

Microbiological water quality for Drinking Water

Colilert test for *Escherichia coli* and total coliforms

<1 *E. coli*/100 mL of drinking water



Yellow colour = total coliforms



UV fluorescence = *E. coli*

What is the problem?

1,177 drinking water supplies

117 of these supplies issued 188 “boil water” or “do not use” notices in 2022,
with 40 of these long-term advisories for up to 7 years

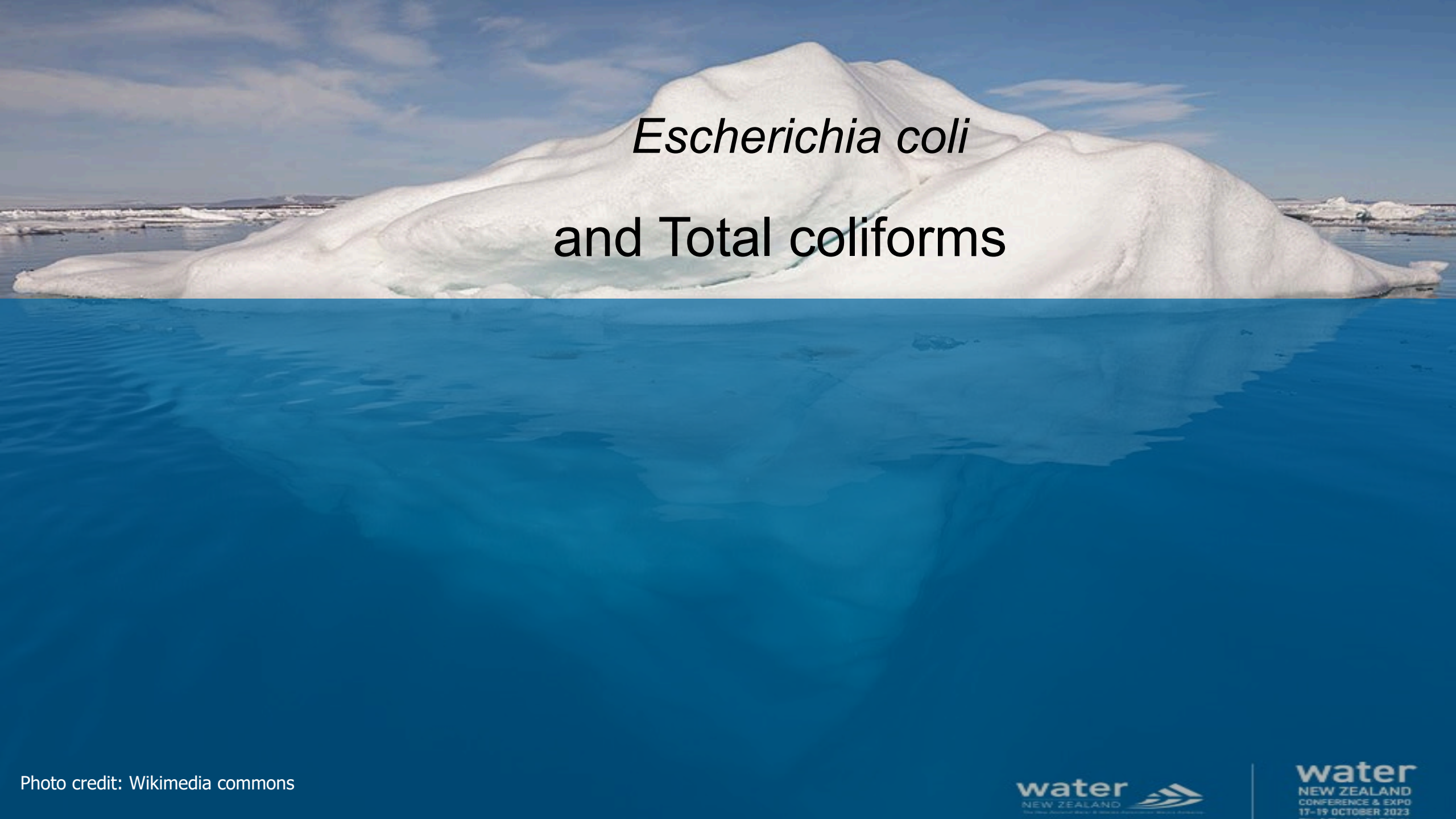


103 of these supplies had detections of *E. coli*, including 45 school supplies.




Escherichia coli

<1 *E. coli*/100 mL of drinking water



Escherichia coli
and Total coliforms

A large iceberg floats in the ocean. The visible part above the surface is a white, rounded mass. Below the surface, a much larger, dark blue-green submerged portion is visible, illustrating the concept of 'the bigger picture'.

**To make informed decisions to deliver safe drinking water
from source to tap**

Water managers need the bigger picture



**To make informed decisions to deliver safe drinking water
from source to tap**

Water managers need the bigger picture

We need to see more:

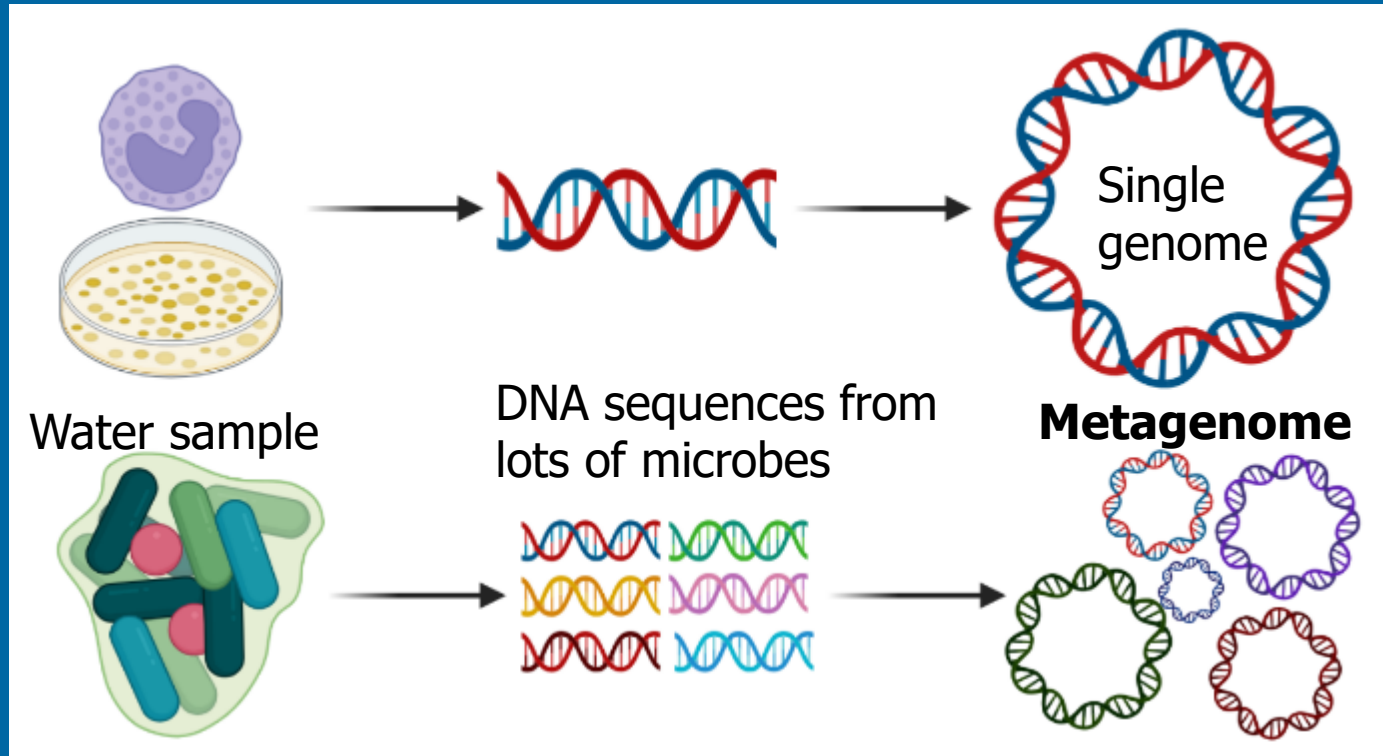
Faecal pathogens

Non-faecal pathogens

biofilms in pipework

Environmental microbes- the good guys

Metagenomics versus genomics



Single organism

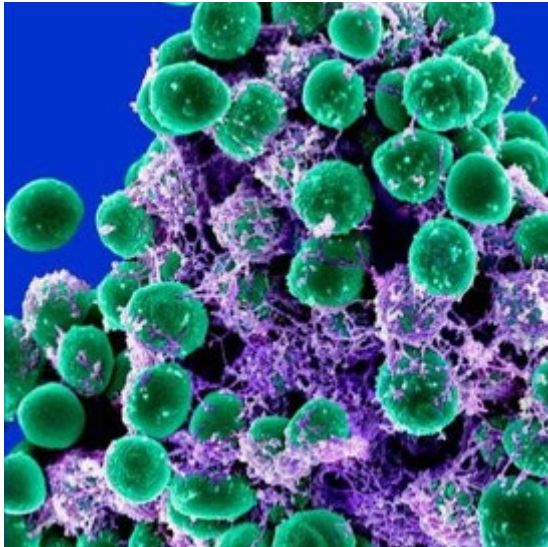
Meta = many
Multiple organisms with
DNA sequences
in a single sample

Metagenomics: the exploration of the entire bacterial community within a sample

Human Microbiome: We are walking apartment buildings for microbes

Find **over a million Microbes** in a teaspoon of soil
The **bacterial cells** that live on the body
10-100 times greater than the **10 million cells**

or a **gram of faeces.**
weigh 1-3 kg
that make up the human body.



Staphylococcus epidermidis, a common bacterium on our skin

Credit NIAID, Agriculture Department, via Associated Press



<https://cosmosmagazine.com/science/biology/how-many-genes-in-the-human-microbiome/>



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Our hypothesis:

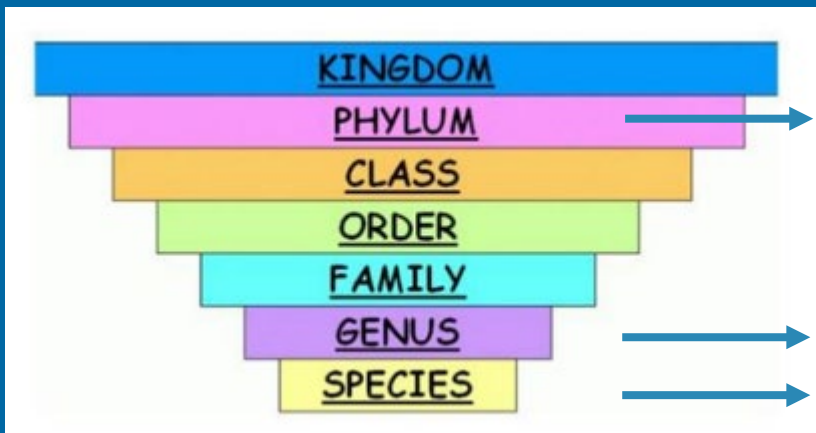
Metagenomic analysis can be used to better understand and support management of microbial health risks in drinking water

We are targeting 16S rRNA gene to identify bacterial taxa in drinking water

Using Oxford Nanopore technology (ONT) to target full length 16S rRNA gene to provide:

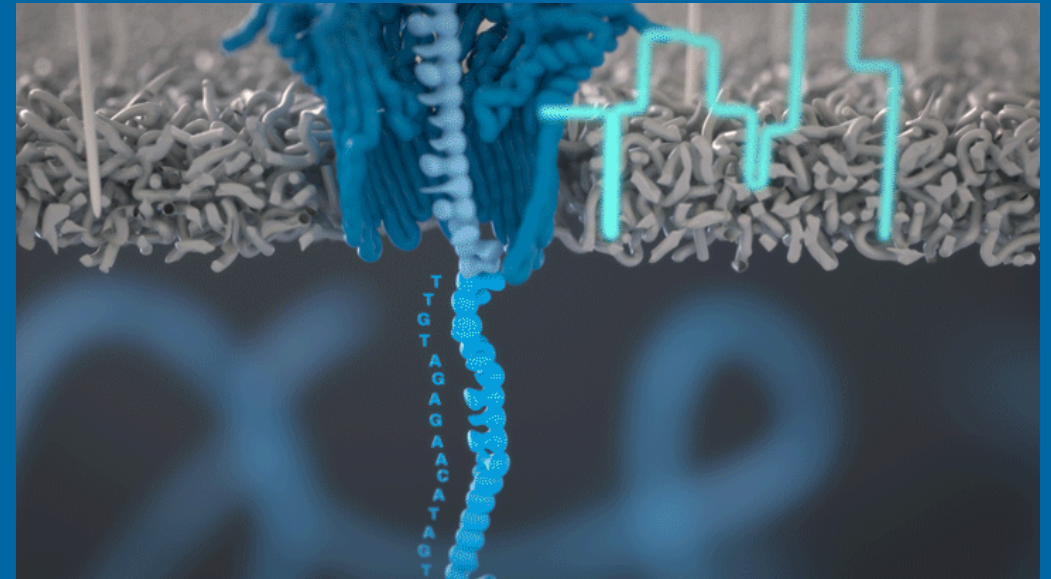
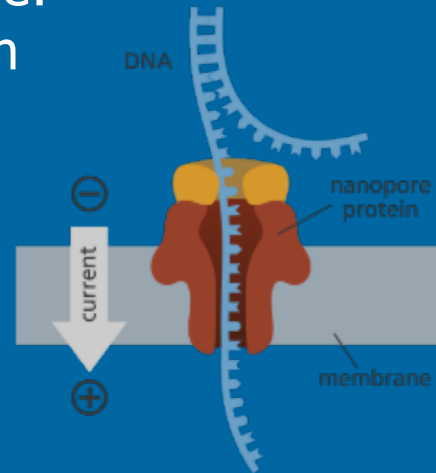
- greater accuracy for identification
- cost-effective
- timely

Seven levels for bacterial classification



Bacteroidota

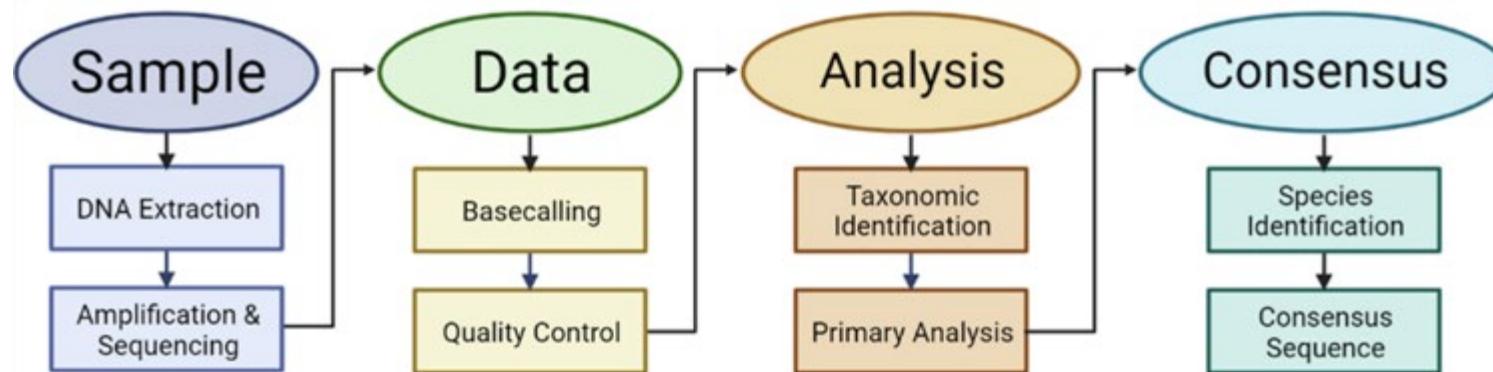
Bacteroides
Bacteroides fragilis



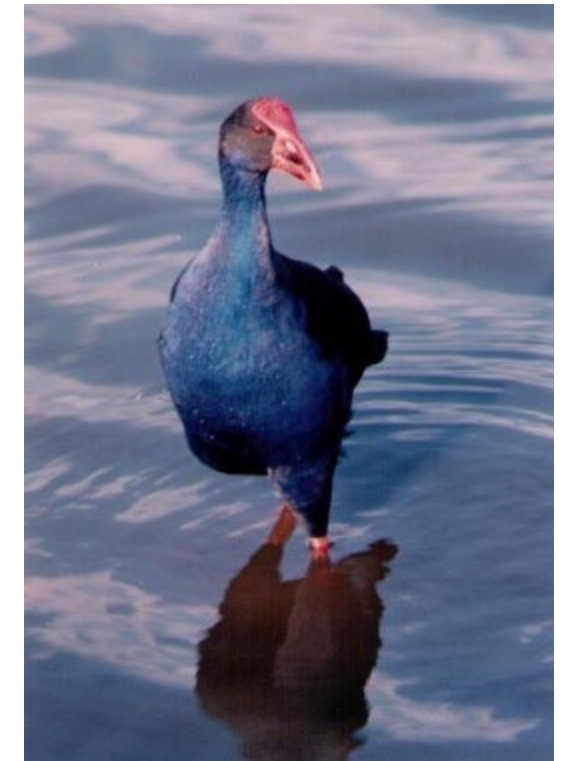
<https://nanoporetech.com/support/how-it-works>

The sequencing process: target 16S rRNA gene to identify bacterial taxa in drinking water

- Filter water
- Extract DNA
- Amplify 16S rRNA gene
- Sequence: Oxford Nanopore technology
 - Quality control processes for sequence data
- Assign: bacterial taxonomy classifications

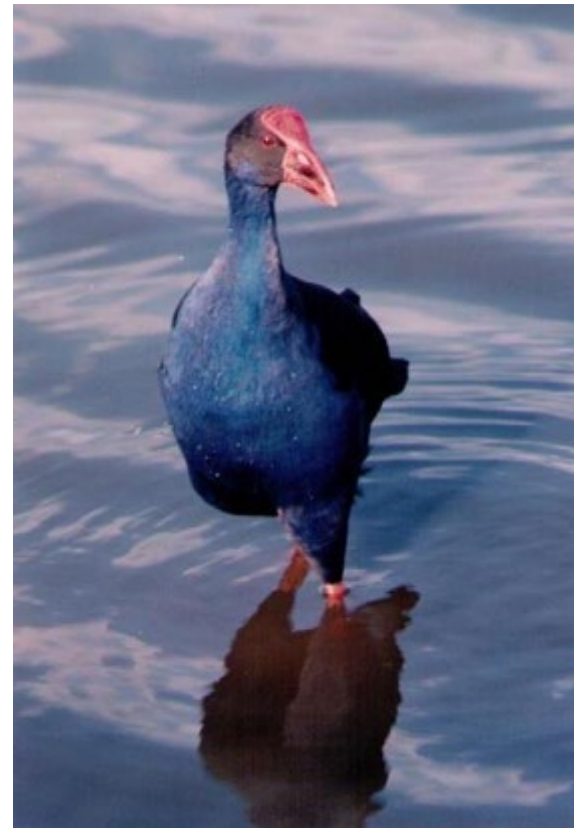
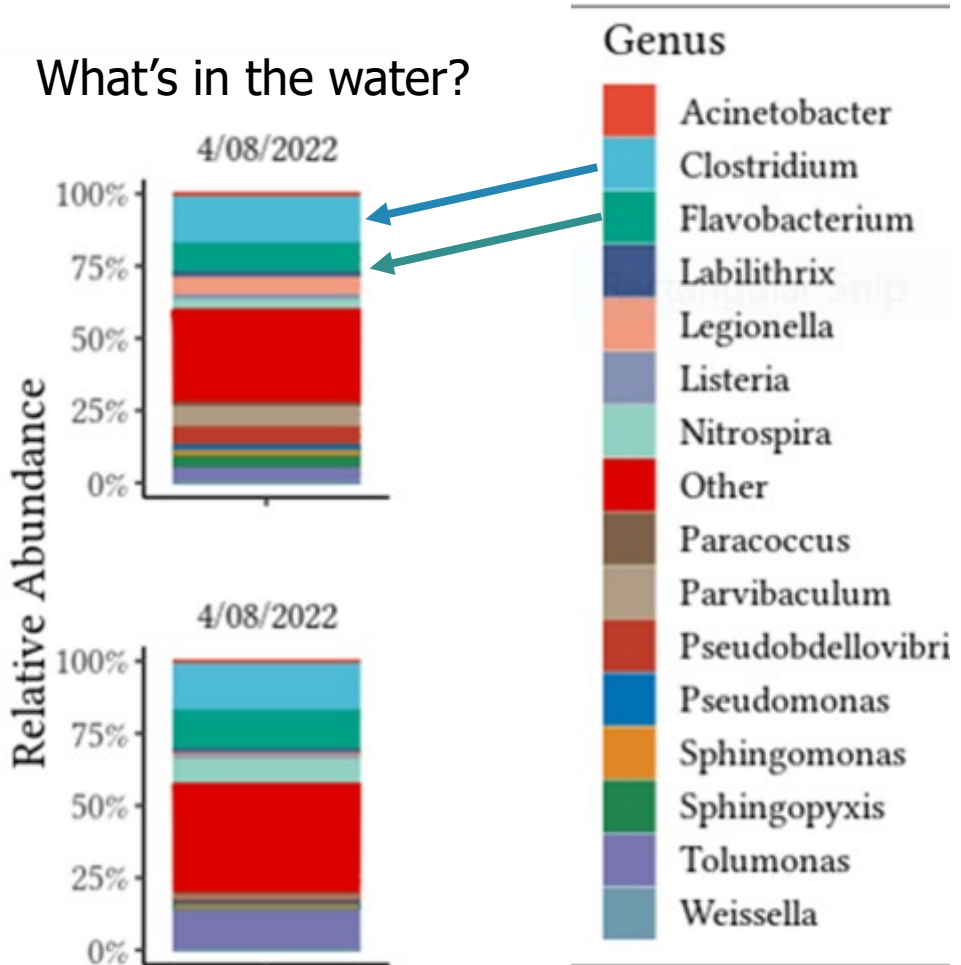


What's in the water?

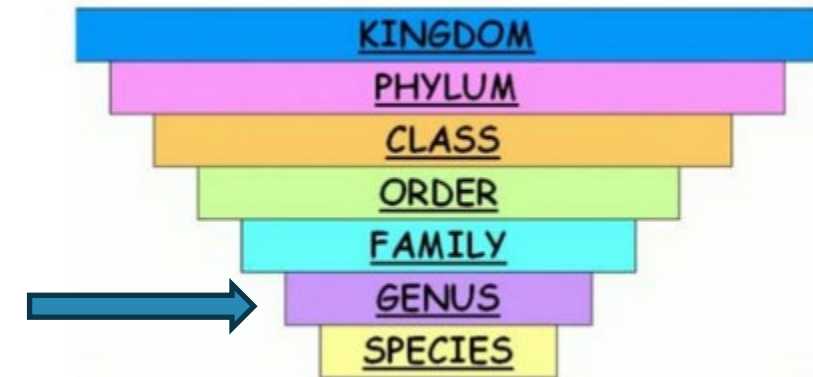


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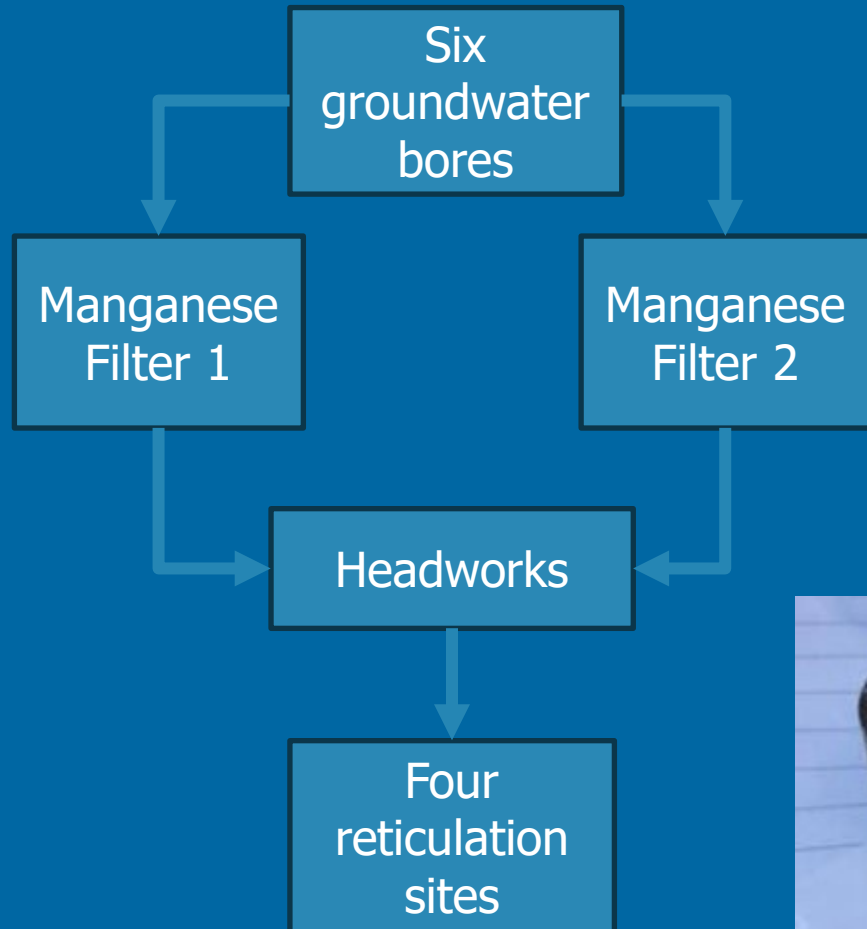
The sequencing process: target 16S rRNA gene to identify bacterial taxa in drinking water



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Case study 1: Bacterial community associated with manganese filters (20th and 28th September 2022)



Groundwater wells 140-250 metres
Manganese

MAV <0.4 mg/L

Aesthetic values <0.04 mg/L

-black/brown stains

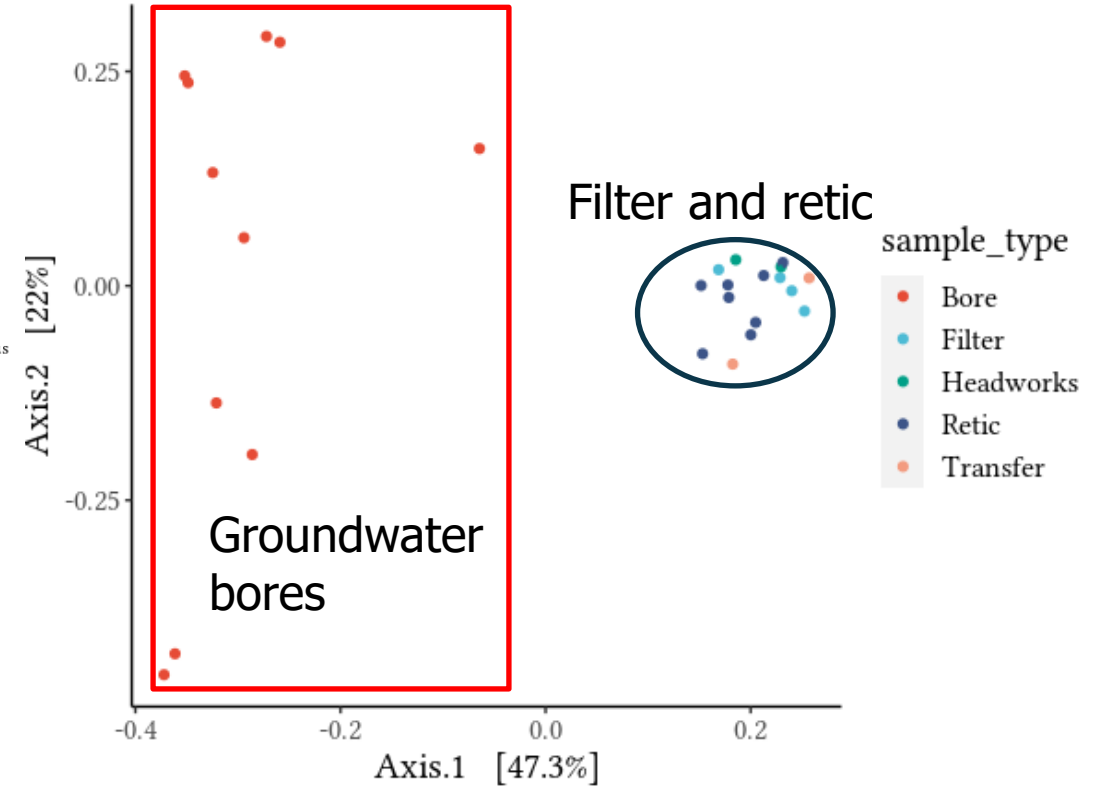
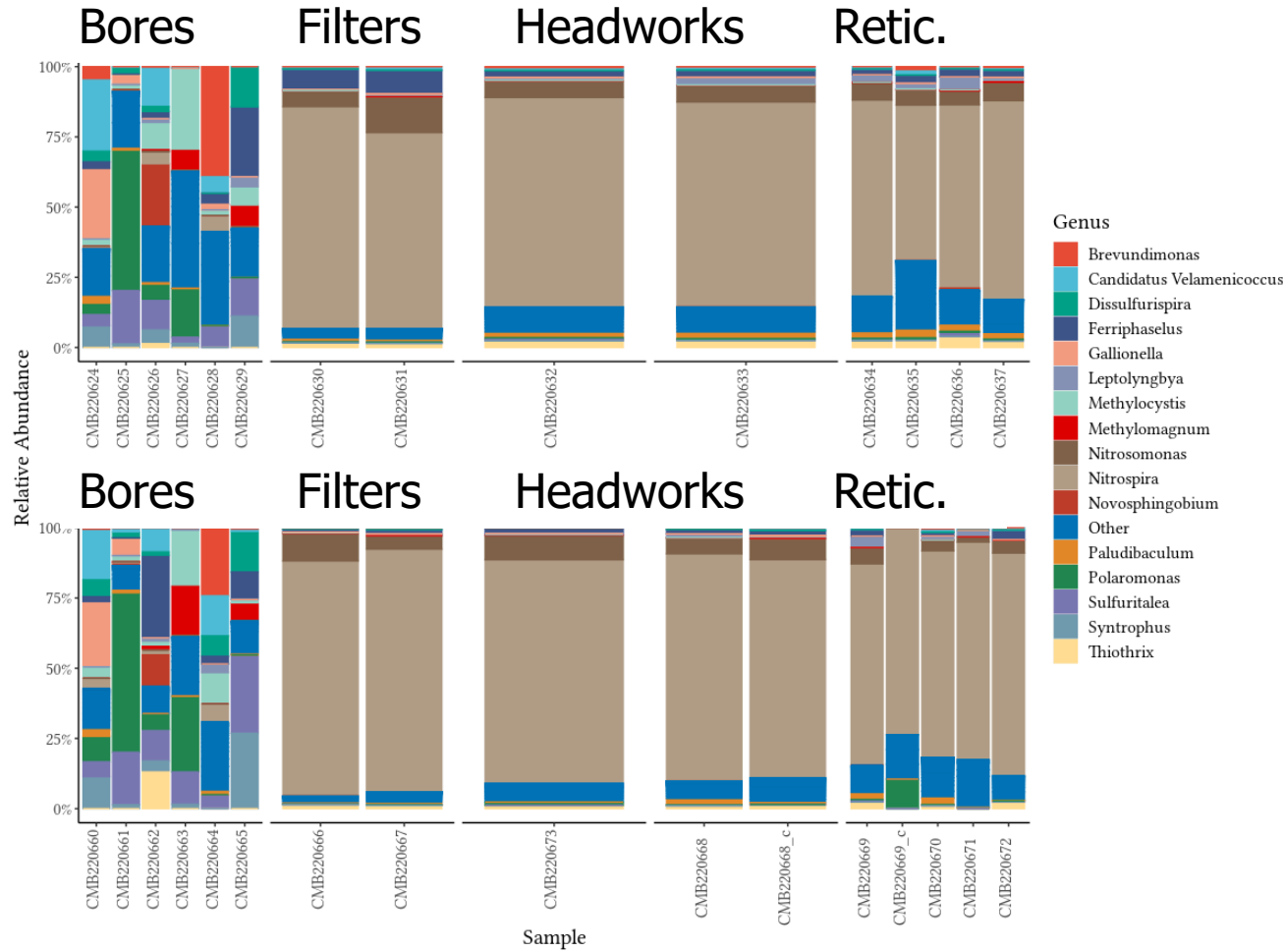
-metallic taste

Post-filters Mn <0.0005 mg/L



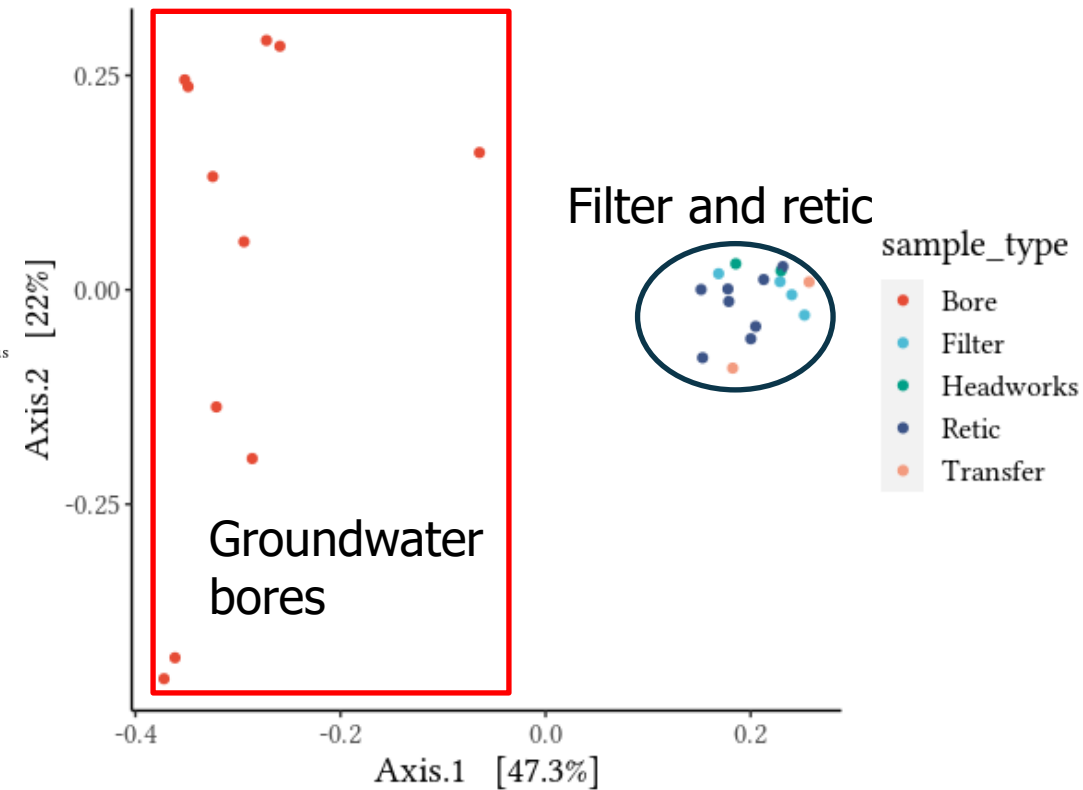
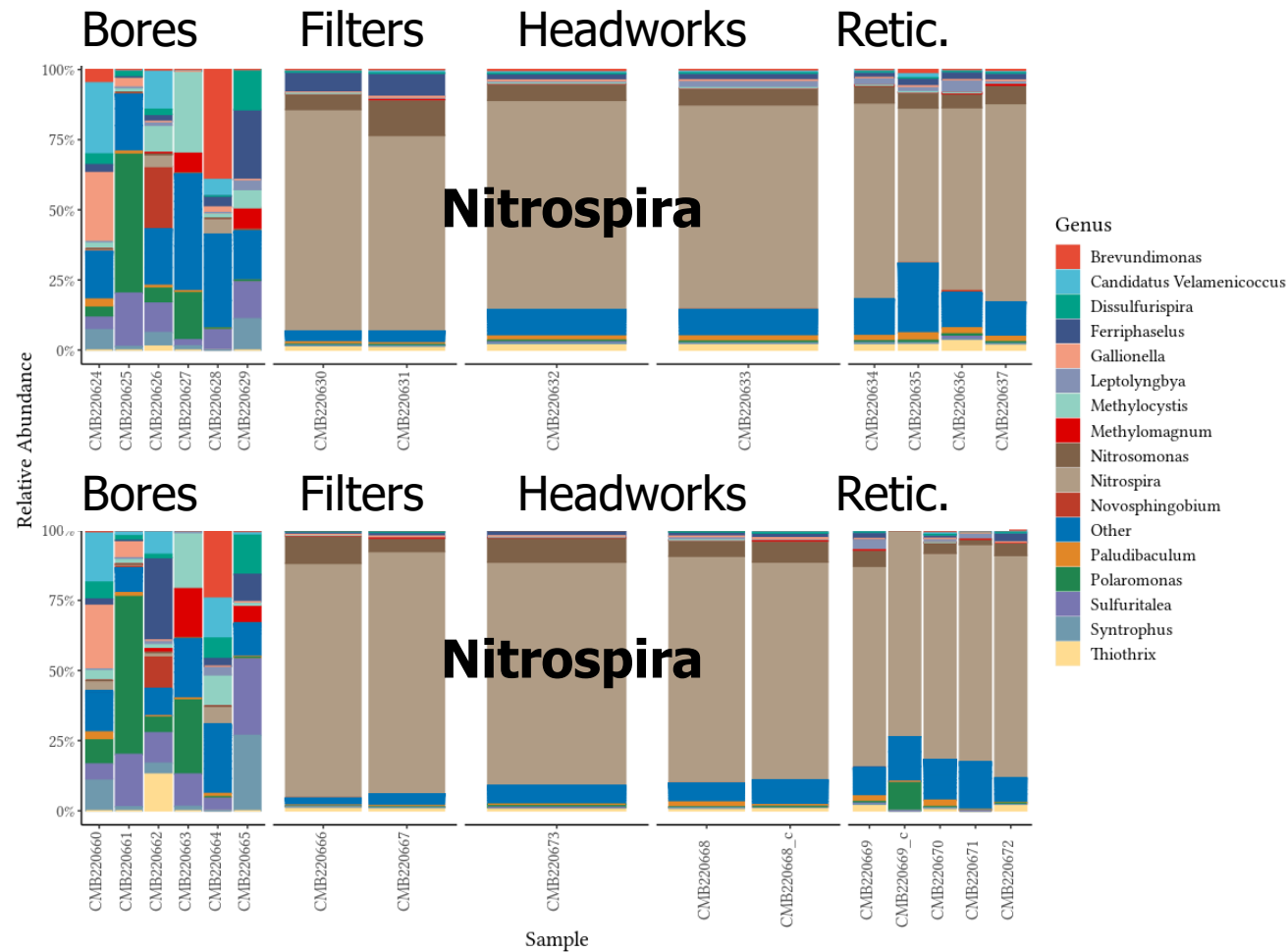
Black flecks of manganese in filters

Case study 1: Bacterial community associated with manganese filters (September 2022)



Drinking water Sample Diversity

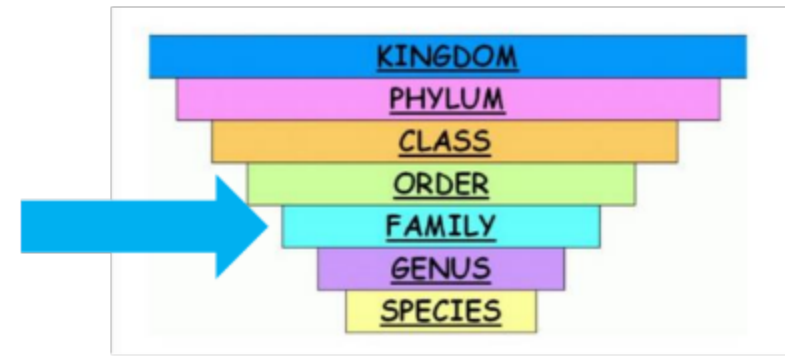
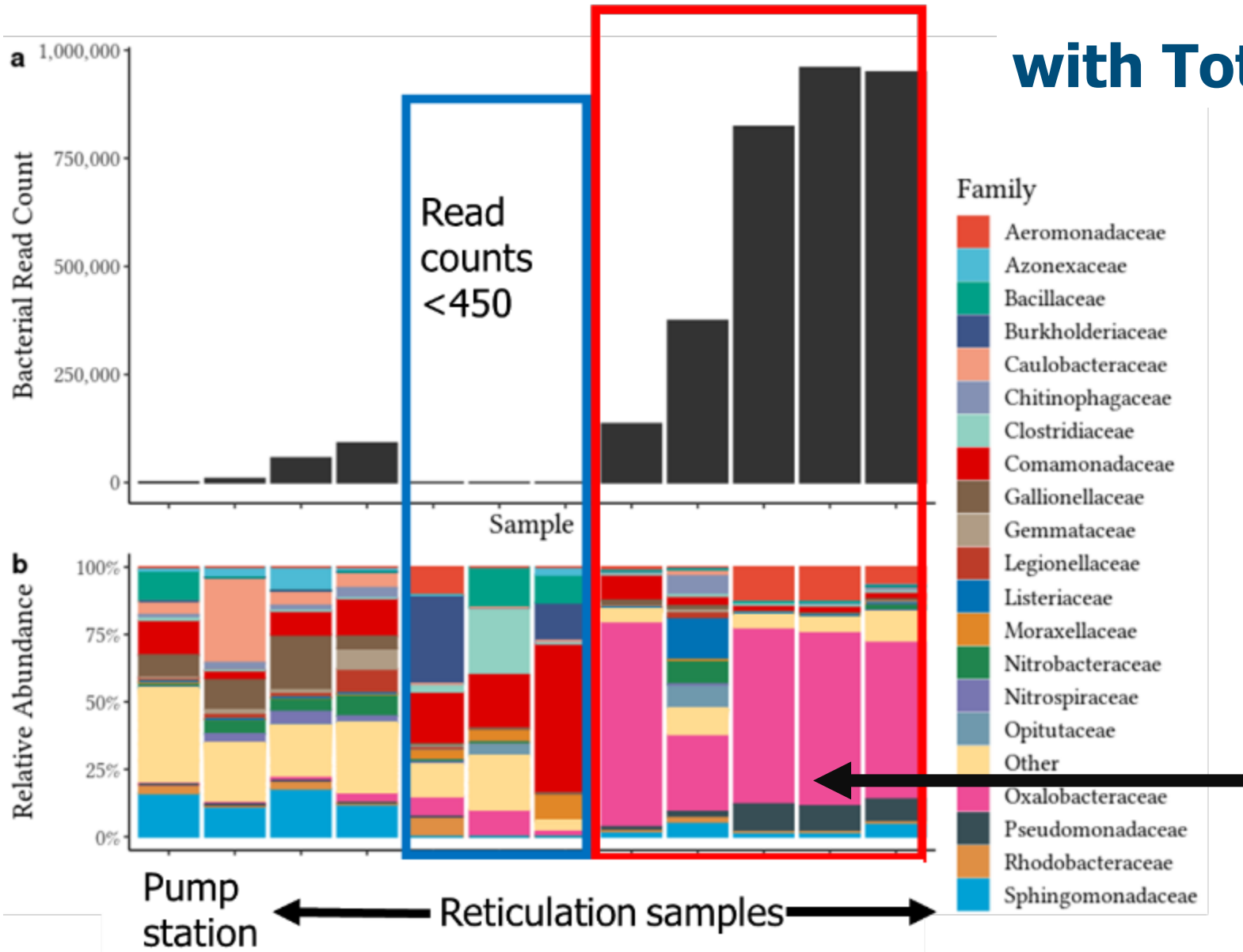
Case study 1: Bacterial community associated with manganese filters (September 2022)



Drinking water Sample Diversity

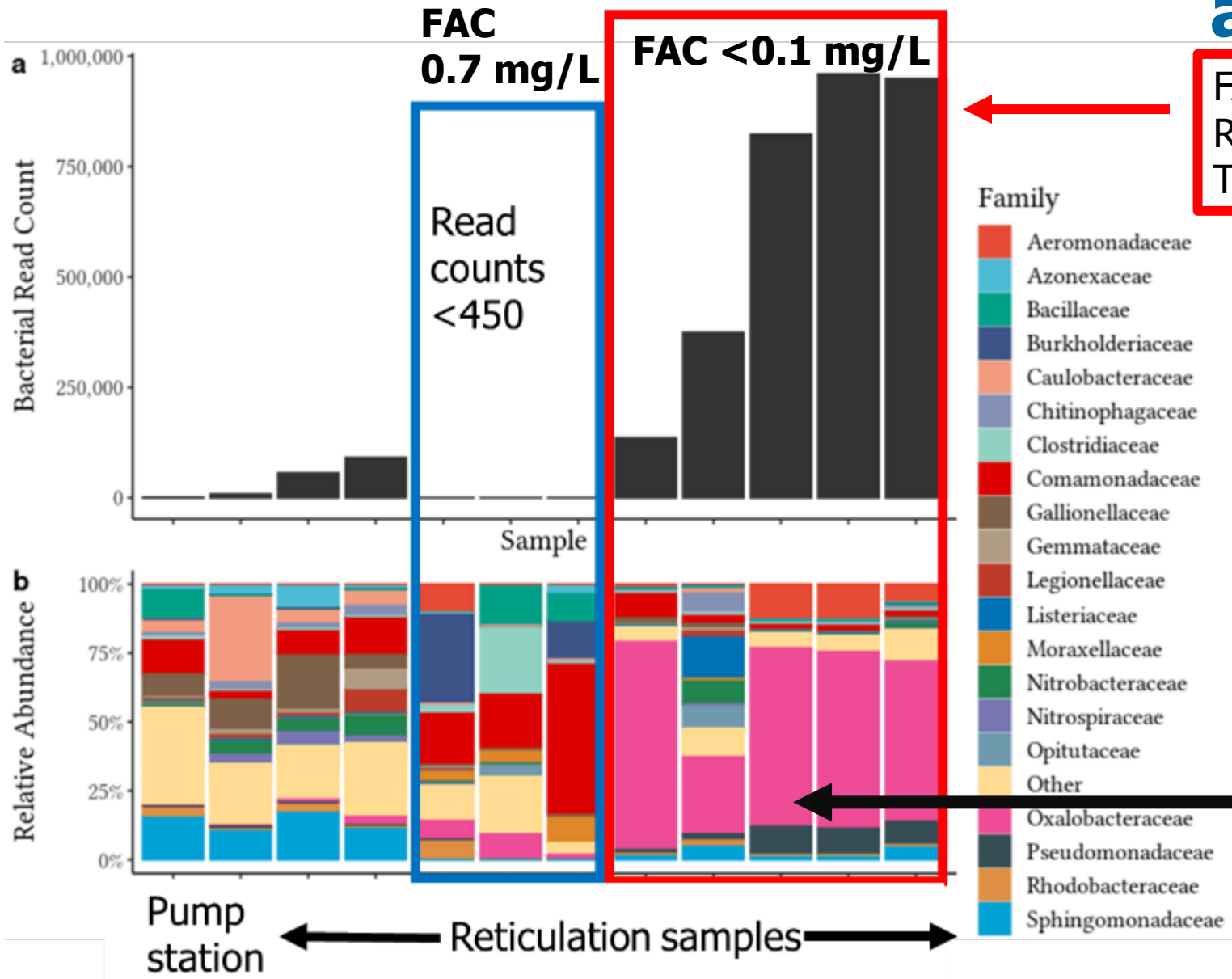
Case study 2: municipal water supply

with Total Coliform detections

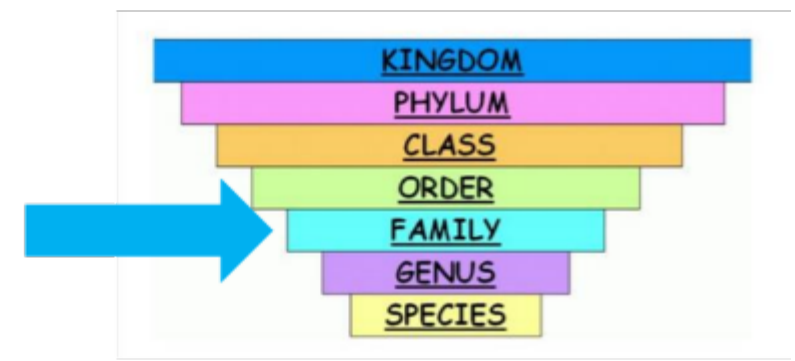


environmental bacteria:
Oxalobacteraceae

Case study 2: Supply with Total Coliforms & Free available chlorine

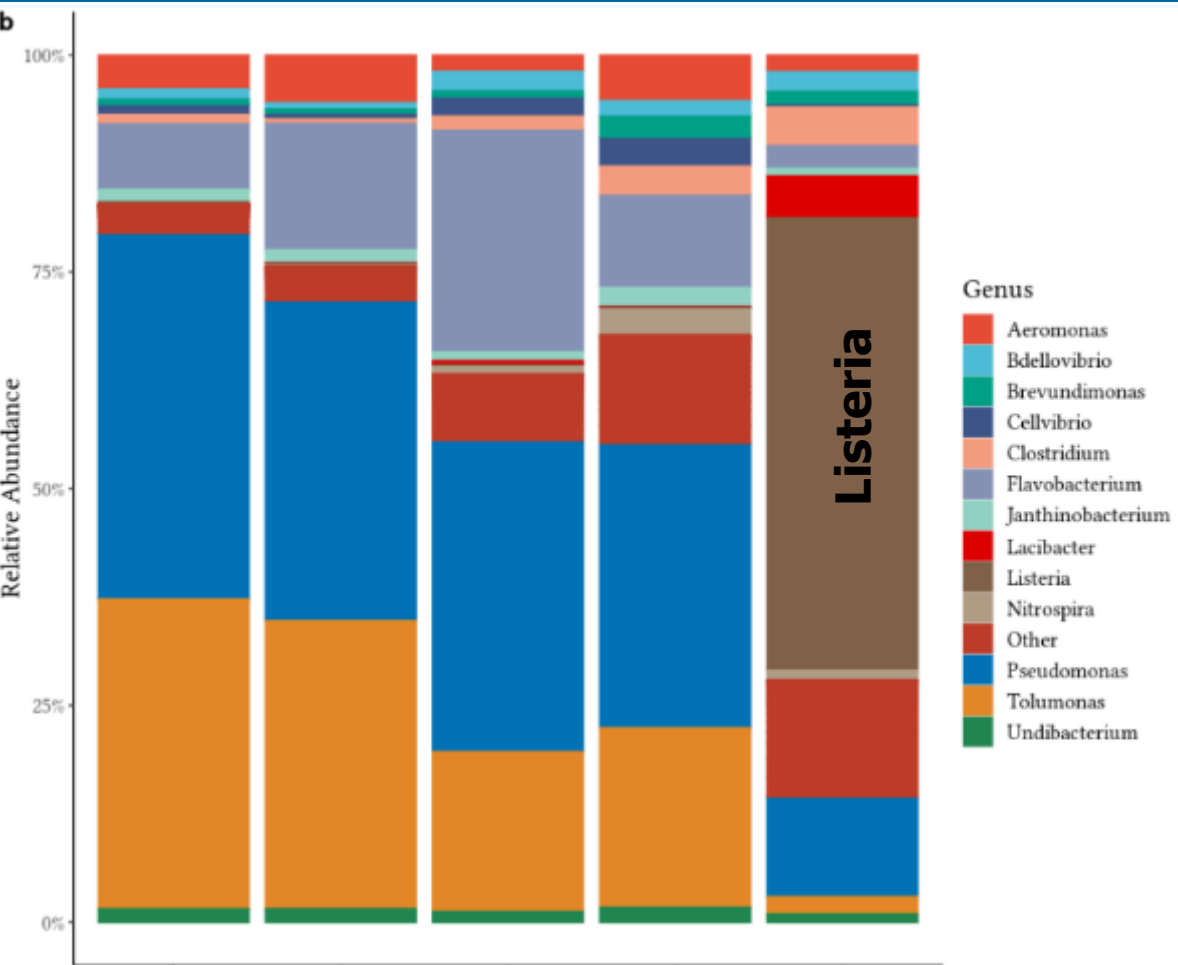


FAC < 0.1 mg/L
 Read counts = ≤ one million
 Total coliforms 8 - 250 TC/100 mL)

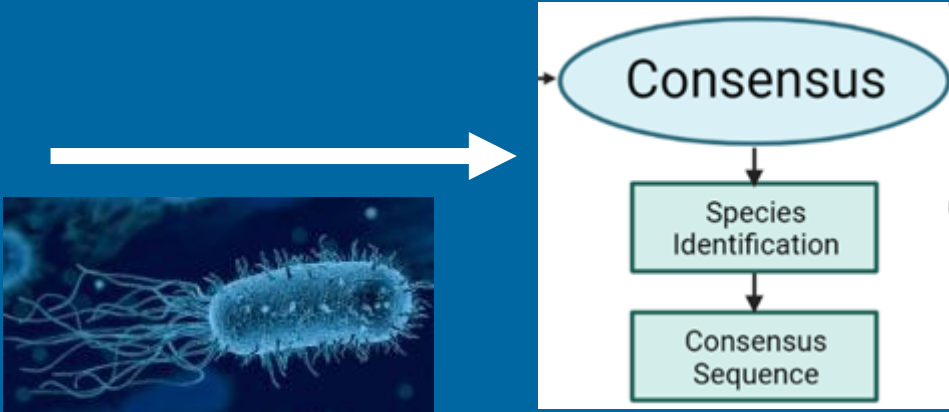
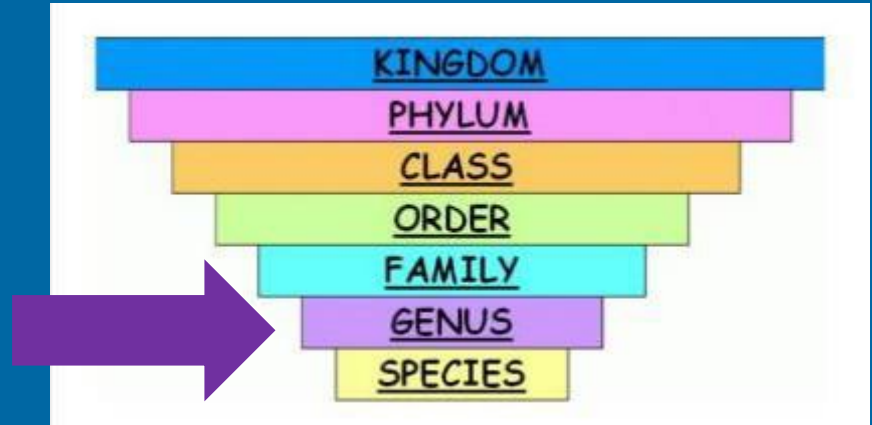


environmental bacteria:
Oxalobacteraceae

Case study 2: Identification of Listeria -is it a pathogen?

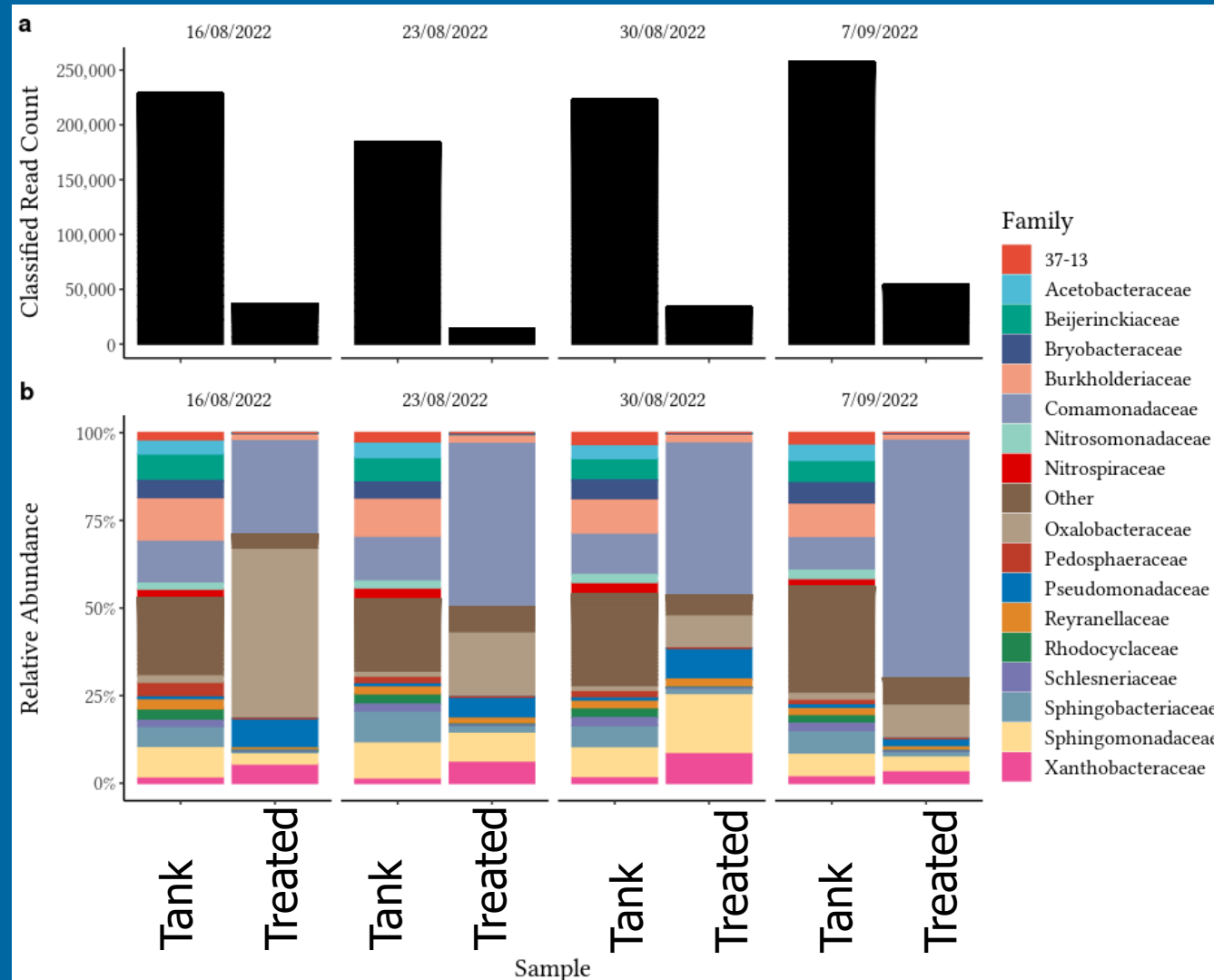


Reticulation water sites



Listeria innocua

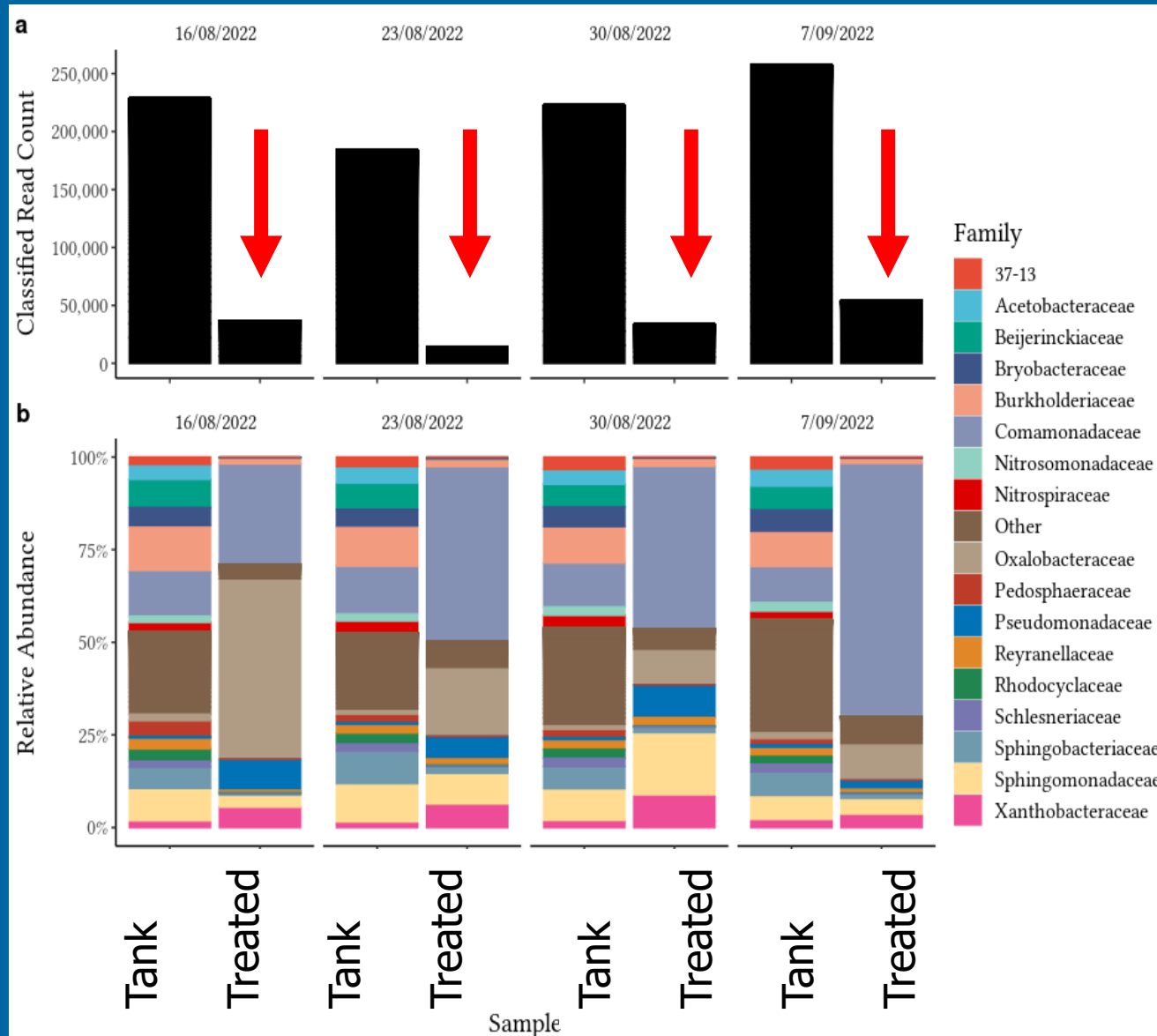
Case study 3: Are we detecting DNA from dead bacteria?



Comparing bacterial counts in rainwater tank and post UV and cartridge filtration

Treated water:
environmental bacteria
Comamonadaceae
Oxalobacteraceae

Viability issues for DNA detection

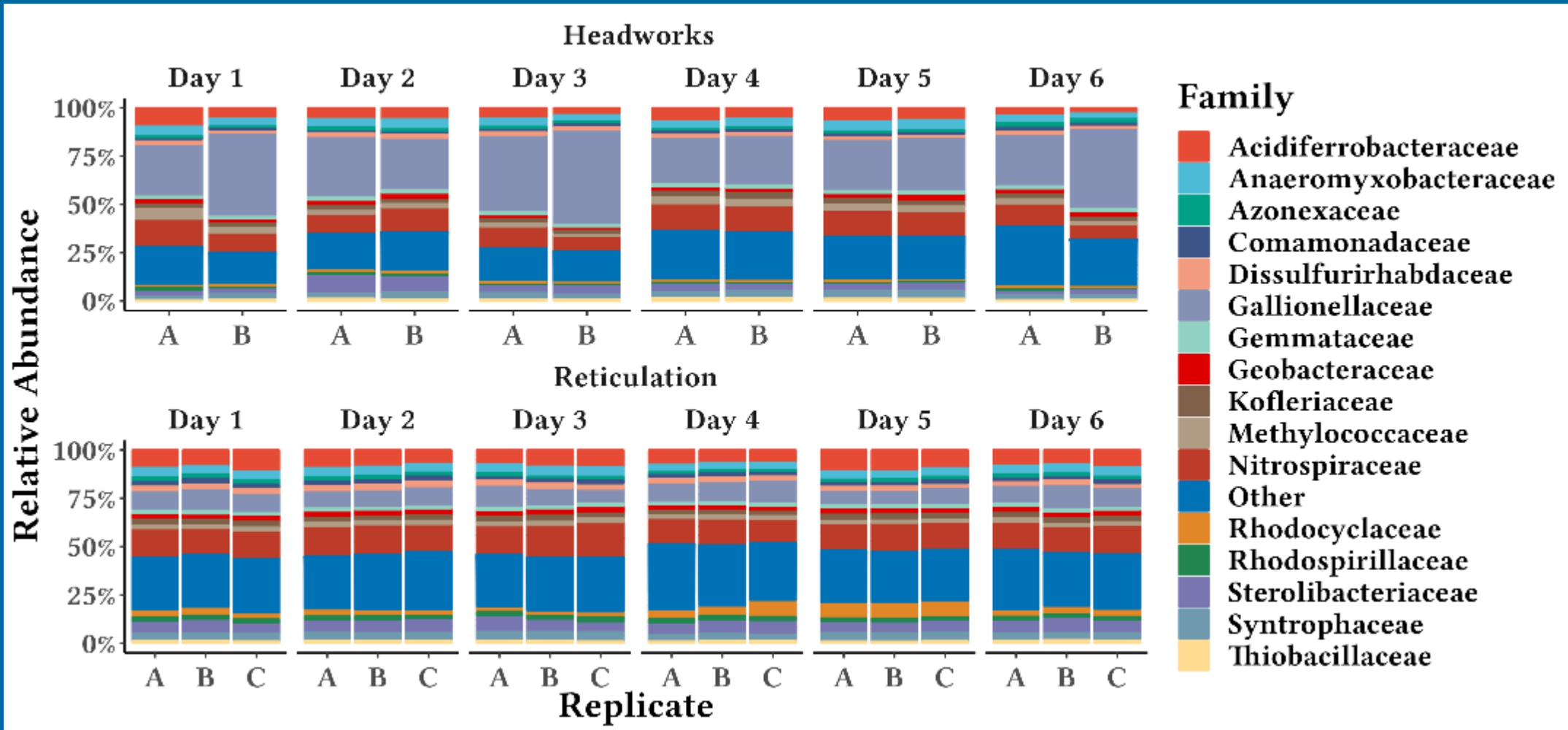


Comparing bacterial counts in rainwater tank and post UV and cartridge filtration

Treated water:
environmental bacteria
Comamonadaceae
Oxalobacteraceae

Markedly lower read counts in treated drinking water

Replicate experiment: Temporal stability of bacterial community within the distribution system



Conclusion

- ❖ **Stable biological population**
- ❖ **Changes in bacterial composition in the water supply**
- ❖ **Monitor treatment processes**
chlorination & UV irradiation/filter cartridge
- ❖ **Detection and confirmation of pathogens**

Future Directions

Drinking water research in Aotearoa NZ

How can we help water supply managers to improve monitoring tools for microbiological water quality?

- **Source water characterisation**
- **Effectiveness of treatment processes**
- **Evaluate the entire microbial population in drinking water:
Total coliforms and *E. coli* as indicators of faecal pathogens**

Acknowledgements

- Taumata Arowai
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Drinking water research in Aotearoa NZ

How can we help water supply managers to improve monitoring tools for microbiological water quality?

More ideas from the water sector

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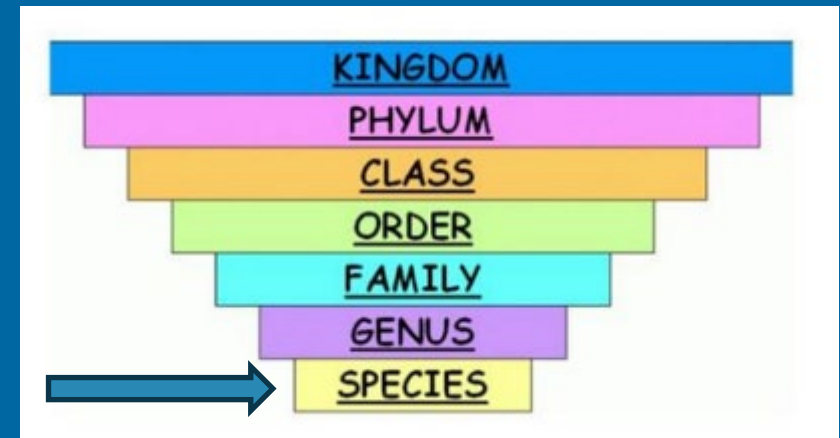


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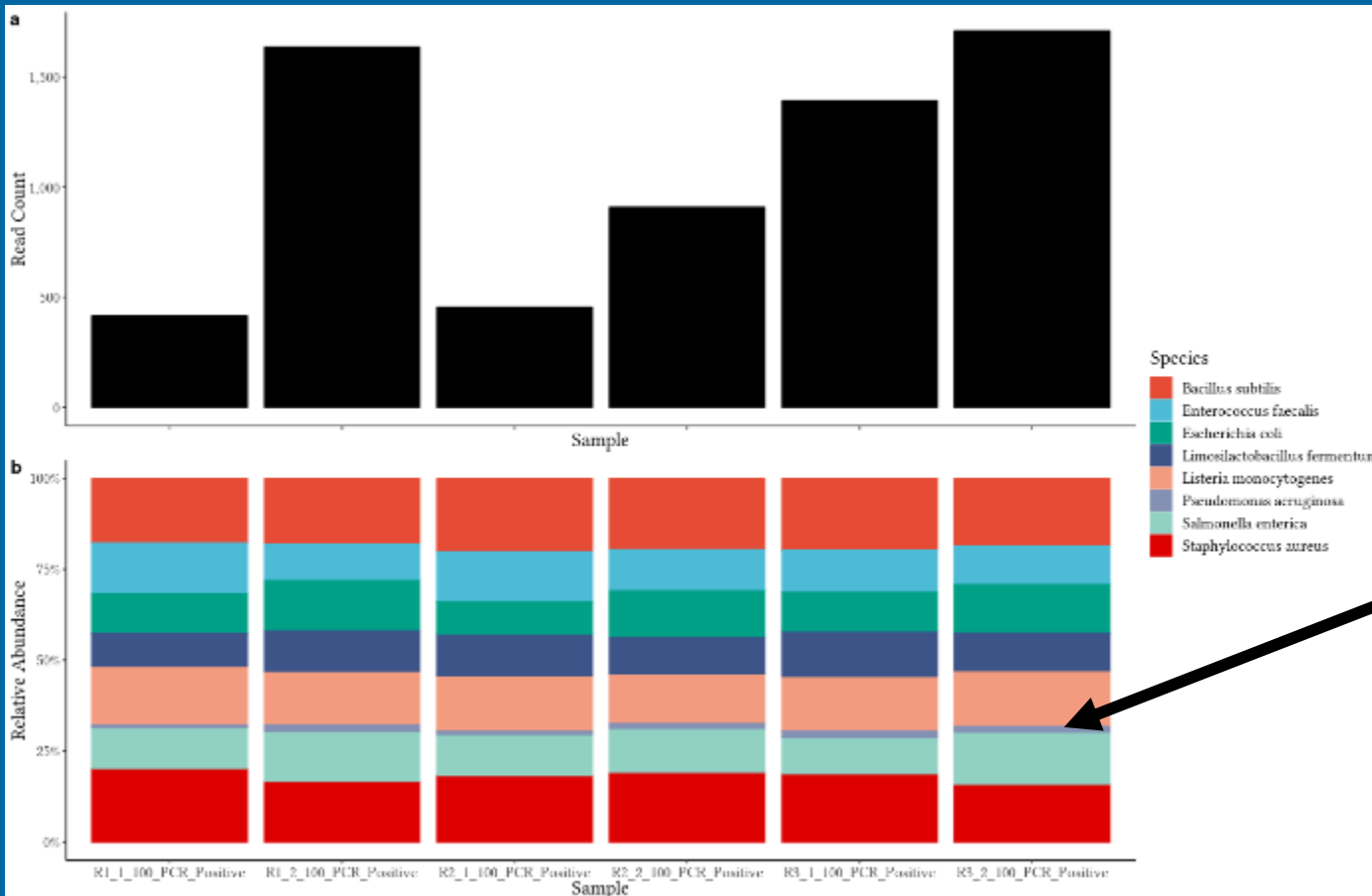
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Quality Control of sequencing data for 16S rRNA Bacterial taxonomy



Each sequencing run contains positive and negative controls Including sample processing controls

Positive controls (mock community) in different sequencing runs to show reliability between sequencing runs



Case study 1: Temporal sampling over months reveals stable communities in bores and Manganese filters

Changes in bacterial community in April 2023

