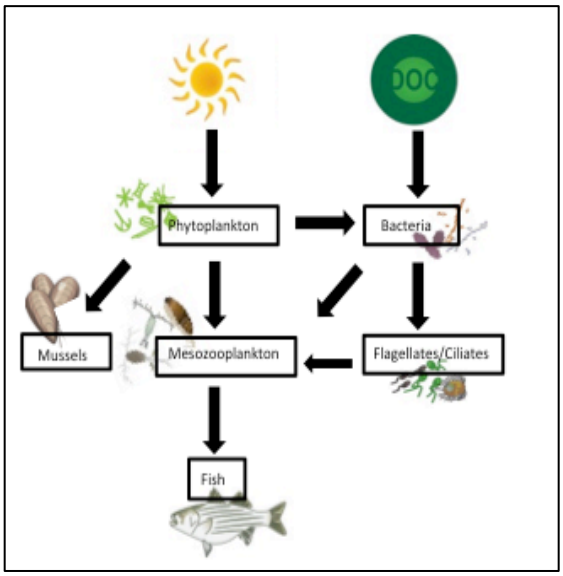


Fig. 1. Simplified Lake Michigan food web showing the potentially major role of allochthonous DOC input.

To examine how the capacity to process DOM varies as a function of initial community composition, both taxonomic and genetic, we targeted the following questions:

1) How do Lake Michigan microbial communities compare between near- to offshore sites and sites experiencing different nutrient inputs?

2) Which organisms are breaking down and assimilating various DOM sources?

3) How is the response to a DOM pulse transmitted through the community over time?

• Project Narrative

Sample collection

Samples were collected in 2015 on a CSMI cruise on board the R/V Lake Guardian across a nearshore to offshore transect of Lake Michigan that has a natural organic matter gradient due to its proximity to the mouth of Kalamazoo River. Surface water (10-15L) was filtered directly from the creek through a 5.0 μm pore size polypropylene cartridge filter followed by a 3.0 *μ*m pore size, 293 mm diameter and then 0.22 *μ*m pore size, 293 mm Poretics polycarbonate membrane filter. Immediately after filtration, filters were frozen for DNA and RNA extraction. Temperature, salinity, and oxygen concentrations were measured at the time of sampling.

Natural DOC amendments

Axenic cultures of *Cryptomonas pyrenoidifera* (CCMP1167) and *Dinobryon cylindricum* (CCMP2766) were obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton Cultures. Cultures were grown on DV-Y media to stationary phase and cells were filtered onto combusted (500°C overnight) GF/F filters and then blended. Centrifugation followed by a second filtration was then used to remove cell and filter debris. Additional cultures of *Chlamydomonas reinhardtii, Anabaena,* and *Oscillatoria* were used.

Leaf litter was collected, rinsed with sterile DI water, and broken into small fragments. Leachate was prepared by placing the plant material in 10 L DI water in an acid washed carboy and incubating the mixture in the dark for 5 d. Cell debris was then removed by centrifugation and filtration. Concentrates of phytoplankton and plant organic matter were stored in acid

washed containers at –20°C until use. Subsamples were removed from both for DOC analysis, measured by high-temperature catalytic oxidation at Northwestern University.

Phytoplankton- or vascular plant-derived DOM were added to a final [DOC] of 2.5 mg L^{-1} in 10 L water collected from each station. Duplicate microcosms were incubated *in situ* and subsampled at 2h and 19h. at which point they were filtered on to 0.22 μm filters for RNA collection.

RNA extraction and processing

RNA was extracted using the RNAqueous-Midi kit with modifications to the manufacturer's instructions. Immediately following filtration, 0.22 μm filters were placed into a 50 ml tube containing RNase-free beads from the PowerSoil Total RNA Isolation Kit (MoBio, Carlsbad, CA) and 6 ml of lysis/binding solution provided by the RNAqueous-Midi kit and vortexed for 10 min. The samples were then centrifuged at ~10,000 rpm for 10 min to clarify the lysate. RNAqueous-Midi kit ethanol solution (5 ml) was added and the supernatants and passed several times through an 18-gauge needle to shear genomic DNA. The mixture was then filtered directly onto a glass fiber filter syringe filter (provided with the kit), washed and eluted according to the manufacturer's instructions. On average, 5 μg RNA were obtained from each filter. RNA was treated with DNase using the TURBO DNA-*free* kit followed by enzymatic treatment with the mRNA-ONLY Prokaryotic mRNA Isolation Kit. RNA libraries were prepared using the SMARTer Stranded RNA-Seq kit and sequenced at the UIC UIC DNA Services lab using the NextSeq platform.

DNA extraction, ssrRNA gene amplification and clone library construction

DNA was extracted from filters with the PowerMax Soil Mega Prep DNA Isolation Kit (MoBio). Small submit rRNA (ssrRNA) library were constructed following the recommended

protocol recommended by the Earth Microbiome Project. Briefly, 16S rRNA genes were sequenced at the UIC DNA Services lab using the MiSeq platform.

Results

In this study, we aim to analyze the microbial community across a nearshore to offshore transect of Lake Michigan that has a natural organic matter gradient due to its proximity to the mouth of Kalamazoo River. The riverine inputs introduce nutrient and dissolved organic matter (DOM) into the lake that can augment nearshore bacterial activity and thereby have potential implications for the lake secondary production and food web dynamics. Using deeply sequenced metagenomes, we first analyzed the bacterial community from both the nearshore and offshore locations to compare the microbial community diversity, structure and functional potential between the two sites that likely have different nutrient and DOM levels. To further understand how bacterial communities across the transect would respond to a pulse of terrestrially derived DOM (120 µM), we set up microcosm experiments where we incubated water from both nearshore and offshore sites amended with terrestrially derived DOM (leaf litter extract: t-DOM). Unamended microcosms using water from both the sites were also incubated alongside, which served as control. Water was subsampled from the microcosms at 2 h and 19 h after incubation, analyzed for microbial community composition (16S rRNA gene amplicons) and functional potential (mRNA seq).

The metagenome based analysis of unamended bacterioplankton reveal that both the nearshore and offshore communities are composed of known freshwater taxa with similar relative abundances, however certain taxa such as *Pelagibacteria* and *Acidimicrobiales* differed in their relative abundance across the transect (and with depth at offshore), and also the nearshore bacterial community exhibited a higher alpha diversity as compared to offshore

communities. Furthermore, microcosm incubations of the offshore bacterioplankton revealed a significant increase (~12%, $p < 0.05$) in the relative abundance of bacteria classified within *ACK-M1* family of Actinobacteria over time in t-DOM amended water as compared to control. However, microcosms of nearshore bacteria showed only a small increase (~4%) in the relative abundance of the same taxa. Most of the other abundant freshwater taxa did not show any notable shifts in response to the t-DOM treatment for both the locations. The specific growth response of freshwater Actinobacteria to the DOM pulse may be associated with the organism's ability to assimilate recalcitrant plant derived DOM, and the DOM preparation methodology employed in this study (see below) likely contributed to a significant refractory component in the t-DOM used. These results when seen in the context of possibly differing DOM levels between nearshore and offshore locations of the study may suggest that the nutrient and DOM limited offshore bacteria showed a stronger response to t-DOM pulse as compared to the same taxa in the nearshore water. The higher background levels of DOM at nearshore location perhaps contributed to the lack of a strong growth response in the nearshore microbiome over time. Results from the mRNA seq experiment from the microcosm incubations can shed more light on the specific taxa possibly assimilating the various t-DOM derived compounds across the transect. Overall, this study characterizes the nearshore and offshore bacterioplankton across a gradient of natural organic matter in Lake Michigan, and highlights the possible role of terrestrially derived DOM in assimilation by abundant freshwater bacteria especially in nutrient limiting conditions.

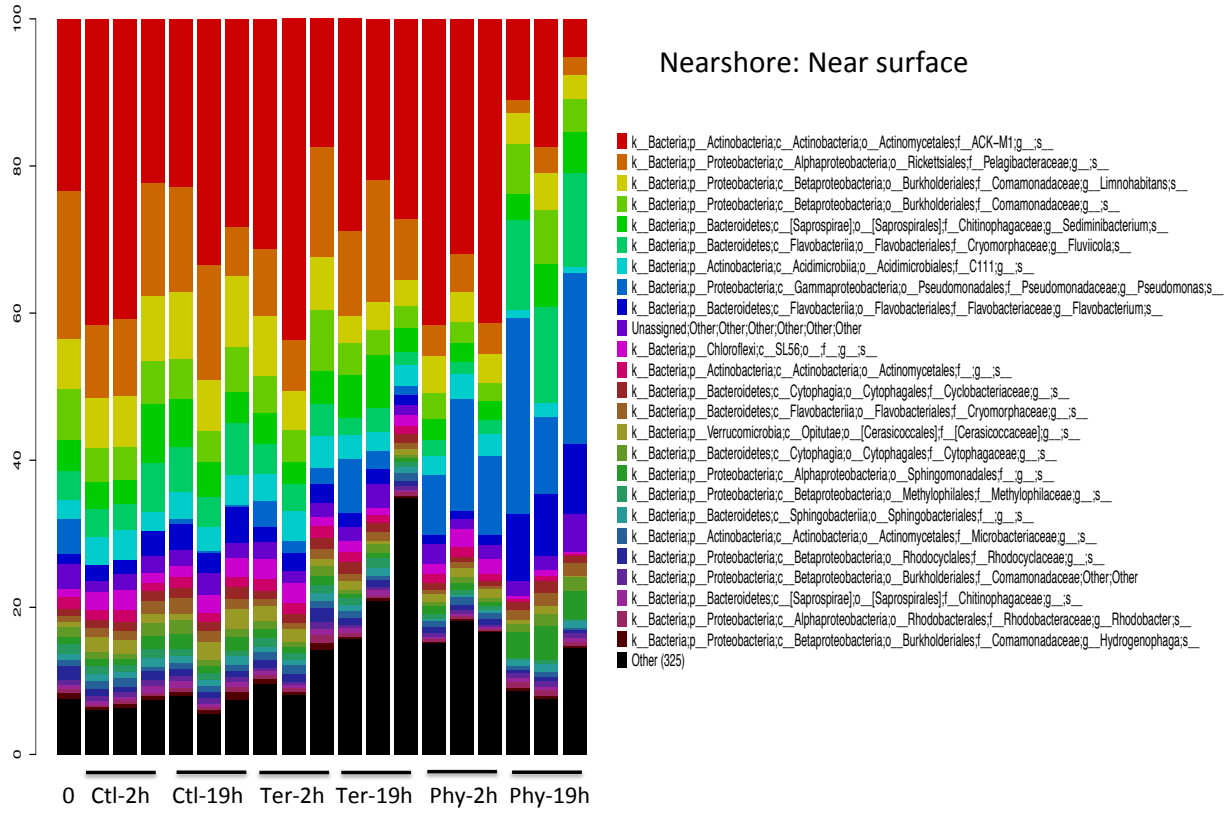
To prepare the terrestrial DOM, senescent and dry leaves collected from a Chicago preserve were incubated in sterile DI water in the dark for 6 days, followed by filtration through GF/F filters (0.7 µm). This methodology is similar to some previous studies where dried/washed and ground plant matter was incubated in the dark for 2-5 days (Poretsky et al. 2010, McMeans

et al. 2015), and to some extent it probably resembles the naturally occurring leaching and decomposition process of leaf litter in the river before inflow to the lake. However, our results indicate that the bacteria on the leaves during the leaching process might have caused bacterial uptake of the more labile components of the DOM and thus a significant portion of the DOM remaining after incubation could be refractory in nature.

Unknown taxa within *Acinetobacter* dramatically increased in relative abundance from being a rare member at 2 h to about 15% relative abundance in the nearshore community at 19 h. While it is difficult to know if this organism actually constituted the rare fraction of the native bacterioplankton, we are not sure if its increase over time could affect the t-DOM availability to the abundant freshwater taxa such as Actinobacteria, which are relatively slow growing. So the final interpretation of the t-DOM treatment in the nearshore community may also need to include the response of this rare organism in the discussion.

In the results described above, we focus on the response of the offshore surface water community and not the depth sample microcosms, as there was not a strong response to t-DOM for it.

We observed a substantial *Pseudomonas* signal in the phytoplankton DOC additions in the offshore samples, which we suspect is due to contamination since this organism is not present at such levels in the background samples. We anticipate learning more about the potential source of this group of organisms once we obtain the metatranscriptomic data.


Nearshore: Near surface
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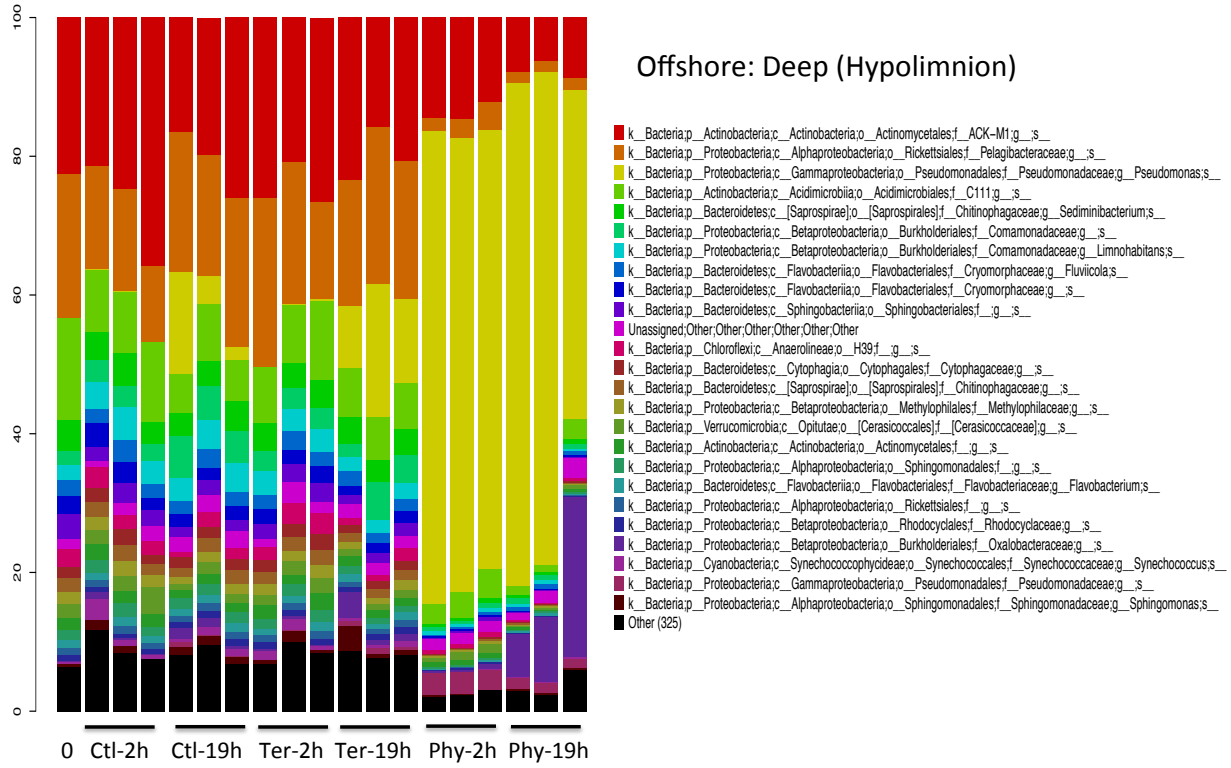
Other (325)


Offshore: Deep (Hypolimnion)
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Unassigned;Other;Other;Other;Other;Other;Other
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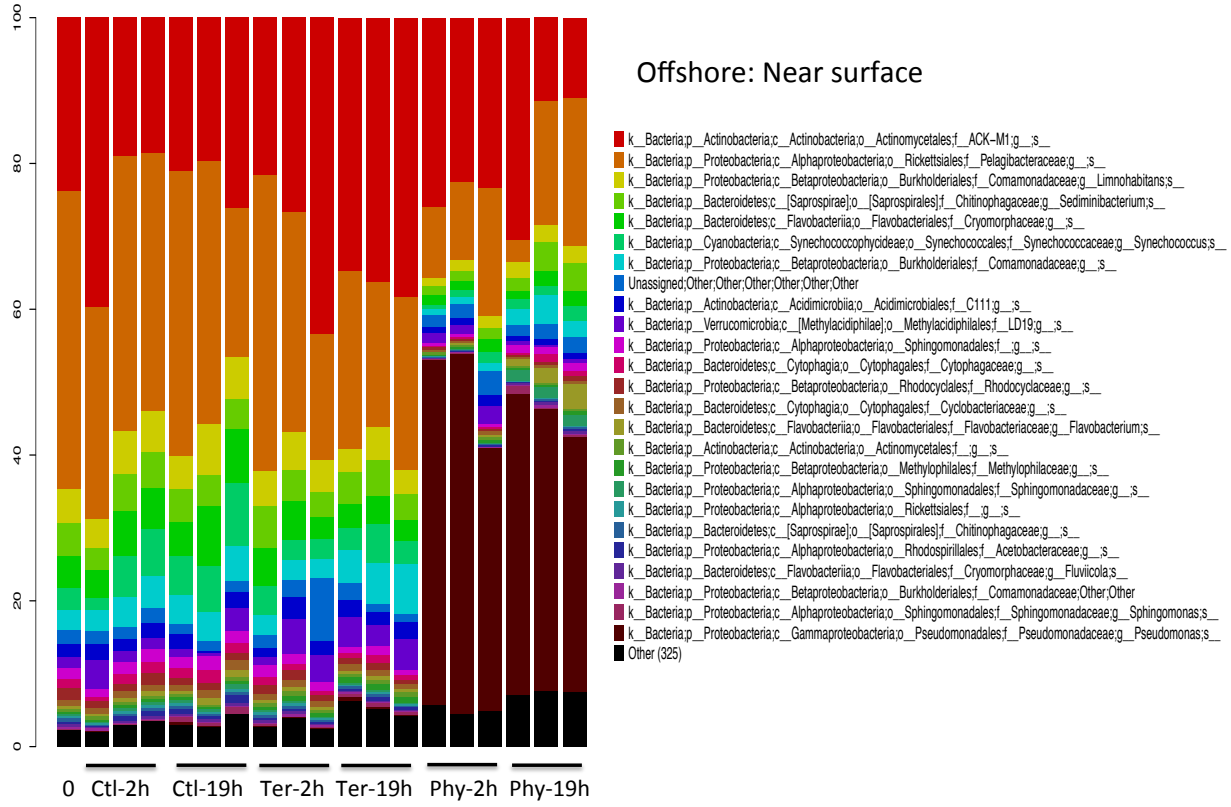
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Sphingomonas;s__
Other (325)



Section C. Outputs

• Publications

We are preparing the first manuscript based on 16S rRNA sequences now. The second will report our findings from the metatranscriptomic data.

• Undergraduate/Graduate Names and Degrees

Adit Chaudhary, PhD candidate, expected graduation 2018

• Project Partnerships

Partnership with Bo Bunnell (USGS) and Joel Hoffman (EPA).

Partnership with the John G. Shedd Aquarium Microbiome Project and Great Lakes research area to disseminate our results to the public (including at Science Pub events in the summer 2017).

Potential collaborations/conversations started with Maureen Coleman (UChicago) and Vincent Denef (UMichigan).

We are also pursuing follow-up research on Great Lakes carbon dynamics with Karl Rockne (UIC), who has preliminary data using isotopic tracers of carbon in Lake Michigan sediments. We are planning to pursue DOE funding for this project. In February 2017, Poretsky submitted an NSF Dimensions of Biodiversity proposal with a colleague at Northwestern University (George Wells) to pursue similar near-to-offshore microbial ecology work in Lake Michigan.

- Awards and Honors

  Rachel Poretsky was nominated for the **2017 Blavatnik National Awards for Young Scientists** and a **UIC Rising Star in Basic Life Sciences** award.

Section D. Metadata for Data Management Plan

All sequence data have been deposited in NCBI's SRA. We will add the metatranscriptomic data when we receive that.

Freshwater microbial community response to autochthonous and allochthonous dissolved organic carbon

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Introduction

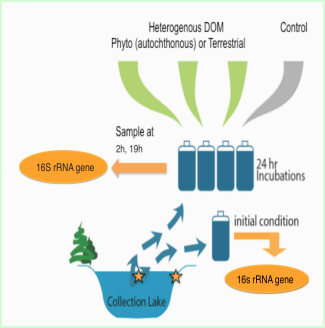
- By assimilation and production of constituents of Dissolved Organic Matter (DOM) pool, freshwater bacterioplankton play an important role in global carbon cycle and the pelagic food web.
- Bacterioplankton are highly sensitive to changes in nutrient regimes, which is critical given the rising impact of anthropogenic activities on lake ecosystems and observed changes in phytoplankton growth/abundance.

Objectives

- Compare microbial communities in Lake Michigan which experience different nutrient inputs by studying the taxonomic and genetic diversity of bacterioplankton from both near-shore and off-shore locations.
- Evaluate which microbes breakdown and assimilate the various DOM sources by assessing the microbial community response to pulses of autochthonous versus allochthonous DOM using samples collected from both near-shore and off-shore locations.

Methods

- Water samples collected along a coastal-to-offshore transect with a natural DOM gradient beginning at an inflow point from the Kalamazoo River.
- Samples also used in microcosms amended with either autochthonous (phytoplankton exudate) or allochthonous (terrestrial leaf litter leachate) DOM, along-with unamended control.
- Water from reference (0 hour) samples, and from microcosms (2 hour and 19 hour time-points) processed for microbial DNA extraction and 16S rRNA gene amplicon sequencing.
- Microbial community diversity and composition analysed with QIIME v1.8.0.



Comparing nearshore and offshore Lake Michigan microbial communities

Fig1. Sampling points (circled) along a coastal-to-offshore transect across Lake Michigan

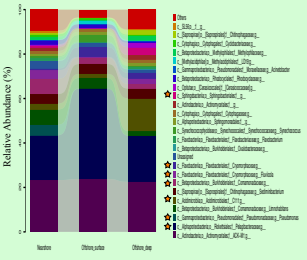


Fig 2. Taxonomic profile for reference samples (0 hr) for nearshore and offshore locations.

➡ **Differences in relative abundance of certain groups (highlighted with '★' in figure key) between the locations can be observed.**

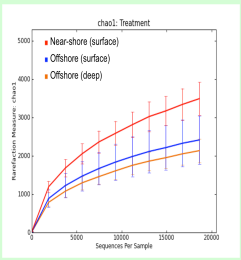


Fig 3. Chao1 richness metric based on 16S rRNA gene amplicon OTUs.

➡ **Higher microbial diversity observed for the nearshore samples as compared to the offshore samples.**

Microbial community response to allochthonous (terrestrial) and autochthonous (phytoplankton exudate) DOM amendments

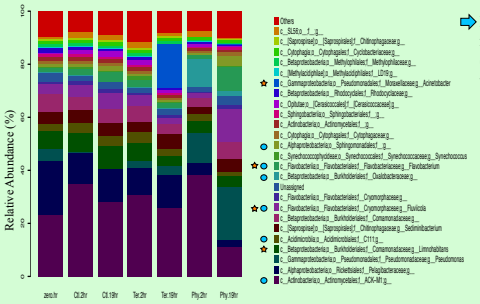
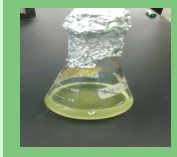
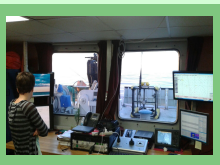


Fig 4. Microbial community profile at genus level for microcosms using **Nearshore** water samples. Barplots for the 2 hr and 19hr time-points represent average values for biological triplicates.

➡ Differening patterns of shifts in relative abundance of bacterial groups ($p < 0.05$ w.r.t. control, t-test) over time observed in the 2 treatments:

- Terrestrial DOM: *Acinetobacter* ($p < 0.1$), *Limnohabitans, Fluviicola, Flavobacterium* (see '★' in figure key)
- Phyto-exudate DOM: *Flavobacterium, Fluviicola, Actinobacteria ACK-M1, Acidimicrobiales, Pelagibacteraceae, Sphingomonadales, Oxalobacteraceae* (see '●' in figure key)





Conclusion & Future Work

- Differences in microbial community composition and diversity observed between the near-shore and offshore locations.
- Microcosm experiments reveal differences in response to the two DOM treatments for the nearshore samples, highlighting possible substrate partitioning among certain bacterial groups.
- Future work to compare the results of microcosms of nearshore samples with offshore sample-microcosms.
- Integrate 16S rRNA taxonomy results with functional data (metagenomics/metatranscriptomics) for these samples.

Acknowledgements

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We would like to thank the personnel of research vessel Lake Guardian for assistance with sample collection from Lake Michigan.

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