The Metropolitan
Water Reclamation District
of Greater Chicago

WELCOME
TO THE AUGUST EDITION
OF THE 2016
M&R SEMINAR SERIES

BEFORE WE BEGIN

- **SAFETY PRECAUTIONS**
  - **PLEASE FOLLOW EXIT SIGN IN CASE OF EMERGENCY EVALUATION**
  - **AUTOMATED EXTERNAL DEFIBRILLATOR (AED) LOCATED OUTSIDE**
- **PLEASE SILENCE CELL PHONES OR SMART PHONES**
- **QUESTION AND ANSWER SESSION WILL FOLLOW PRESENTATION**
- **PLEASE FILL EVALUATION FORM**
- **SEMINAR SLIDES WILL BE POSTED ON MWRD WEBSITE (www. MWRD.org: Home Page ⇒ Reports ⇒ M&R Data and Reports ⇒ M&R Seminar Series ⇒ 2016 Seminar Series)**
- **STREAM VIDEO WILL BE AVAILABLE ON MWRD WEBSITE (www.MWRD.org: Home Page ⇒ MWRDGC RSS Feeds)**

M. Cristina Negri

Current: Principal Agronomist/Environmental Engineer, Argonne National Laboratory

Education: **D**octor in Agricultural Sciences from the University of Milan, Milan, Italy.

Experience: During her more than 20 years at Argonne, M. Cristina Negri conducted and directed laboratory to full-scale multidisciplinary projects developing technologies and concepts for environmental remediation and stewardship, including soil remediation and water treatment.
Principal Investigator of MWRD's microbial source tracking project since 2013

Professional: Senior Fellow with the Energy Policy Institute at the Harris School
Fellow of the Institute of Molecular Engineering at the University of Chicago.
Fellow of the Northwestern Univ.—Argonne Institute of Science and Engineering

Award: WIST Diversity Award, ANL 2014;
Outstanding Postdoctoral Mentor Award Honorable Mention, ANL, 2013;
U.S. Department of Energy, Office of Science Outstanding Mentor Award, 2004

Jack A Gilbert

Current: Director of The Microbiome Center, Professor of Surgery at University of Chicago, and Group Leader at Argonne National Laboratory

Education: Ph.D. from Nottingham University, UK

Experience: Senior Fellow at the Marine Biological Laboratory, Woods Hole; Fellow of the Field Museum, Associate Director of the Institute of Genomic and Systems Biology. Dr. Gilbert is currently applying next-generation sequencing technologies to microbial metagenomics and metatranscriptomics to test fundamental hypotheses in microbial ecology.

Professional: Founding Editor in Chief of mSystems Journal. On the Advisory board of the Genomic Standards Consortium (www.gensc.org), co-founded The BioCollective, and is currently the Chief Scientific Advisor, Crain's Business Chicago's 40 Under 40 List, one of the 50 most influential scientists by Business Insider 2015, and Brilliant Ten by Popular Scientist 2015. The Altemeier Prize 2016 (Surgical Infection Society), and the WH Pierce Prize 2016 from the Society for Applied Microbiology.

Publication: More than 230 peer reviewed publications and book chapters on microbial ecology and microbiology.

MICROBES OF THE CAWS

Jack A. Gilbert
M. Cristina Negri

Argonne National Laboratory

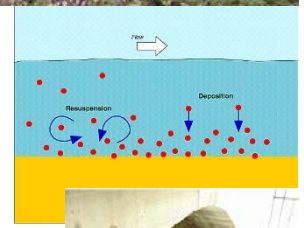
Argonne NATIONAL LABORATORY

STUDY OBJECTIVES

- CAWS are 78 miles long, man made and natural, drain 645 mi^2
- 70% of flow typically effluent from reclamation plants, but there are many other sources of microbial communities

Objectives:

- To understand which microbial communities live in the CAWS in relation to space and time
- To determine their likely sources (host, spatial location and physical source)
- To determine the impacts of disinfection and TARP on microbial communities in the CAWS
- To develop a model to predict variations in CAWS's microbial communities based on weather, flow and other physical variables.



Phase	2013	2014	2015	2016	2017	2018	2019
I- Pre-disinfection, Pre-TARP completion, Calumet and Chicago River systems	-	----	----				
II – Post-Disinfection, Pre-TARP reservoir completion, Chicago River System				----	----		
III – Post disinfection and post-TARP reservoir completion, Calumet River system (2016-19) and Chicago River System (2018-19)				----	----	----	----

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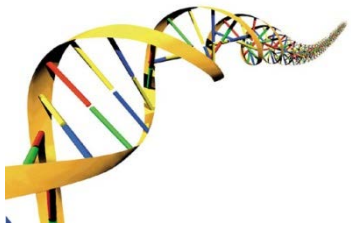
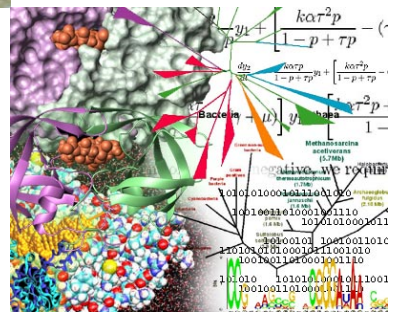
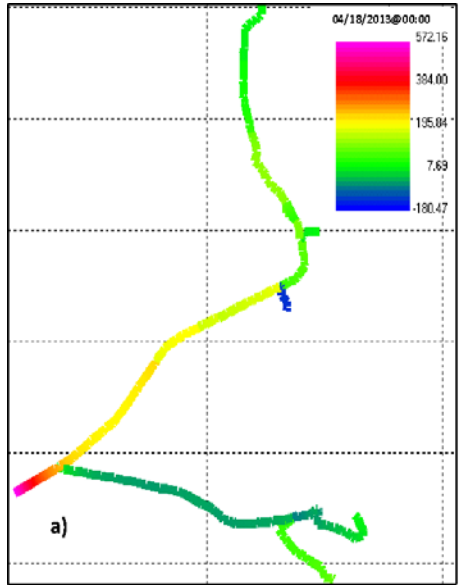
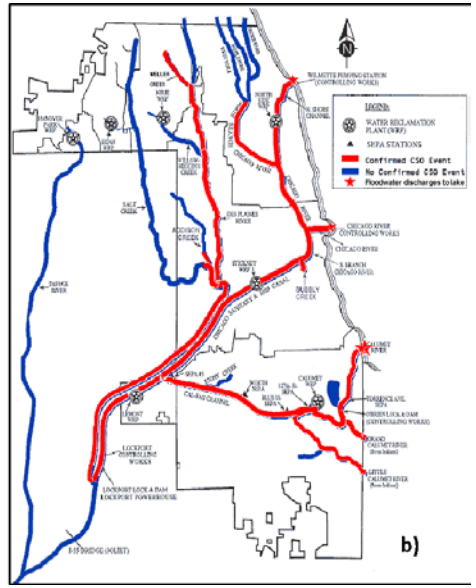
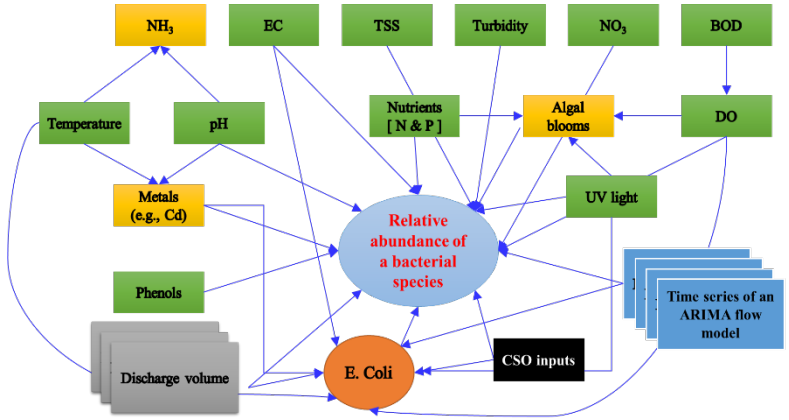
IN PRACTICE











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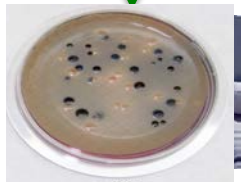
A NEW WAY OF EXPLORING THE CAWS MICROBES!

Environmental samples

Sediment

Water Filters



Fecal coliform cultures

DNA extraction

(PowerSoi
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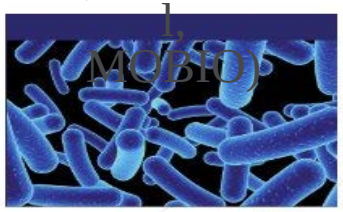
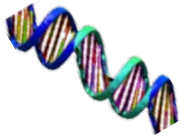
MOBIO)





DNA extraction

16S rRNA PCR amplification

Shotgun (Meta)Genomics

(Nextera XT)

Miseq (Illumina)

Hiseq (Illumina)

qiime Quantitative Insights Into Microbial Ecology

R: Vegan

- ***Phylogenetic Analysis***
(Metaphlan)
- ***Assembly***
Idba, (meta)-velvet
- ***Mapping***
Bowtie, GASSST
- ***Annotation***
RAST, MGRAST

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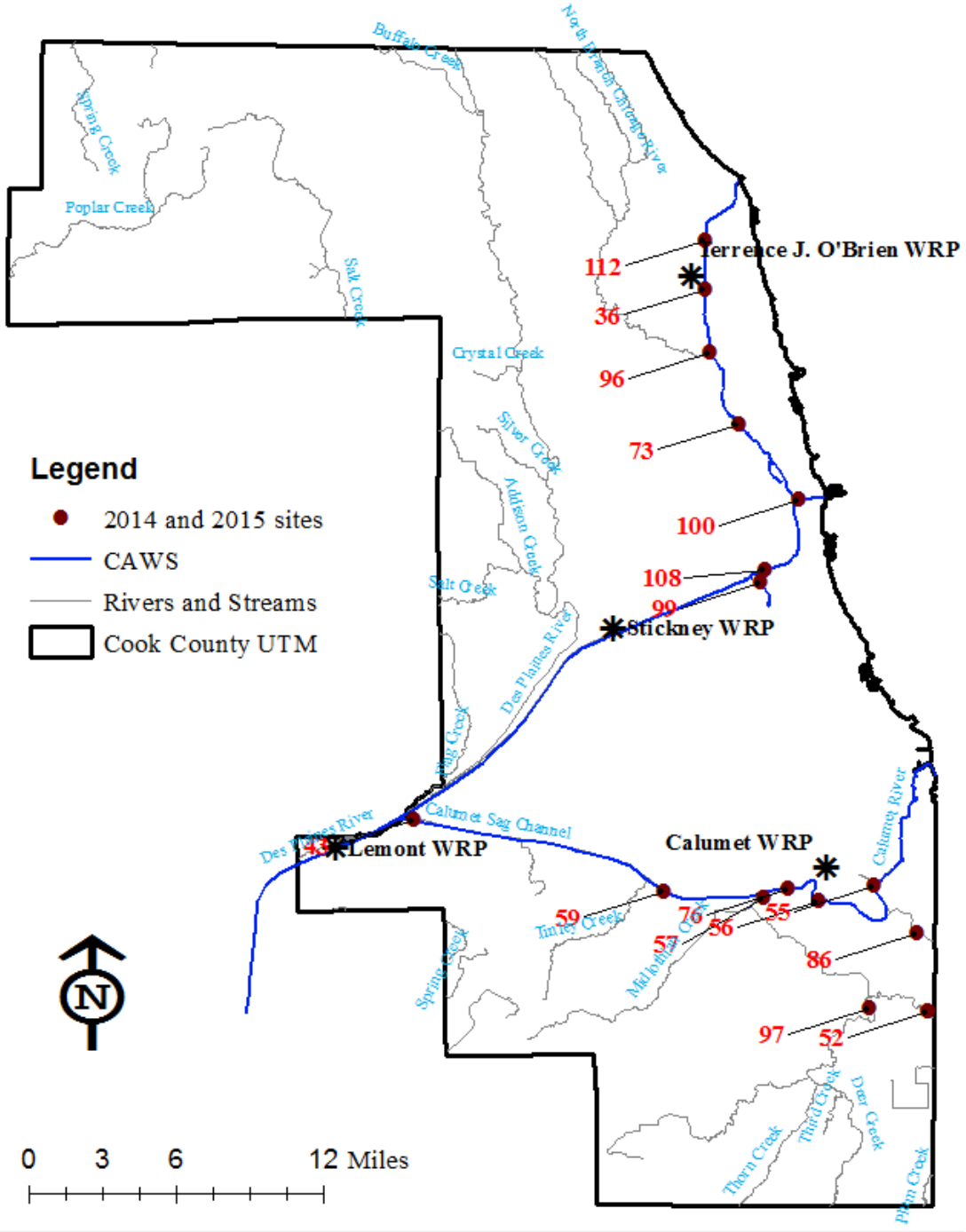
MICROBIAL WORK TO DATE

- Received samples from 2013, 2014, 2015 and 2016 from water and sediment, plus some extraneous sampling sites (beaches, fish, water filters, treated sewage, etc.)
- Collected 196 blank (equipment, filter) samples, 9 Bioball®-spiked samples, 24 fish gut samples, 24 fish mucus samples, 278 sediment samples, and 429 water column samples from 17 sites in the Chicago River and related man-made waterways, together with 22 influent sewage, 10 mixed liquor, and 190 secondary treated final effluent samples from two Water Reclamation Plants (WRP, O'Brien and Calumet) sampled during 2013, 2014, and 2015
- Processed 1,269 for 16S rRNA sequencing – telling us what bacteria are there.
- Processed 112 for shotgun metagenomics – telling us what virulence, antibiotic resistance and other functional genes are there.
- Analyzed the genomes of E.coli organisms isolates by MWRD from the CAWS
- Received 429 samples for 2016 so far, and are currently processing these.

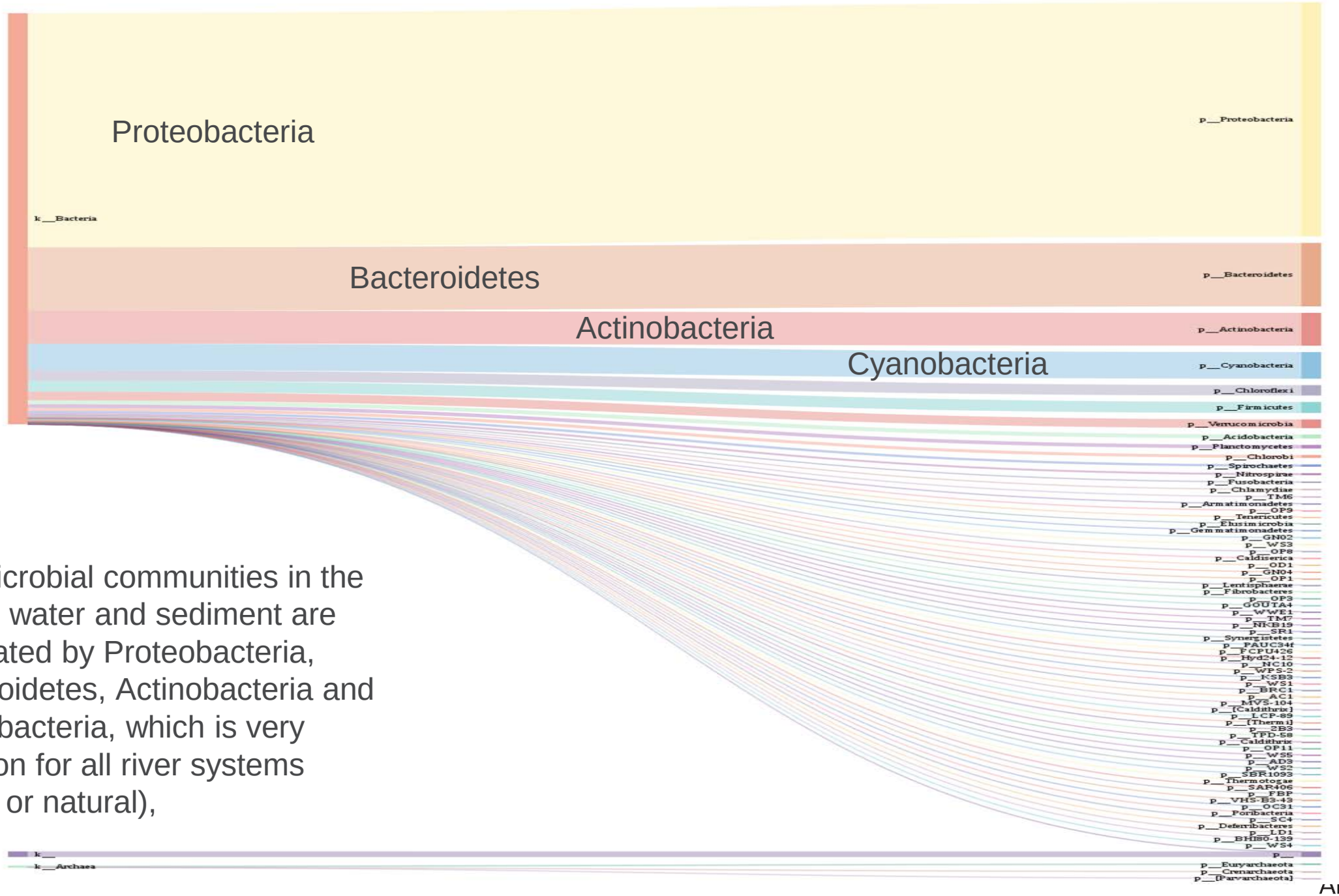
WHAT QUESTIONS ARE WE ASKING?

- Does microbial species diversity show differential geographic and temporal structure?
  - Are these differences observed by sampling medium (sediment vs. water column vs. effluent)?
  - Are these differences observed by sampling time points (year and month)?
  - Are these differences observed by sampling site? And in particular, is there an effect of sampling site-location (upstream or downstream of a WRP)?
- What is the relative abundance of fecal indicator organisms (FIOs)?
  - Does FIO abundance decay with distance from point sources?
  - What are the functional attributes of potential FIOs?
- What are the potential sources of microbial organisms at different points in the CAWS?
  - Does source apportionment vary with season or year for a particular location?
  - Are sources highly local or more general across the CAWS?
- What is the influence of land-use on microbial community structure?
  - Does land-use influence physicochemical properties in the CAWS?
  - Do different land-types influence source apportionment?



Sixteen sites monitored monthly during the spring, summer and fall for 2013,2014,2015.

Site	Address
36	North Shore Channel @ Touhy Ave.
43	Cal-Sag Channel @ Route # 83
52	Little Calumet River @ Wentworth Ave.
55	Calumet River @ 130th St.
56	Little Calumet River @ Indiana Ave.
57	Little Calumet River @ Ashland Ave.
59	Cal-Sag Channel @ Cicero Ave.
73	North Branch Chicago River @ Diversey Ave.
76	Little Calumet River @ Halsted St.
86	Grand Calumet River @ Burnham Ave.
94	Des Plaines River @ Empress Casino
96	North Branch Chicago River @ Albany Ave.
97	Thorn Creek @ 170th St.
99	South Fork, South Branch Chicago River @ Archer Ave.
100	Chicago River Main Stem @ Wells St.
108	South Branch Chicago River @ Loomis St.
112	North Shore Channel @ Dempster Street



The microbial communities in the CAWS water and sediment are dominated by Proteobacteria, Bacteroidetes, Actinobacteria and Cyanobacteria, which is very common for all river systems (urban or natural),

Figure S1A: Sankey Diagram highlighting abundant bacterial phlya across all CAWS samples.

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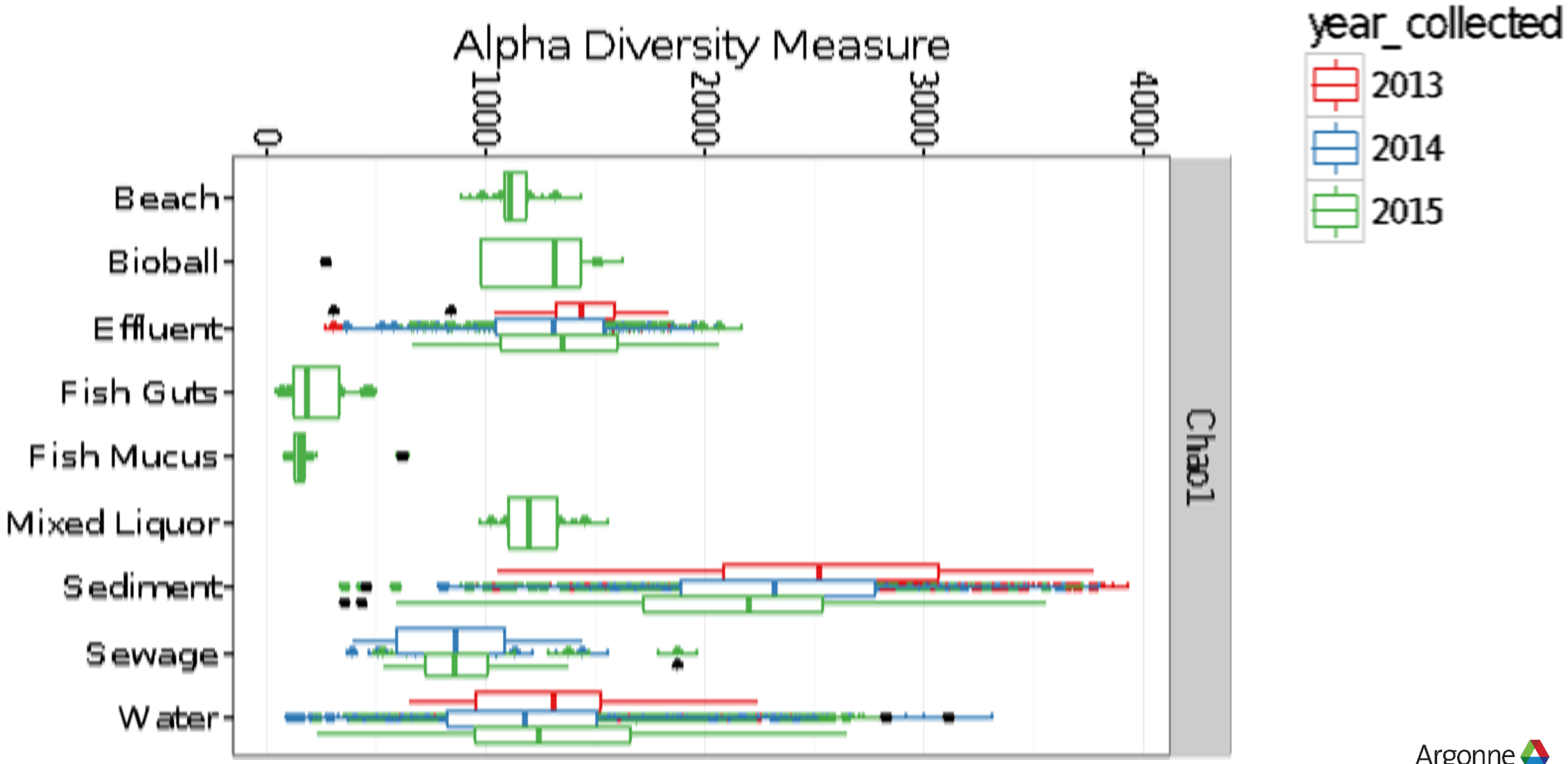
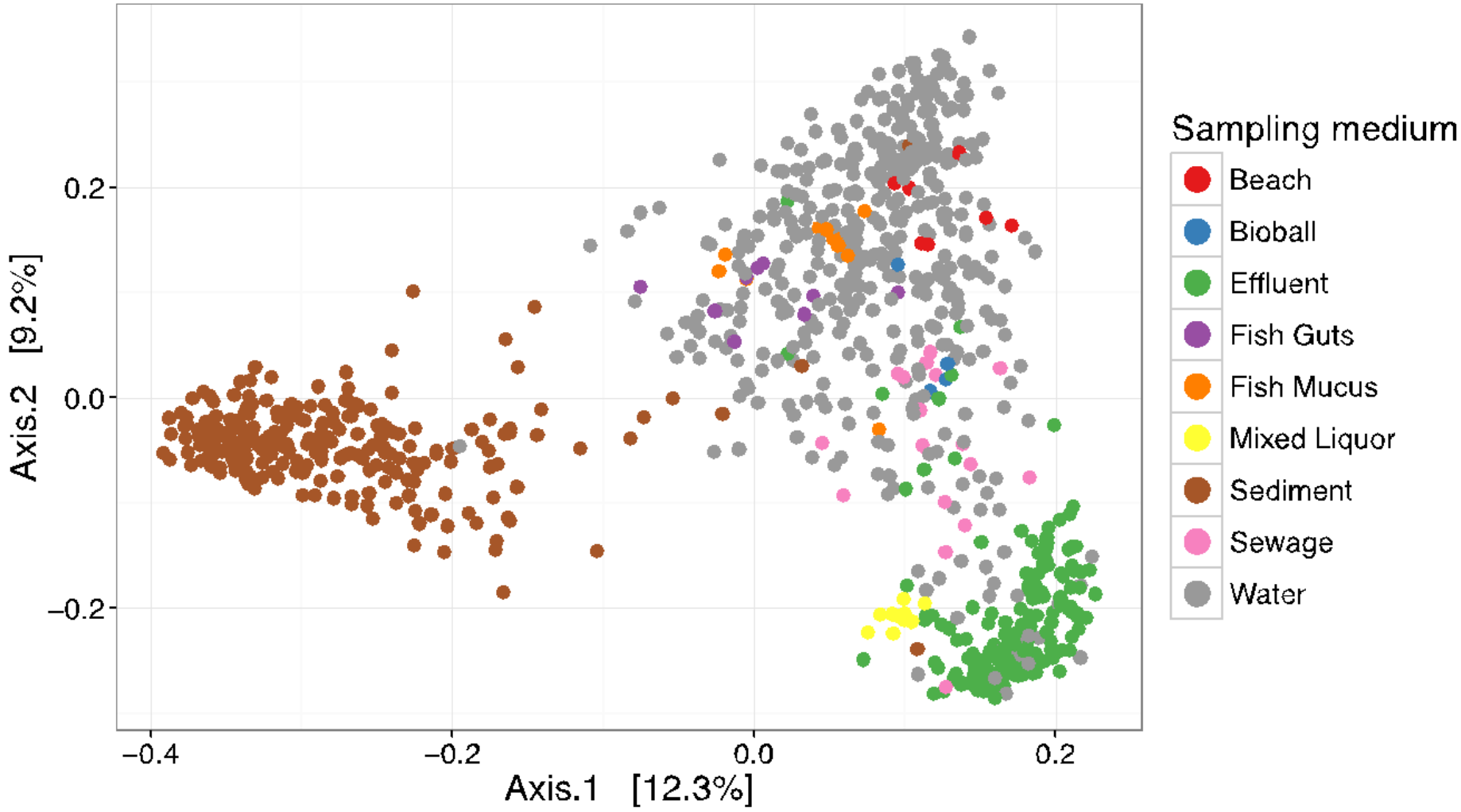
MICROBIAL DIVERSITY VARIES BY SAMPLED MEDIUM



MICROBIAL COMMUNITY STRUCTURE DIFFERS BY MEDIUM



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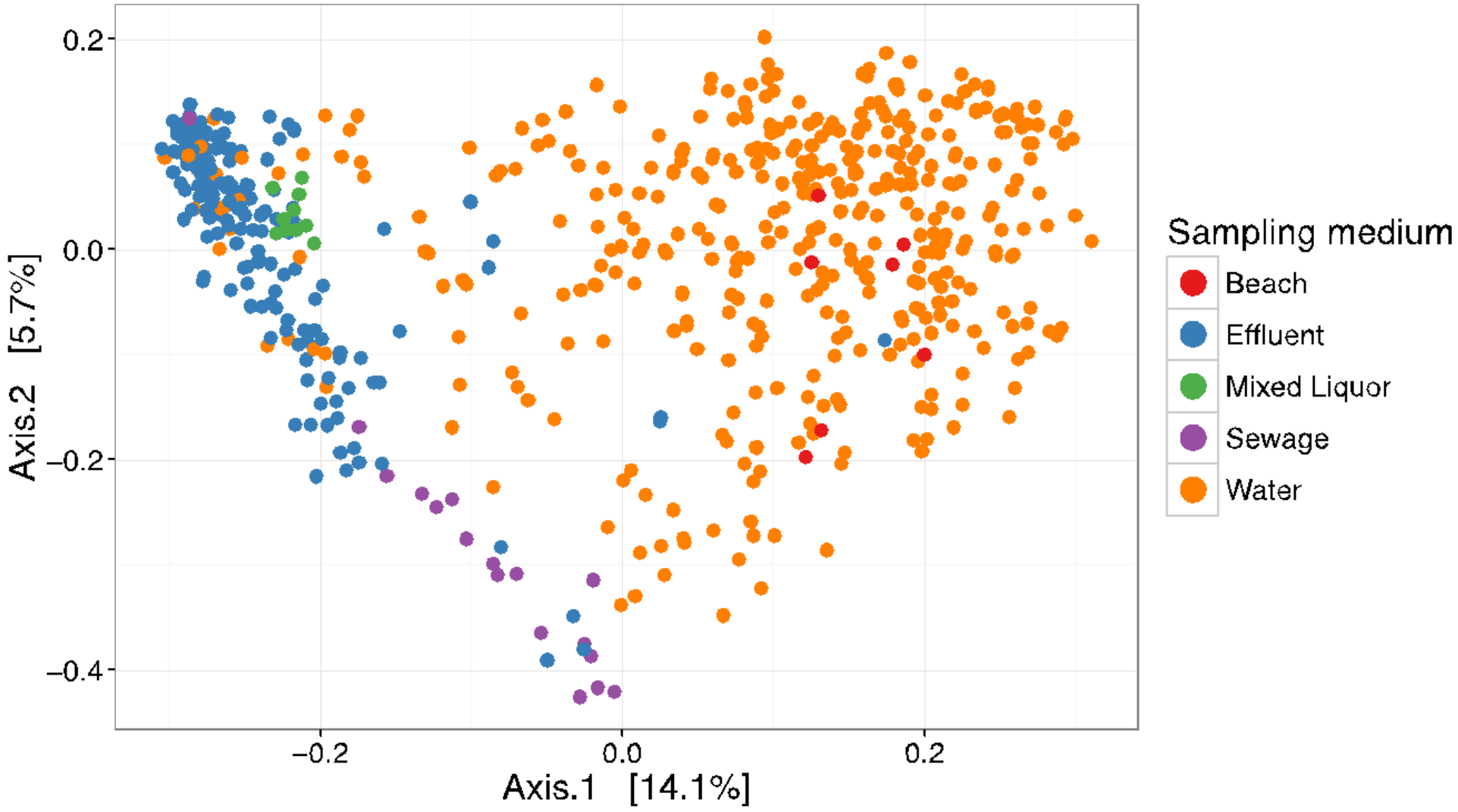

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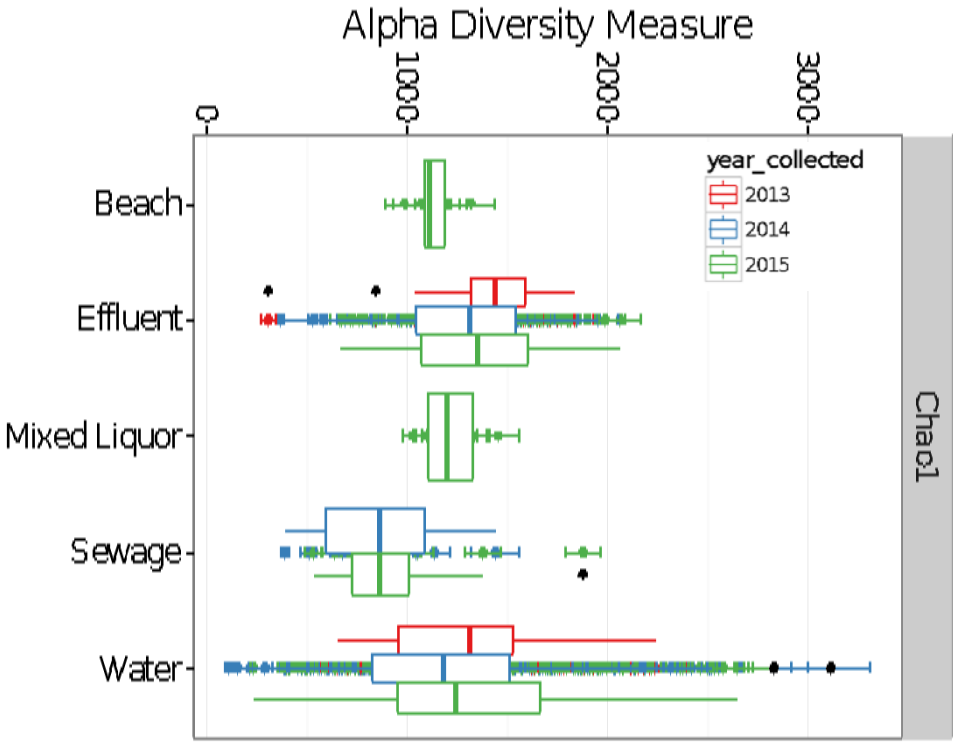
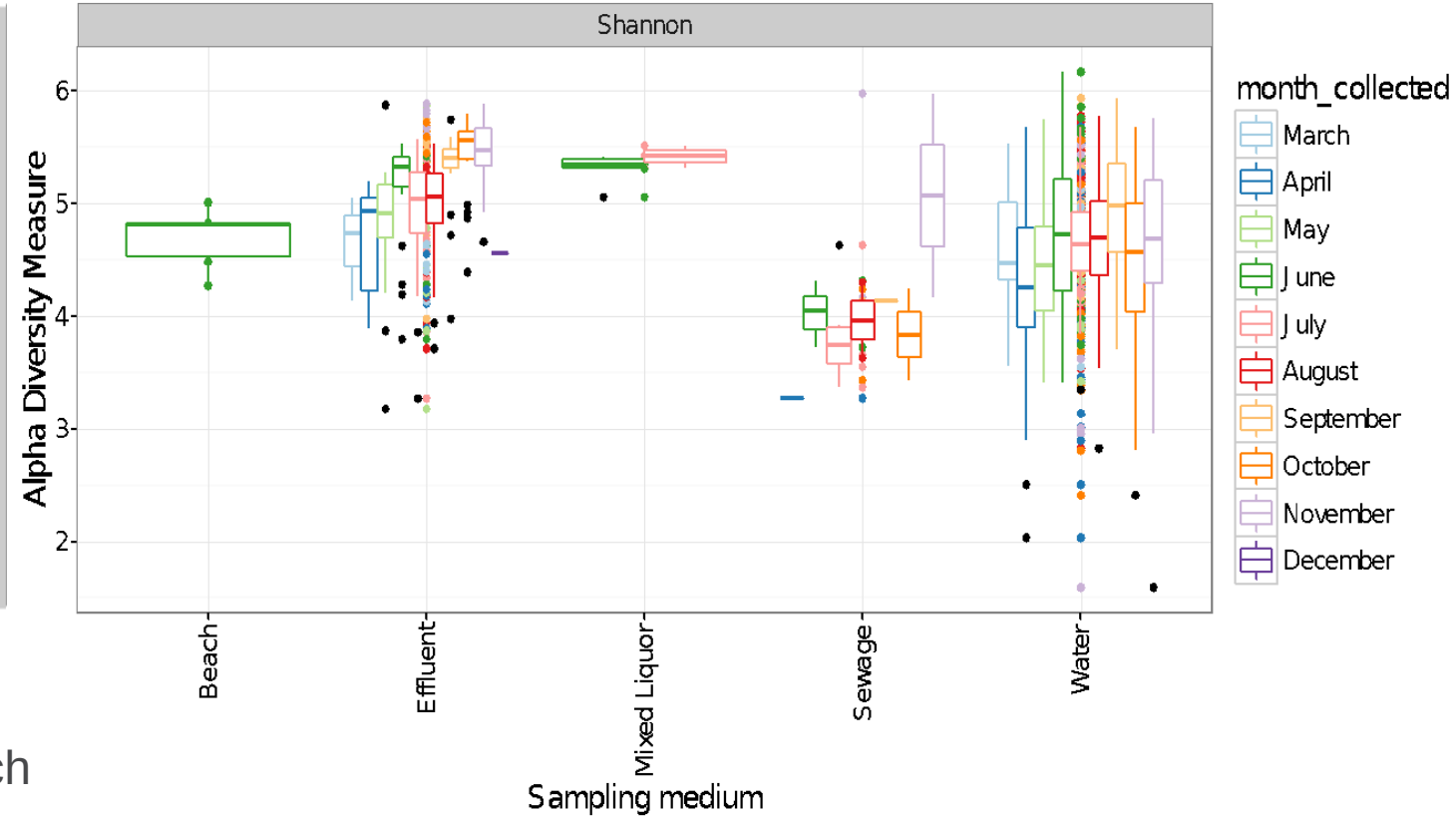
MICROBIAL DIVERSITY DOES NOT DIFFER BY YEAR OR MONTH





Alpha diversity for water column samples collected in April was typically lower at each sampling site than those collected in other months.

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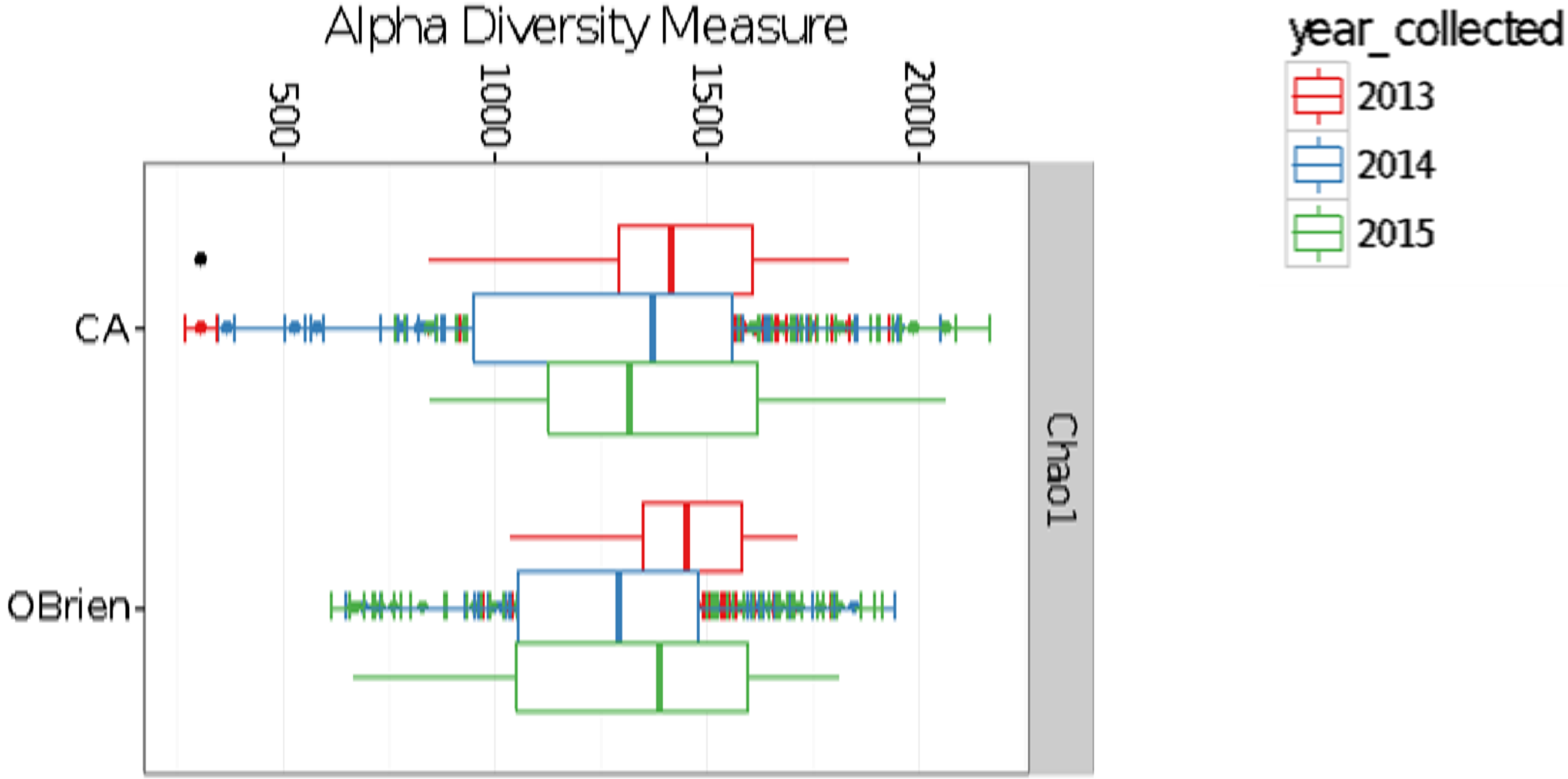
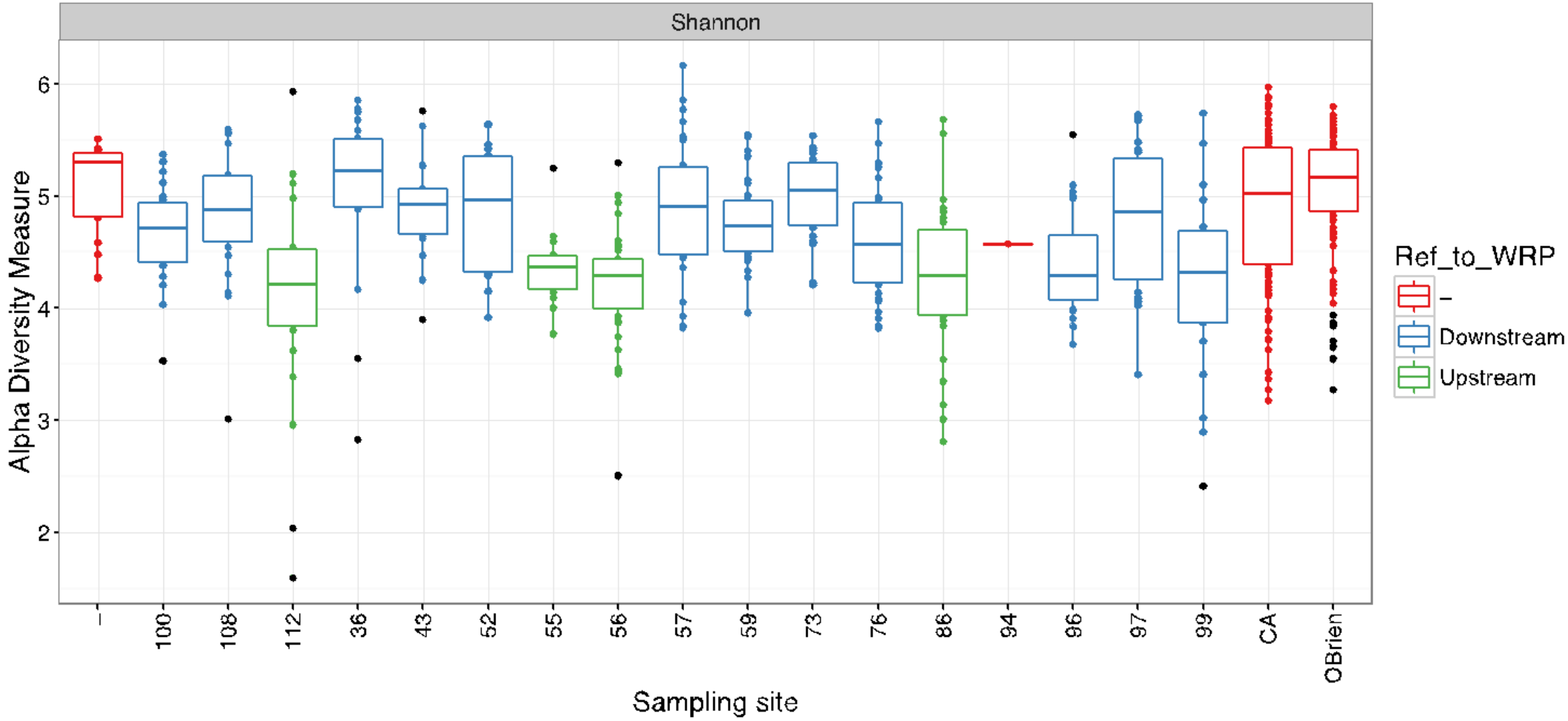
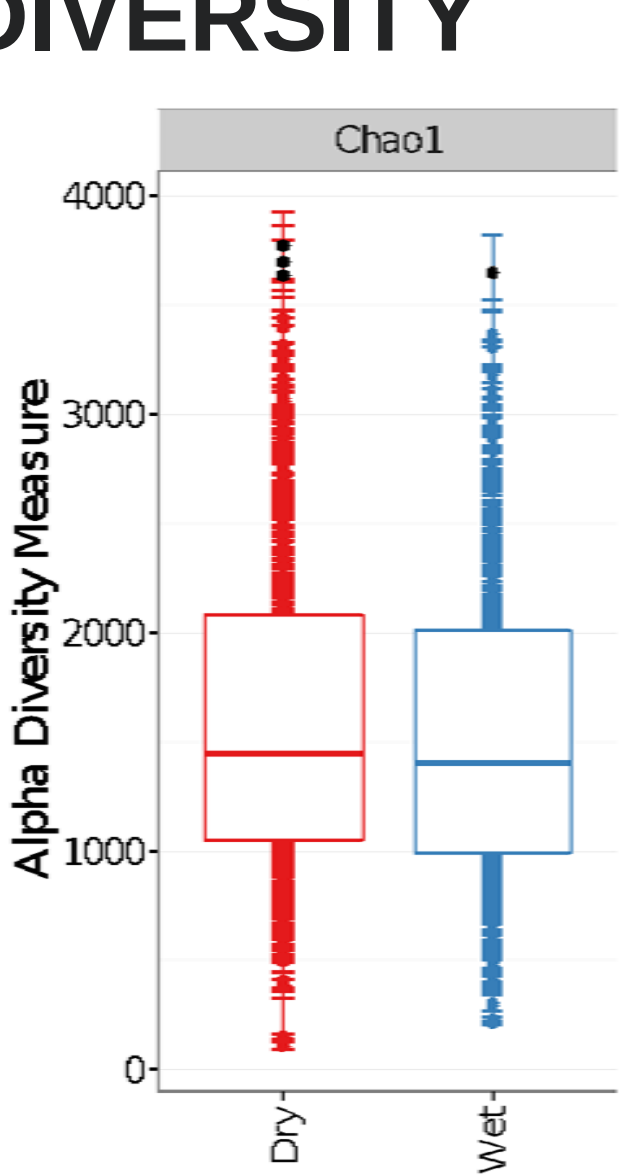
SECONDARY TREATED EFFLUENT DIVERSITY HARDLY CHANGES

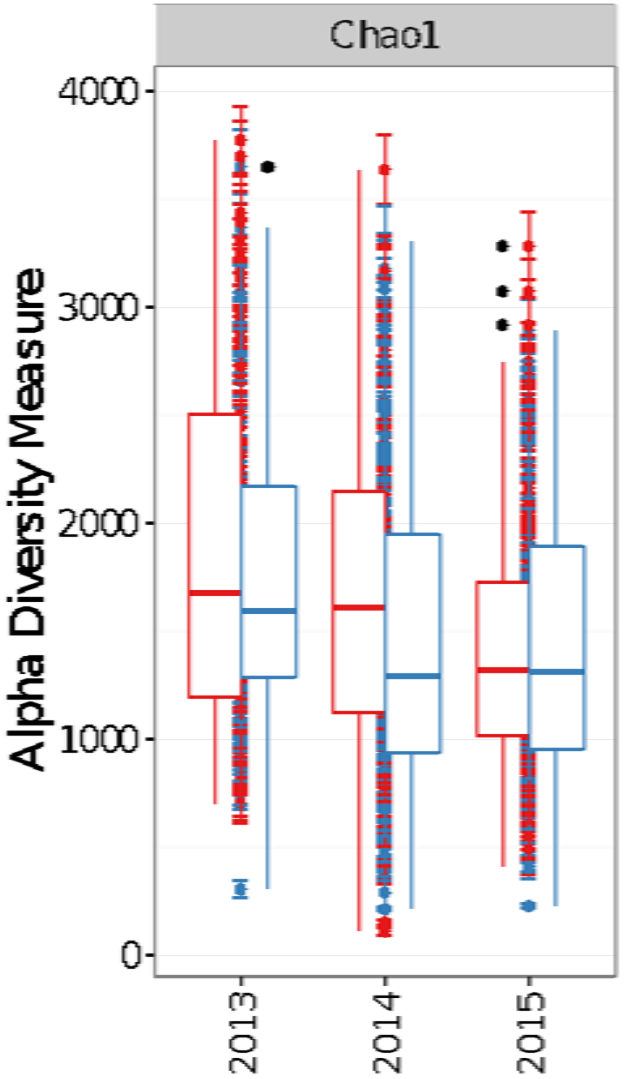


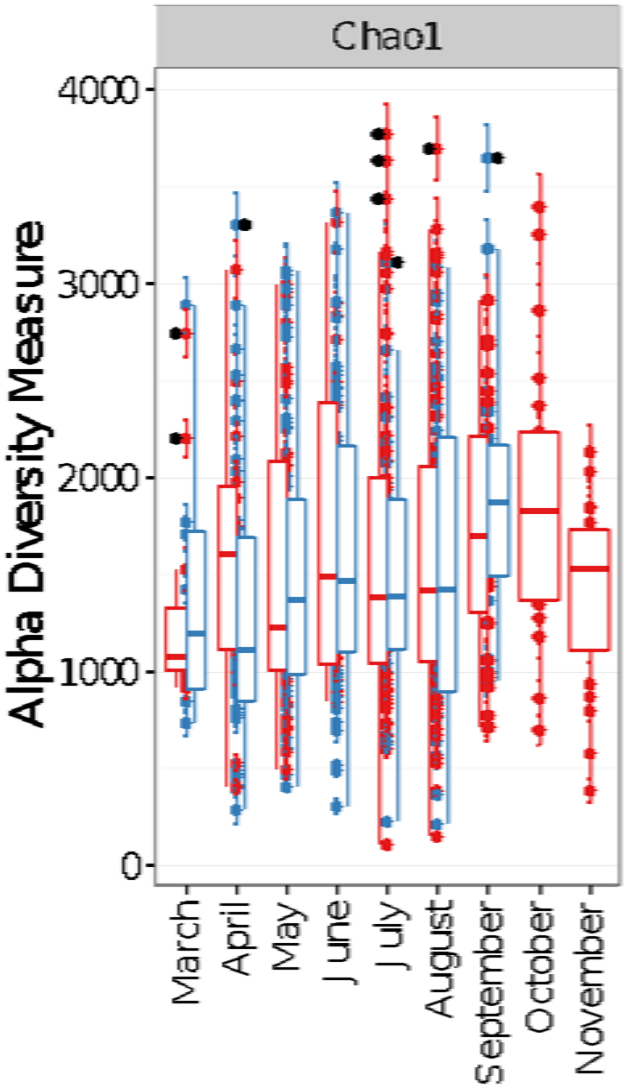
MICROBIAL DIVERSITY IS GREATER DOWNSTREAM OF AN OUTFALL



DRY AND WET WEATHER DOESN'T ALTER THE DIVERSITY







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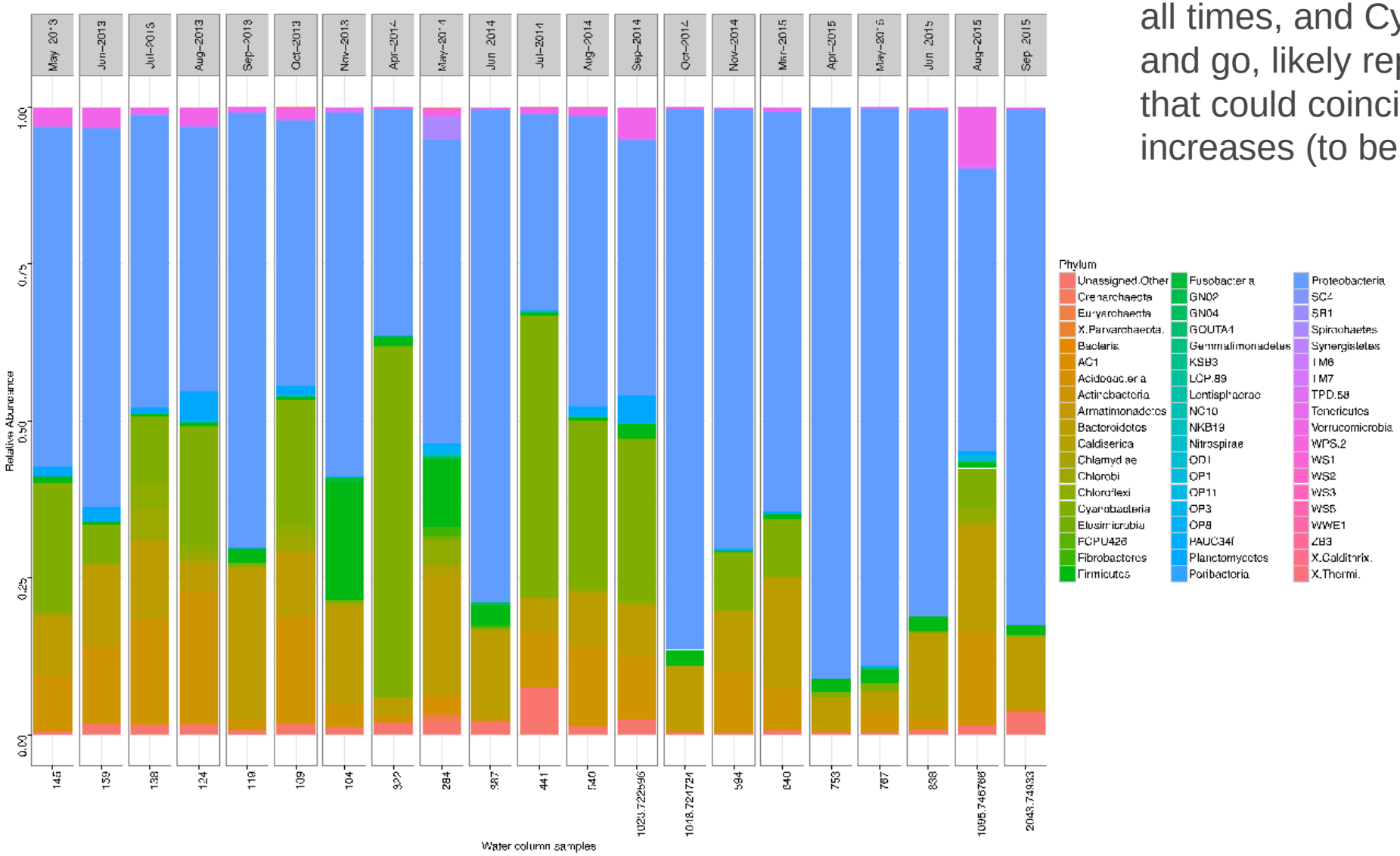
BUBBLY CREEK OVER TIME



There are no seasonal changes that are robust across all 3 years of analysis. Proteobacteria dominate at all times, and Cyanobacteria come and go, likely representing blooms that could coincide with nutrient increases (to be determined).

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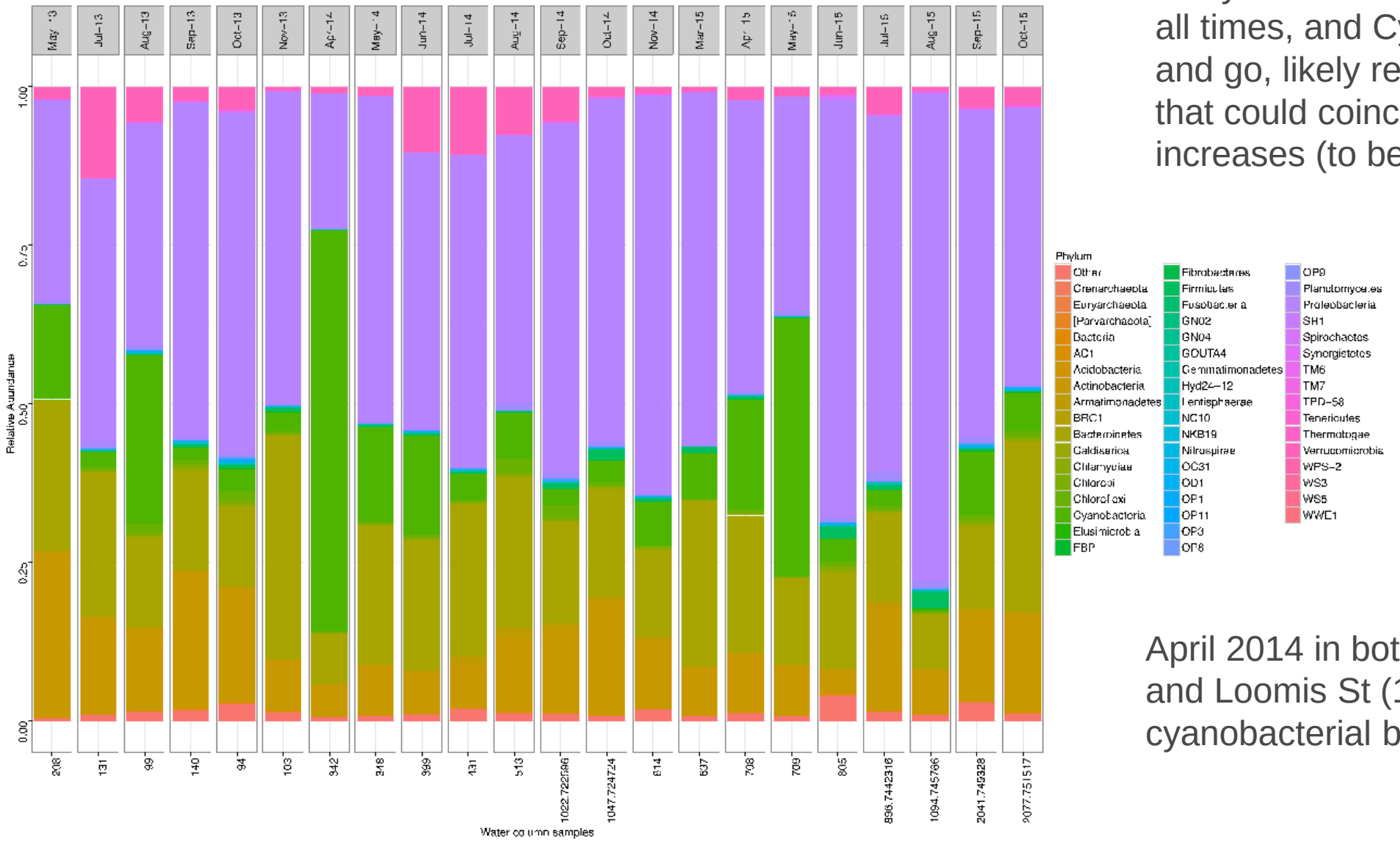
LOOMIS ST CHICAGO RIVER S



There are no seasonal changes that are robust across all 3 years of analysis. Proteobacteria dominate at all times, and Cyanobacteria come and go, likely representing blooms that could coincide with nutrient increases (to be determined).

April 2014 in both Bubbly Creek (99) and Loomis St (108) has a big cyanobacterial bloom in April 2014

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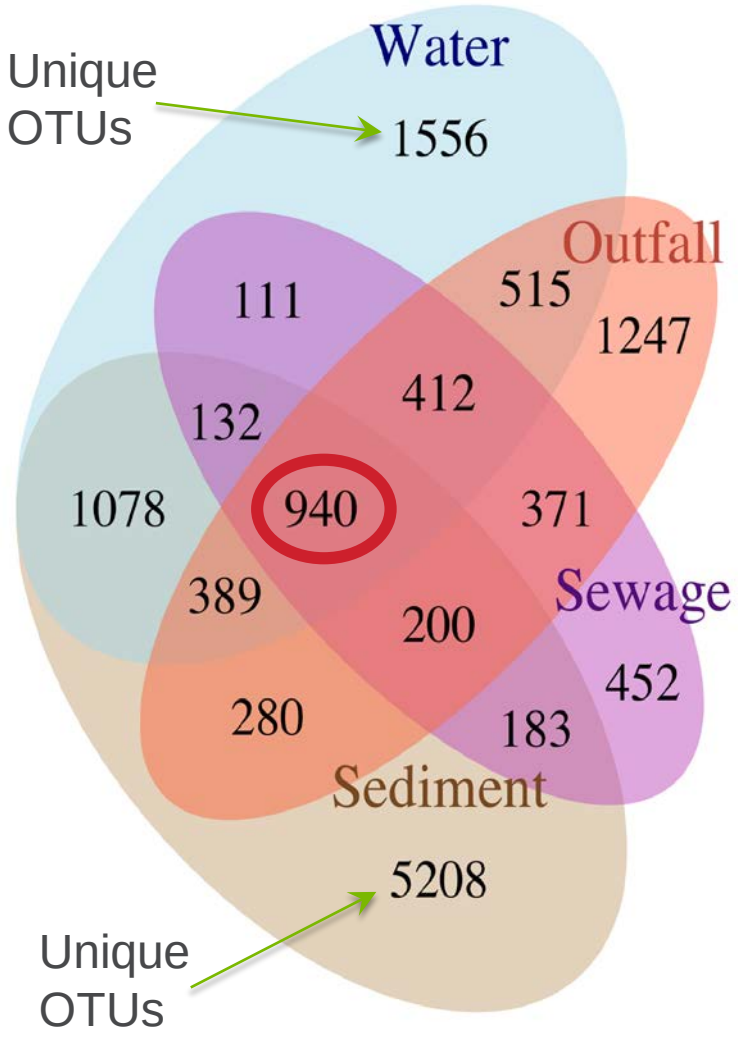
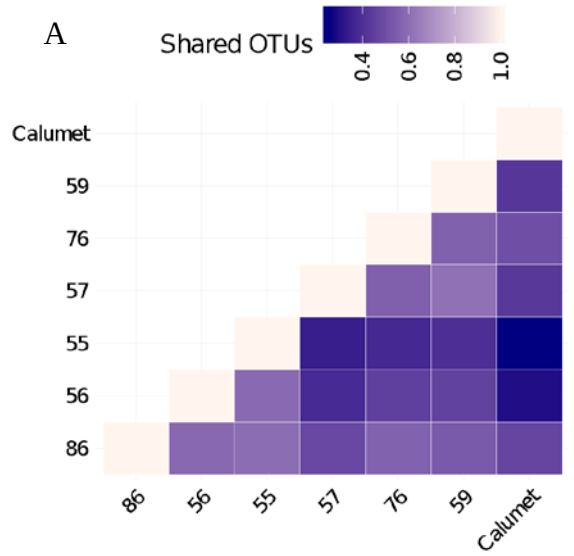
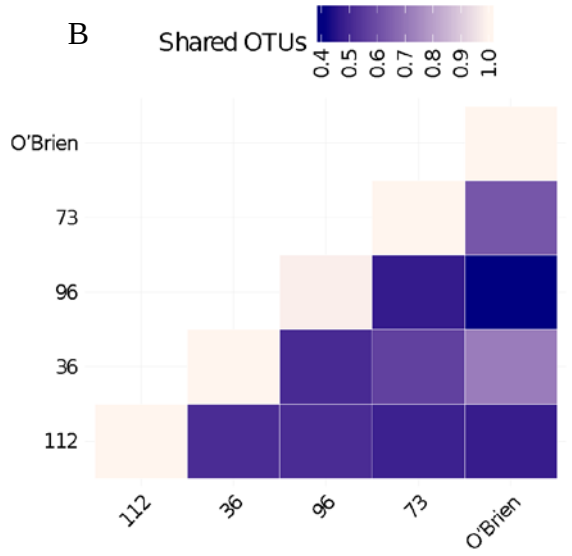
BACTERIAL TAXA SHARED ACROSS SAMPLE TYPES.







Shared OTUs between the different sampling sites located near the two WRPs at Calumet (A) and O'Brien (B).

This is displayed as a heatmap above wherein the quantity of shared OTUs is colored by a gradient with increased OTUs represented by light purple.

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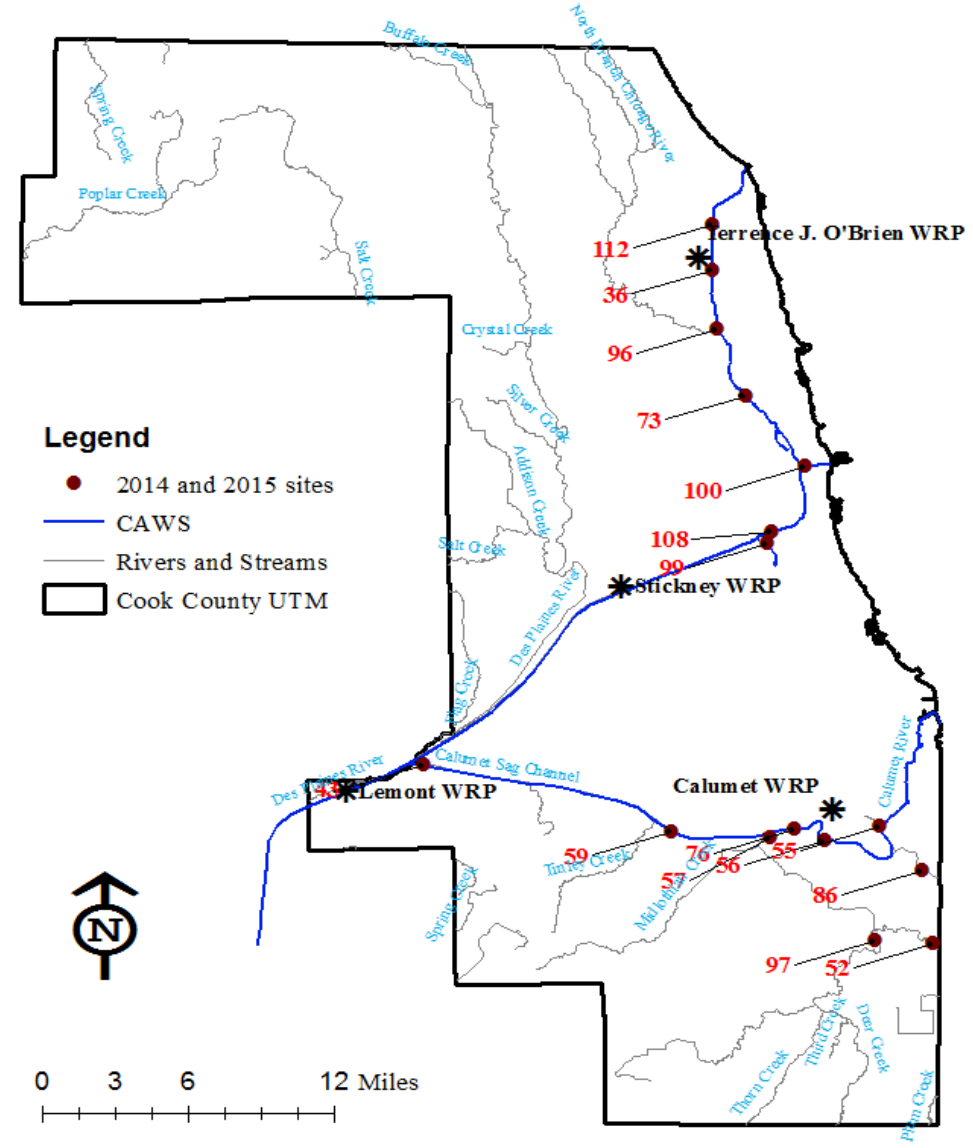
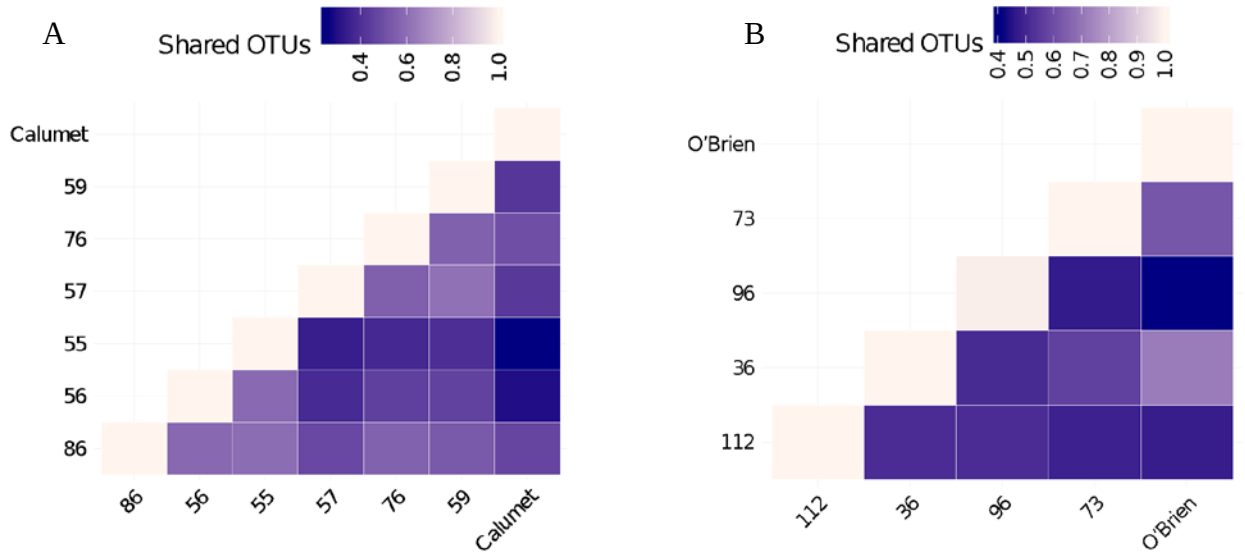
BACTERIAL TAXA SHARED ACROSS SAMPLE TYPES.





Shared OTUs between the different sampling sites located near the two WRPs at Calumet (A) and O'Brien (B).

This is displayed as a heatmap wherein the quantity of shared OTUs is colored by a gradient with increased OTUs represented by light purple.

55 and 56 are upstream of Calumet WRP and share the least with the outfall.
96 and 112 are upstream of O'Brien WRP and share the least with the outfall.

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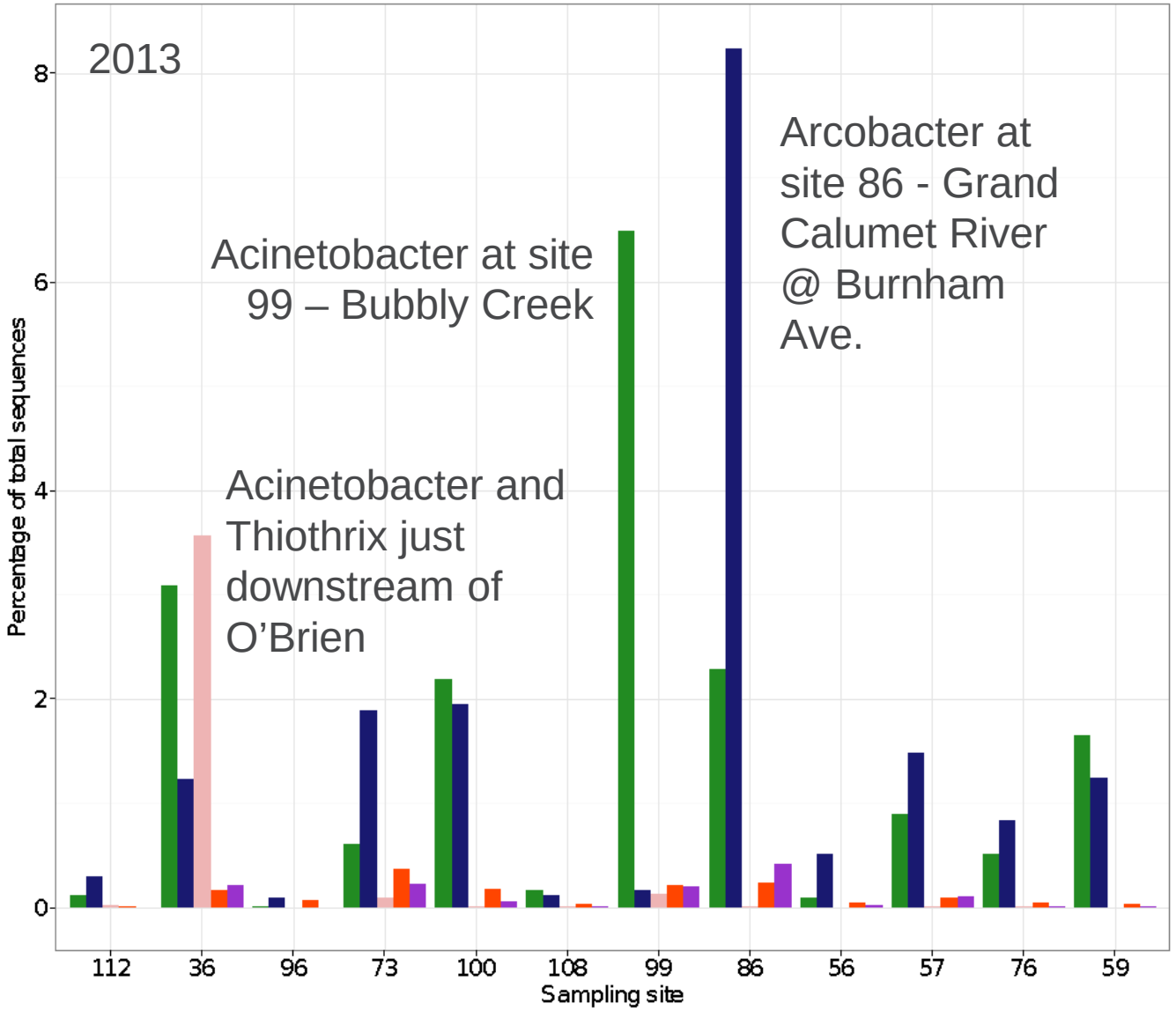
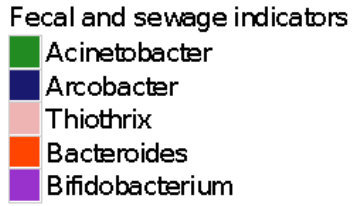
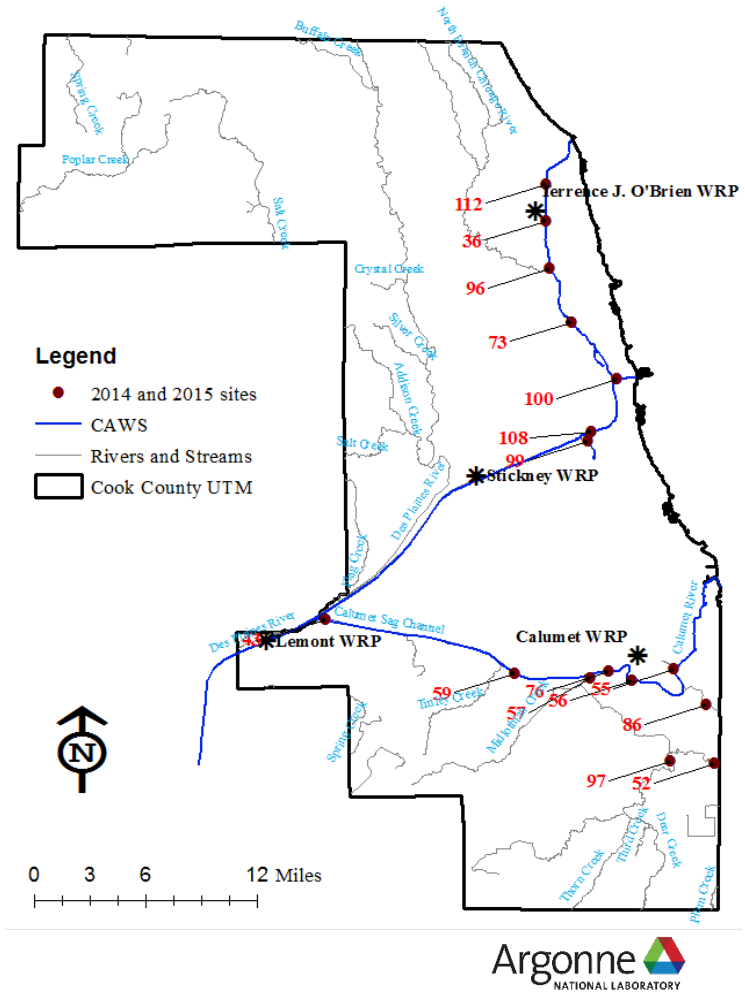
TRACKING THE ABUNDANCE OF FECAL INDICATOR TAXA

Lowest sites – 56, 96 and 112 – all upstream of off branch from outfalls







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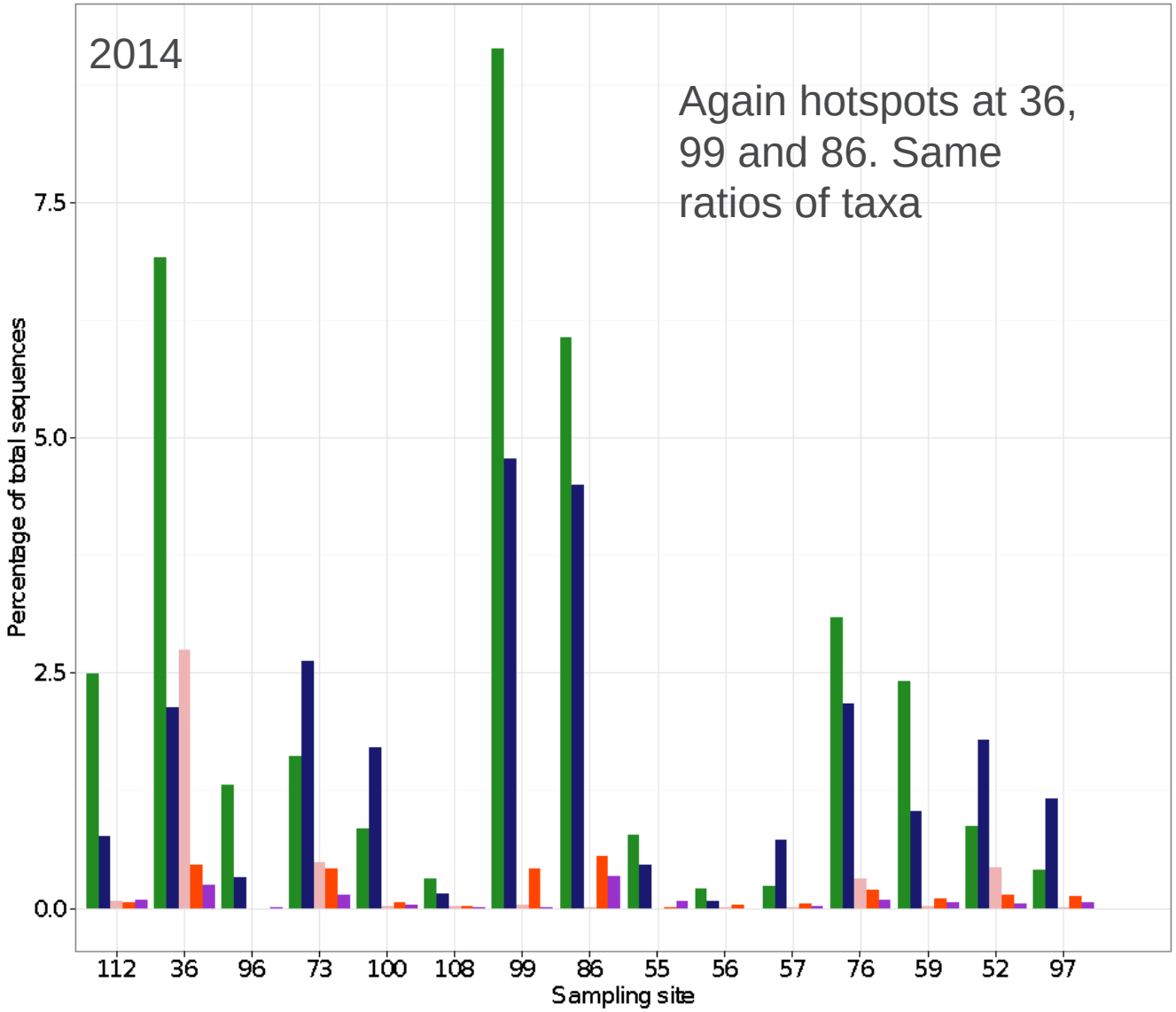
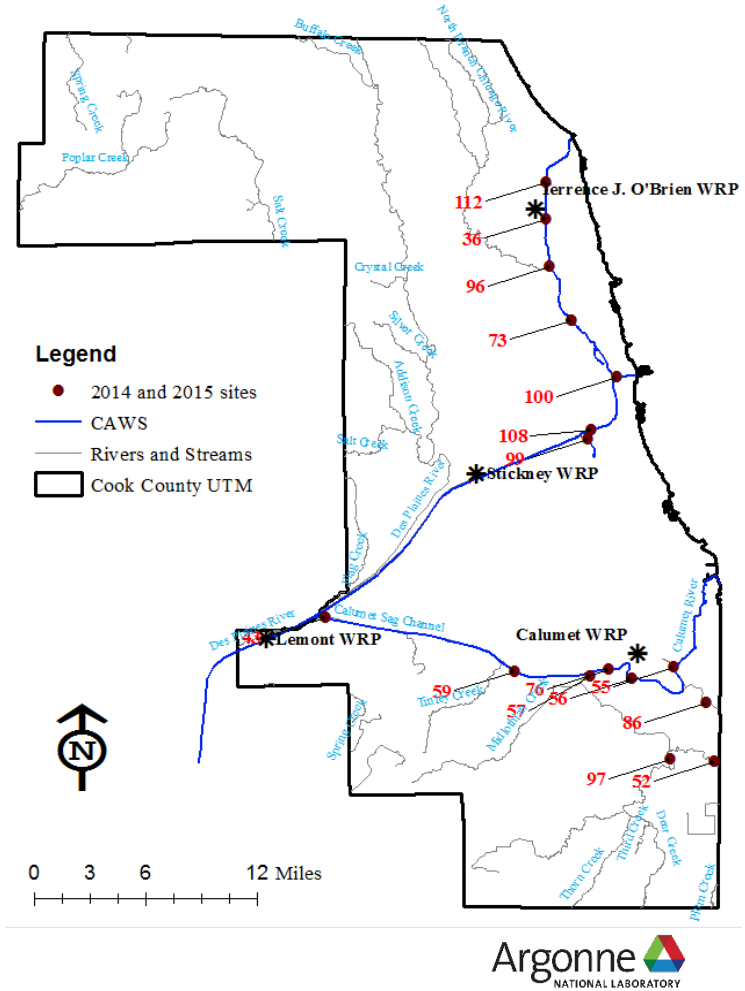
TRACKING THE ABUNDANCE OF FECAL INDICATOR TAXA

Lowest sites – 55, 56, 57, 96 and 112 – all upstream of off branch from outfalls



Fecal and sewage indicators
- Acinetobacter
- Arcobacter
- Thiothrix
- Bacteroides
- Bifidobacterium



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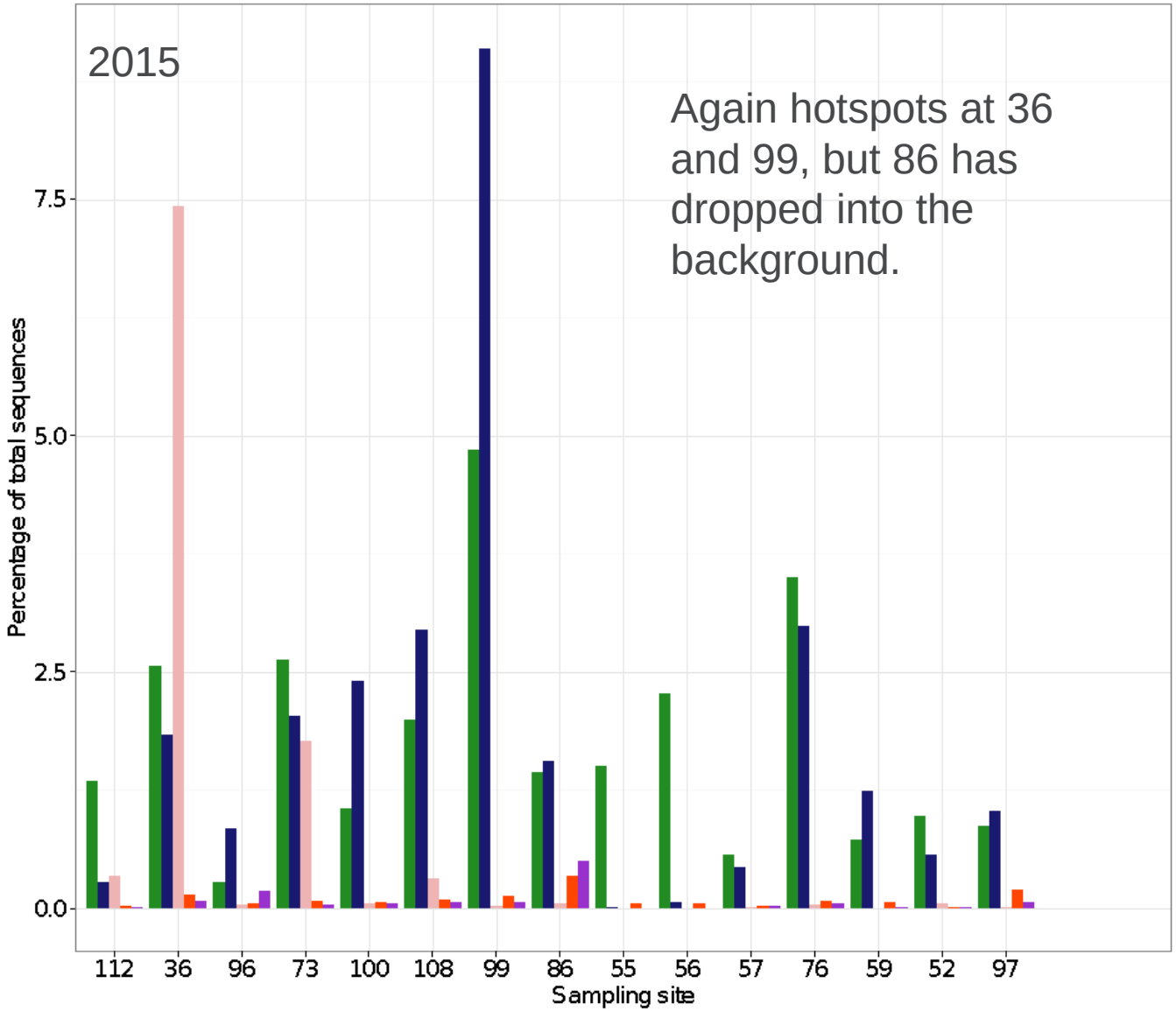
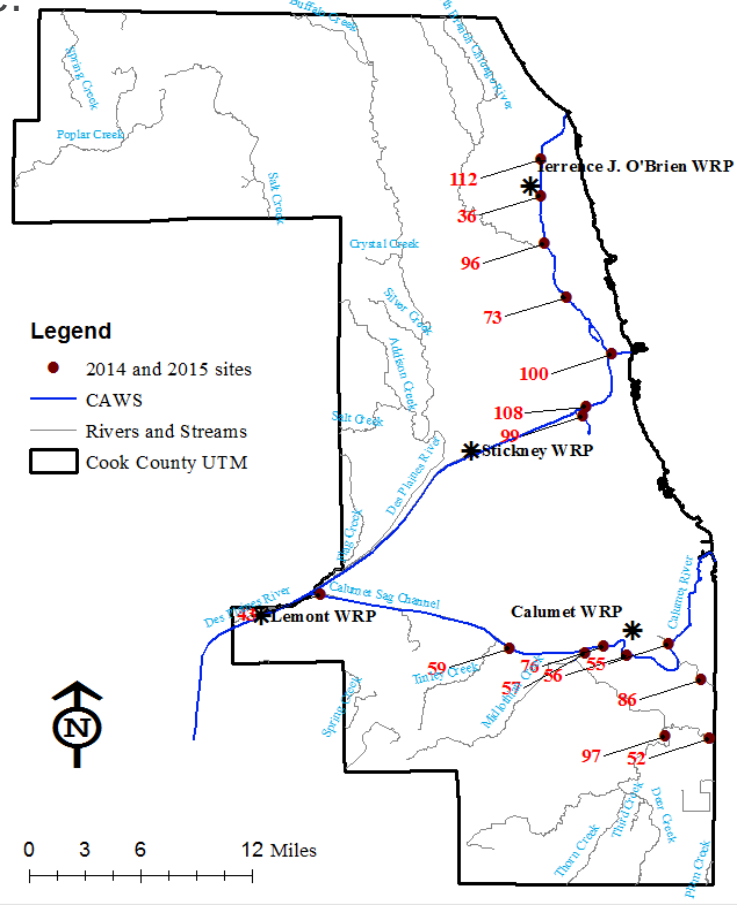
TRACKING THE ABUNDANCE OF FECAL INDICATOR TAXA



Lowest sites – 55, 56, 57, 96 and 112 – all upstream of off branch from outfalls.

But Acinetobacter is more abundant everywhere.

Fecal and sewage indicators
- Acinetobacter
- Arcobacter
- Thiothrix
- Bacteroides
- Bifidobacterium



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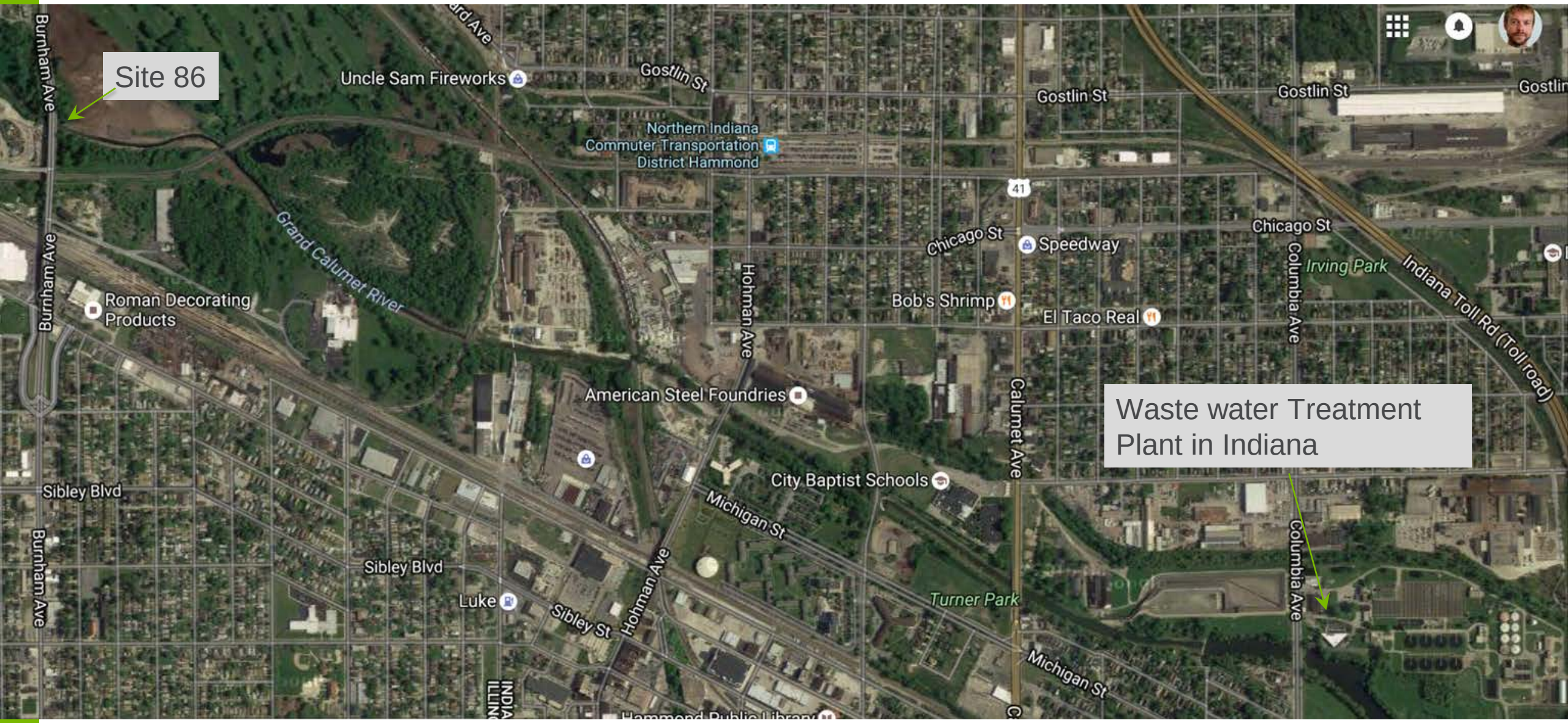
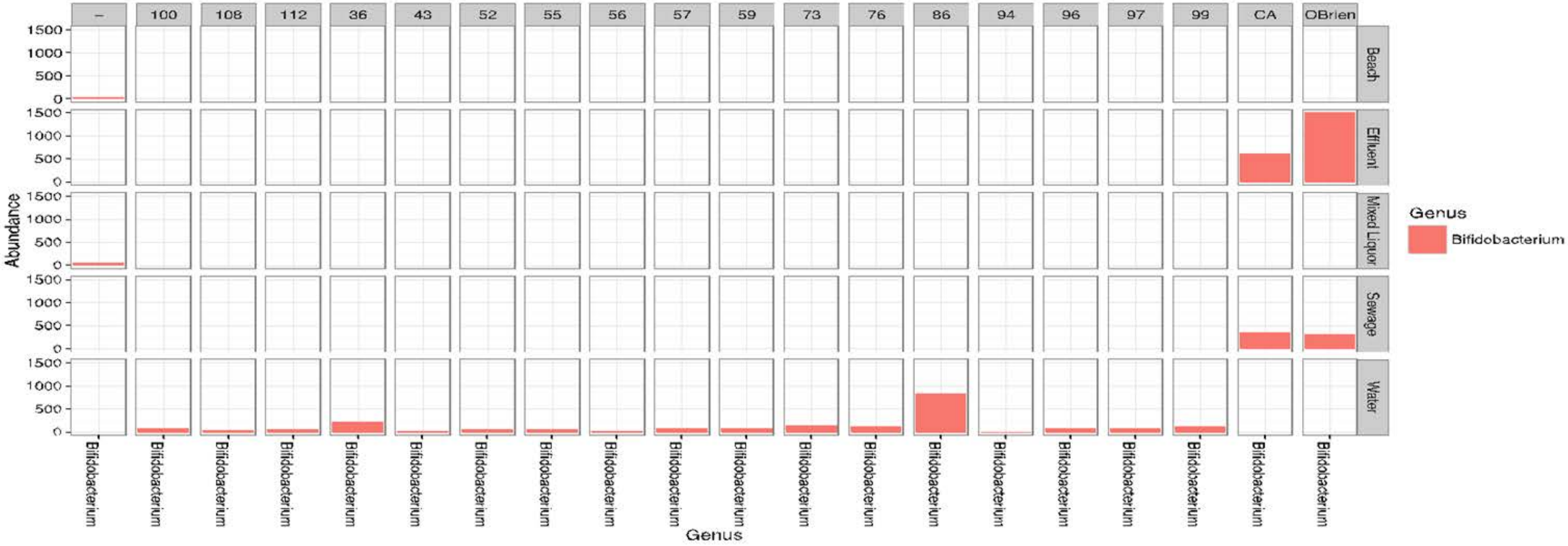
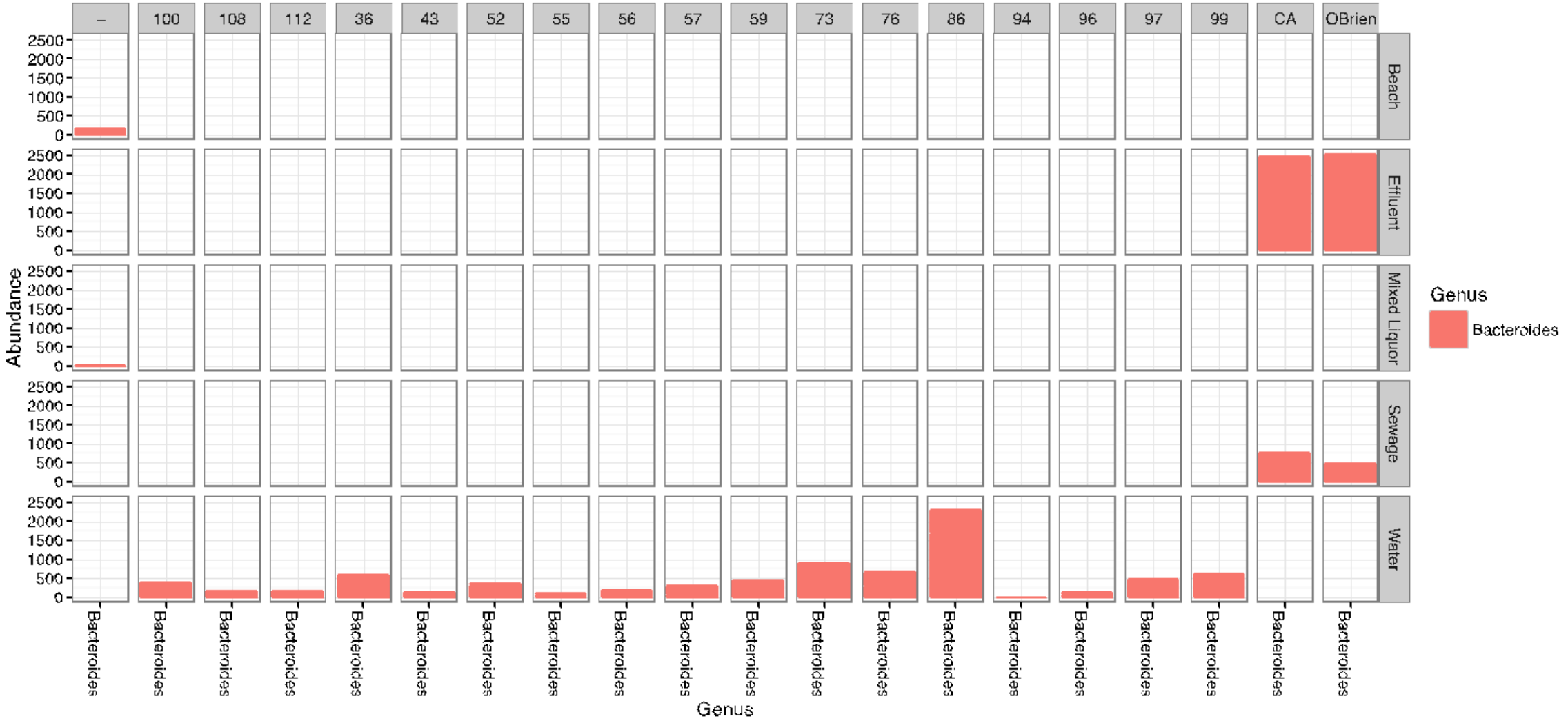

Site 86
Waste water Treatment Plant in Indiana
Burnham Ave
Uncle Sam Fireworks
Gostlin St
Northern Indiana Commuter Transportation District Hammond
Grand Calumet River
Roman Decorating Products
Hohman Ave
American Steel Foundries
Chicago St
Speedway
Bob's Shrimp
El Taco Real
Irving Park
Columbia Ave
Indiana Toll Rd (Toll road)
Calumet Ave
City Baptist Schools
Michigan St
Sibley Blvd
Luke
Sibley St
Turner Park
Hammond Public Library

FIO TRACKING BY ORGANISM - BIFIDOBACTERIUM



FIO TRACKING BY ORGANISM - BACTEROIDES



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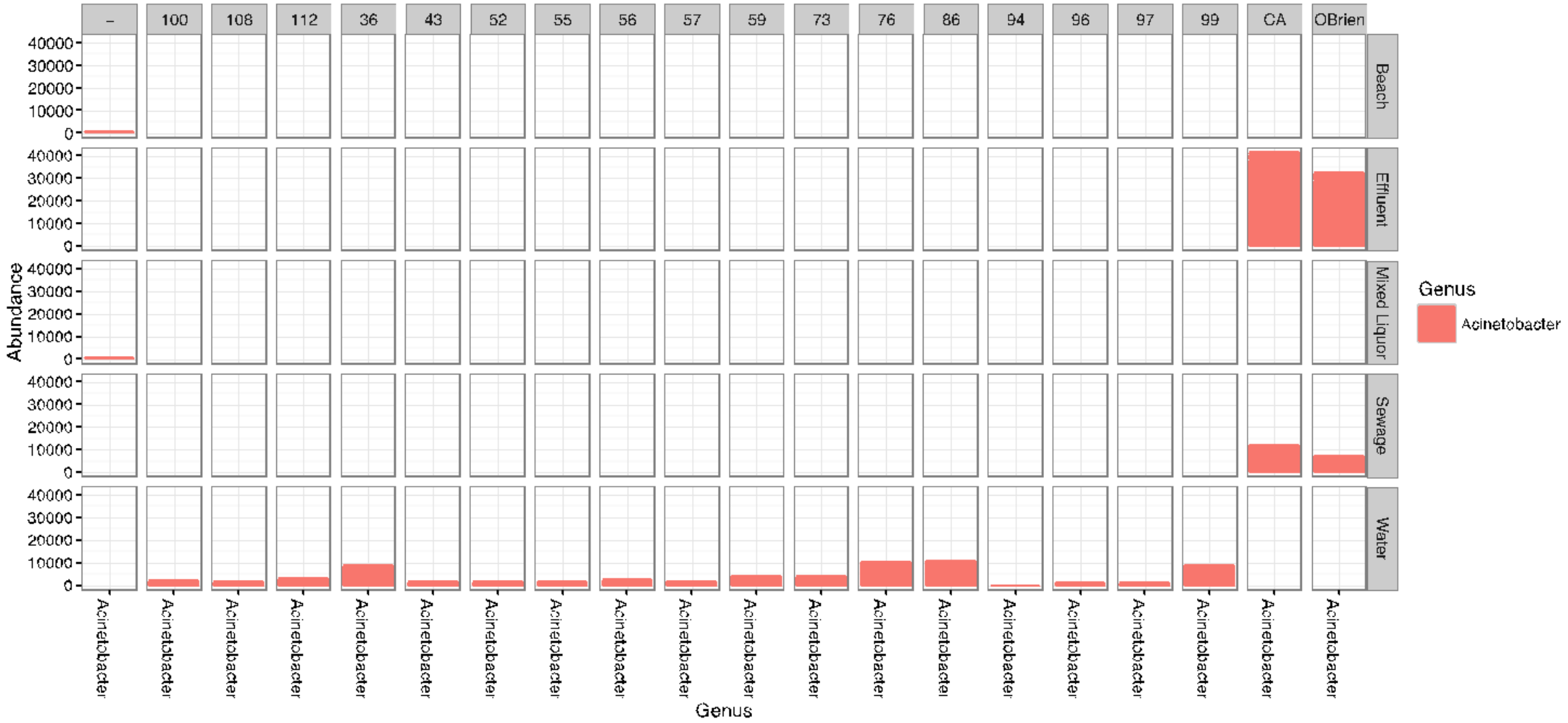
FIO TRACKING BY ORGANISM – ACINETOBACTER



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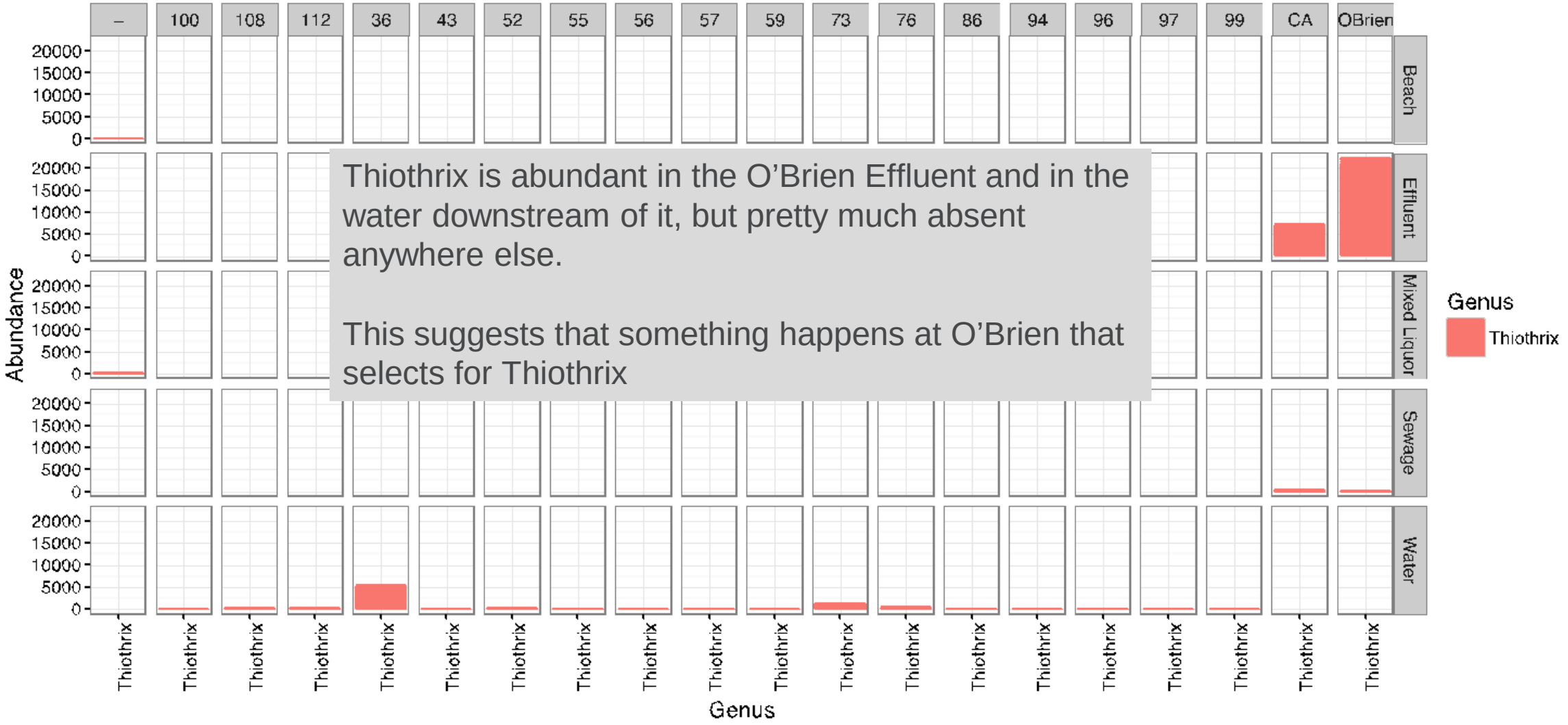
FIO TRACKING BY ORGANISM - THIOTHRIX



Thiothrix is abundant in the O'Brien Effluent and in the water downstream of it, but pretty much absent anywhere else.

This suggests that something happens at O'Brien that selects for Thiothrix

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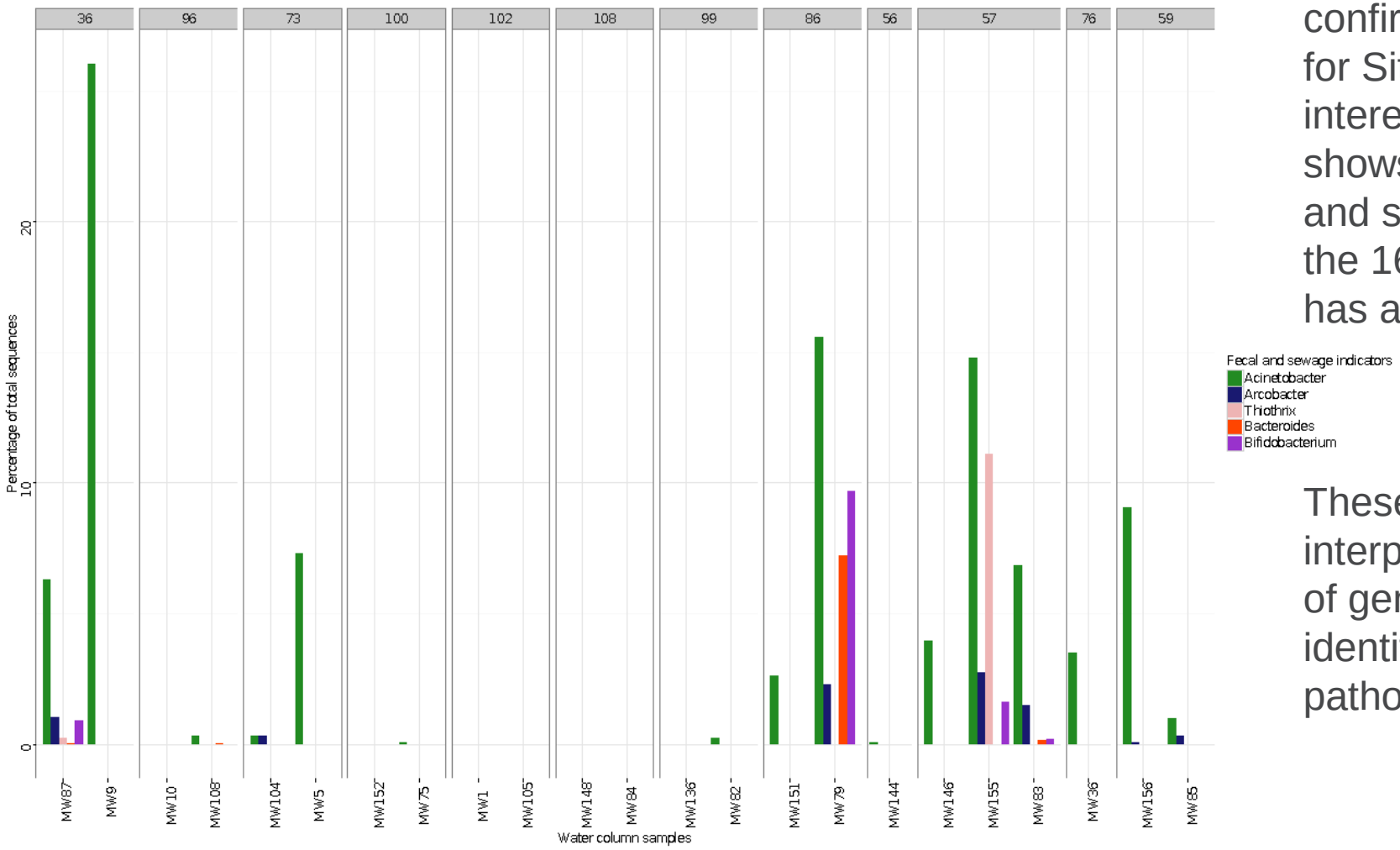
METAGENOMIC FIO TRACKING



For 2013 – shotgun metagenomes confirms the results from the 16S for Sites 36 and 86. But interestingly site 99 (Bubbly Creek) shows virtually no FIO genomes, and site 57, which was very low for the 16S, is now much greater and has a bloom of Thiothrix.

These analyses will take longer to interpret, but the refined resolution of genotyping should help us to identify the exact strains and their pathogenicity.

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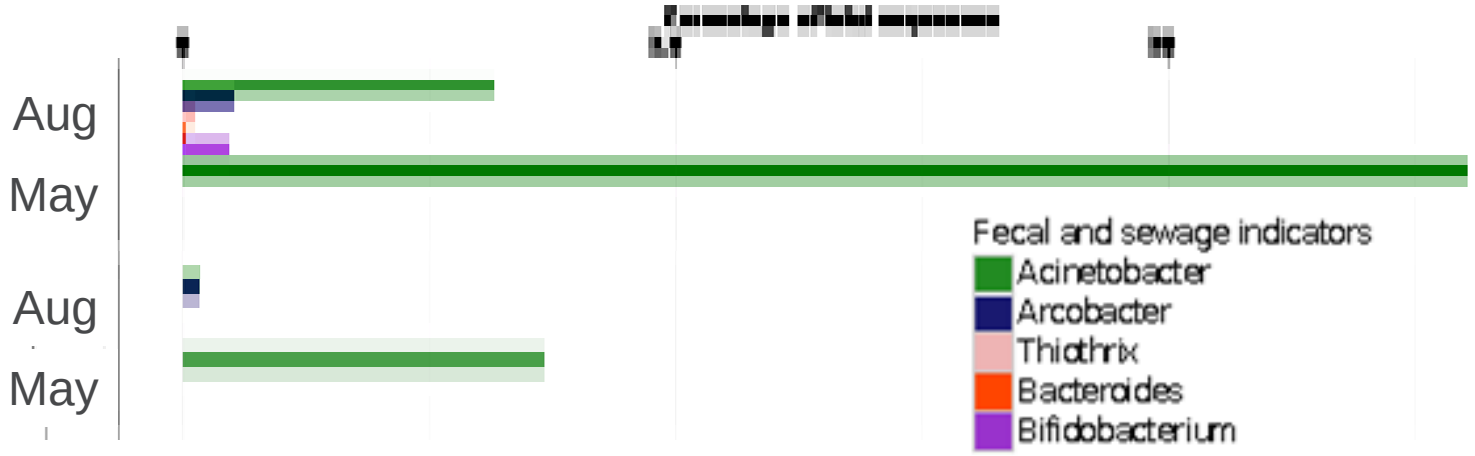
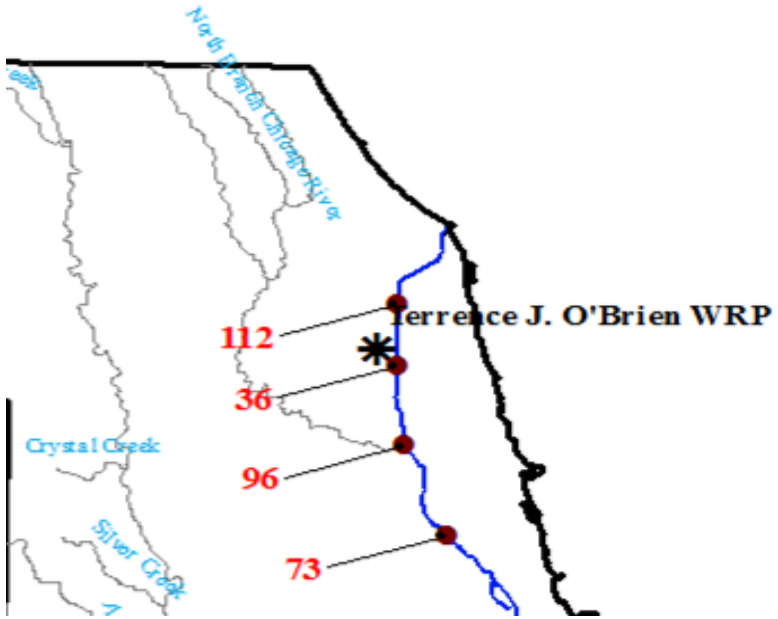
METAGENOMIC FIO TRACKING





Site 36 is dominated entirely by Acinetobacter in the spring and then overall less abundance but more diverse FIOs in the late summer

Site 73 shows a similar pattern – greater abundance of Acinetobacter in the spring, and then this dies down in the later summer.

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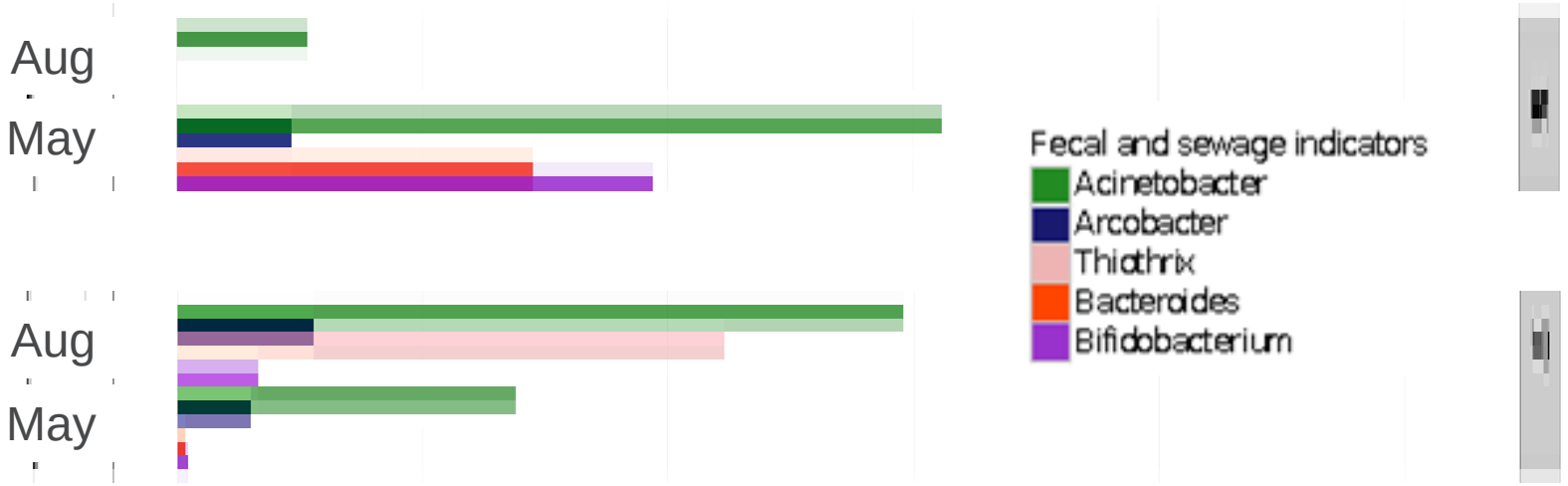
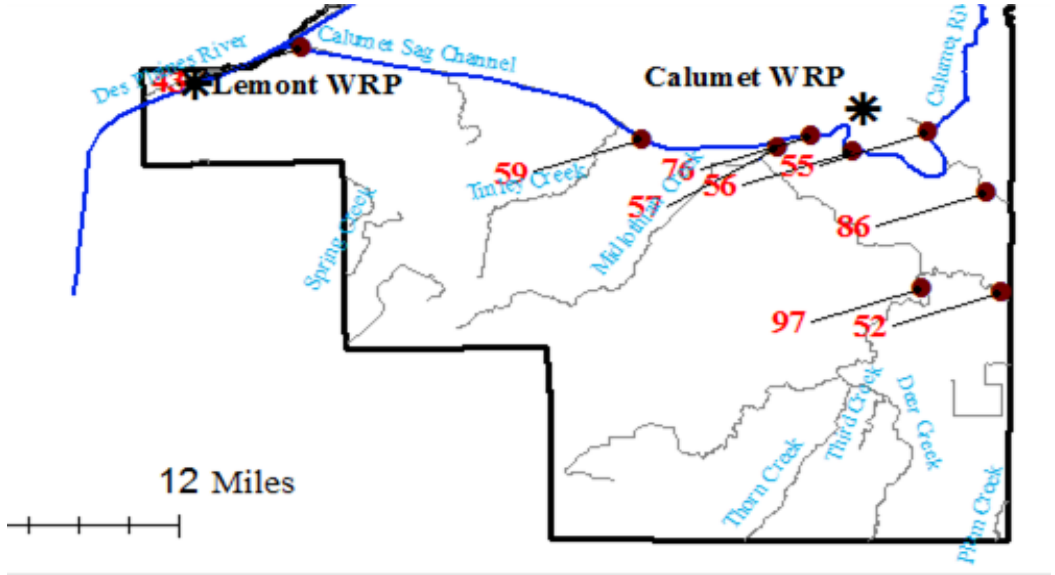
METAGENOMIC FIO TRACKING





Site 86 has a great abundance of Acinetobacter, Bacteroides and Bifidobacterium in the spring, which dies away late summer.

Site 57 shows the reverse with some levels all year round, but a greater abundance of Acinetobacter and Thiothrix in the late summer.

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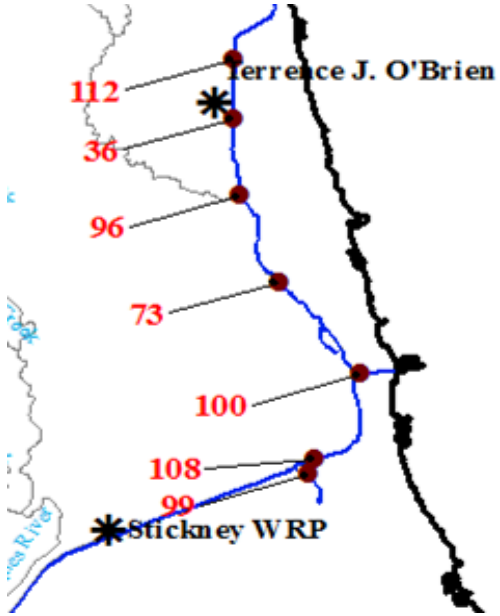
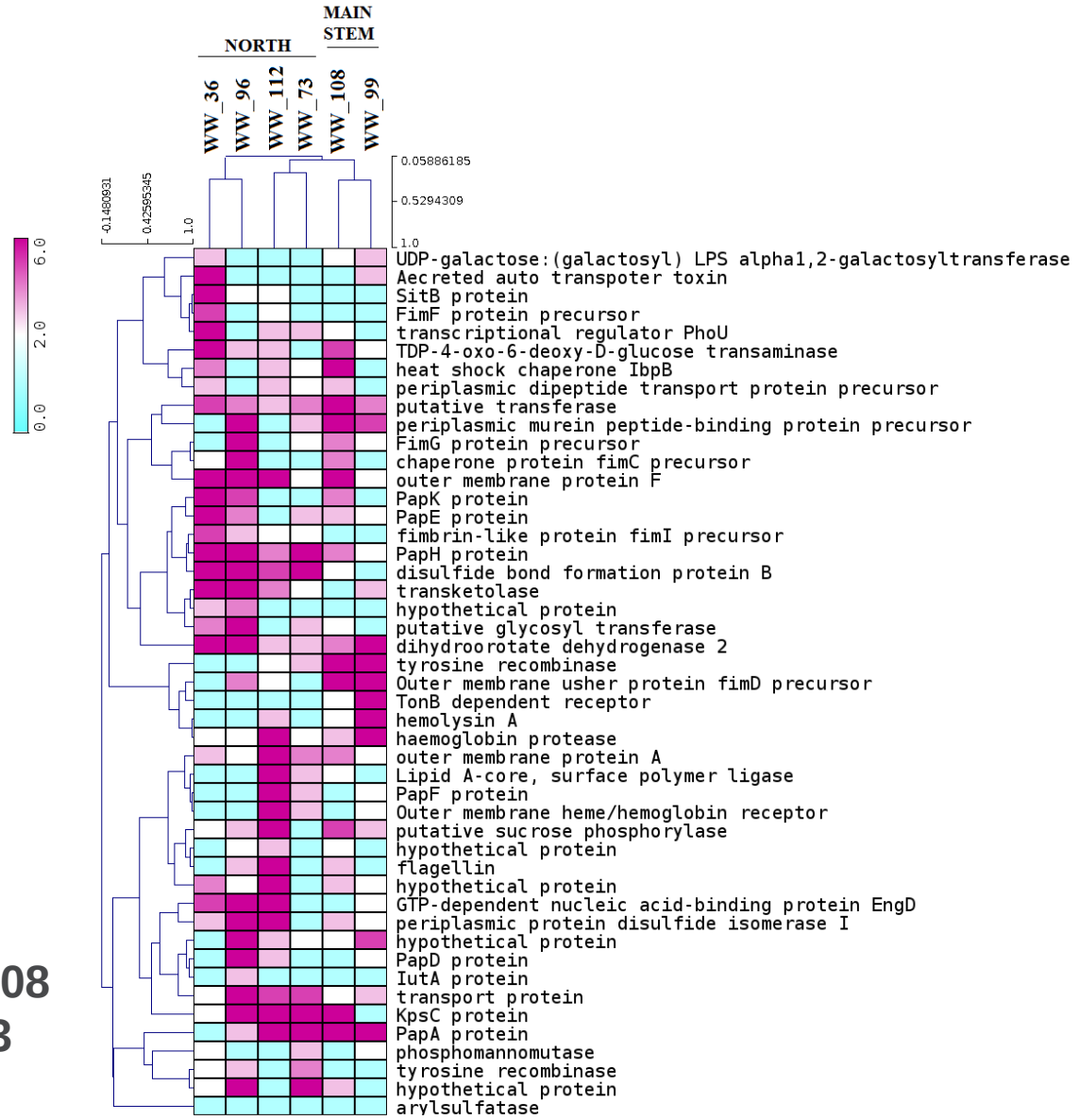
RELATIONSHIPS WITH E.COLI OR FECAL COLIFORMS

- E.coli were rare in metagenomes and 16S data.
- E.coli virulence did not match outfall locations

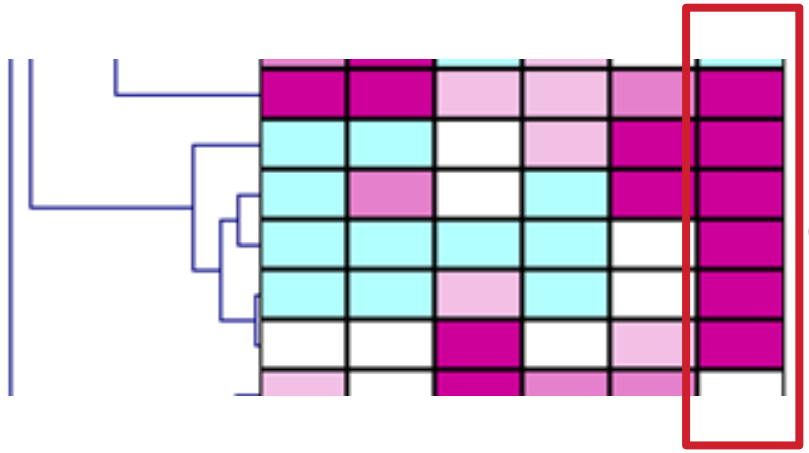


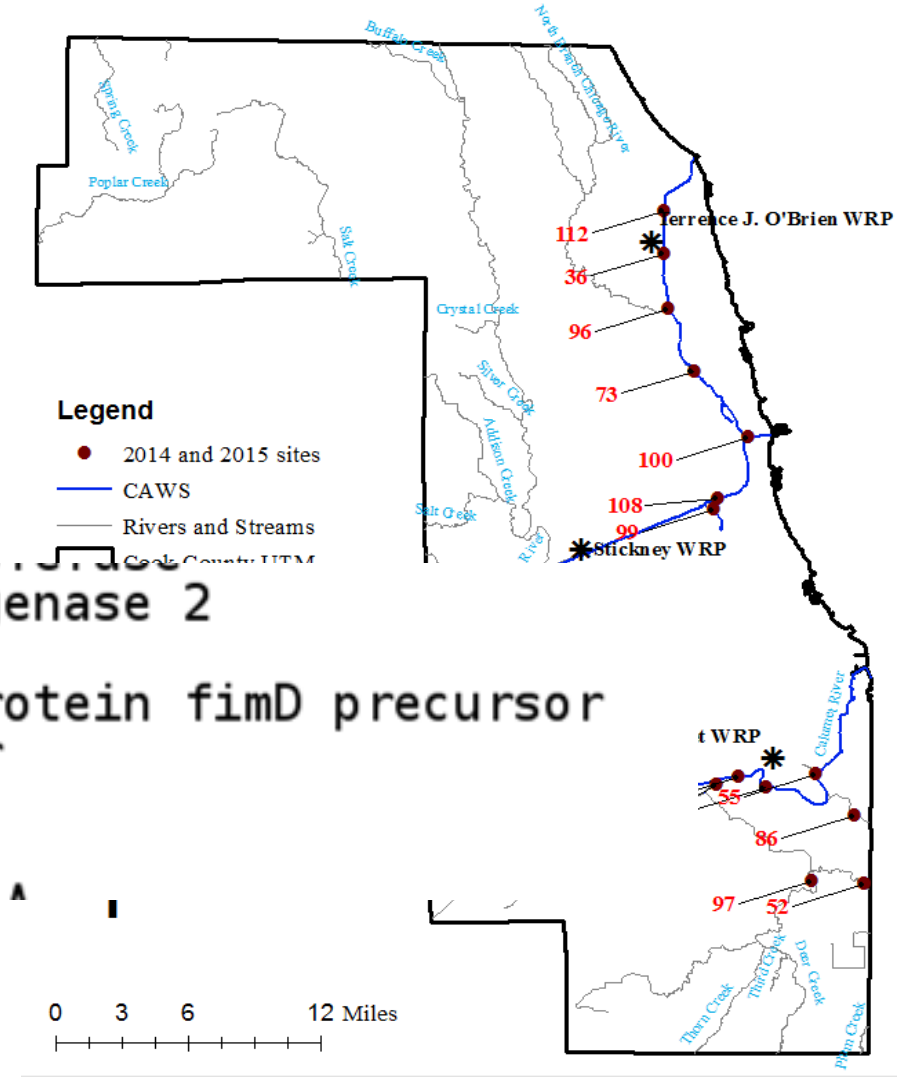
	Site36	Site96	Site73	Site112	Site99	Site108
Total	124	143	165	152	190	113



RELATIONSHIPS WITH E.COLI OR FECAL COLIFORMS

- E.coli abundance was greatest at site 99 in all years.
- Hemolysin A abundance was also highest here.
- Could suggest a persistent contamination source





	Site36	Site96	Site73	Site112	Site99	Site108
Total	124	143	165	152	190	113

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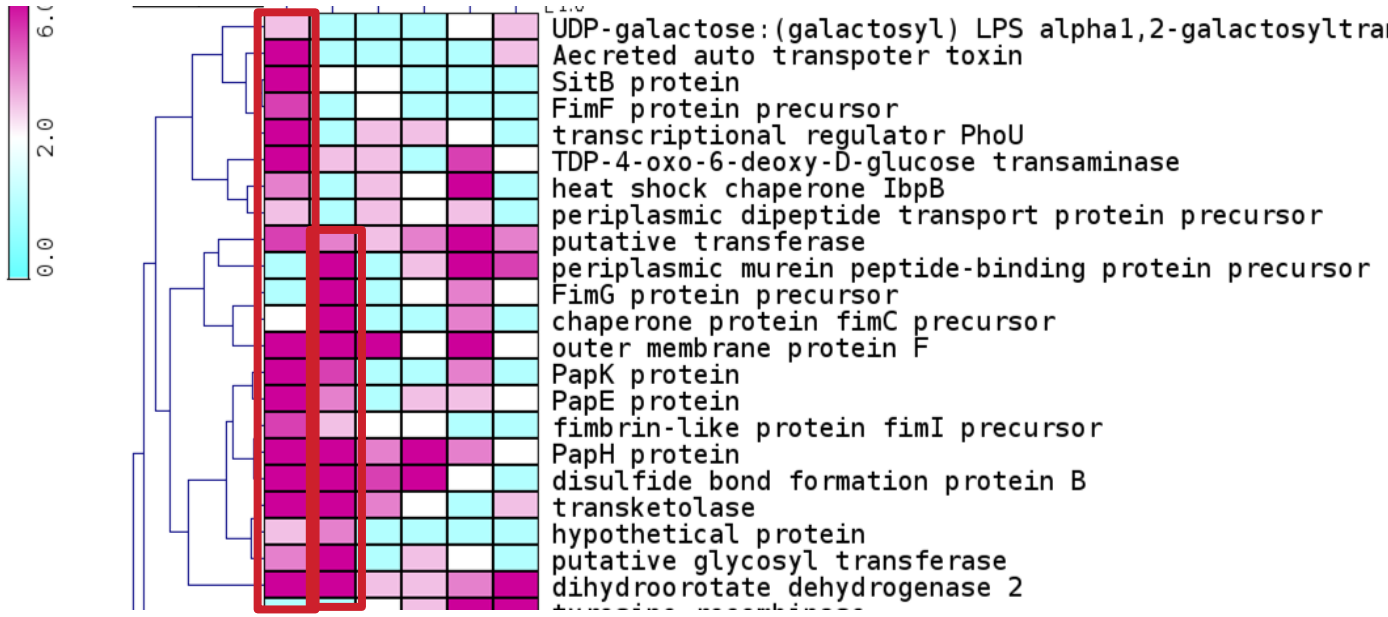
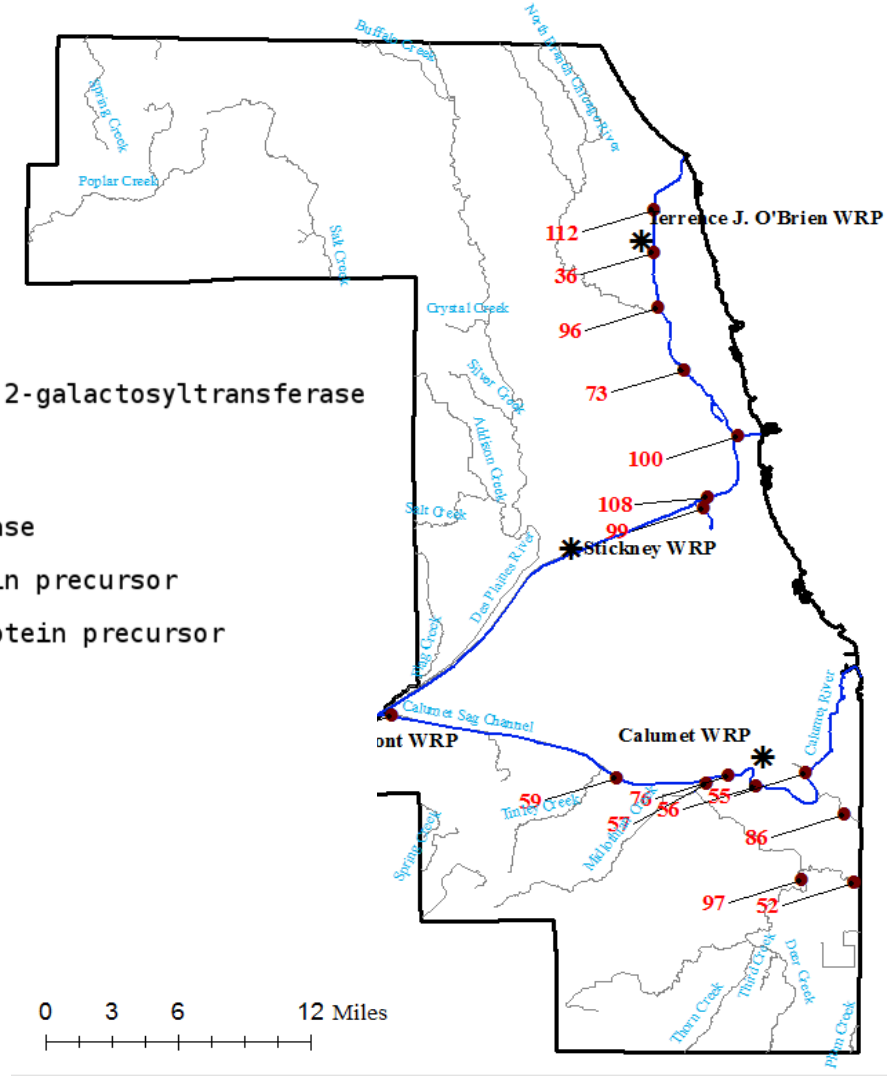
RELATIONSHIPS WITH E.COLI OR FECAL COLIFORMS

- E. coli abundance was lowest at site 36.
- Very different pathways, different toxins.
- Very similar to site 96 – which shows no FIOs and is not downstream of an outfall and has no toxins.



	Site36	Site96	Site73	Site112	Site99	Site108
Total	124	143	165	152	190	113



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SEQUENCING E.COLI GENOMES FROM O'BRIEN WASTE WATER TREATMENT EFFLUENT

Table 5 – Summary of the genome annotation results of seven samples without marker duplication.

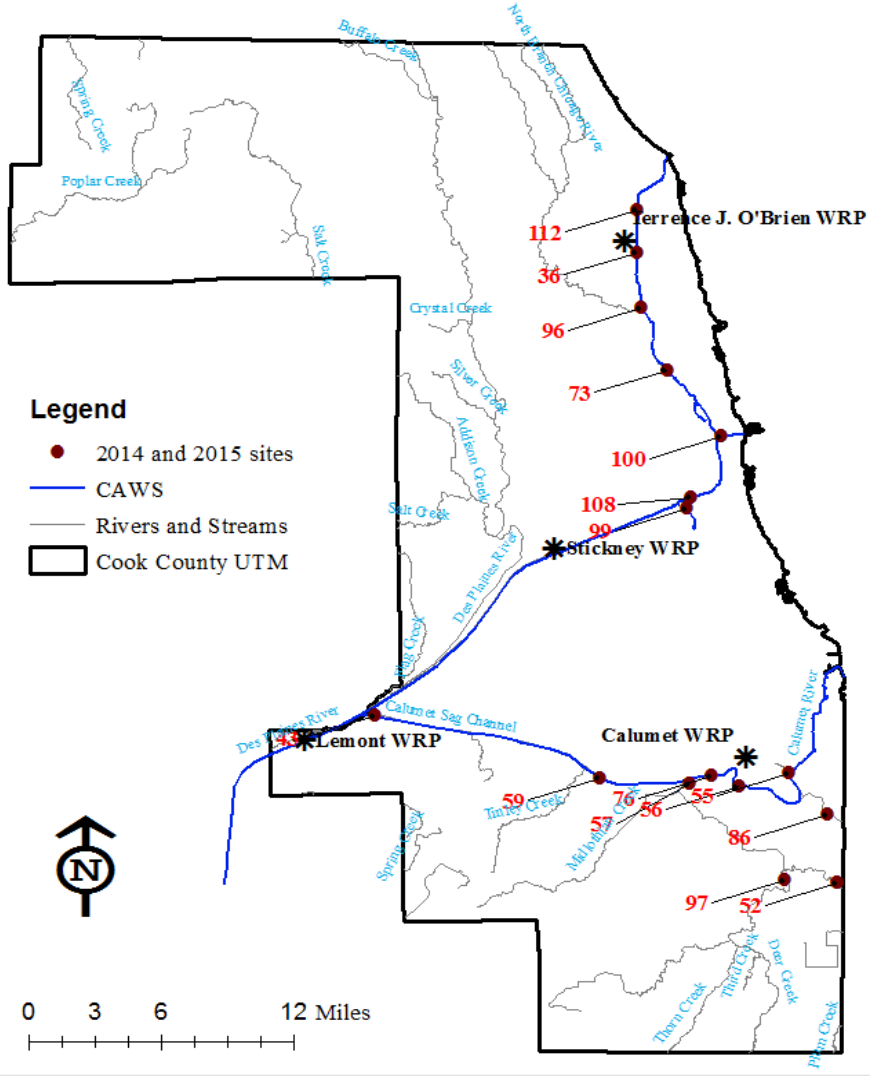
	Obrien, May (mTEC)	Obrien, May (mTEC, 1/10)	Obrien, May (mFC, 1/10)	Obrien, April (mTEC)	Obrien, April (mTEC, 1/100)	Obrien, April (mTEC, 1/100)	Calumet, April (mTEC)
Genome Size (bp)	4,502,378	4,758,466	5,023,097	4,589,399	4,791,369	4,722,285	5,535,441
No. of Contigs	192	249	182	150	234	339	2870
No. of Subsystems	579	576	583	581	586	585	496
No. of Coding seqs.	4301	4672	4942	4429	4650	4615	5655
No. of RNAs seqs.	29	31	29	50	57	66	30
Closest Neighbor	*E. coli* 88.1467	*E. coli* 88.0221	*E. coli* AA86	*E. coli* 88.1467	*E. coli* PCN033	*E. coli* 88.1467	*E. coli* PCN033

Average of 0.03% genes annotated to "Virulence, disease and defense" subsystem.

No Hemolysin A found.

Likely non-pathogenic commensal E.coli.

SOURCE TRACKING



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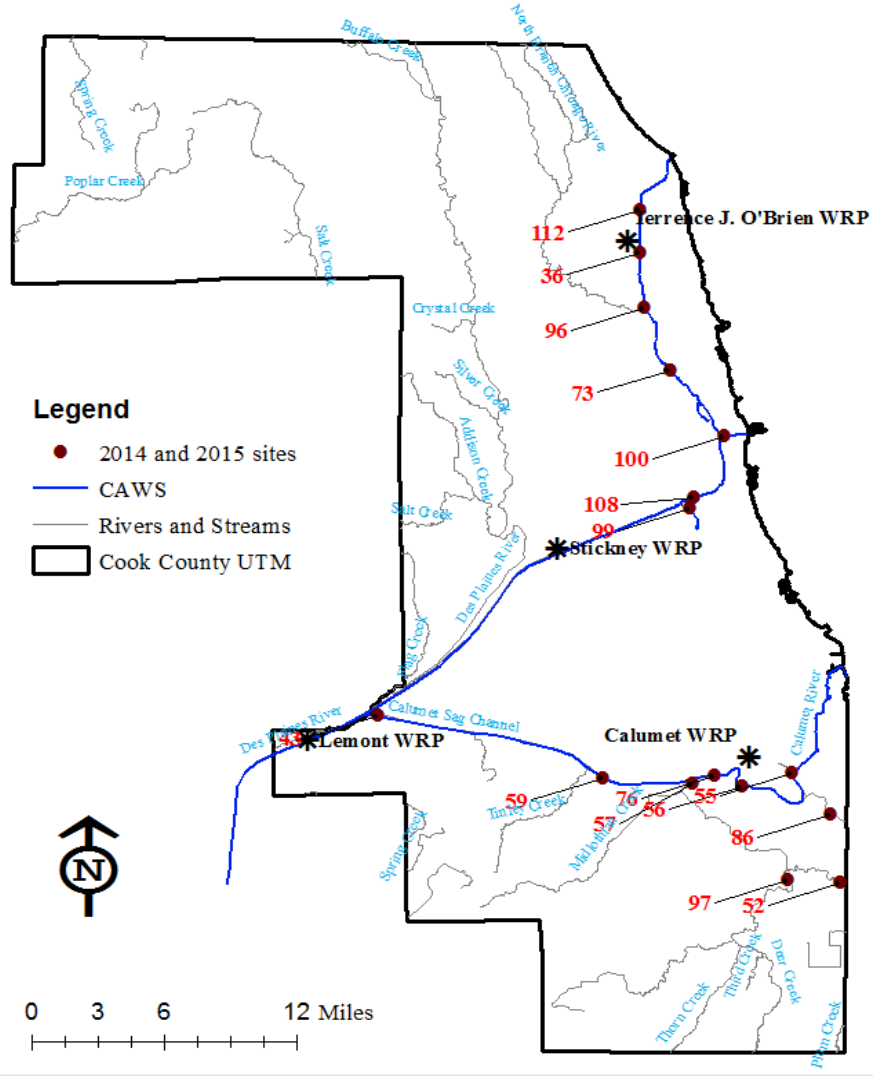
HOW DOES WATER CHEMISTRY DIFFER ACROSS SITES AND YEARS?

- 2013 and 2014:
  - Site 86 had significantly greater total organic carbon (TOC) lower Dissolved Oxygen (DO).
- 2015:
  - Sites 86 & 57 showed significantly higher concentrations of SO_4



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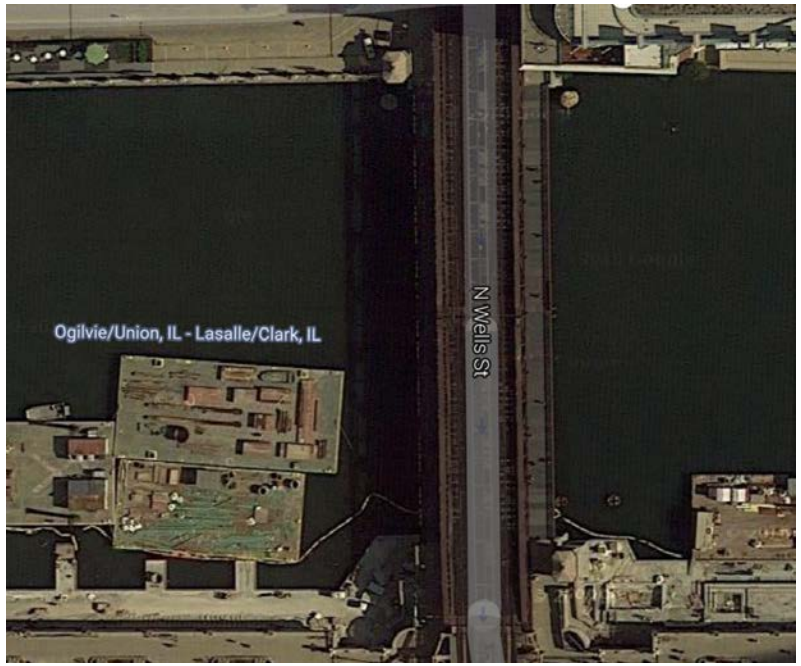
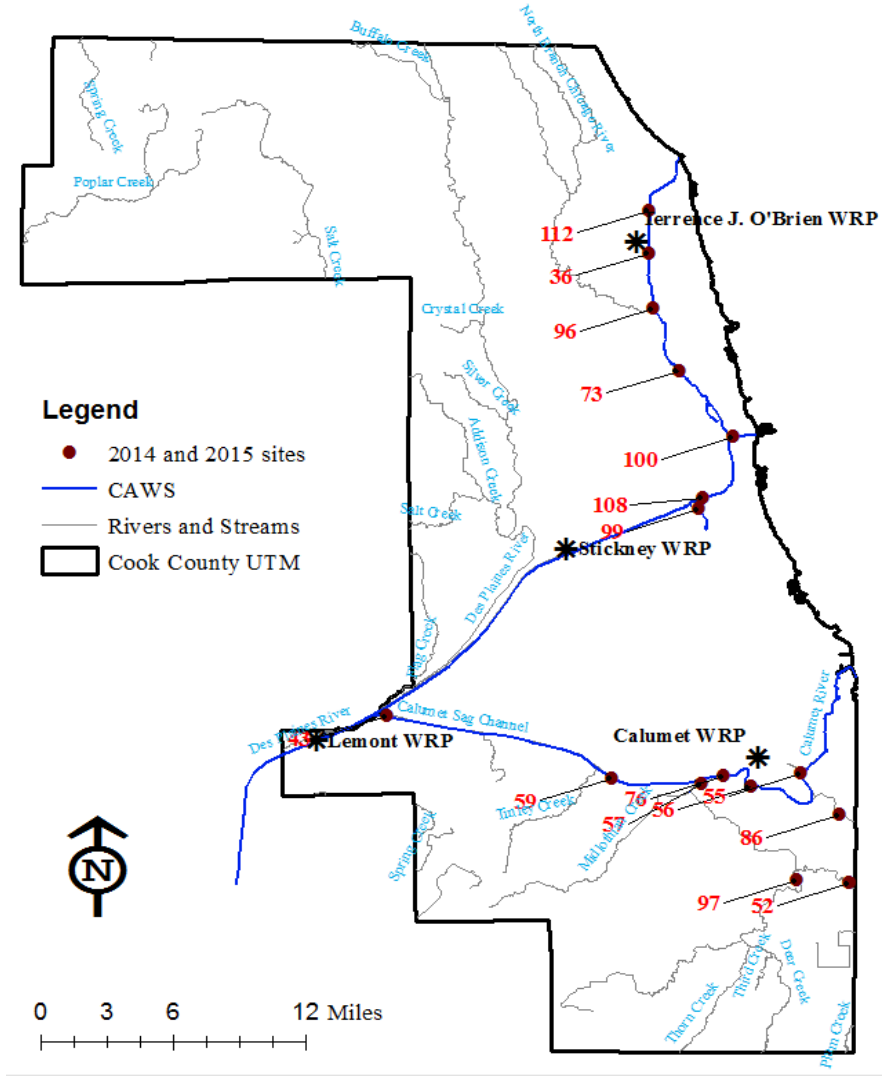
HOW DOES SEDIMENT CHEMISTRY DIFFER ACROSS SITES AND YEARS?

- 2013, 2014 and 2015:
  - Site 100 had greater concentrations of Cd, Ag, Cr, Ni, and Pb than all other CAWS sites.





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DOES LAND USE TYPE DRIVE CHEMISTRY IN SEDIMENT AND WATER?

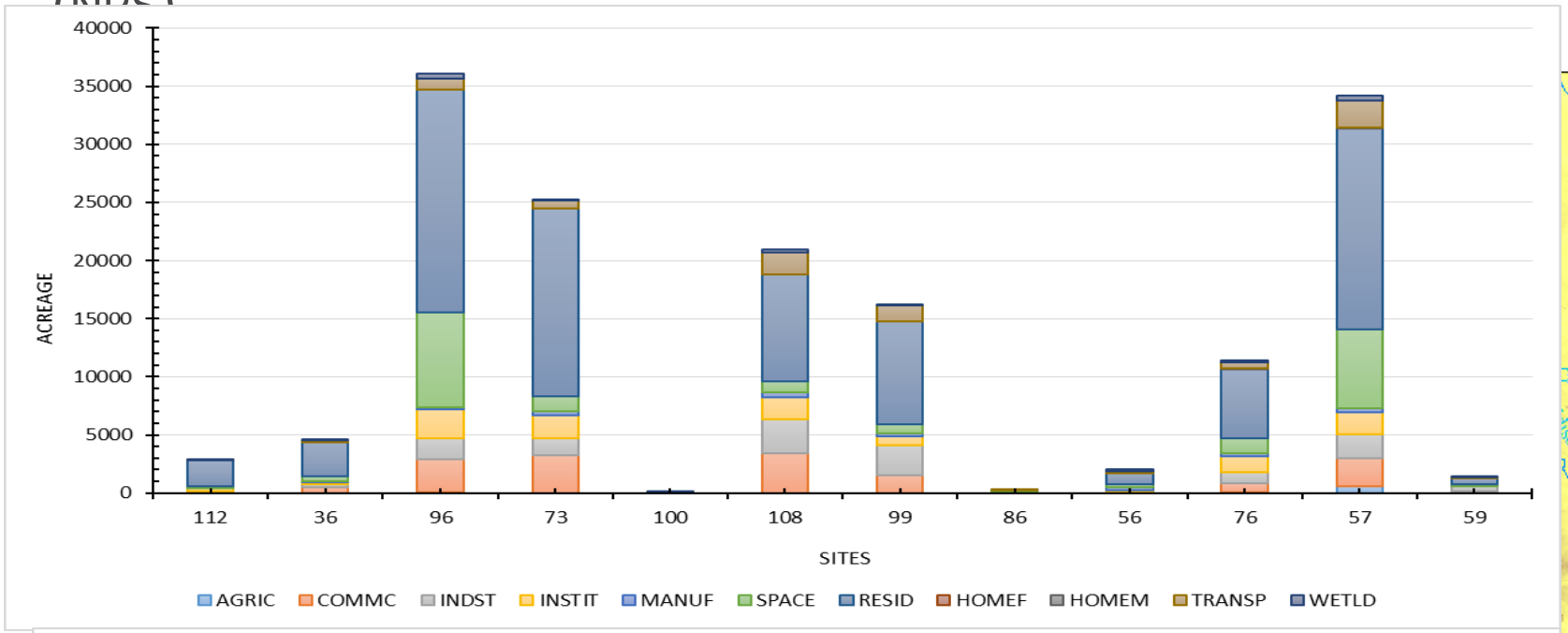
- Water Chemistry:
  - Road, residential, and open-space significantly influenced water-associated properties ($p<0.05$)
  - Dissolved oxygen (DO) and sulphate (SO_4) were significantly correlated with road, residential, and open-space land-use types ($p<0.05$)

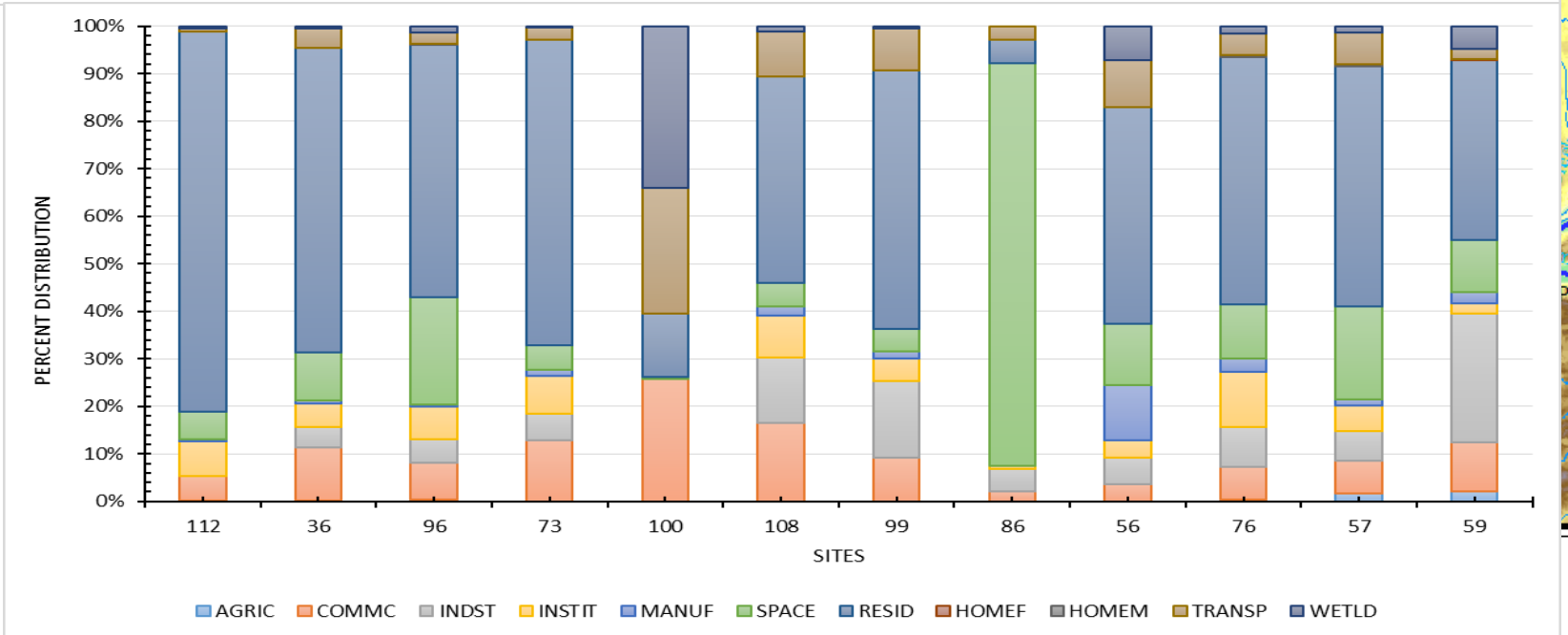
- Sediment Chemistry:
  - Commercial, institution, road, residential, and transport/utility significantly influenced sediment-associated properties.
  - Ag, Cd, Cr, Pb, and Zn were significantly correlated with commercial, institution, road, residential, and transport/utility land-use types.

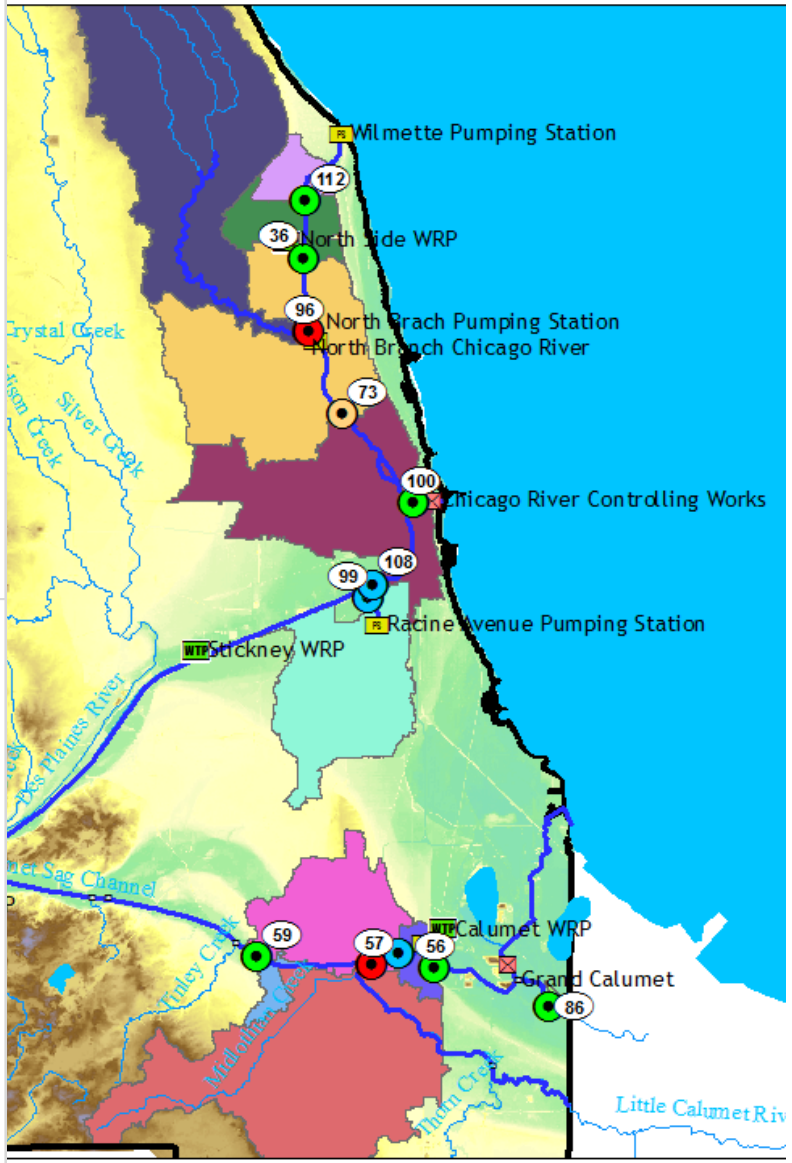
WHAT QUESTIONS HAVE WE ANSWERED?

- Does microbial species diversity show differential geographic and temporal structure?
  - ~~Are these differences observed by sampling medium (sediment vs. water column vs. effluent)?~~
  - ~~Are these differences observed by sampling time points (year and month)?~~
  - ~~Are these differences observed by sampling site? And in particular, is there an effect of sampling site-location (upstream or downstream of a WRP)?~~
- What is the relative abundance of fecal indicator organisms (FIOs)?
  - ~~Does FIO abundance decay with distance from point sources?~~
  - What are the functional attributes of potential FIOs?
- What are the potential sources of microbial organisms at different points in the CAWS?
  - Does source apportionment vary with season or year for a particular location?
  - Are sources highly local or more general across the CAWS?
- What is the influence of land-use on microbial community structure?
  - ~~Does land-use influence physicochemical properties in the CAWS?~~
  - Do different land-types influence source apportionment?

Land use land cover distribution to characterize site differences in drivers of non-point sources (NPS)







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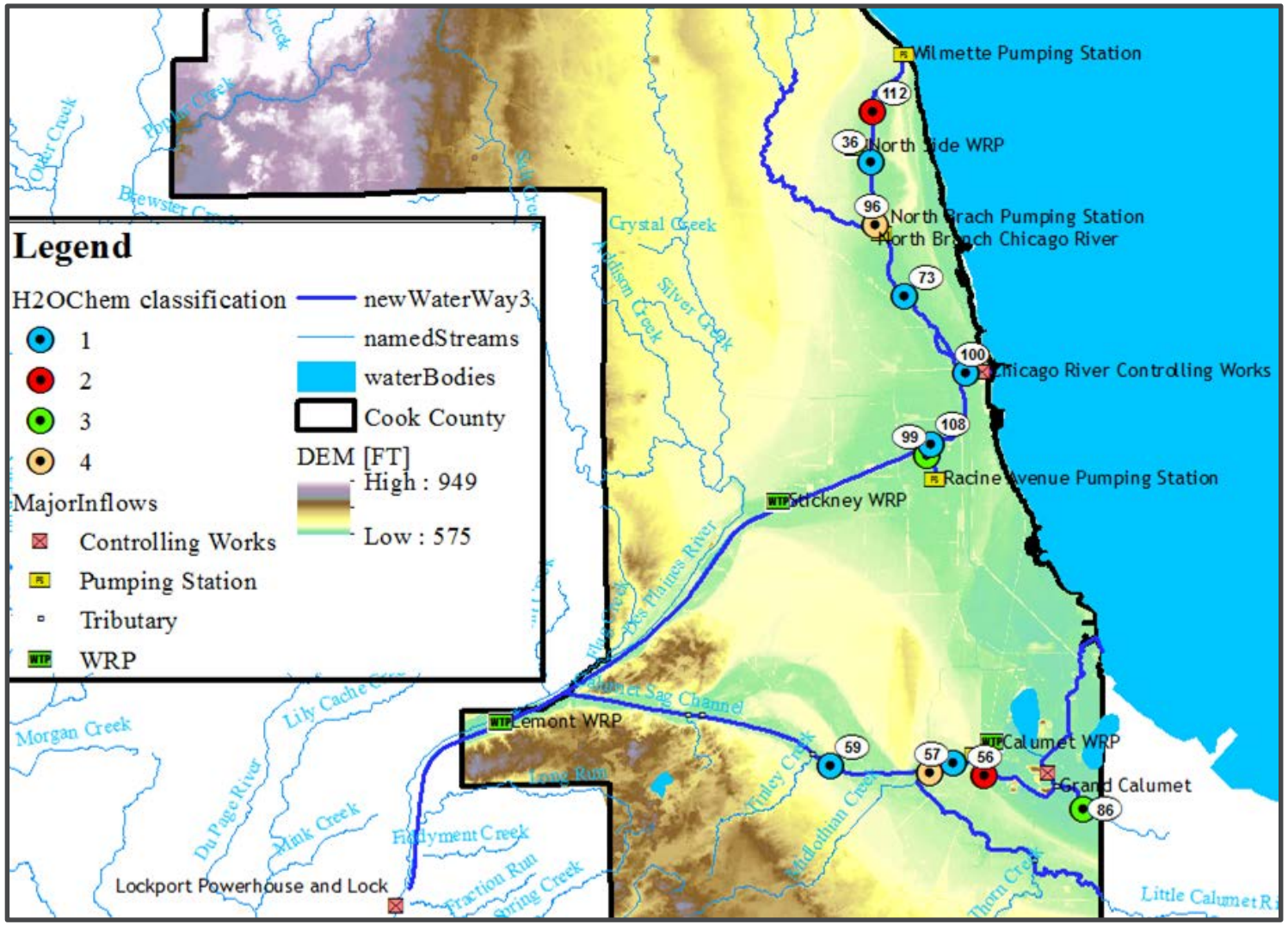
SITE CLASSIFICATION BASED ON WATER CHEMISTRY

Observations

The site classification is to identify sites that are relatively homogeneous with respect to the above attributes (classification is based on k-means clustering algorithm with no spatial constraint such that similarity is not restricted to proximity to either the TJ O'Brien (North Branch) WRP or the Calumet WRP).

1. Water quality (WQ) of tributaries NBCR [96] and LC [57] are similar:
   - ➢ The main tributaries (NBCR and LC) do not influence WQ of the CAWS
2. WQ from GC [86] and after the RAPS [99] are similar
3. WQ changes after both WRPs (number iDs) and is similar downstream for both WRPs



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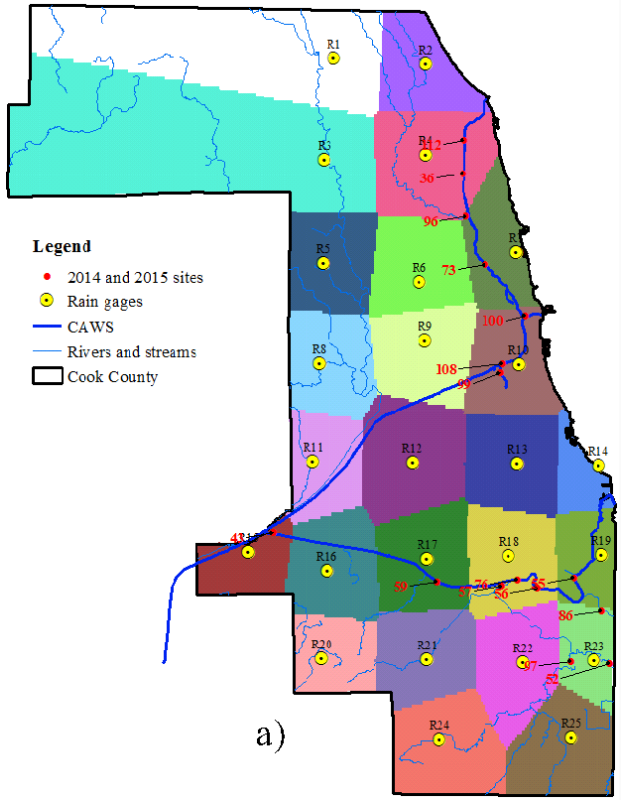
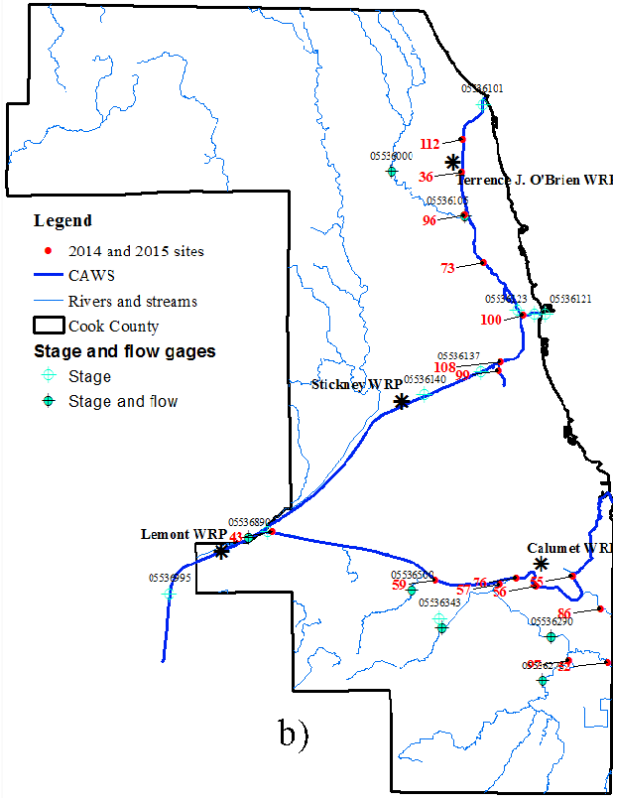
DUFLOW MODEL

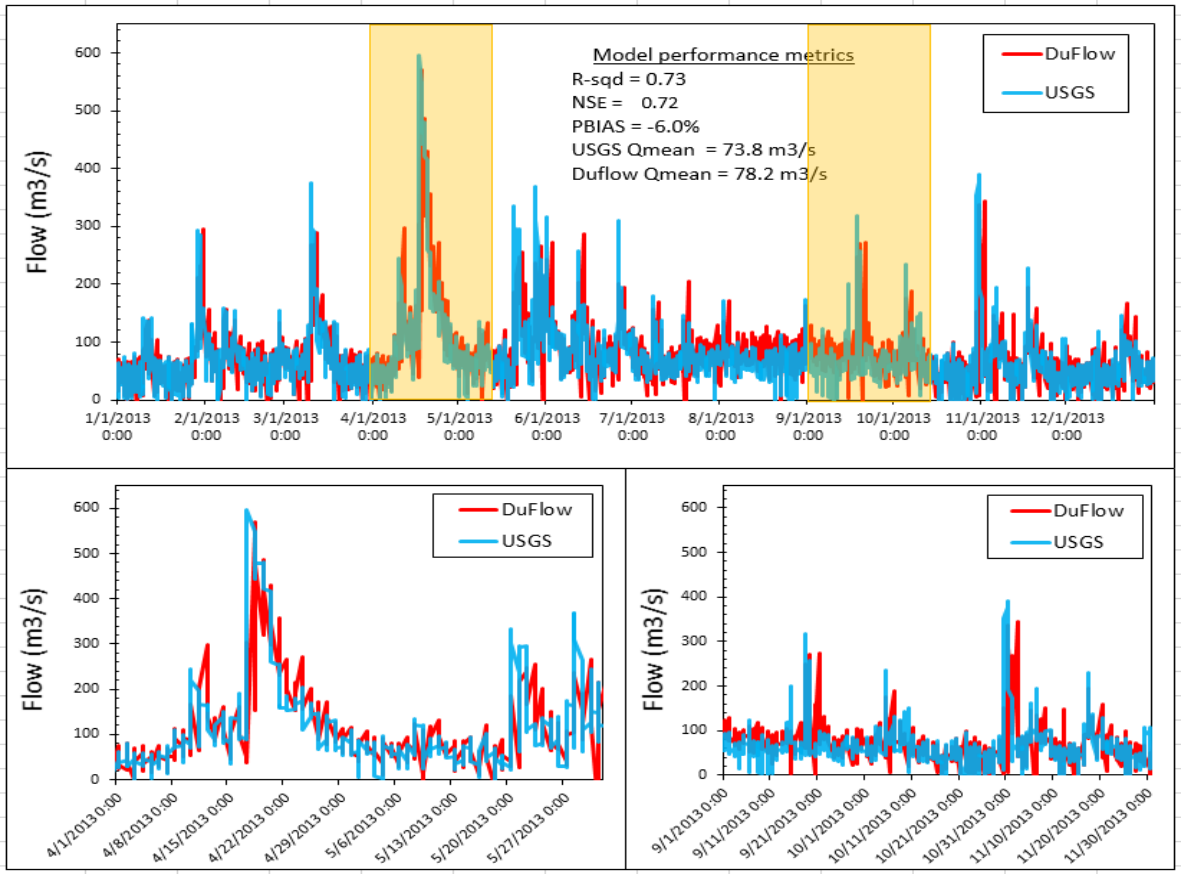
Extracting flow and hydraulic data at specific sampling locations to obtain complete datasets at each location

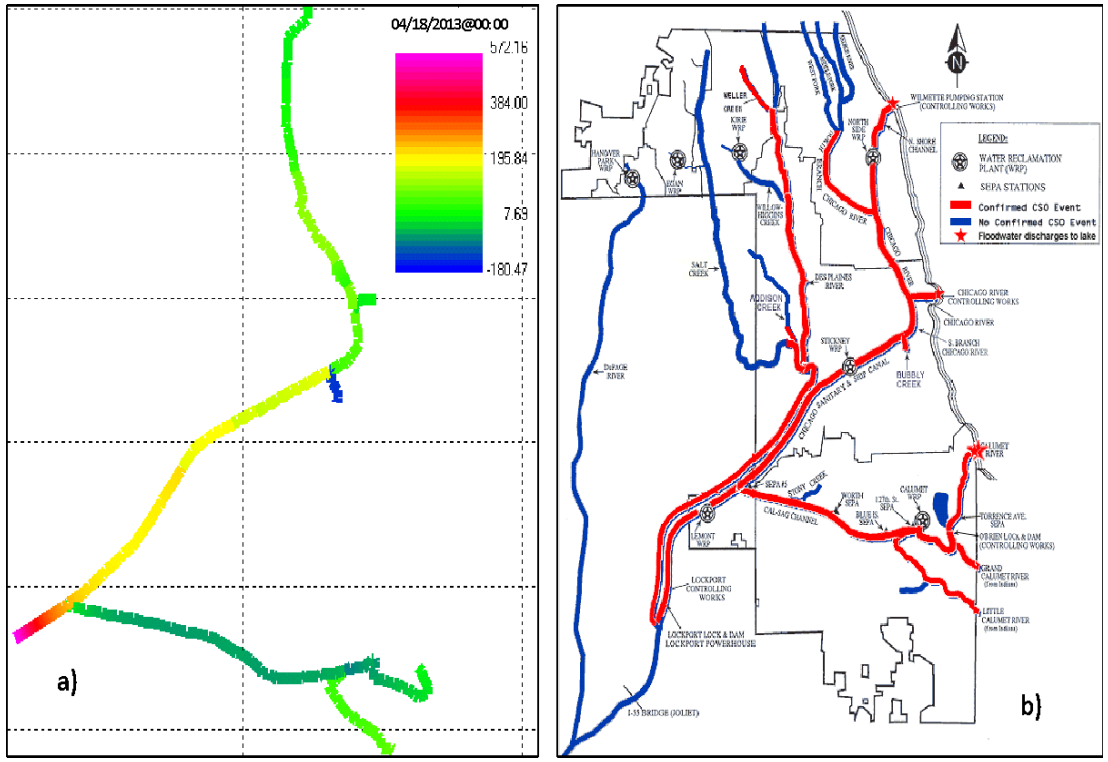
Model developed for 2013, will be extended to other years when interface is complete.





RESULTS SHOW A ROBUST MODEL PERFORMANCE





Spatial difference of stream flow (m^3/s) on April 18, 2013 and CAWS sections with confirmed CSO event as modeled by DuFlow.

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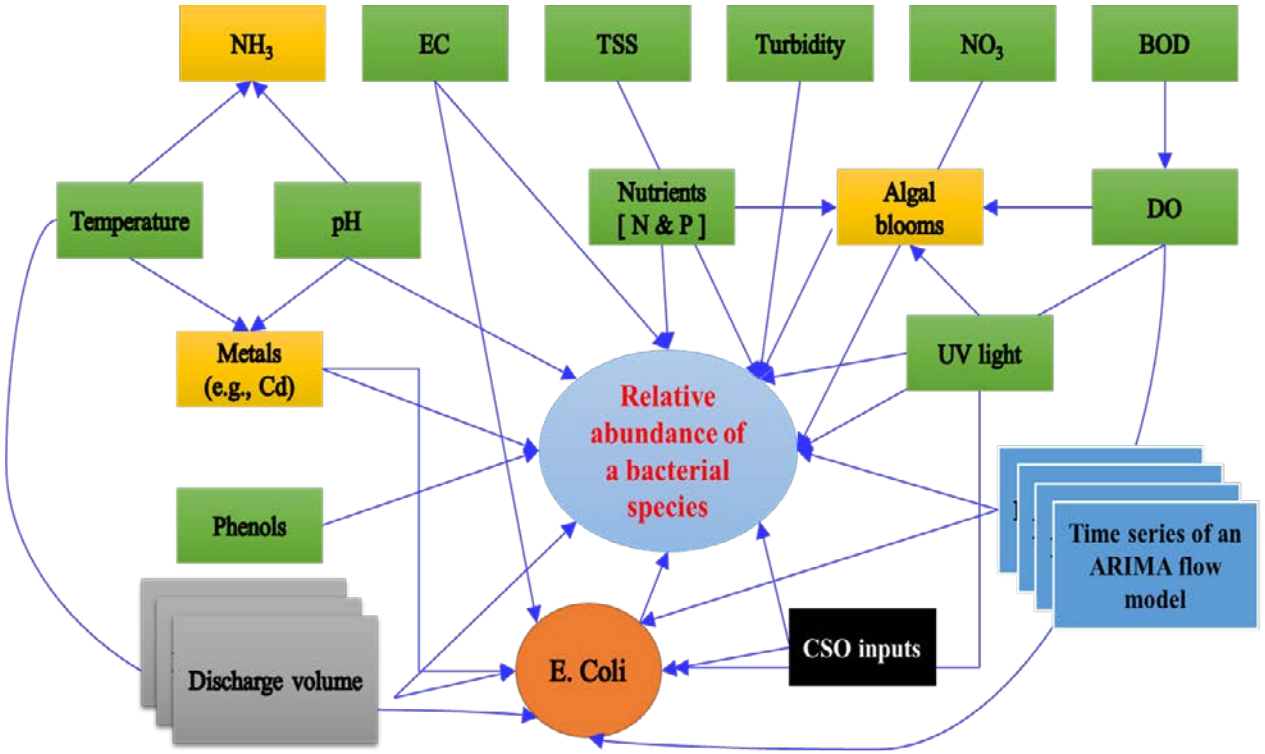
PATH FORWARD

- Develop an interface e between the 2013 model data and predictive neural network-based model incorporating selected microbial genera
- Obtain prototype microbial predictive model
- Expand to other years.



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In summary

- We are shedding light on the microbial communities that live in the CAWS, and provided a baseline for assessing the impacts of future changes in water management on the CAWS microbial composition
- Microbial communities show a distinct distribution pattern across the different sampling locations and media
- They appear to be stable with time
- We can track microorganisms from effluent sources downstream
- Modeling will allow us to relate microbial communities with water flow and transport, thus allowing to develop predictive frameworks for microbial presence in the CAWS.