Metropolitan Water Reclamation District of Greater Chicago

Welcome to the September Edition of the 2020 M&R Seminar Series

NOTES FOR SEMINAR ATTENDEES

- All attendees' audio lines have been muted to minimize background noise.
- A question and answer session will follow the presentation.
- Please use the WebEx Q/A feature to ask a question via text. Please submit the question to all Panelists.
- The presentation slides will be posted on the MWRD website after the seminar.

M. Cristina Negri, Ph.D.
Division Director, Environmental Science
Argonne National Laboratory

- Dr. Negri is the Director of the Environmental Science (EVS) Division. In her more than 25 years as a scientist at Argonne, she conducted and directed laboratory to full-scale multidisciplinary projects developing technologies and concepts for environmental remediation and stewardship, including soil remediation and water treatment.
- Cristina is a Fellow with CASE at the University of Chicago. She is also a Fellow of the Northwestern University—Argonne Institute of Science and Engineering. She earned her Doctor Degree in Agricultural Sciences at the University of Milan in Milan, Italy.
- Prior to joining Argonne, Cristina worked in private industry in Italy as a research and development manager and as a liaison with universities and other Italian national research organizations. Her research focused on developing methods for the sustainable, beneficial reuse of industrial and urban waste and for pollution mitigation in agriculture.
- She also served as the Convener of a CEN (the European Standardization Organization) Working Group, leading experts from European Union Nations toward the creation of European environmental standards for agricultural commodities.

Jack A Gilbert, Ph.D.
Professor, Department of Pediatrics and Scripps Institution of Oceanography

- Dr. Gilbert is a Professor in Pediatrics and the Scripps Institution of Oceanography with University of California San Diego. Dr. Gilbert uses molecular analysis to test fundamental hypotheses in microbial ecology. He cofounded the Earth Microbiome Project and American Gut Project.
- Professor Gilbert earned his Ph.D. from Unilever and Nottingham University, UK in 2002, and received his postdoctoral training at Queens University, Canada.
- From 2005-2010 he was a senior scientist at Plymouth Marine Laboratory, UK; and from 2010-2018 he was Group Leader for Microbial Ecology at Argonne National Laboratory, a Professor of Surgery, and Director of The Microbiome Center at University of Chicago. In 2019, he moved to San Diego, CA.
- Dr. Gilbert was recognized on Crain's Business Chicago's 40 Under 40 List in 2014, and in 2015 he was listed as one of the 50 most influential scientists by Business Insider, and in the Brilliant Ten by Popular Scientist. In 2016 he won the Altemeier Prize from the Surgical Infection Society, and the WH Pierce Prize from the Society for Applied Microbiology for research excellence.
- In 2019 he was elected to the Philosophical Society of Washington. He also co-authored "Dirt is Good" published in 2017, a popular science guide to the microbiome and children's health.

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THE RESPONSE OF MICROBIAL COMMUNITIES IN THE CHICAGO AREA WATERWAY SYSTEM TO MAJOR INFRASTRUCTURE CHANGES IN THE REGION

FINAL REPORT PRESENTATION
September 25, 2020

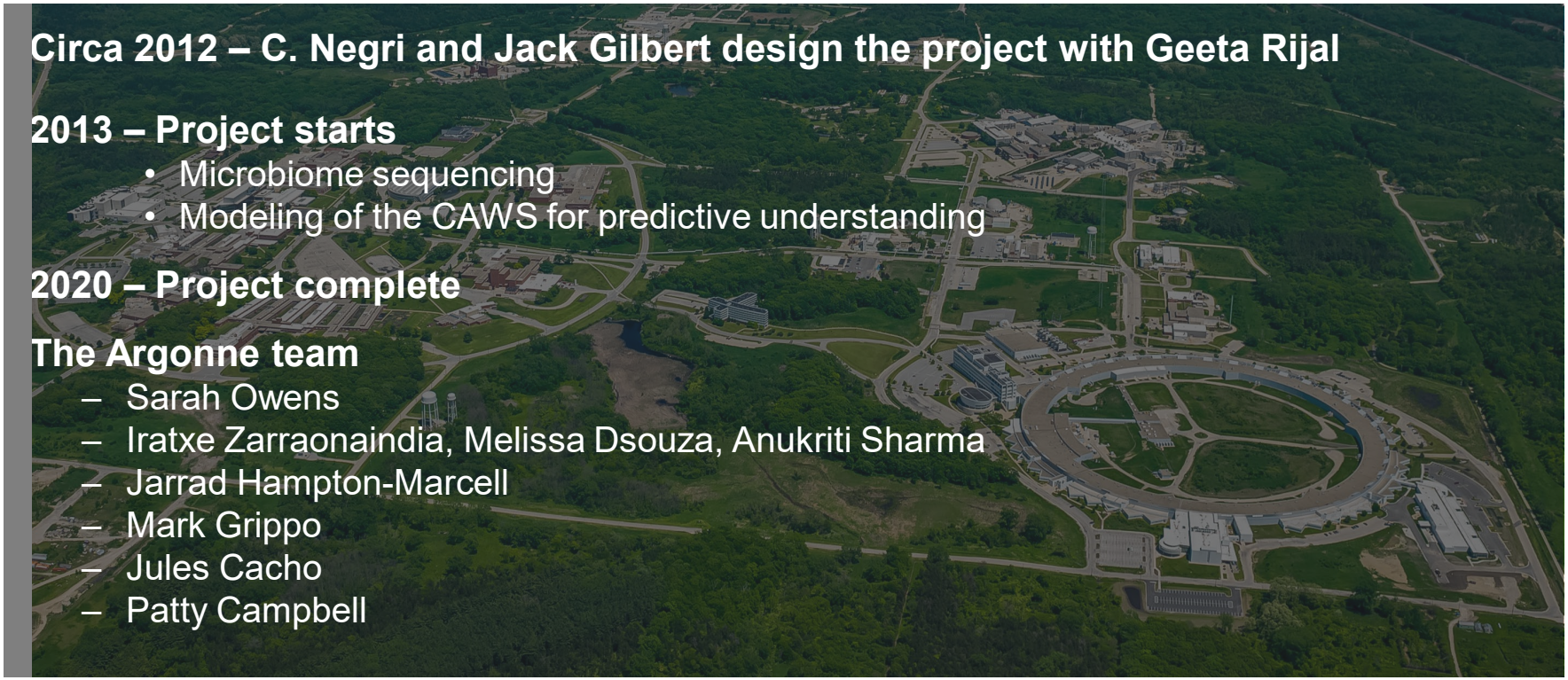
JACK GILBERT

Current affiliation:
University of California San Diego

CRISTINA NEGRI

Division Director
Environmental Sciences Division

HISTORY AND ACKNOWLEDGEMENTS



Circa 2012 – C. Negri and Jack Gilbert design the project with Geeta Rijal

2013 – Project starts

- Microbiome sequencing
- Modeling of the CAWS for predictive understanding

2020 – Project complete

The Argonne team

- Sarah Owens
- Iratxe Zarraonaindia, Melissa Dsouza, Anukriti Sharma
- Jarrad Hampton-Marcell
- Mark Grippo
- Jules Cacho
- Patty Campbell

CHICAGO AREA WATERWAY SYSTEM MICROBIOME RESEARCH

Final Report Presentation

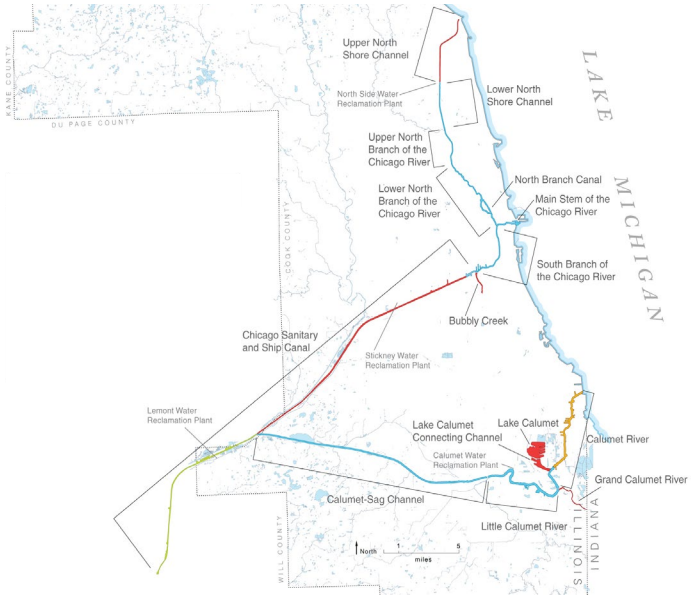
25th Sep, 2020

Jack Gilbert
Principal Investigator

Introduction

- This is a seven-year investigation of the microbial communities within the Chicago Area Waterway System (CAWS).
- The study coincides with the Metropolitan Water Reclamation District of Greater Chicago's (MWRD) efforts to implement disinfection and storm water reservoir control management.

- CAWS is 78 miles long, man made and natural, draining 645 mi^2
- 75% of flow typically treated effluent from water reclamation plants
- Sampling started in March, 2013
  - Water
  - Sediment
  - Effluent
  - Sewage
  - Fish associated samples



MWRD's initiatives

i) Terrence J. O'Brien WRP

UV disinfection

2016- Introduced state-of-the-art technology

Seven channel chamber

75 million gallons per day in one chamber

ii) Calumet WRP

Chlorination/de-chlorination

2015- Modified the **existing chlorine contact basin**

- Replaced all interior baffle walls, gates
- Installing liquid sodium bisulfite diffuser piping

iii)

The Calumet Tunnel and Reservoir Plan (TARP) System's Thornton Composite Reservoir (TCR) became operational by October in 2015.

McCook Reservoir operational in December 2017.

CAWS Microbiome - Study 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.

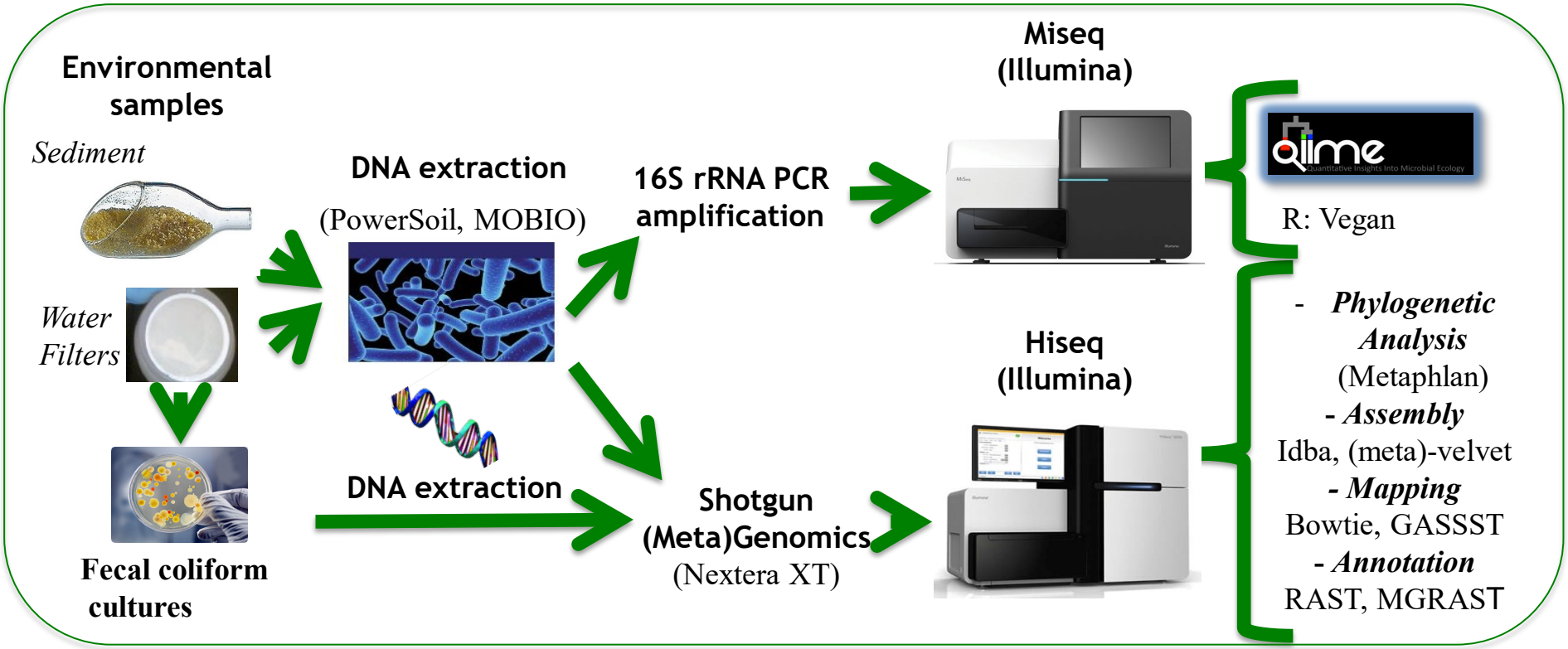
Phase	2013	2014	2015	2016	2017	2018	2019
I- Pre-disinfection, Pre-TARP completion, Calumet and Chicago River systems	-	----	----				
II – Post-Disinfection, Pre-TARP reservoir completion, Chicago River System				----	----		
III – Post disinfection and post-TARP reservoir completion, Calumet River system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

Microbial Molecular Profiling

- **Traditional laboratory-culture methods**
  - **(Fecal indicator bacteria (FIB) counts and PCR)**
  - Inability to resolve the source of fecal and/or sewage contamination
  - Do not completely describe the diversity of microbial communities present
  - Only identify cultivable microbial community
  - Typical culture-based methods that currently only detect approximately 8% of known microbes

- **16S rRNA amplicon and metagenomic sequencing**
  - Can capture uncultivable microbial community
  - Untargeted profiling of the whole community
  - Ability to map the functional potential, including resistance and virulence.

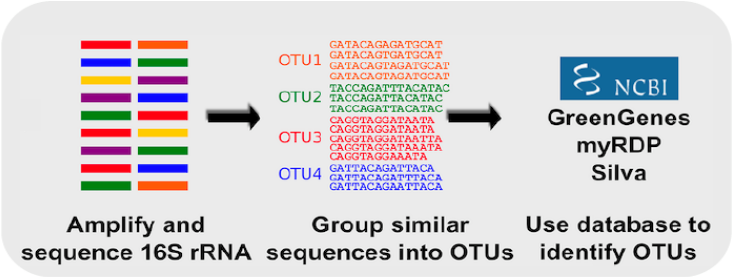
A new way of exploring CAWS microbes



16S rRNA gene amplicon sequencing

Clusters- similarity threshold of 97% sequence identity

PCR sequencing errors?



Multiple similar species- grouped into a single OTU

Individual identifications lost to the abstract of a cluster

Exact Amplicon Sequence Variants

No clustering- so no Blurring!! (Calling Single Nucleotide Variation)

Starts by determining which exact sequences were read and how many times each exact sequence was read

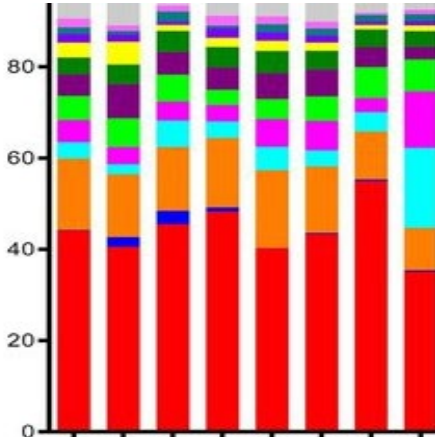
Error model- given read at a given frequency is not due to sequencer error

Microbial Diversity Analyses

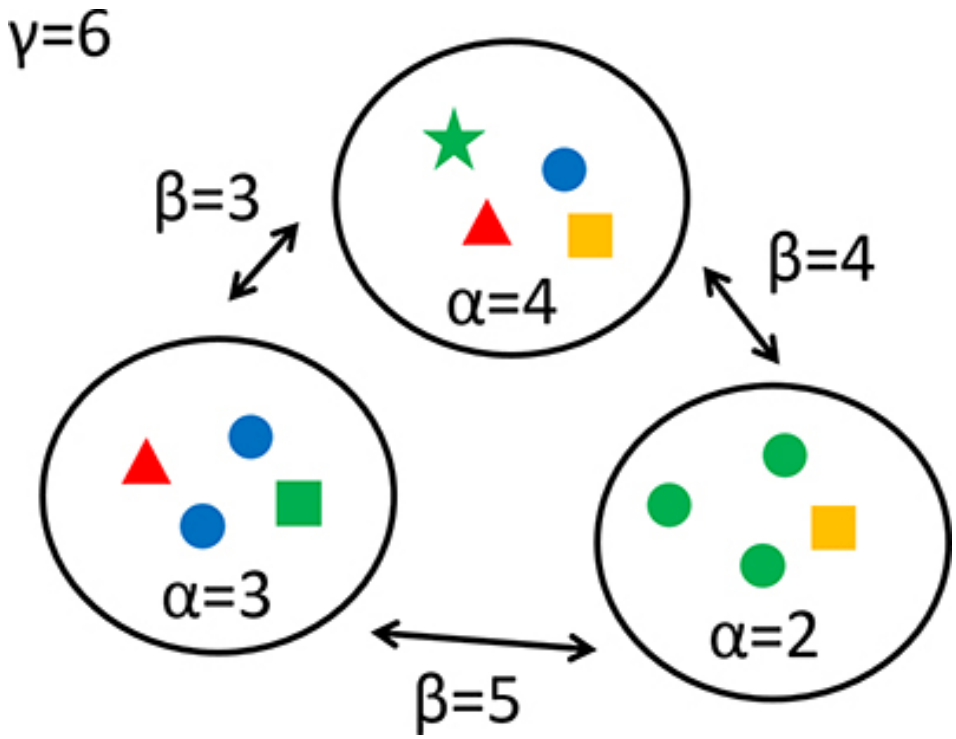
Alpha (α) – diversity within a community, # of species

Beta (β) – diversity between communities (differentiation), species identity and proportion is taken into account

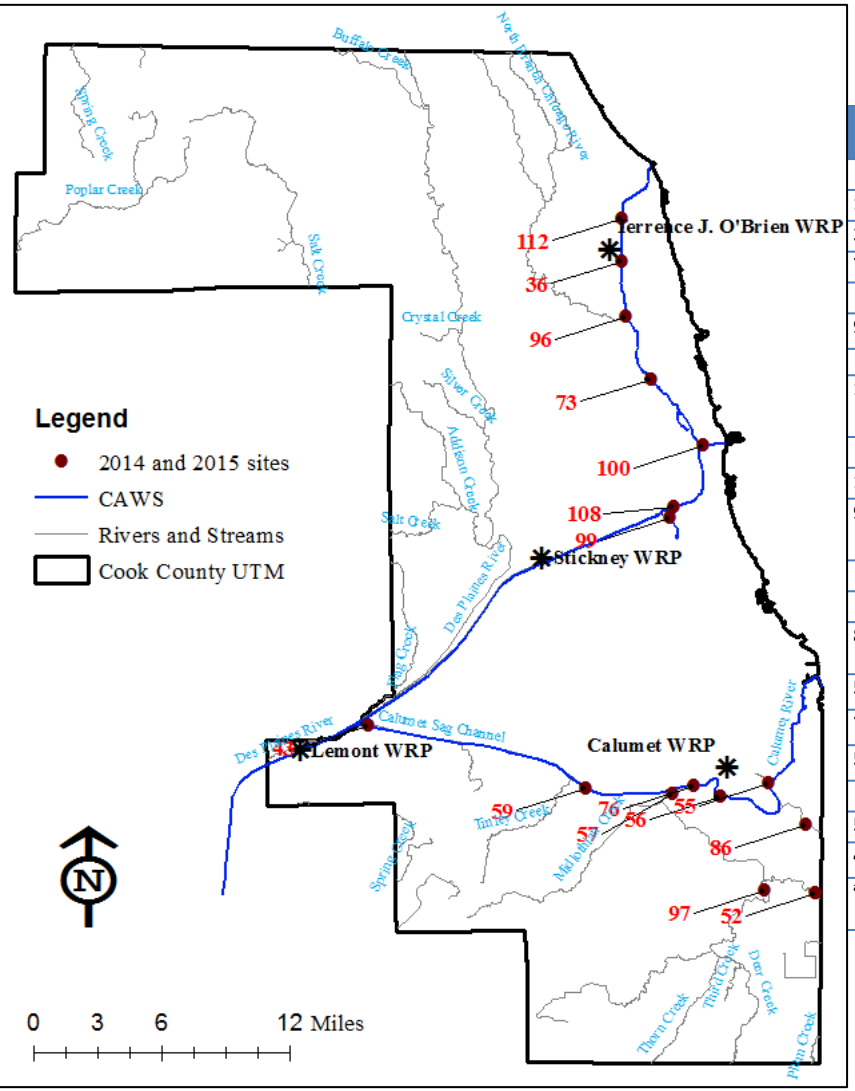
Gamma (γ) – (global) diversity of the site, γ = α×β



Compositional analyses



Sites sampled across CAWS



A. CAWS North			
WRP	O'Brien WRP Disinfected Effluent		UV Disinfected Effluent
112	North Shore Channel (NSC)	Dempster St	~1.5 Miles Upstream from O'Brien WRP
36	North Shore Channel	Touhy Ave.	~0.68 Miles Downstream from O'Brien WRP
73	North Branch Chicago River	Diversey Ave.	~6.5 Miles Downstream from O'Brien WRP
B. CAWS North Tributary			
96	North Branch Chicago River[b]	Albany Ave.	Tributary River ~ 3.2 Miles from O'Brien WRP
C. CAWS Main Stem			
100	Chicago River Main Stem	Wells St.	Downtown Chicago River ~ 11 Miles from O'Brien WRP
D. CAWS South Branch Chicago River			
108	South Branch Chicago River	Loomis St.	~14.5 Miles Downstream from O'Brien WRP
99	SF, South Branch Chicago River	Archer Ave.	South Fork River (~Bubbly Creek receives Racine Avenue Pumping Station Discharge flow)
E. CAWS Calumet River			
WRP	Calumet WRP Disinfected Effluent		Chlorination/dechlorination Disinfected Effluent
86	Grand Calumet River[b]	Burnham Ave.	Upstream Tributary ~ 4.4 Miles from Calumet WRP
56	Little Calumet River[b]	Indiana Ave.	~1 Mile Upstream from Calumet WRP
76	Little Calumet River[b]	Halsted St.	~1.3 Miles Downstream from Calumet WRP
57	Little Calumet River[b]	Ashland Ave.	Tributary River ~ 1.7 Miles from Calumet WRP
F. CAWS Cal-Sag Channel			
59	Cal-Sag Channel	Cicero Ave.	~ 6.4 Miles Downstream from Calumet WRP
43[c]	Cal-Sag Channel	Route #83	~ 17.2 Miles Downstream from Calumet WRP

[a]Miles for a site along the river which correspond to distance from WRP to the point the tributary joins the CAWS.

Number of samples analyzed (2013-2019)

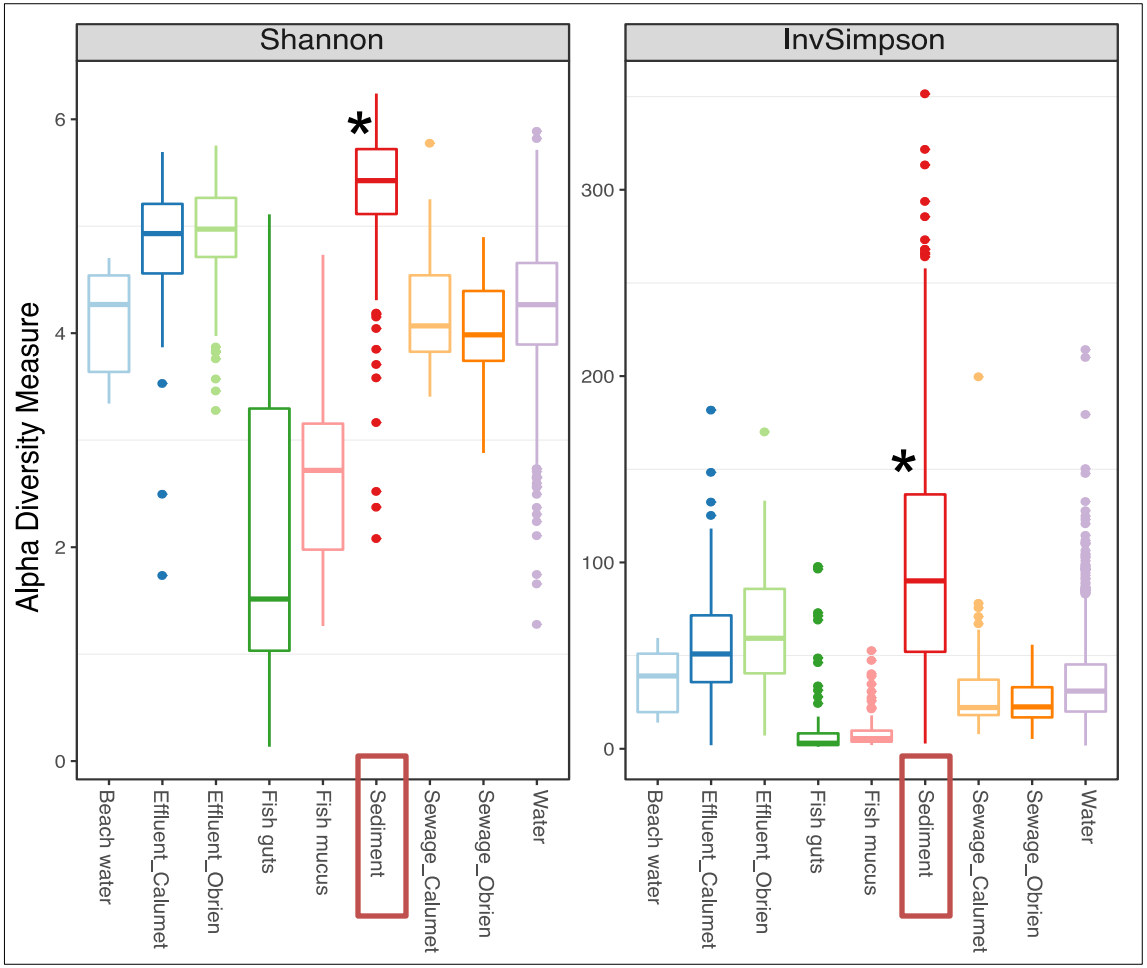
Year	Effluent	Sediment	River	Wet/Dry	Raw Sewage	Controls	Fish	Spiked	Plate	Lake Bypass	Beach	Total
2013	55	78	82	0	0	15	0	0				230
2014	72	84	133	54	9	56	0					408
2015	76	99	109	54	17	103	48	9			7	522
2016	41	104	106	44	19	82	47					443
2017	17	107	107	53	16	83	34		55	2		474
2018	16	100	108	14	16	74	42	0	0	0	0	370
2019	15	0	104	16	15	59	48	0	0	2	0	259
Total	292	572	749	235	92	472	219	9	55	4	7	2,706

THE UNIQUE MICROBIAL DIVERSITY OF DIFFERENT SAMPLE TYPES

ALPHA DIVERSITY
BETA DIVERSITY
COMPOSITIONAL DIFFERENCES

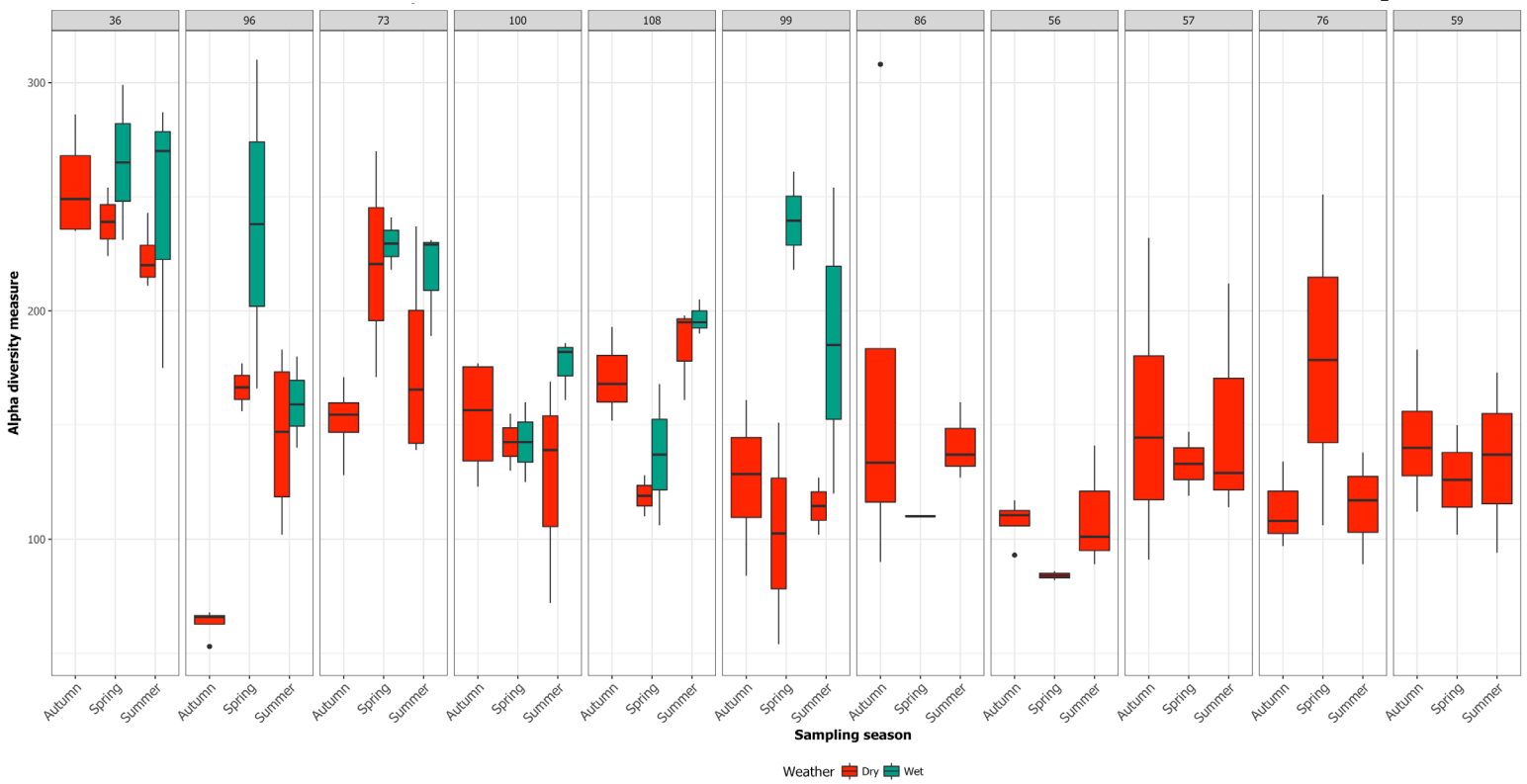
Alpha diversity

Sediments are most diverse

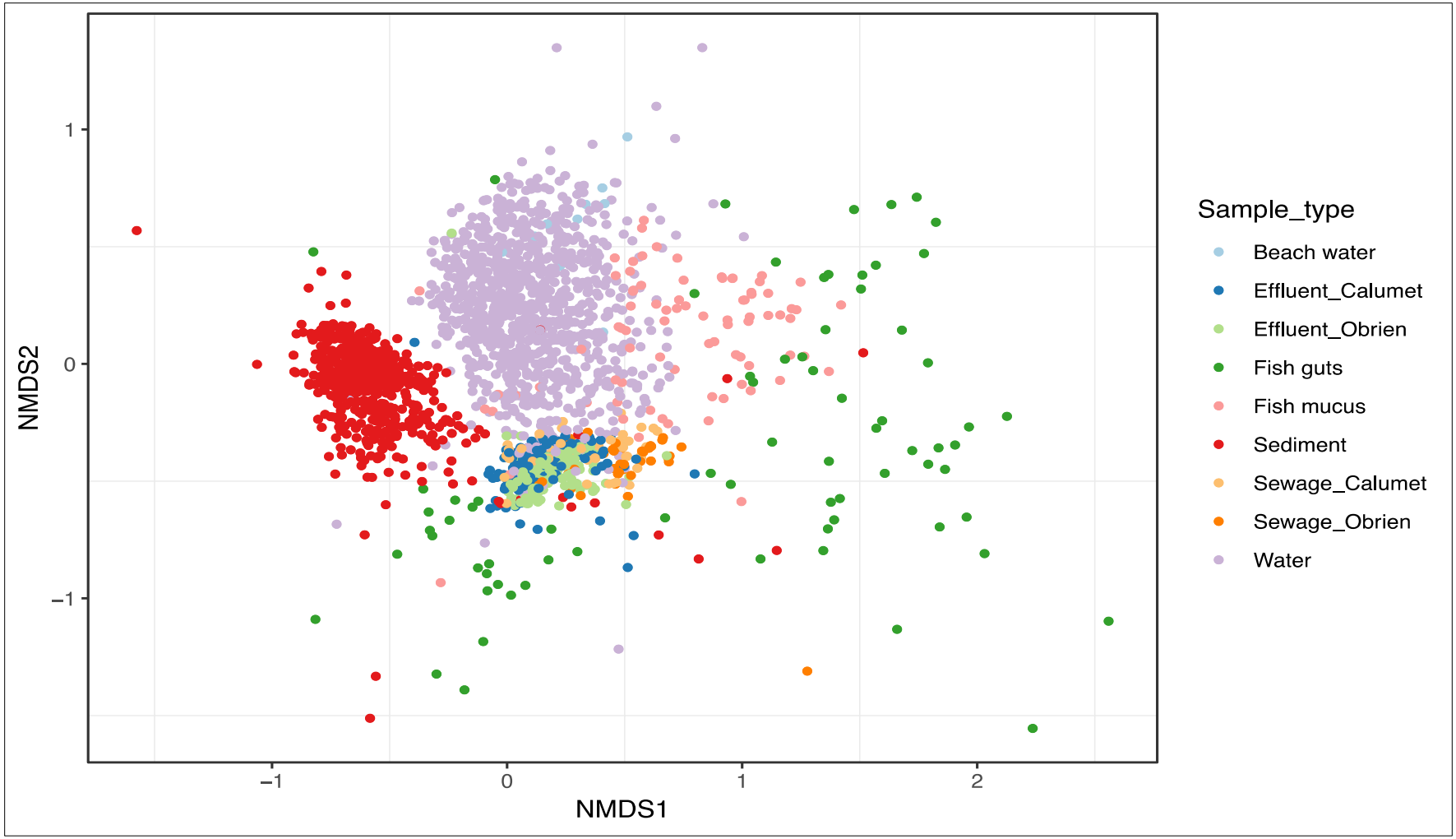


Sediment samples, due to inherent complexity of this medium, **had the greatest alpha diversity**, which was significantly greater than any other medium; **effluent samples** had the second greatest alpha diversity

Microbial richness is affected by Dry and Wet weather in 2016 water columns samples



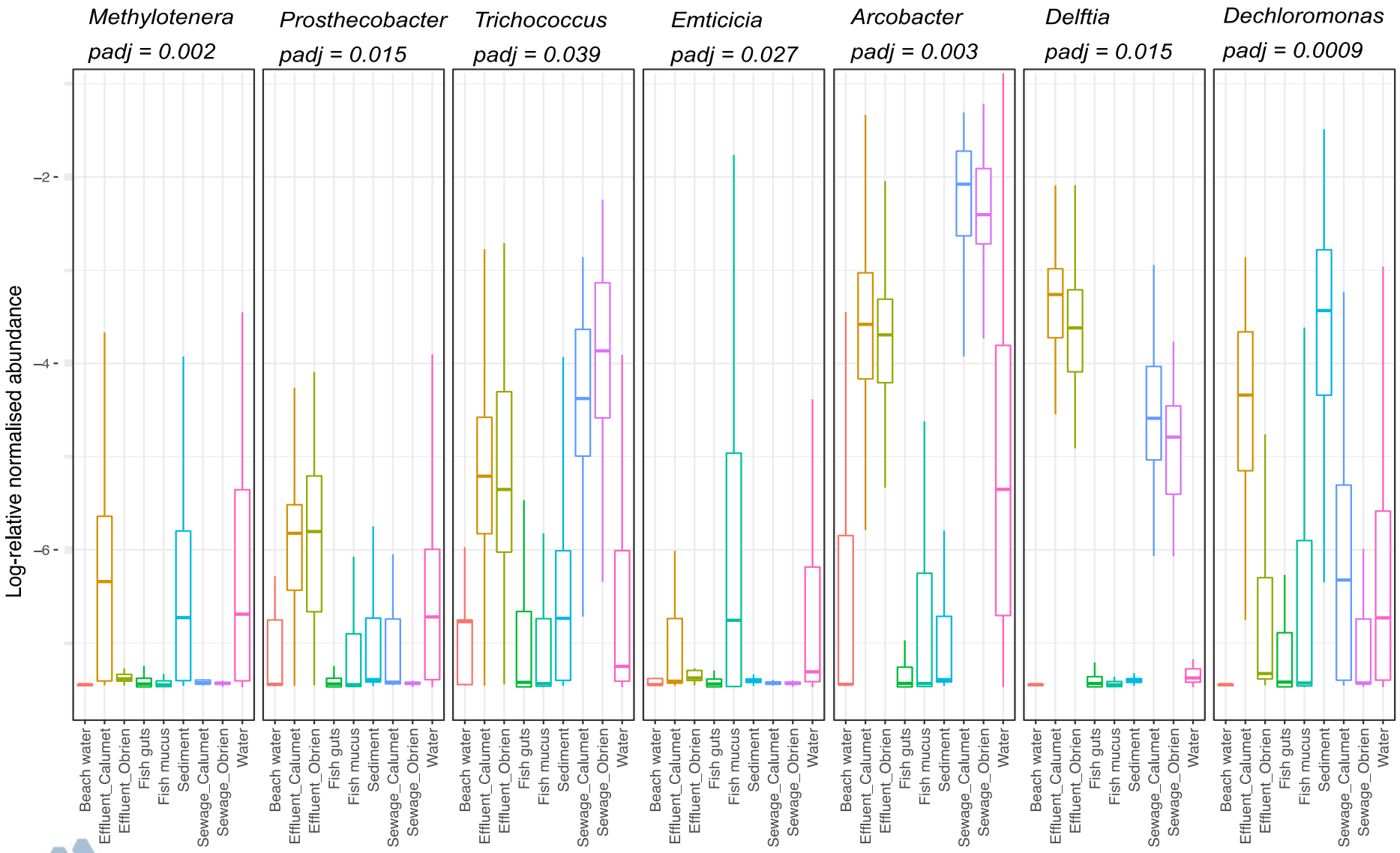
Beta diversity



Beta diversity was significantly different between sample types (weighted UniFrac; $p_{PERMANOVA} < 0.05$).

Water, sediment and effluent samples ordinated into separate clusters.

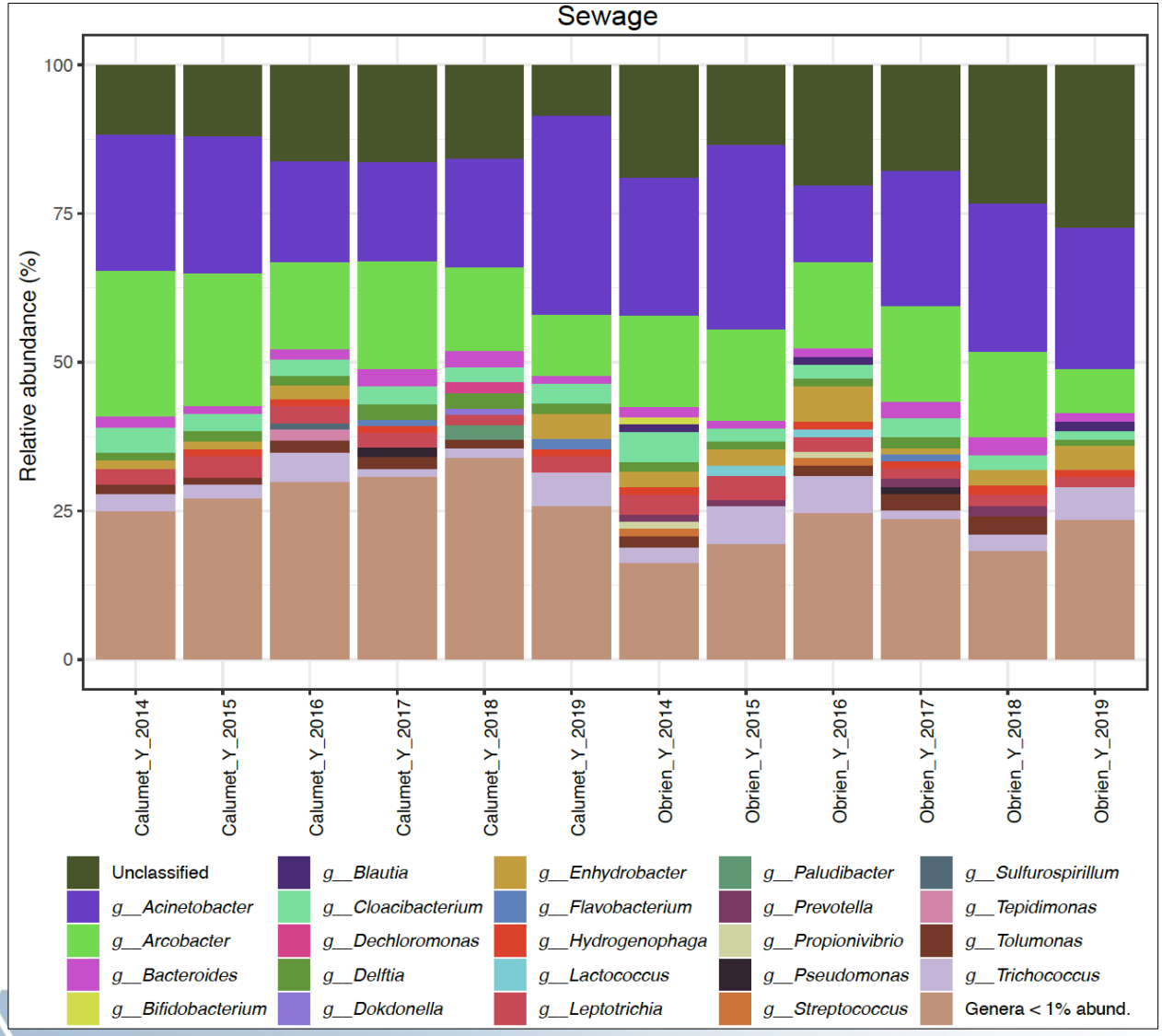
Compositional Differences



IMPACT OF DISINFECTION ON DIFFERENT SAMPLE TYPES

SEWAGE
TREATED EFFLUENT
RIVER WATER

Microbial composition of sewage



Pre-disinfection-
2013-2015
Post-disinfection
2016-2019

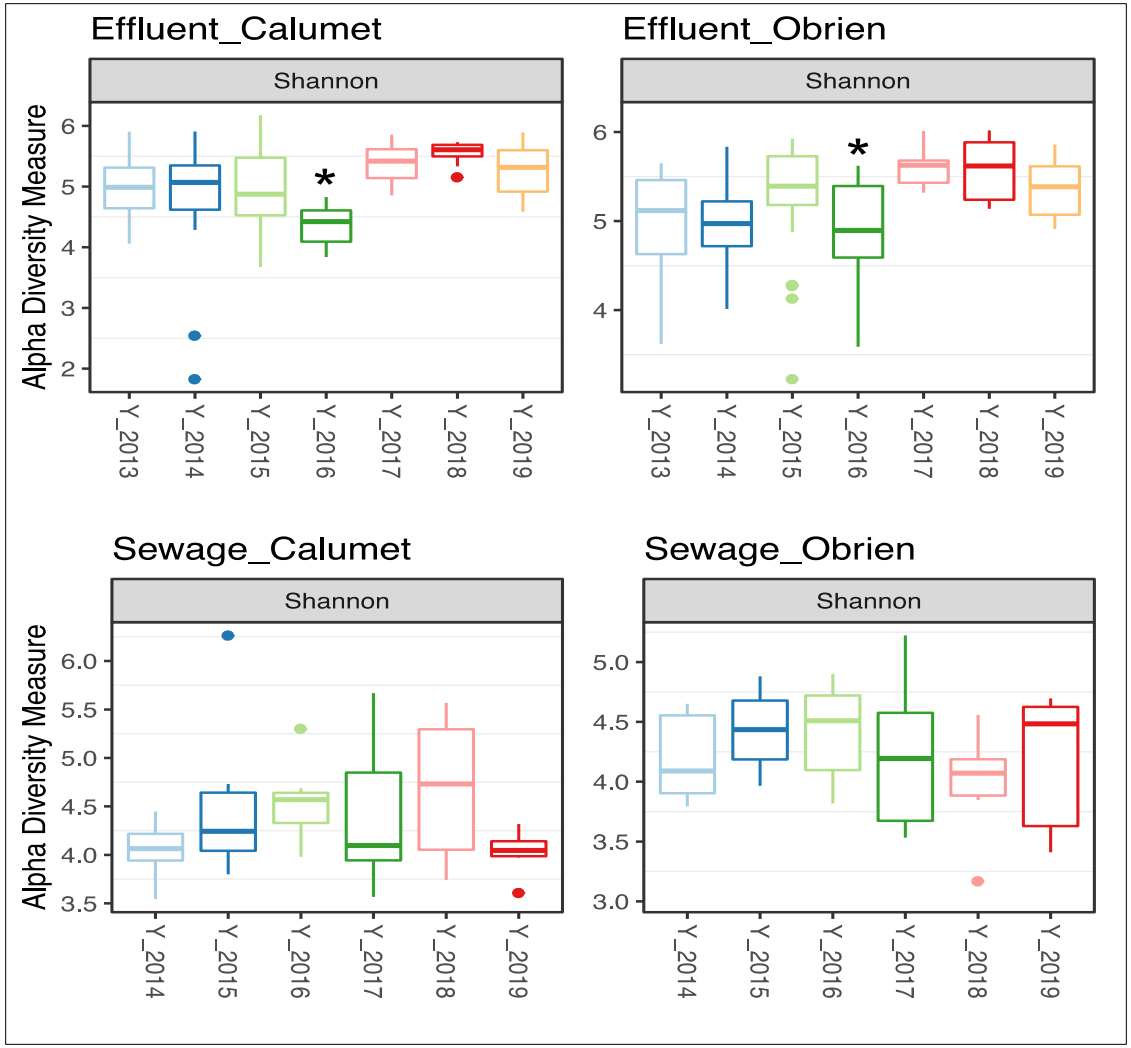
Dominated by sewage indicators-

Acinetobacter
(Cal~18.31%,
O'Brien~19.97%)

Arcobacter
(Cal~ 17.63%,
O'Brien~14.79%)

Across all the years (2013-2019).

Significant reduction in effluent microbial alpha diversity in 2016

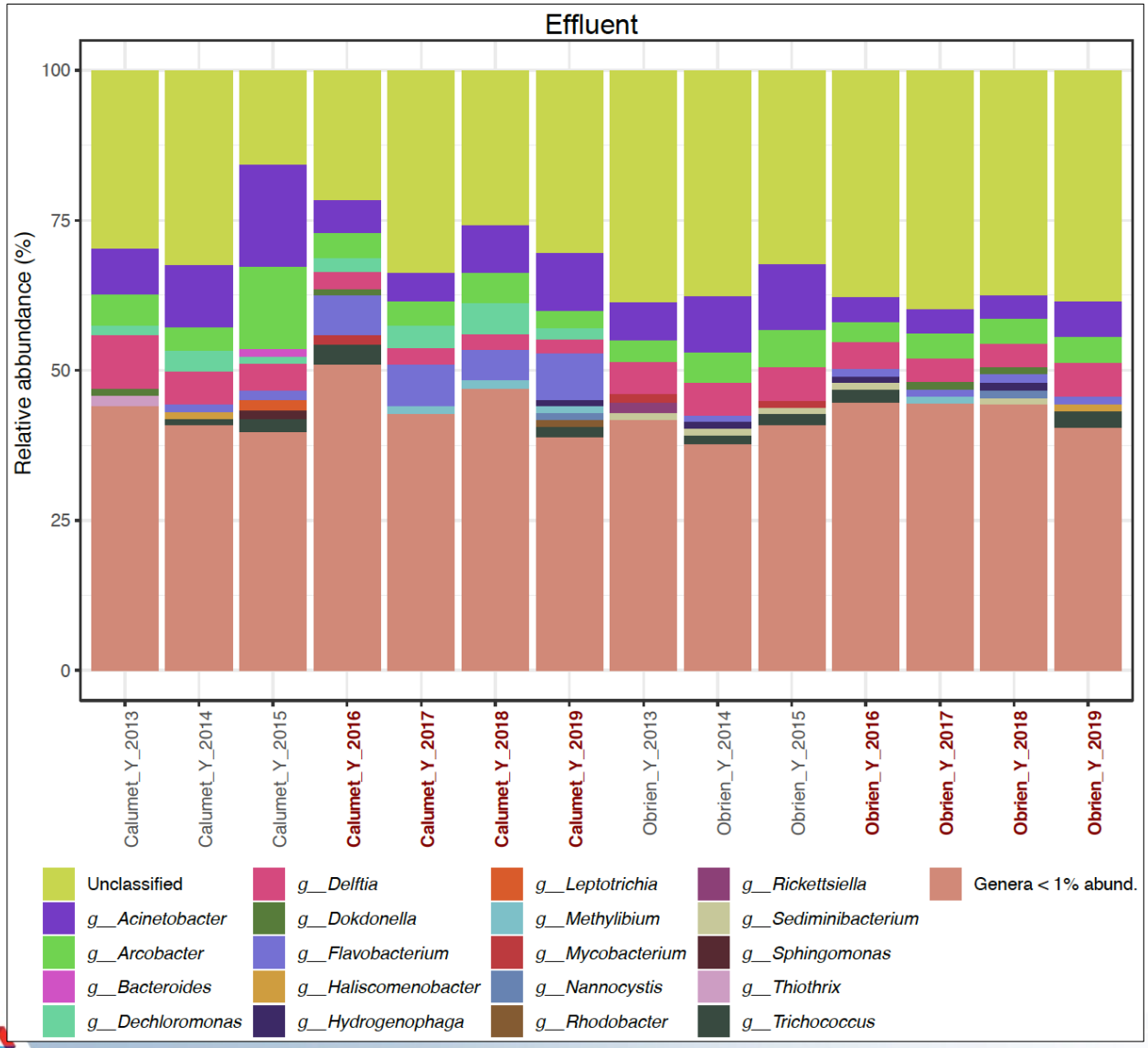


Effluent has greater microbial diversity compared to sewage

Effluent in both Calumet and O'Brien had a significant reduction in 2016 compared to all years.

Sewage showed no change.

Impact of disinfection on the outgoing effluent (Significant changes)



Pre-disinfection-
2013-2015
Post-disinfection
2016-2019

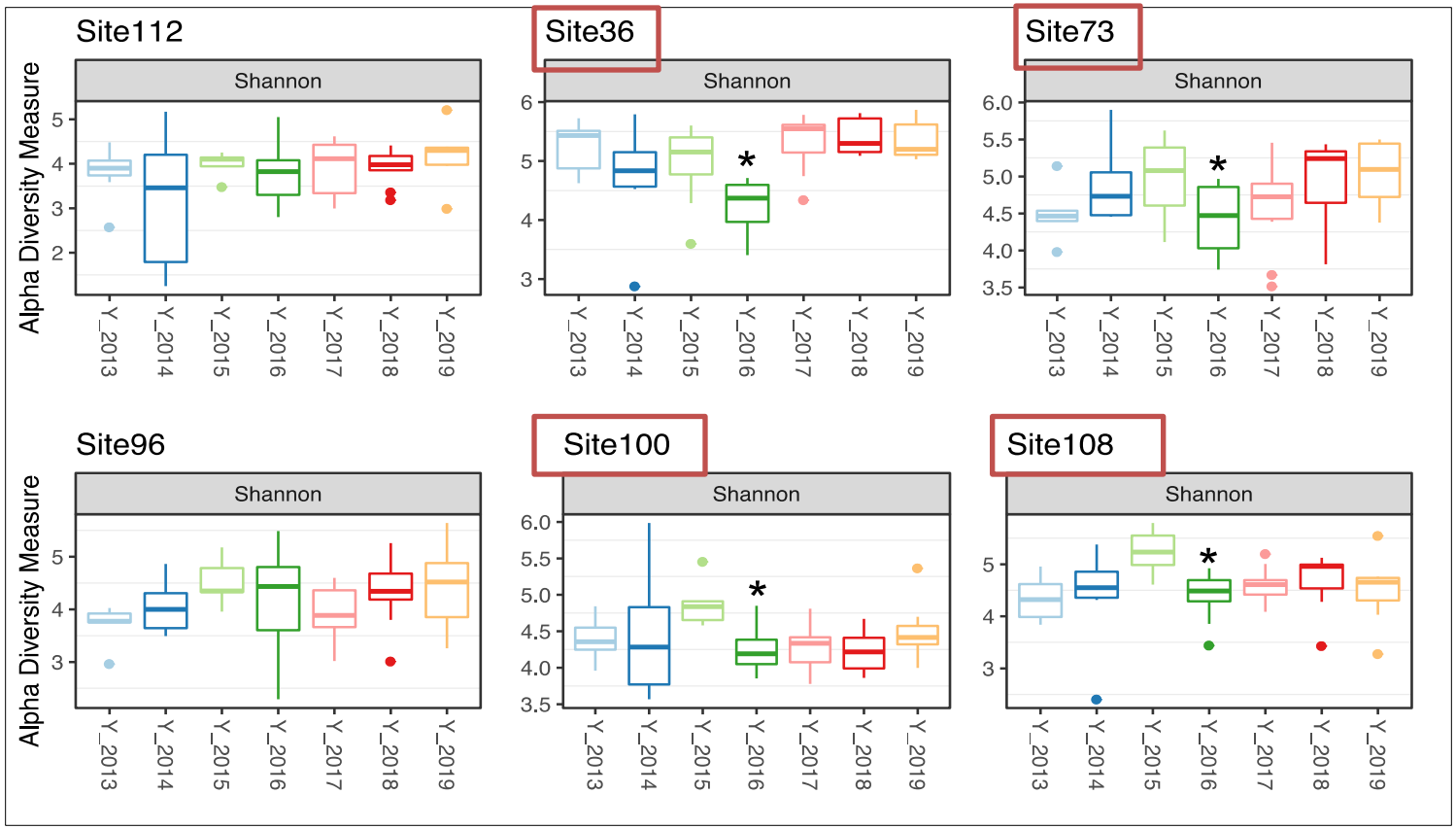
Dominated by sewage indicators-

Acinetobacter
(Cal~7.52%,
O'Brien~6.89)

Arcobacter
(Cal~5.54%,
O'Brien~3.74%)

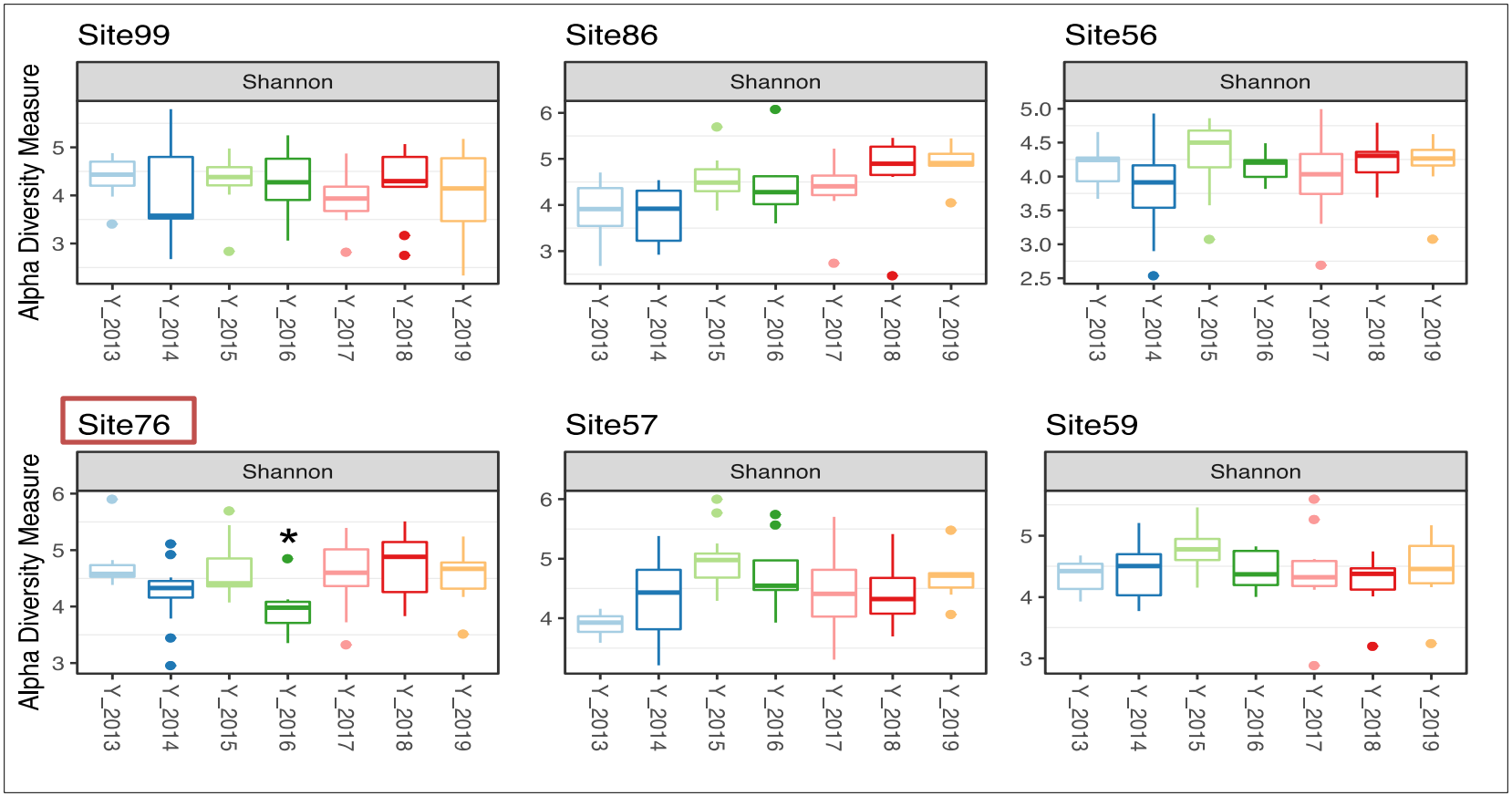
BUT
significantly reduced in the post-disinfection years (2016-2019)

Significant reduction in microbial diversity in 2016 downstream of O'Brien Water samples



Alpha diversity significantly decreased in 2016 (the year of disinfection) for the sites downstream of O'Brien (36, 73) as well as two sites of South Branch (100, 108) which are not directly impacted.

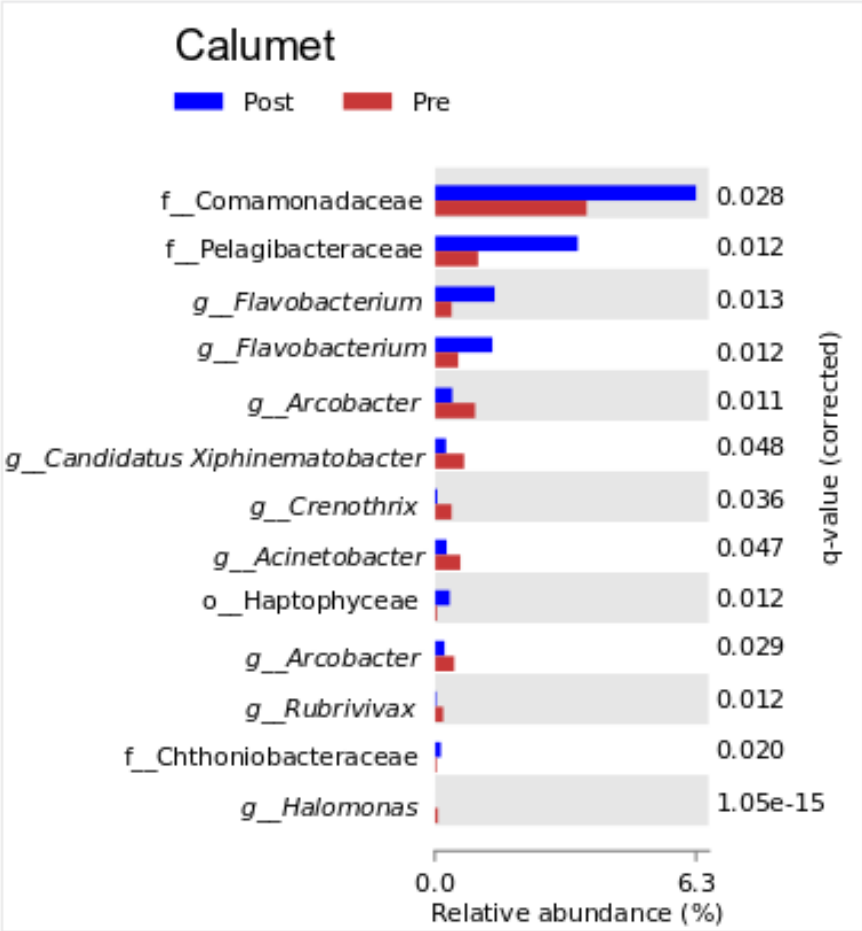
Significant reduction in microbial diversity in 2016 downstream of Calumet Water samples



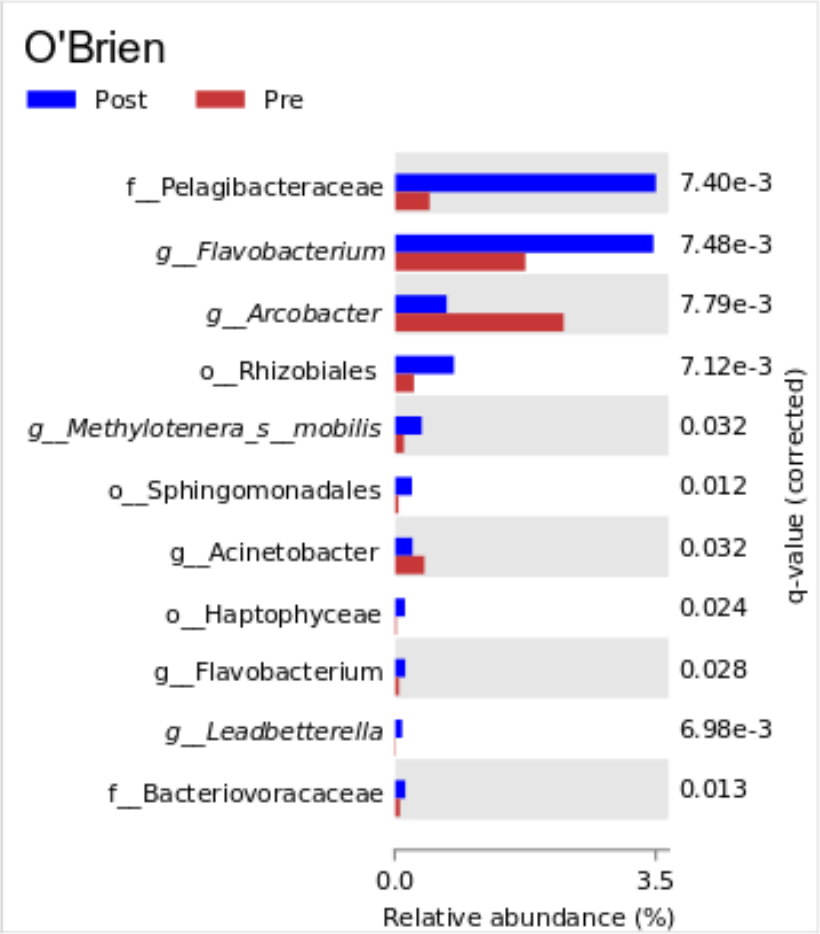
Alpha diversity significantly decreased in 2016 (the year of disinfection) for the sites downstream of Calumet (i.e. 76).

Sewage and fecal indicators decrease post-disinfection at the immediate downstream sites

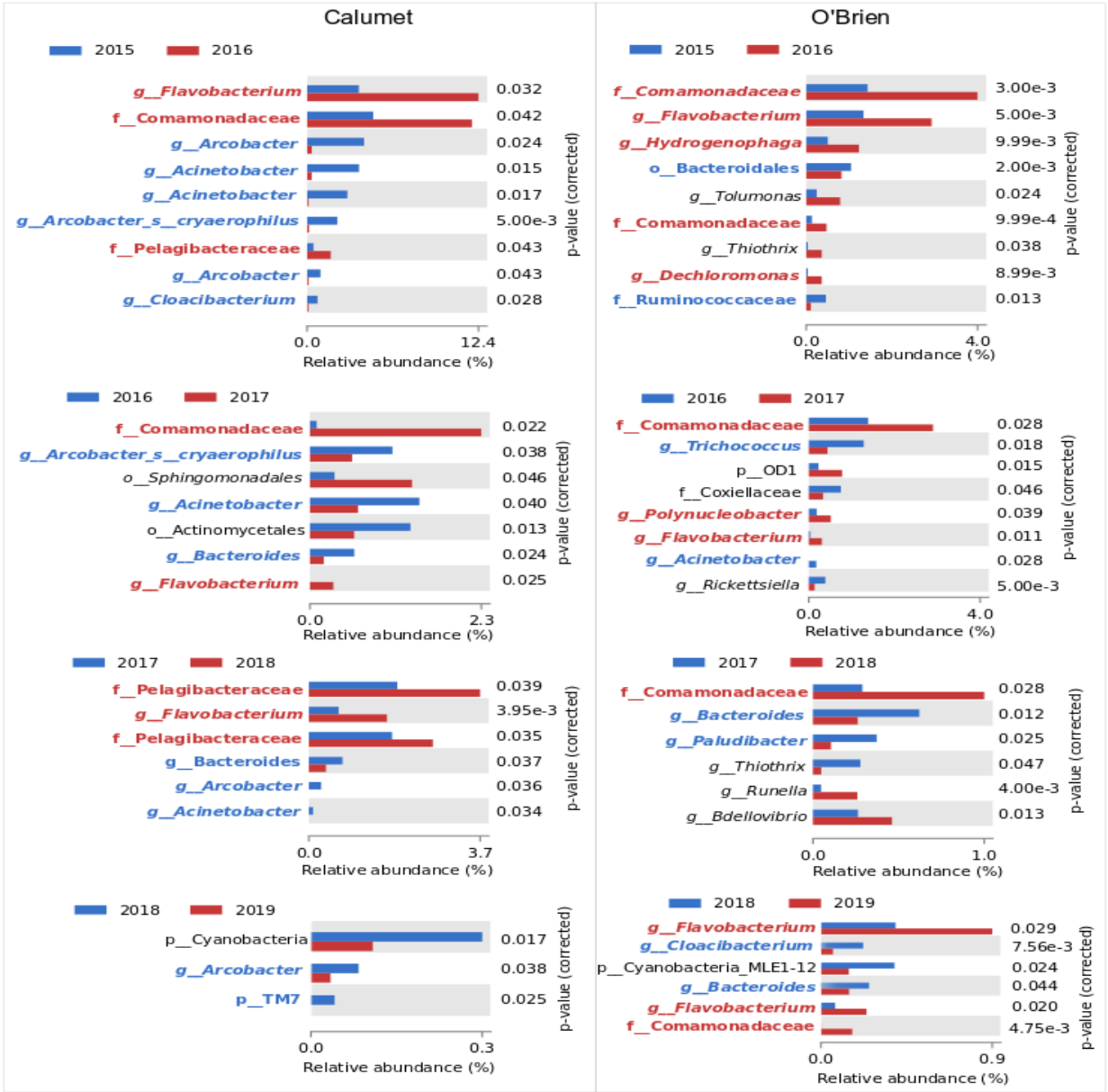
Site 76



Site 36



Sequential decrease of sewage and fecal indicator bacteria in each year post-disinfection

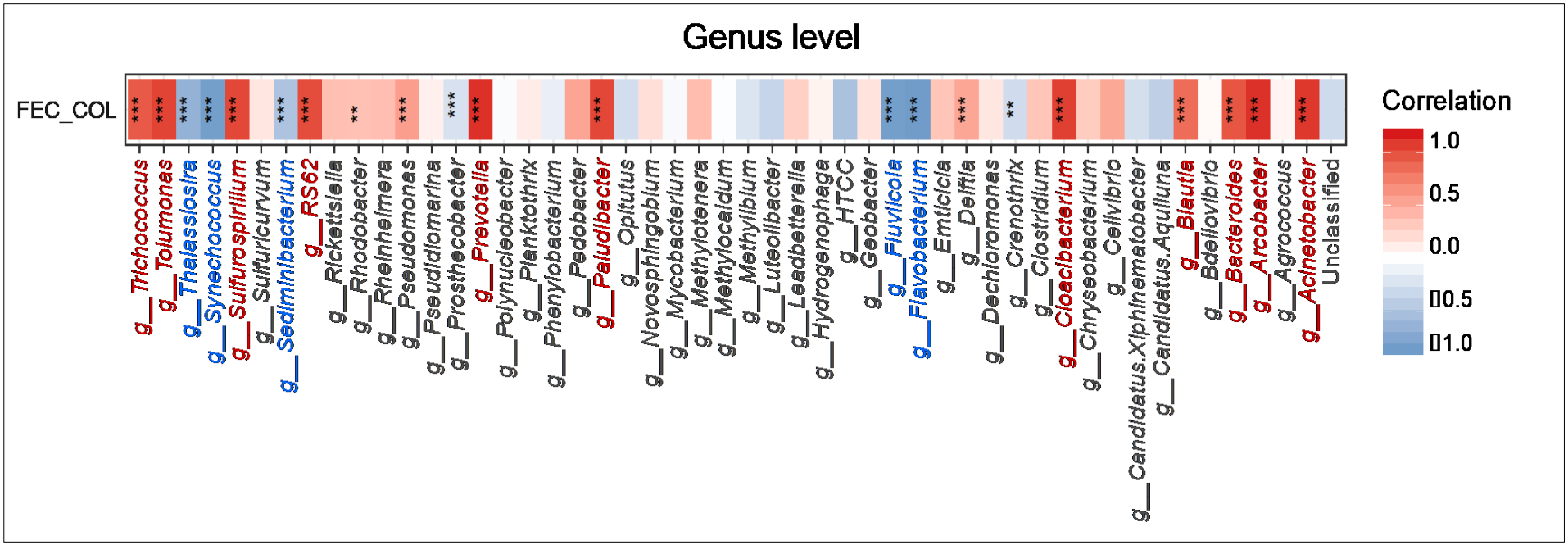


sewage/fecal indicators
'bold blue'
fresh-water **'bold red'**

IMPACT OF RESERVOIR IMPLEMENTATION ON FECAL-COLIFORM ASSOCIATED BACTERIA

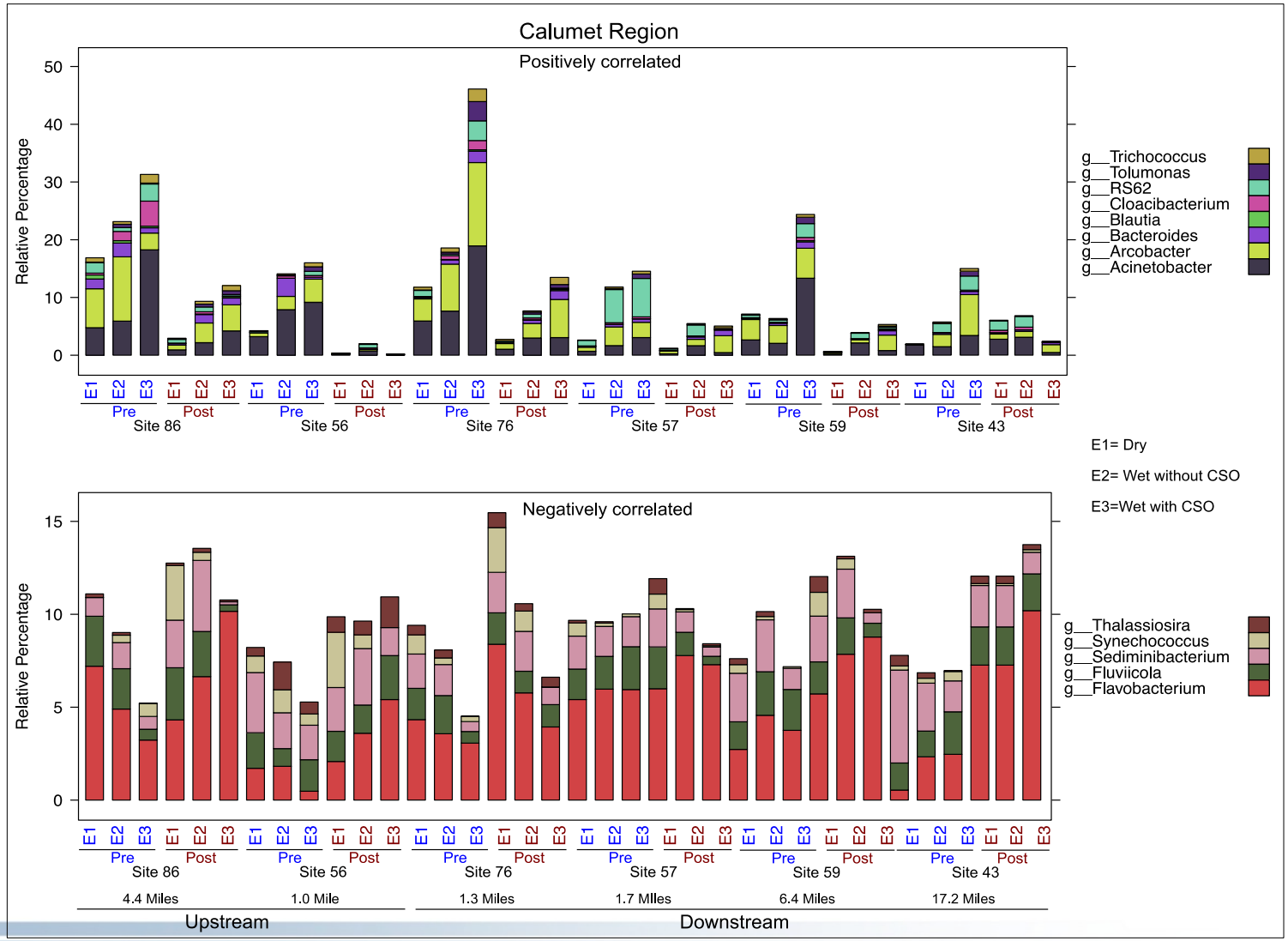
CALUMET-TARP (CALUMET REGION)
MCCOOK (NORTH REGION)

Specific microorganisms strongly correlate with fecal coliform data

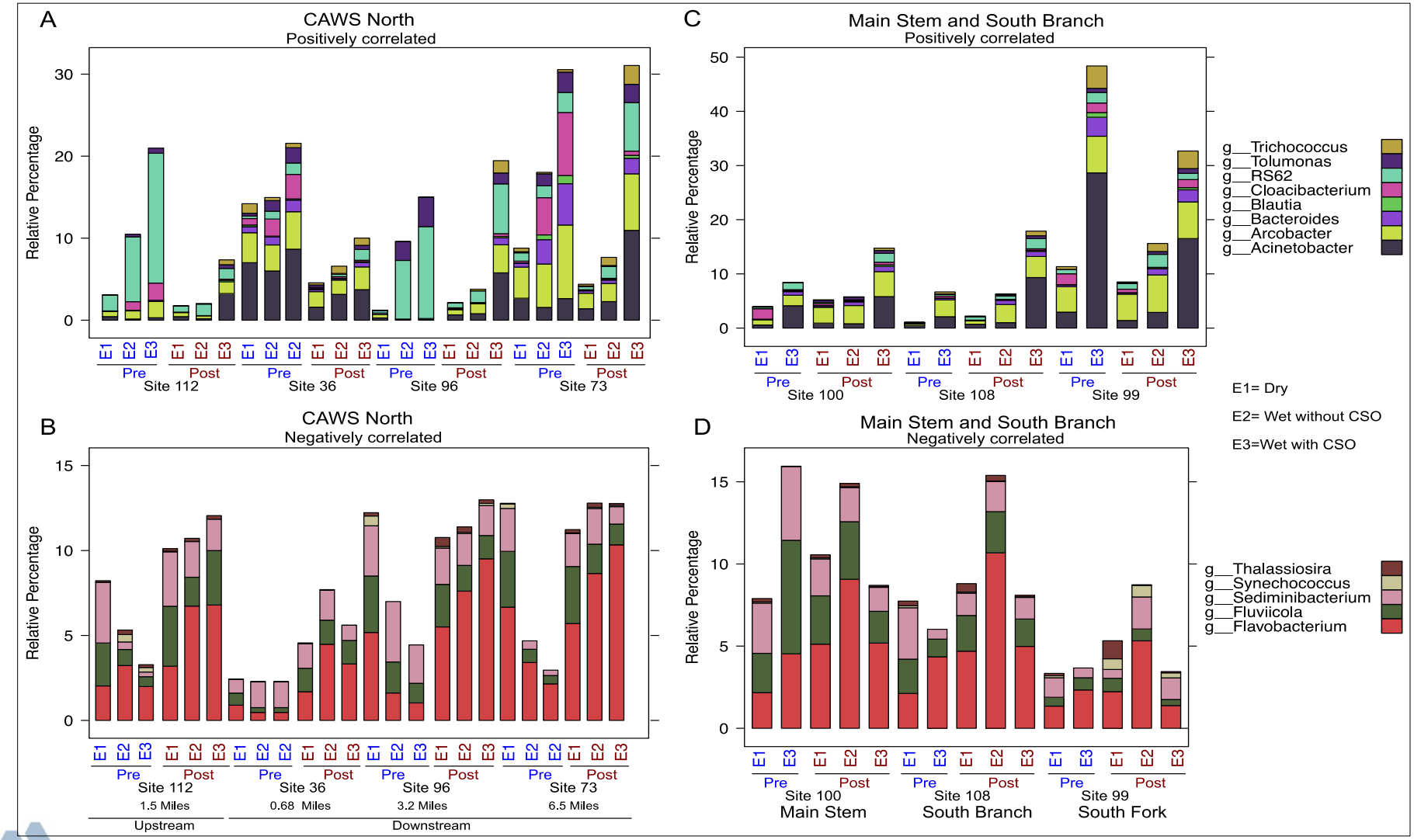


Acinetobacter, Arcobacter, Blautia, Bacteroides, and *Prevotella*, etc. were positively correlated with fecal coliform counts. Whereas *Synechococcus, Sediminibacterium, Fluviicola* (river water indicators) were negatively correlated with coliforms.

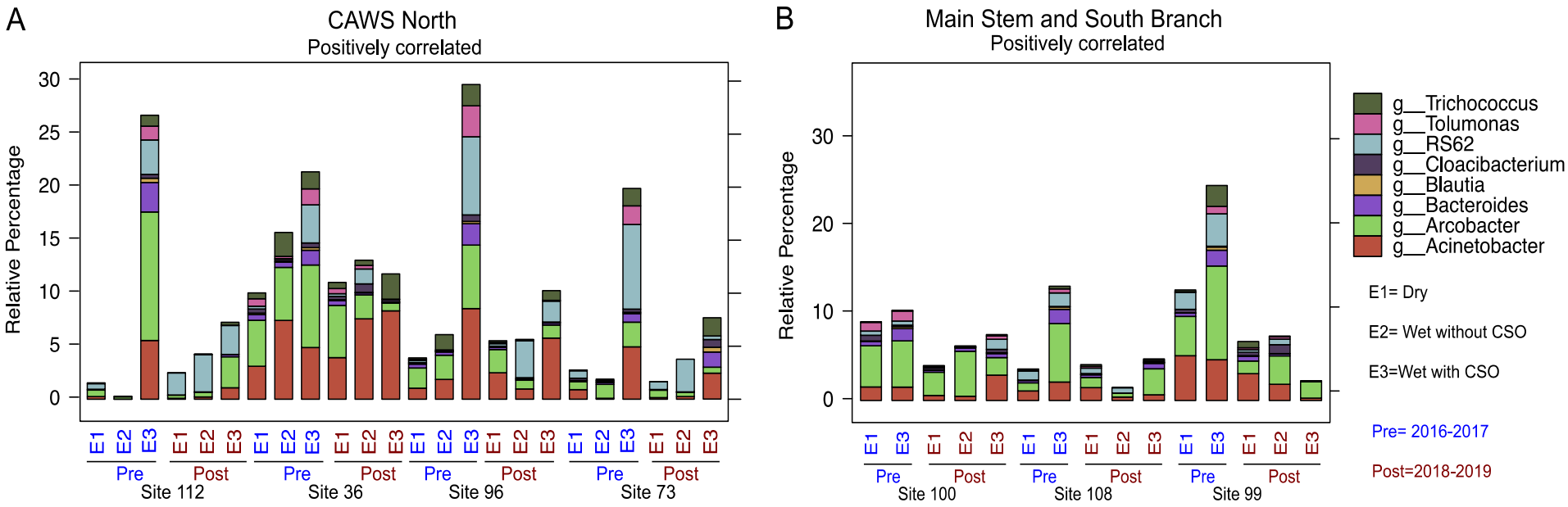
Calumet-TARP controlled the CSOs and thus reduced sewage/fecal indicators in the post-TARP phase in the Calumet region



No significant Calumet-TARP trends in the North Region (and main stem)

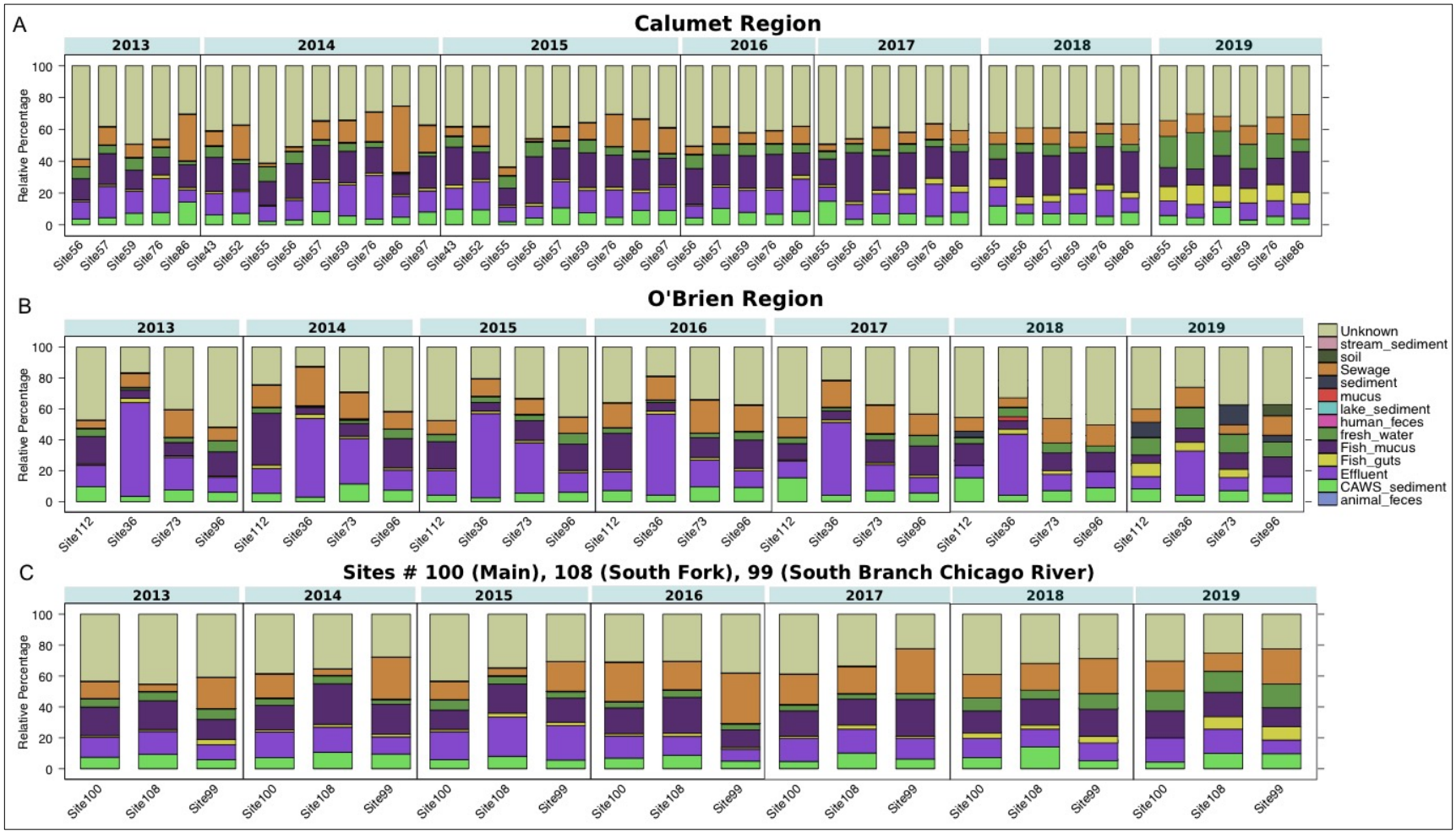


Impact of McCook Implementation-
Reduced sewage/fecal microbial signature
(North Region and Main Stem)



Acinetobacter, Bacteroides, Arcobacter, Cloacibacterium, Tolumonas, decrease significantly during the post-implementation years (i.e. 2018-2019)

Sources of microbial organisms across the CAWS sites



The contribution made by human fecal matter and animal feces across all water column samples was extremely low, i.e. an average of 0.03% and 0.07% of all taxonomic units in each sample, respectively.

Shotgun metagenomics confirmed the 16S patterns

- Shotgun data support the 16S rRNA analysis data, suggesting a low proportion of human fecal indicators such as *Bacteroides* (~0.08%), *Prevotella* (0.05%), *Bifidobacterium* (0.01%), *Cloacibacterium* (0.06%) etc. in the CAWS water.

- *Acinetobacter* and *Arcobacter* species significantly increased in proportion during the wet weather events at the Calumet region, Main and South Branch.

- However, in the Calumet region, we observed a reduction in these indicators post-TARP.

- The most abundant metabolic pathways encoded by the CAWS metagenome included amino acid biosynthesis, aerobic respiration, pyruvate fermentation, nucleoside biosynthesis, acetyl CoA biosynthesis, and photosynthetic light reactions

- Virulence genes formed only 0.25% of the total genetic repertoire.

Conclusions

- The CAWS has greater **than twenty thousand species of microbes** in the water and sediment.
- Compared to the pre-disinfection period (2013-2015), the final effluent from both the Calumet and O'Brien WRPs and river water samples immediately downstream of the WRPs demonstrated a **significant decrease in microbial taxa that are generally associated with sewage and human feces.**
- **Fecal coliform bacteria levels at sites downstream of the T.J. O'Brien WRP and the Calumet WRP showed reduction in the post-disinfection period** (2016-2019) compared to pre-disinfection period (2013 to 2015).
- The Calumet-TARP and McCook reservoir implementation led to a significant reduction in CSO events and thus sewer/fecal signature in the river water samples.

The sewage and wastewater microbes decrease significantly post-disinfection compared to prior (pre) condition without disinfection. The freshwater microbes increase significantly post-disinfection.

Limitations

- 16S rRNA -based techniques have limitations
  - The sequenced read is short, and so contains limited information
  - Limited ability to identify specific species and strains

- Metagenomic sequencing was able to overcome the above biases and hence provided with additional resolution to supplement our results.

- Tracking Sources of Microbial Diversity- this technique is promising, the lack reference databases, geographic variability, the cost and time to build high-throughput sequencing libraries are major limitations to widespread application.

Acknowledgements

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Kim Handley
Cristina Negri
Geeta Rijal
Members of the Gilbert lab

Current Lab

Question and Answer Session

Please use the Q&A feature in Webex to type in your question, and the speaker will respond verbally. Please select All Panelists. Only questions addressed to all panelists will be seen and answered.

Thank you!

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