Muth Lab: Microbiomes of Microplastics and Duckweed

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BUEE 2024 Cohort

Overview of projects

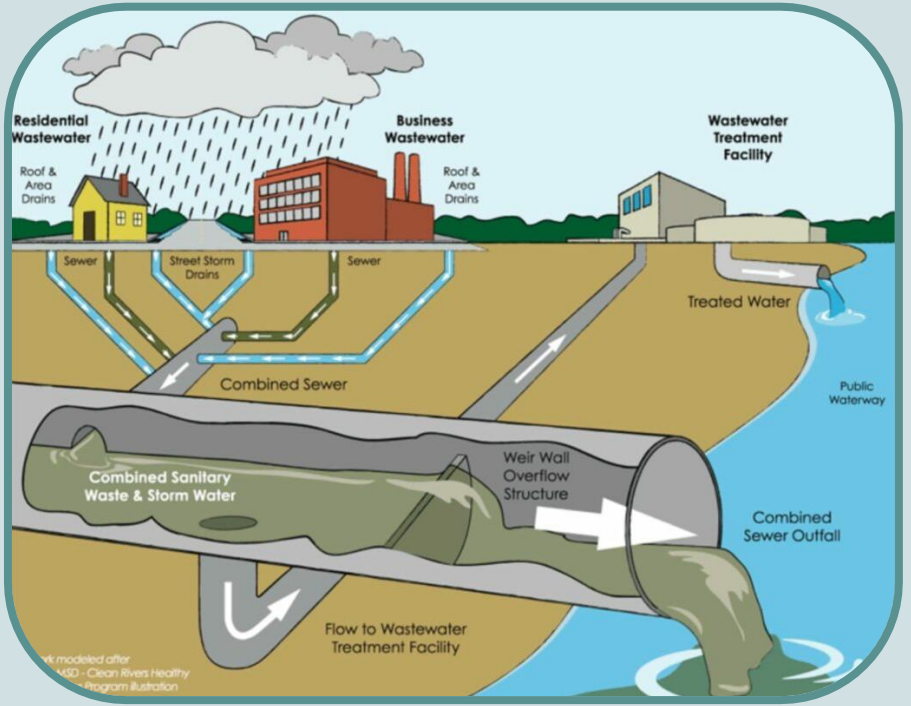
- Analyzing the microbiome of biofilms on microplastics in the Hudson River
- Identifying antibiotic resistant bacteria in the duckweed microbiome

Pier 40
(Hudson River)

Duckweed (Prospect Park)

Background

- NYC’s sewer system struggles to prevent pollution during heavy rain.
- Pollutant runoff flows into the Hudson River and Prospect Park Lake.
- Our studies can help us understand the extent of this pollution
- Potential remediation with duckweed



Background

- Biofilm is the layer of bacteria stuck together and on surface, often with a slimy substance or “extracellular matrix”
- Biofilm formation conditions promote antibiotic resistance and are linked to many diseases (bacterial pathogens)

(Vestby et al., 2020, Oluwole et al., 2022)

Table 1

Biofilm-related bacterial infections

Associated microbe	Biofilm-related infection
Escherichia coli	catheter-associated urinary tract infection, acute and recurrent urinary tract infection, biliary tract infection
Pseudomonas aeruginosa	catheter-associated urinary tract infection, cystic fibrosis lung infection, contact lens-related keratitis, persistent wound infection, recurrent rhinosinusitis, recurrent otitis media
Staphylococcus aureus	recurrent rhinosinusitis, persistent osteomyelitis, otitis media, endocarditis, long-term orthopaedic implants
Streptococcus pneumoniae	recurrent rhinosinusitis, colonization of nasopharynx, long-term obstructive pulmonary disease, recurrent otitis media.
Streptococcus pyogenes	Colonization of oral cavity and nasopharynx, recurrent tonsillitis

Table from Oluwole et al., 2022

Why Do We Care About This Research?

- Microplastic pollution in water is prevalent
- Microplastics can harbor biofilms with antibiotic-resistant bacteria (Marathe & Bank, 2022)
- Aim to highlight the need to prevent microplastic pollution.

(New Scientist)

Microbiomes on Microplastics at the Hudson River: Main objectives

- Sequencing bacterial communities from the river – indicators of water quality
- Examining the connection between microplastics and the presence of antibiotic resistance genes (AGRs) within microbial communities

HUDSON RIVER PK

Methods: Microbiomes on Microplastics

- 16g of microplastic beads into tea infusers
- Placed infuser in minnow traps fastened with zip ties
- The traps were deployed on docks along the Hudson River

Microplastics in the tea infusers

Zip tying infusers into the traps at HRPT Wet Lab

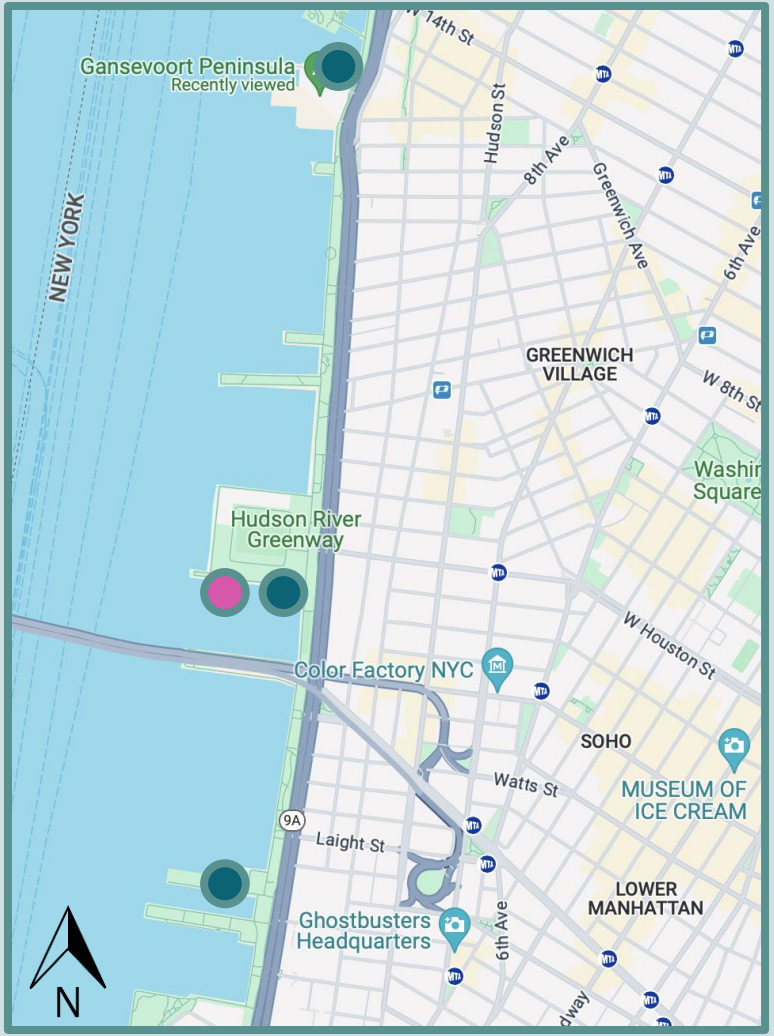
Methods: Microbiomes of Microplastics

- Secured the traps to floating docks
- Waited 10-21 days for biofilm to accumulate
- Collected tea infusers

Collecting traps and diffusers

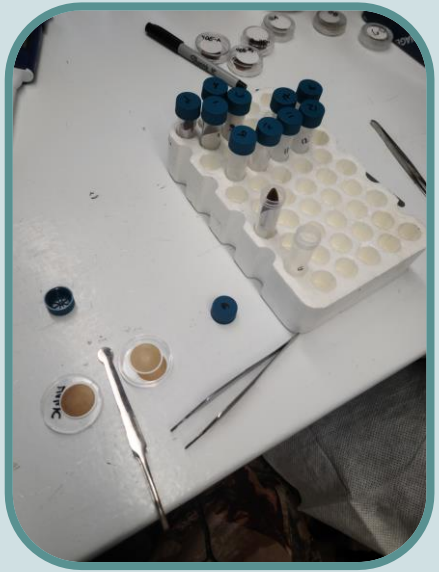
Methods: Microbiomes of Microplastics

- 1st Test: 4 different types of plastic in equal amounts
  - 4 different locations
- 2nd Test: Each tea infuser has 1 of the 4 types of plastic
  - Same location

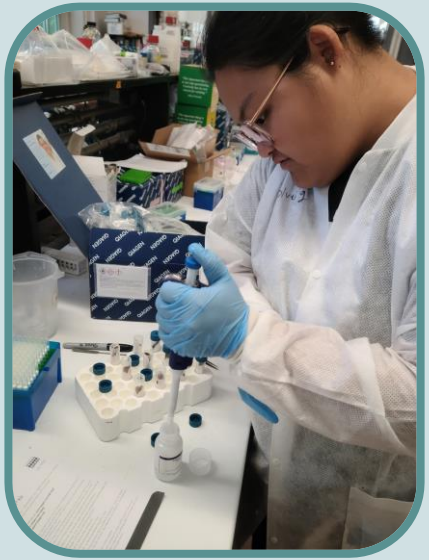


Methods: Microbiomes of Microplastics

- Washed off biofilm with detergent and saline
- Collected bacteria on a 0.22 micron pore filter
- DNA analysis of collected material



Micropore filtering



Preparing DNA samples

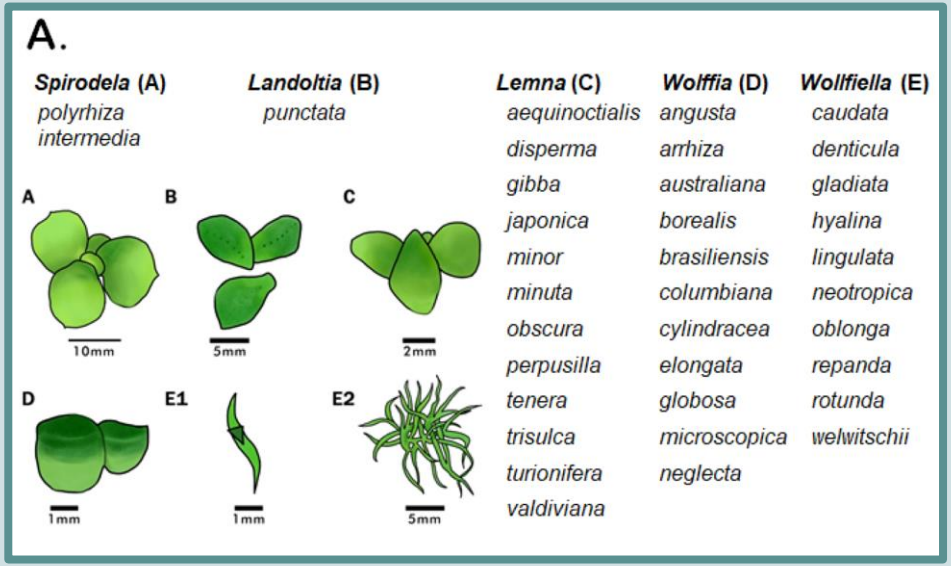
Goals of 2nd Project: Duckweed Microbiome

- Identify antibiotic resistant bacteria in the biofilm duckweed microbiome
- Understand how to better utilize duckweed as a method of bioremediation

Duckweed (Prospect park)

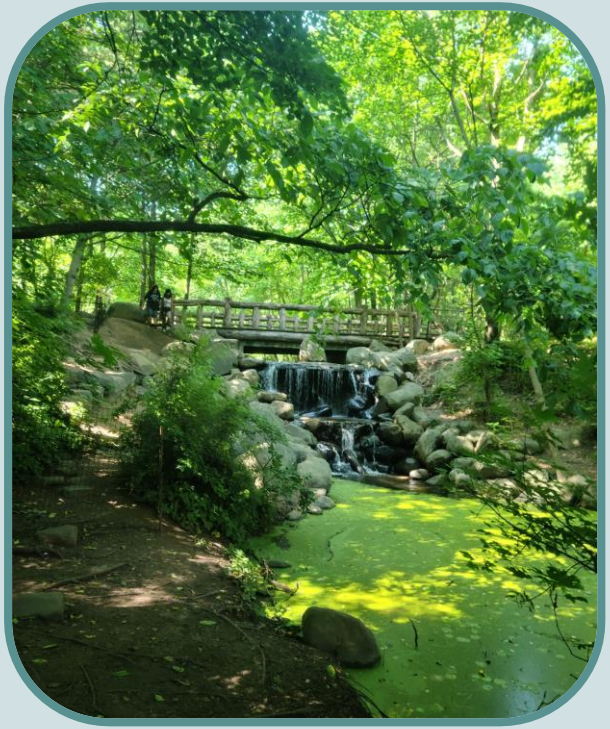
Duckweed

- Duckweed present in several water bodies in NYC, the contiguous US, and globally.
- Duckweed can double its biomass in 2-3 days
(Sowjanya Sree & Appenroth, 2024)
- Duckweed can be grown for food used for bioremediation, and as biofuel



Why Do We Care About This Research?

- Duckweed tolerates water bodies with high levels of nitrogen and phosphorus
- Previous studies have shown that duckweed can process excess nitrogen and antibiotics from polluted water (ex. Toyama et al., 2024)

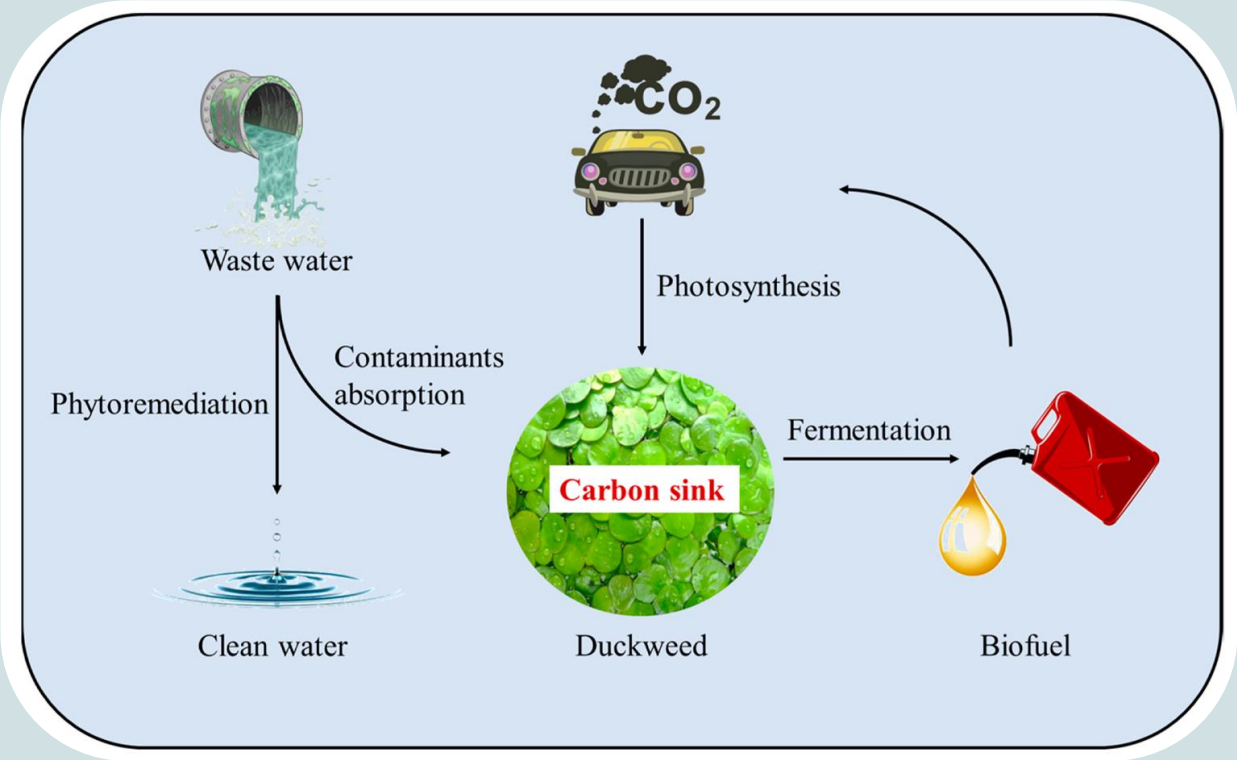


Location: Binnen Bridge

Antibiotic Resistance on Duckweed Microbiomes

- The use of antibiotics is prevalent within numerous fields
- Antibiotic resistance is spread through waste water
- Environmentally safe and effective methods of bioremediation need to be developed

Duckweed as a Form of Bioremediation



Methods: Microbiomes on Duckweed

- Collected samples of duckweed and water from 2 places in Prospect Park

Location: Vale of Cashmere

Location: Binnen Bridge

Methods: Microbiomes on Duckweed

- Skimmed Duckweed off of water surface
- Washed biofilm off duckweed with detergent and saline
- Used a micropore filter to collect bacteria from the duckweed and water samples

Duckweed from Binnen Bridge

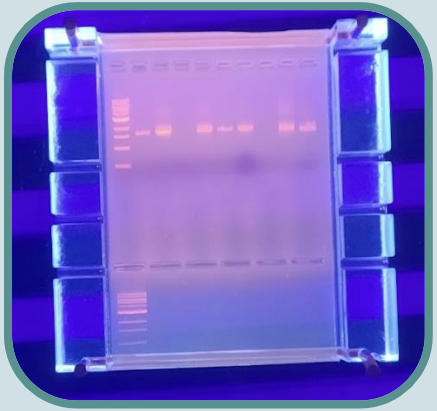
Methods: DNA Analysis

1. Prepared samples with Qiagen PowerSoil kit

2. Completed a PCR

3. Used NanoDrop or Qubit

4. Implemented gel electrophoresis



5. Ran through Nanopore Sequencing

Nanopore Sequencing

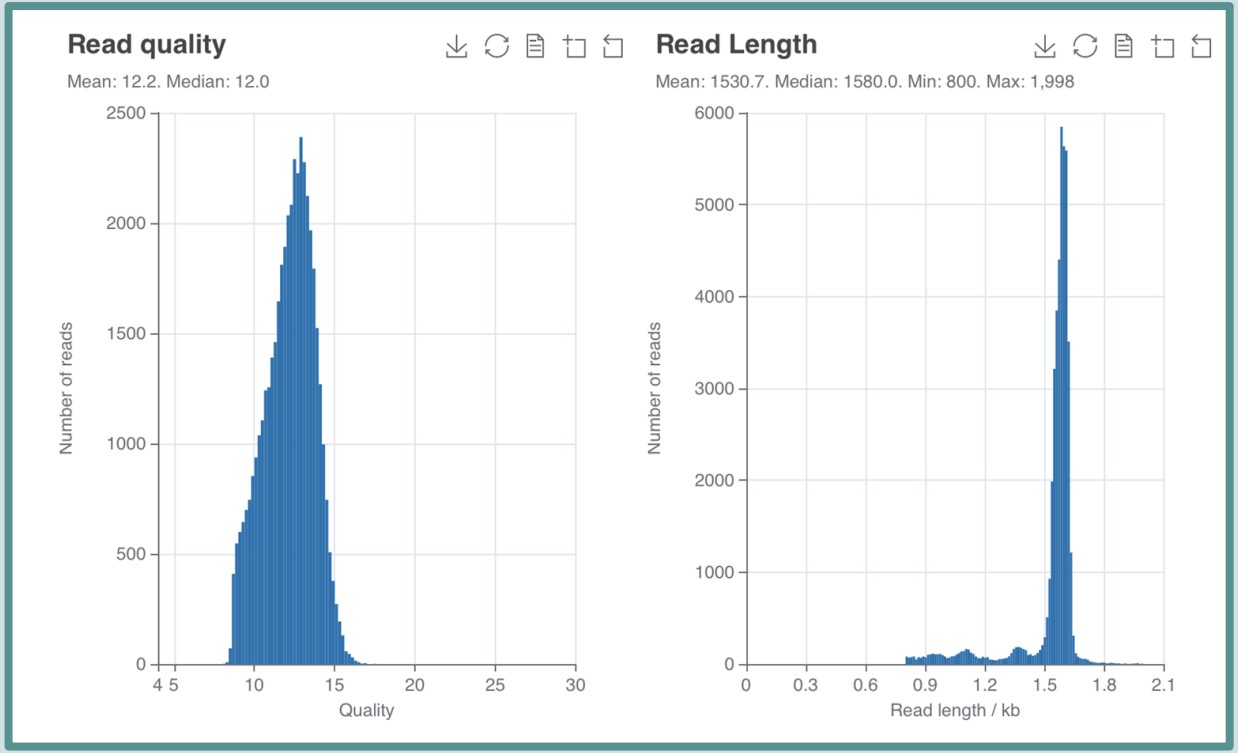
How does nanopore sequencing work?

- DNA is placed on flow cells filling the nanopores
- An electric current is run through the cell causing the different nucleotides within DNA to move

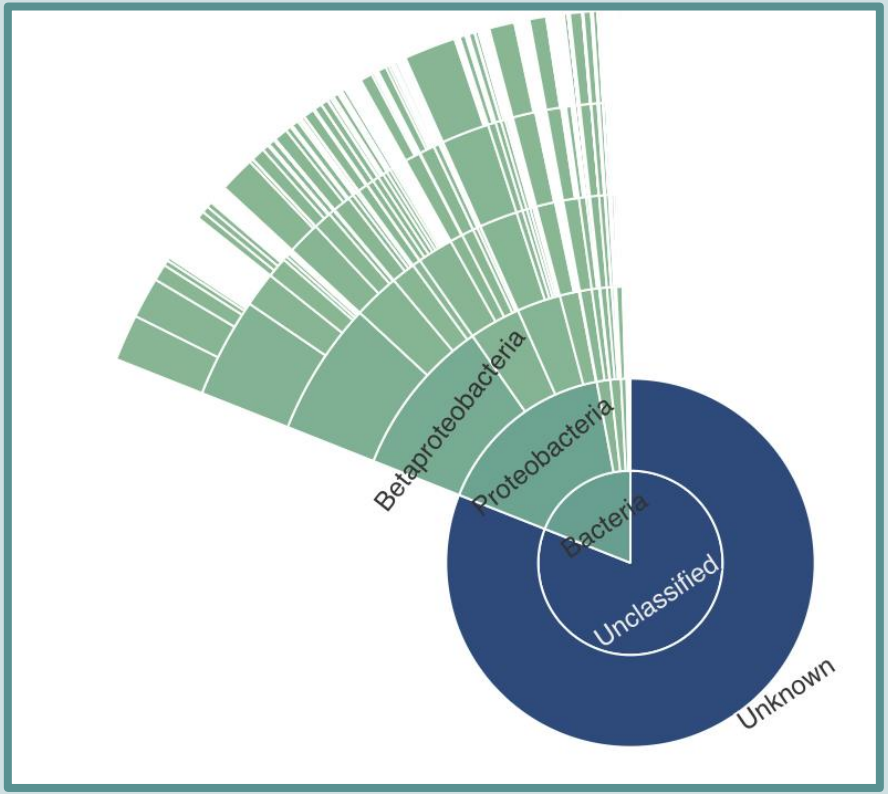
Advantages

- Gives results quickly in real time
- Relatively affordable and accessible

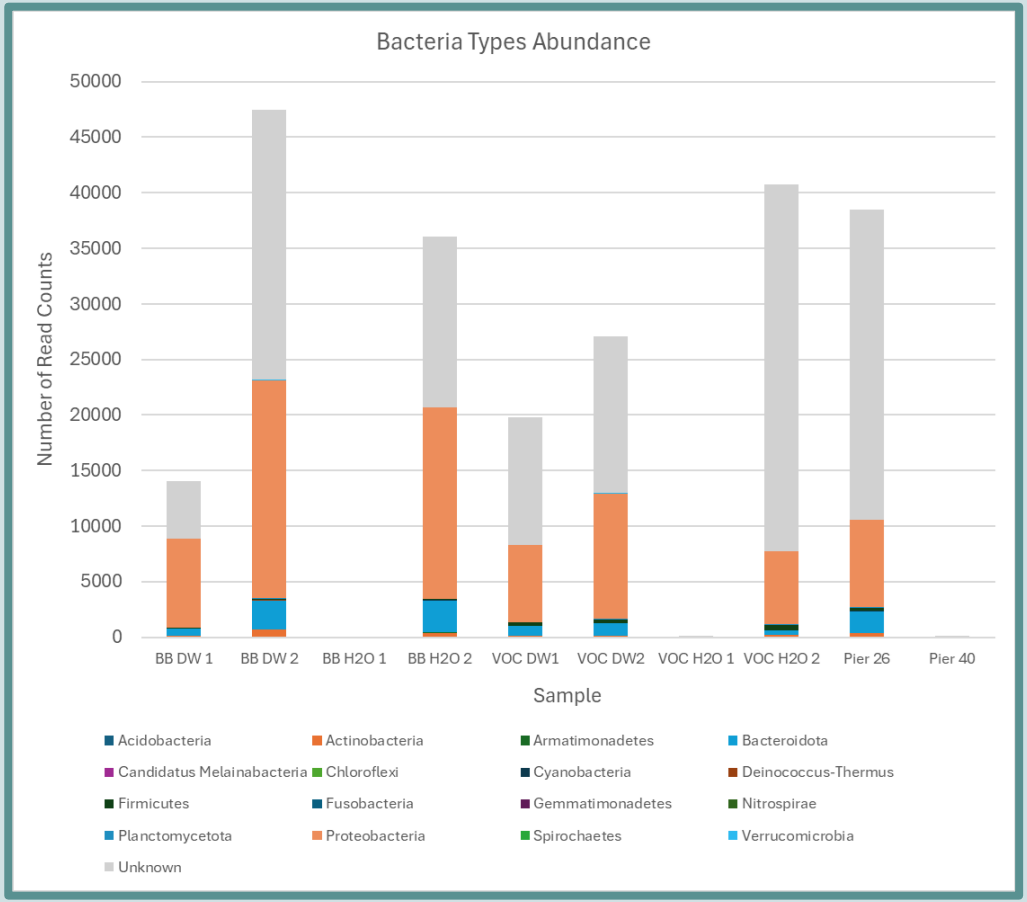
Results So Far:Nanopore Microplastics



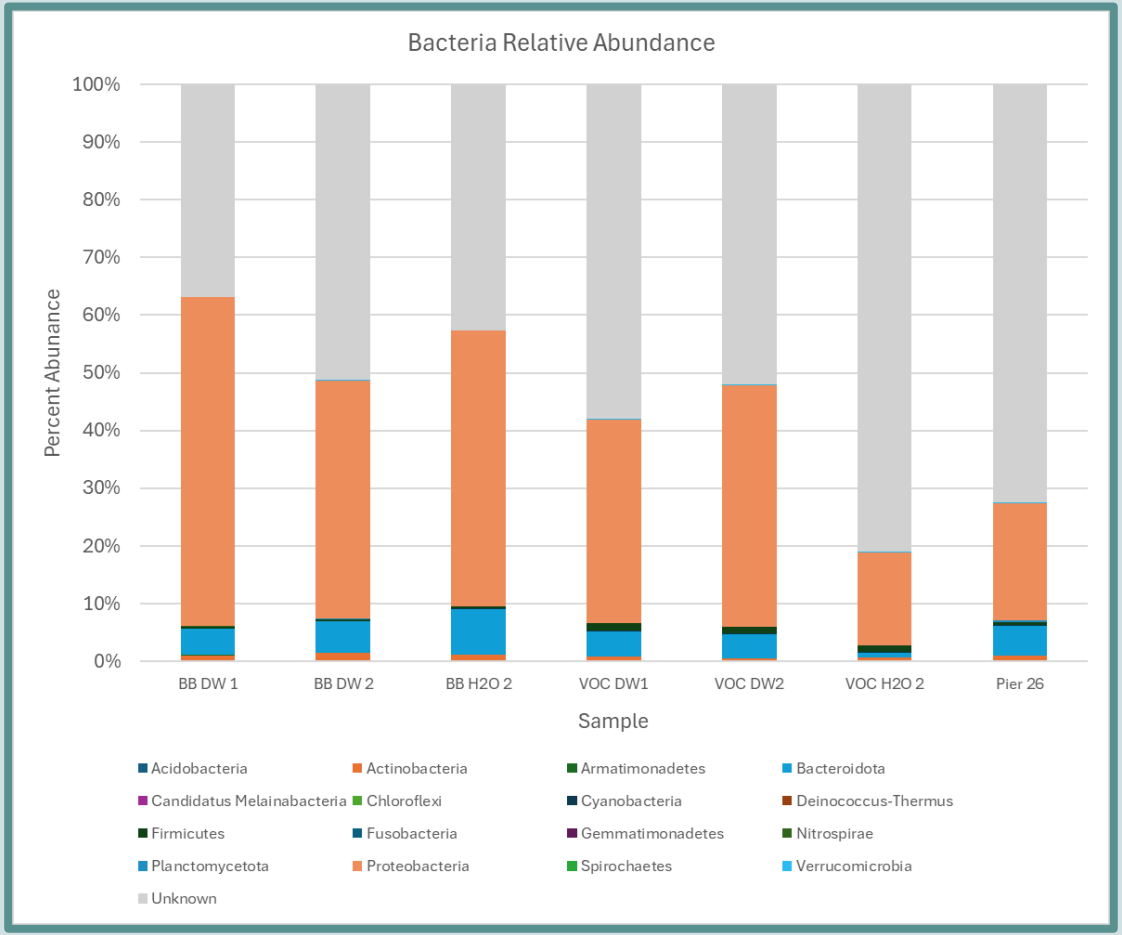
Results So Far:Nanopore Microplastics



Results So Far: Nanopore Duckweed Samples



Results So Far:Nanopore



Results So Far:Nanopore

Sample	BB DW 1	BB DW 2	BB H2O 1	BB H2O 2	VOC DW 1	VOC DW 2	VOC H2O 1	VOC H2O Pier26		Pier 40	
species	barcode05	barcode06	barcode07	barcode08	barcode09	barcode10	barcode11	barcode12	barcode13	barcode14	total
Unknown	1011	7112	1	2180	3860	3549	5	13611	14248	3	77348
Limnobacter humi	1326	2758	0	493	0	0	0	1	1	0	4579
Methylovulum psychrotolerans	115	217	0	77	1640	1387	0	166	0	0	3602
Methylobacter tundripaludum	131	249	0	107	919	734	1	1187	3	0	3332
Acidovorax temperans	559	1667	0	554	126	143	0	17	0	0	3070

Table shows pilot results from the Prospect Park duckweed and water samples

What’s Next?

1. Early results indicate that the water quality is not ideal

1. It is too early to tell if we have indicators of antibiotic resistant bacteria

1. Further data collection and analysis is needed

Sources

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Questions?