

# **The microbial world within water reuse**

Carleton Biology seminar, October 8, 2018  
Rose Kantor, Ph.D.



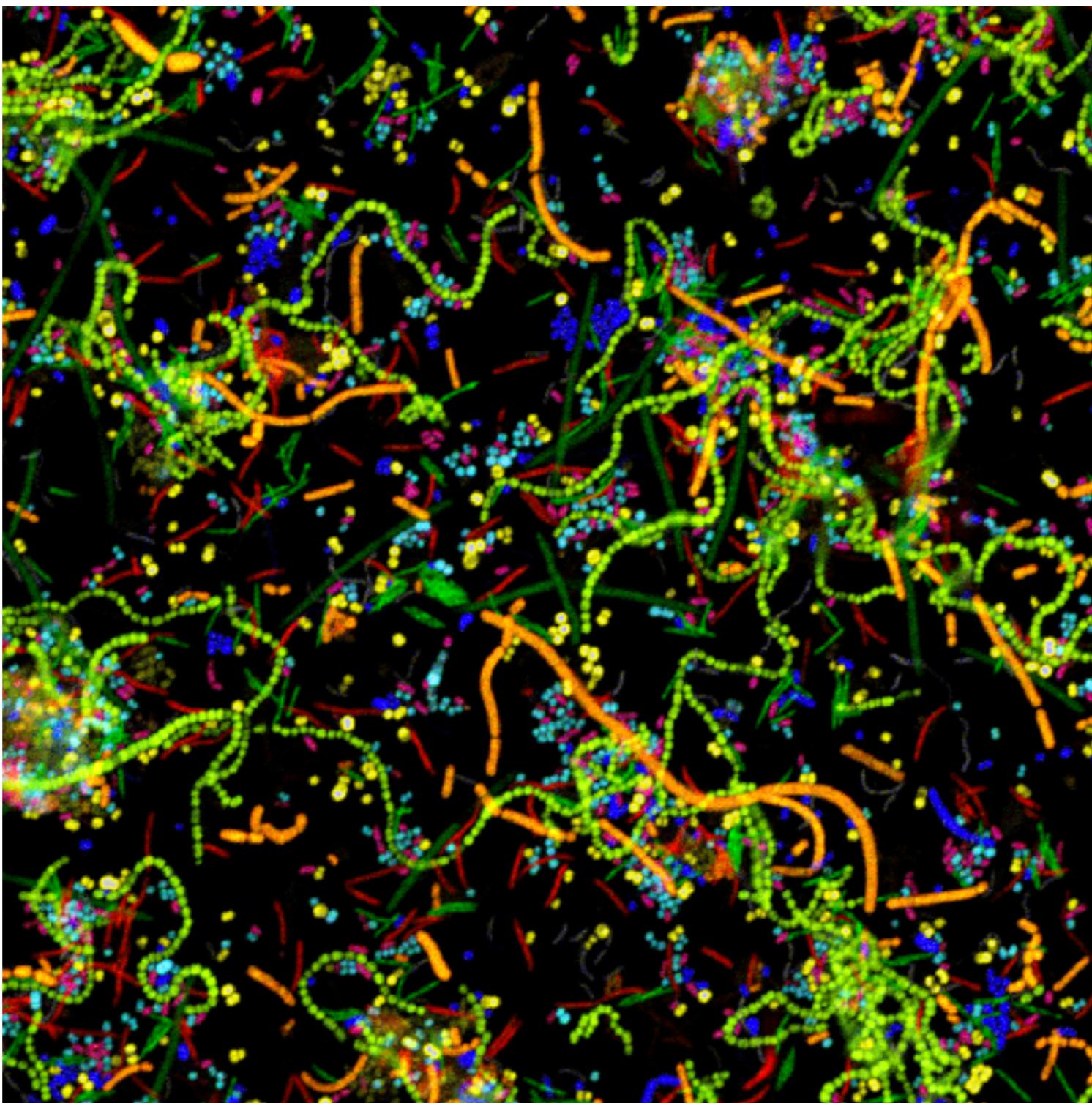
# About me



# Intersection of biology and engineering



# The microbial world



# The microbial world



John Rostron (Wikipedia)

<http://www.global-membrane.com/biofouling/membrane-biofouling/>  
<http://maritime-executive.com/article/intertanko-releases-biofouling-and-coating-guide>  
<http://wqinvestigations.com/water-quality-case-studies/>

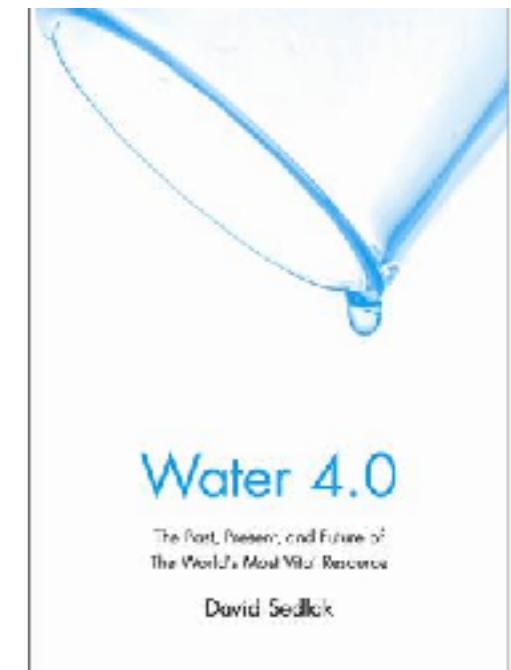
# Drinking water



# Drinking water



# Drinking water



**Water 4.0**

The Past, Present, and Future of  
The World's Most Vital Resource

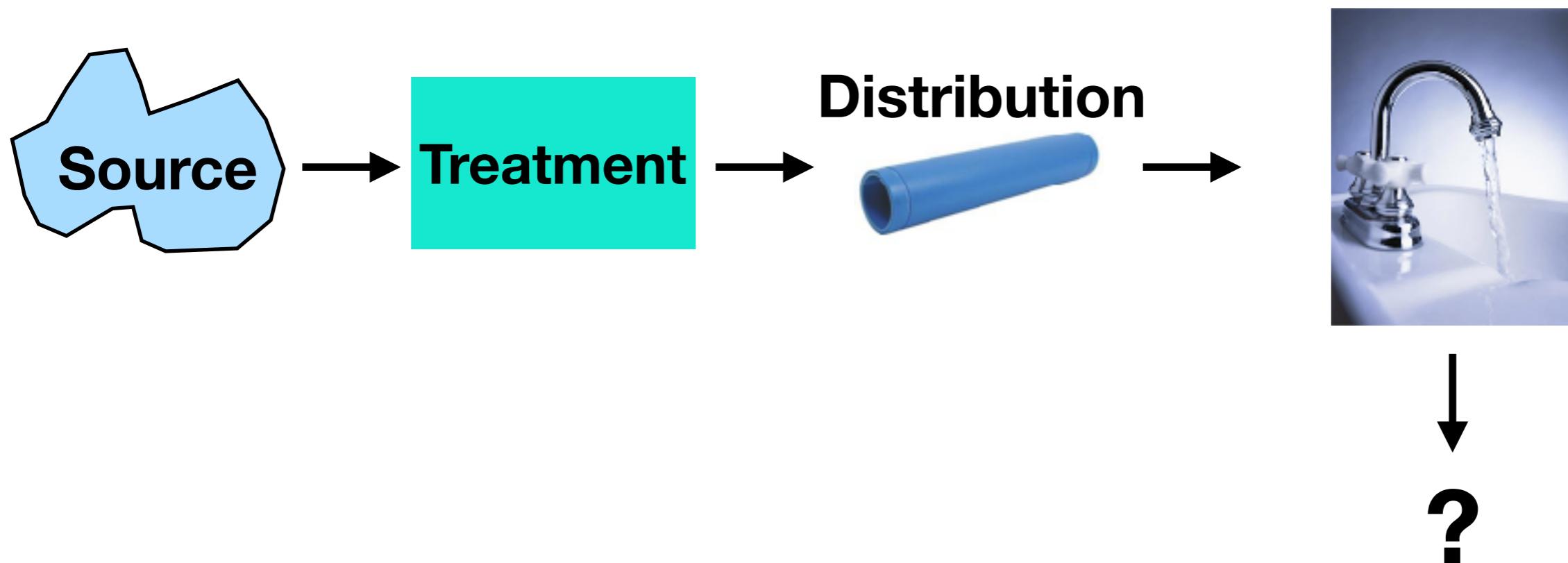
David Sedlak

**Water 4.0**

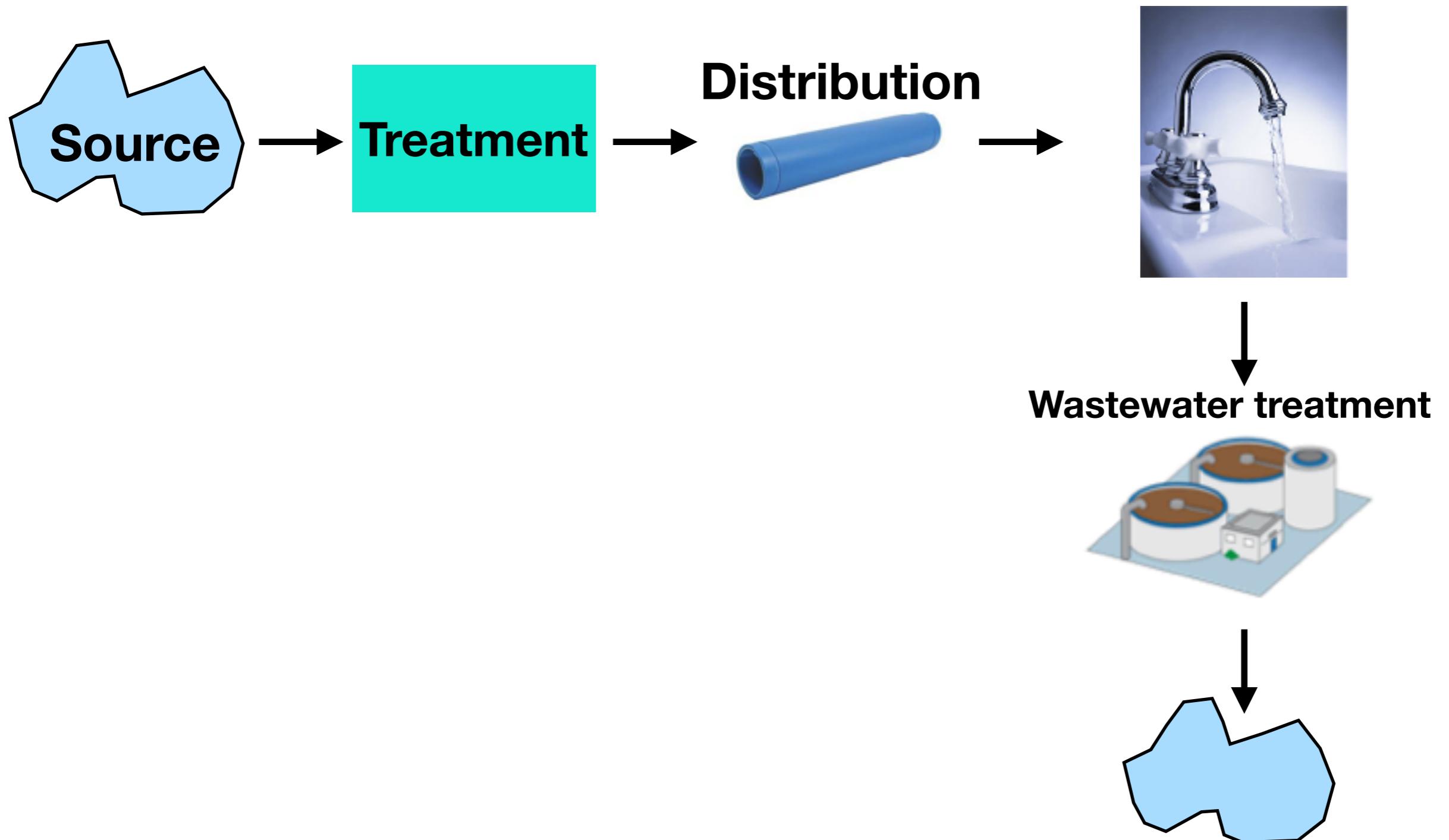
by

**Prof. David Sedlak**

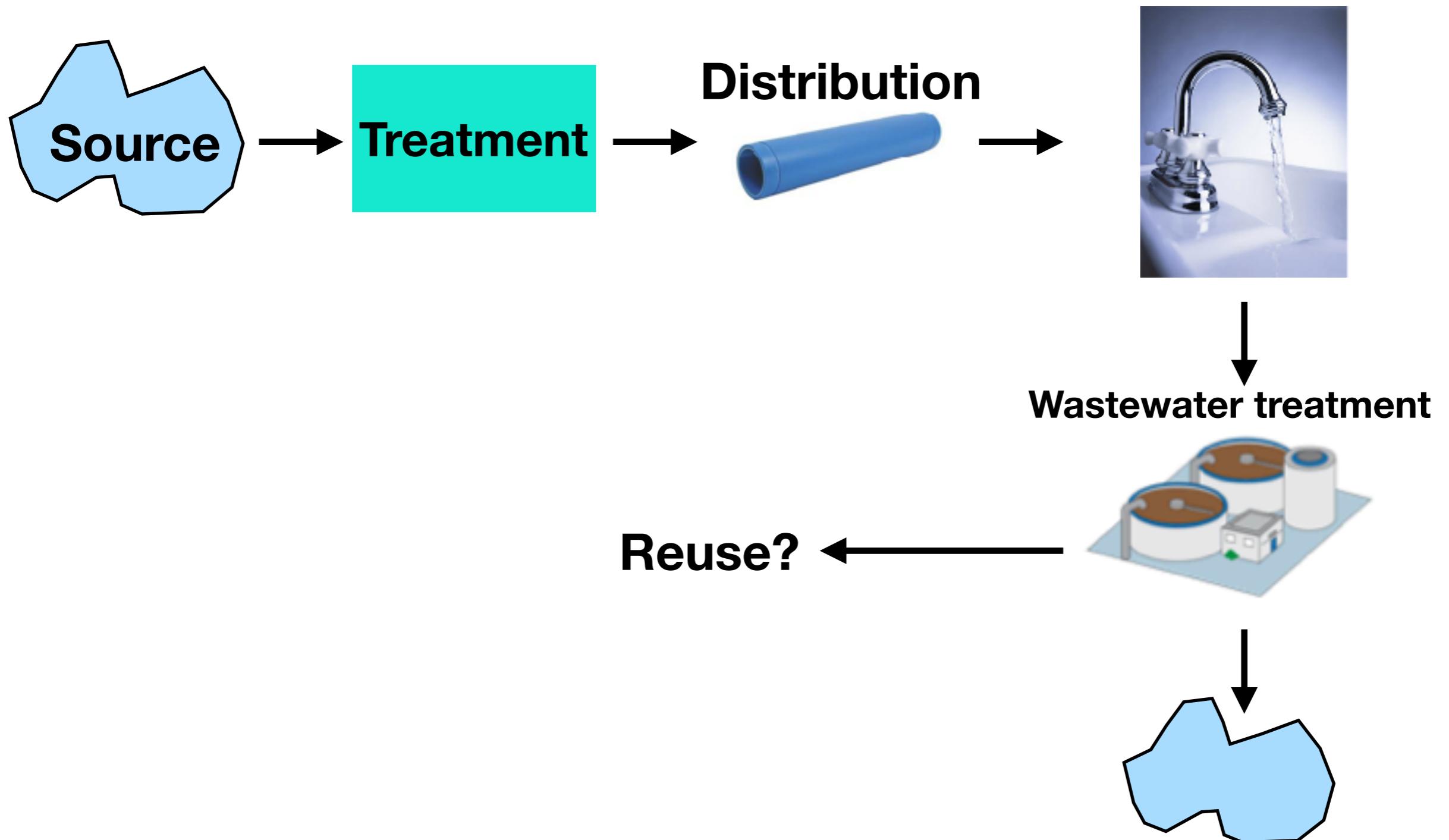
# Drinking water cycle



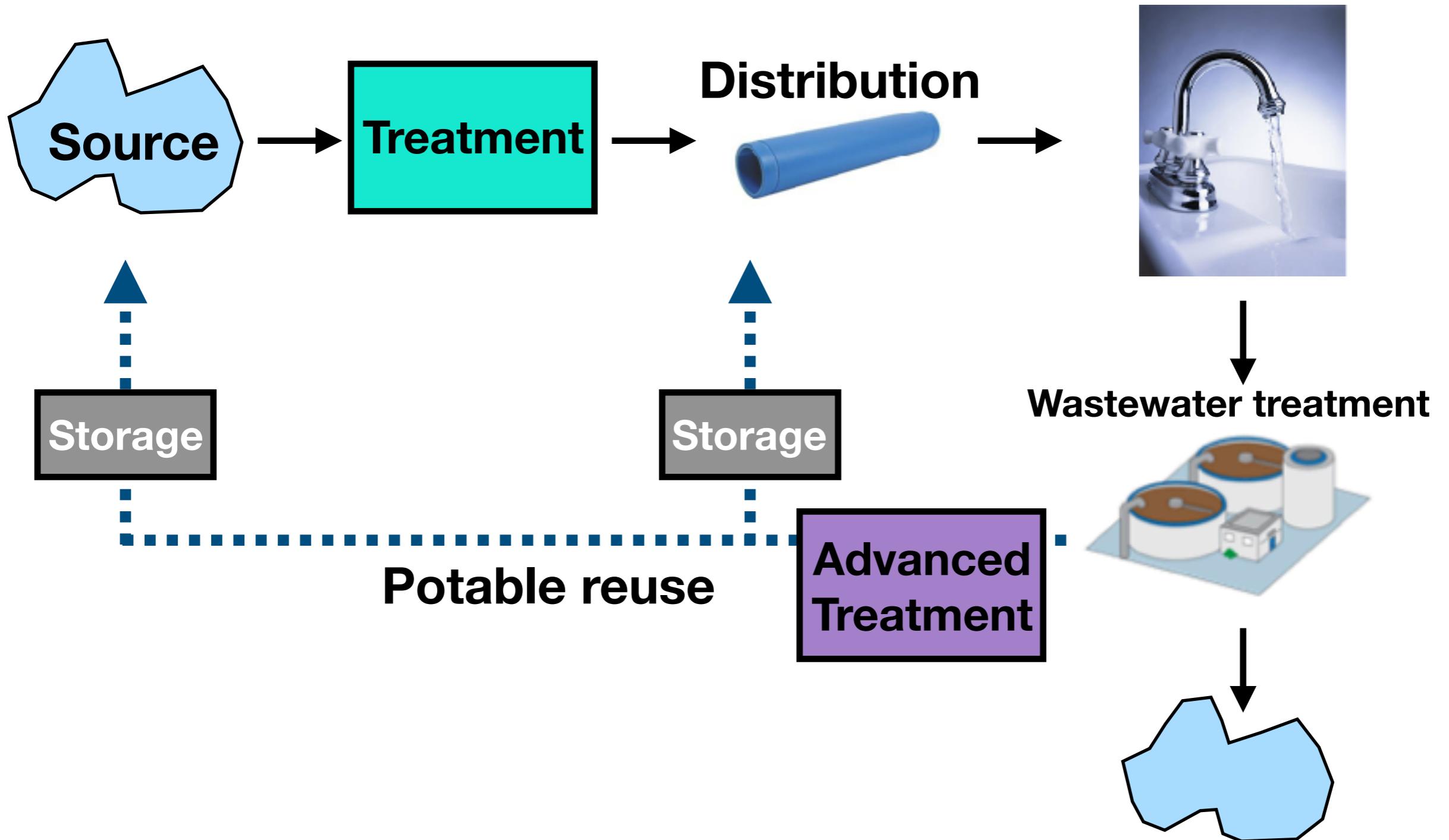
# Drinking water cycle



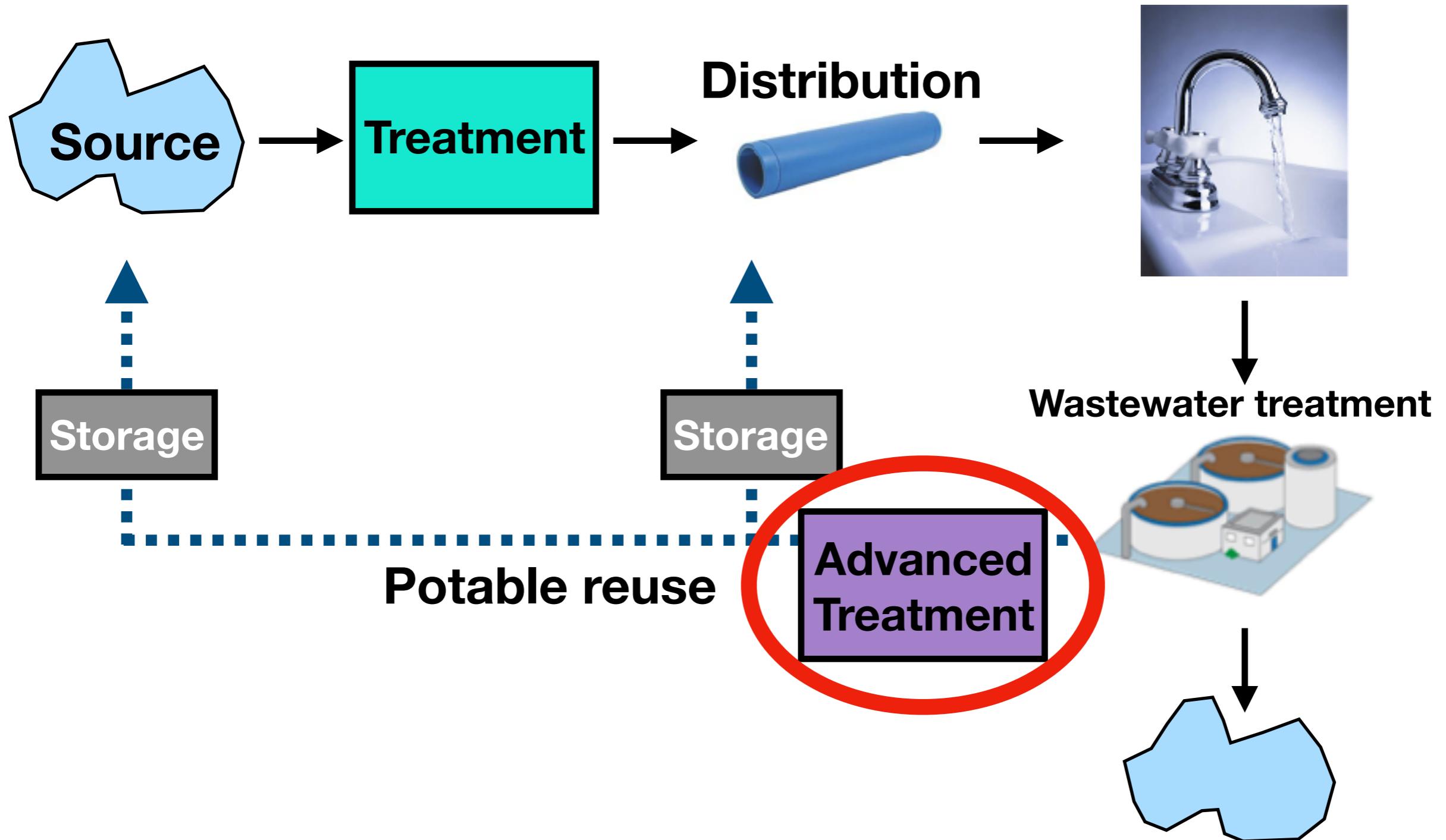
# Drinking water cycle



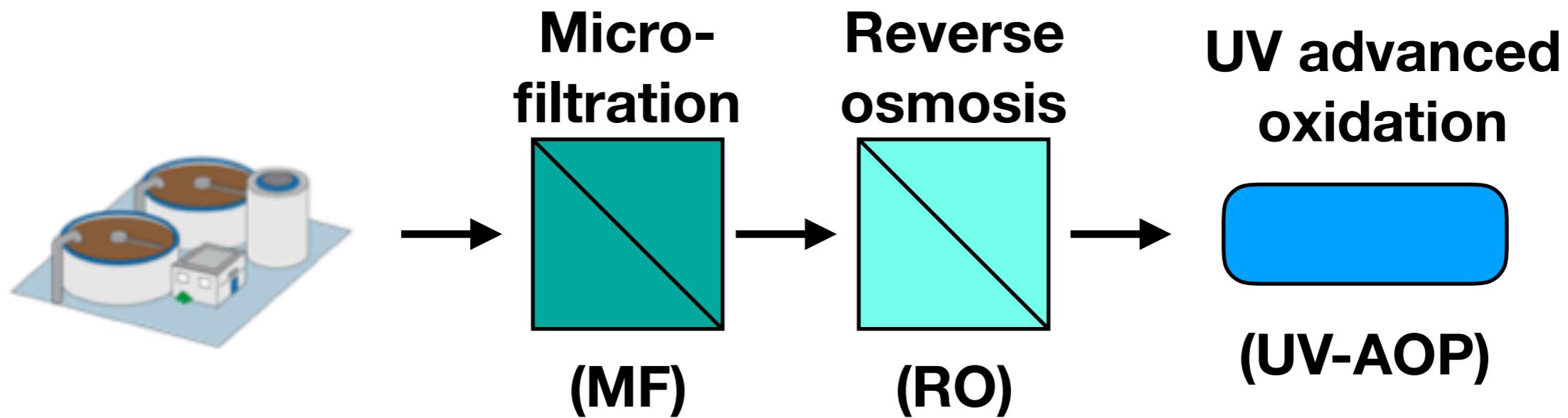
# Drinking water cycle



# Drinking water cycle



# Advanced Treatment



# Research questions

- 1. How does the microbial community change through treatment and simulated distribution?**
- 2. Before and after treatment: Who survives? Are there pathogens? Are there antibiotic resistance genes? How might bacteria survive?**

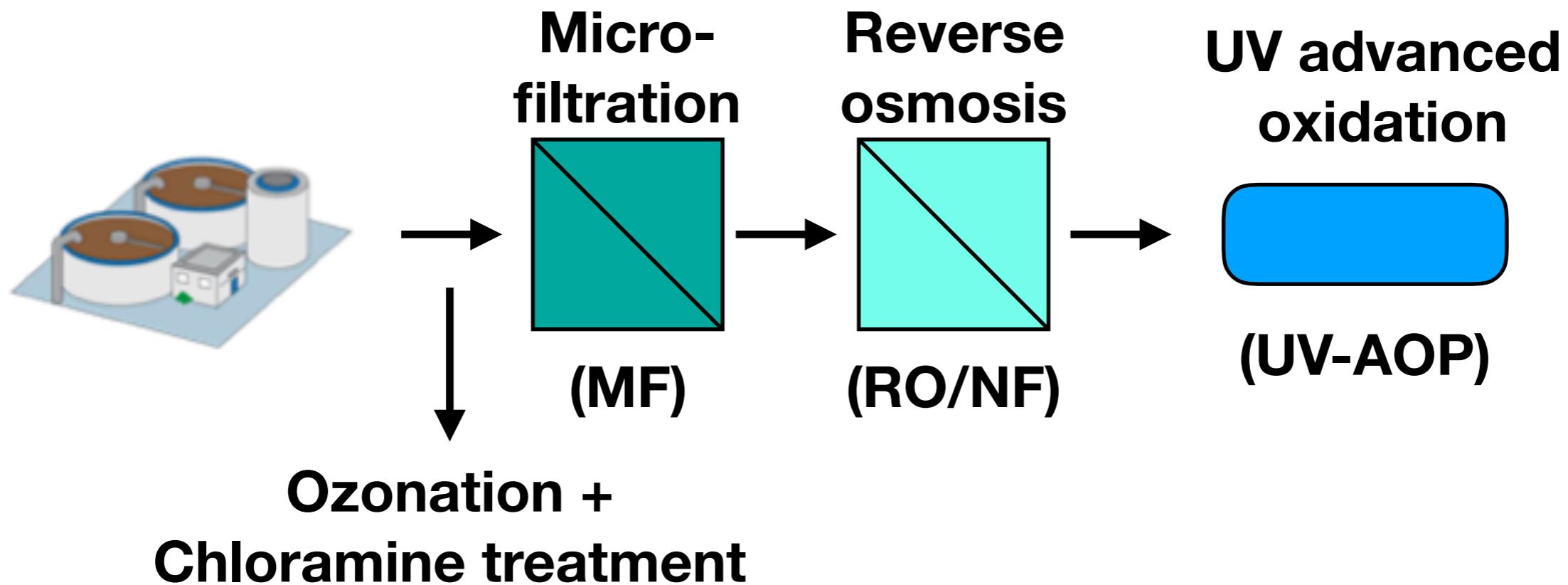
# Research site: El Paso, Texas Pilot-scale Advanced Treatment Facility



Photo from Scott Miller

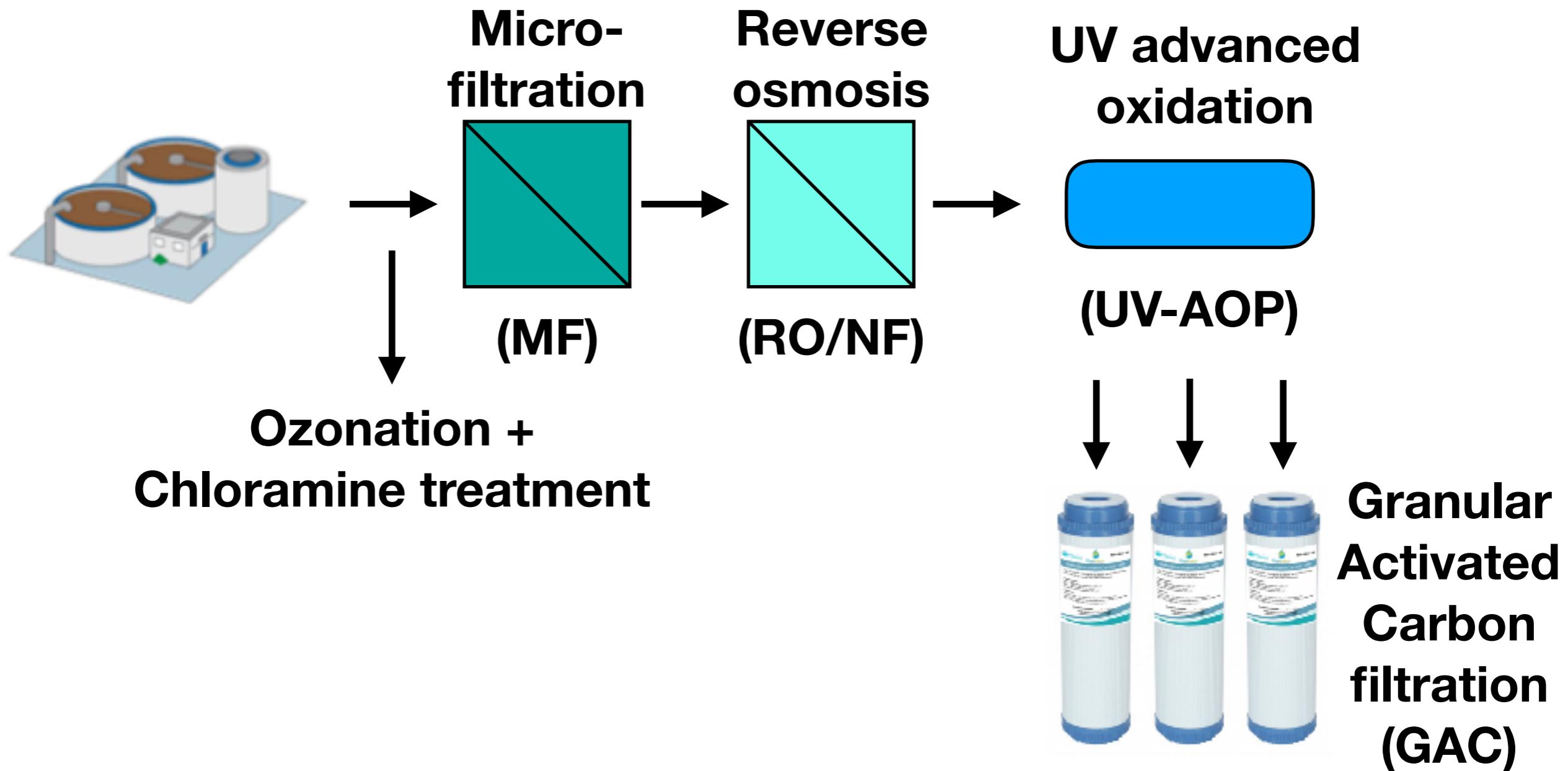
# Research site: El Paso, Texas

## Pilot-scale Advanced Treatment Facility



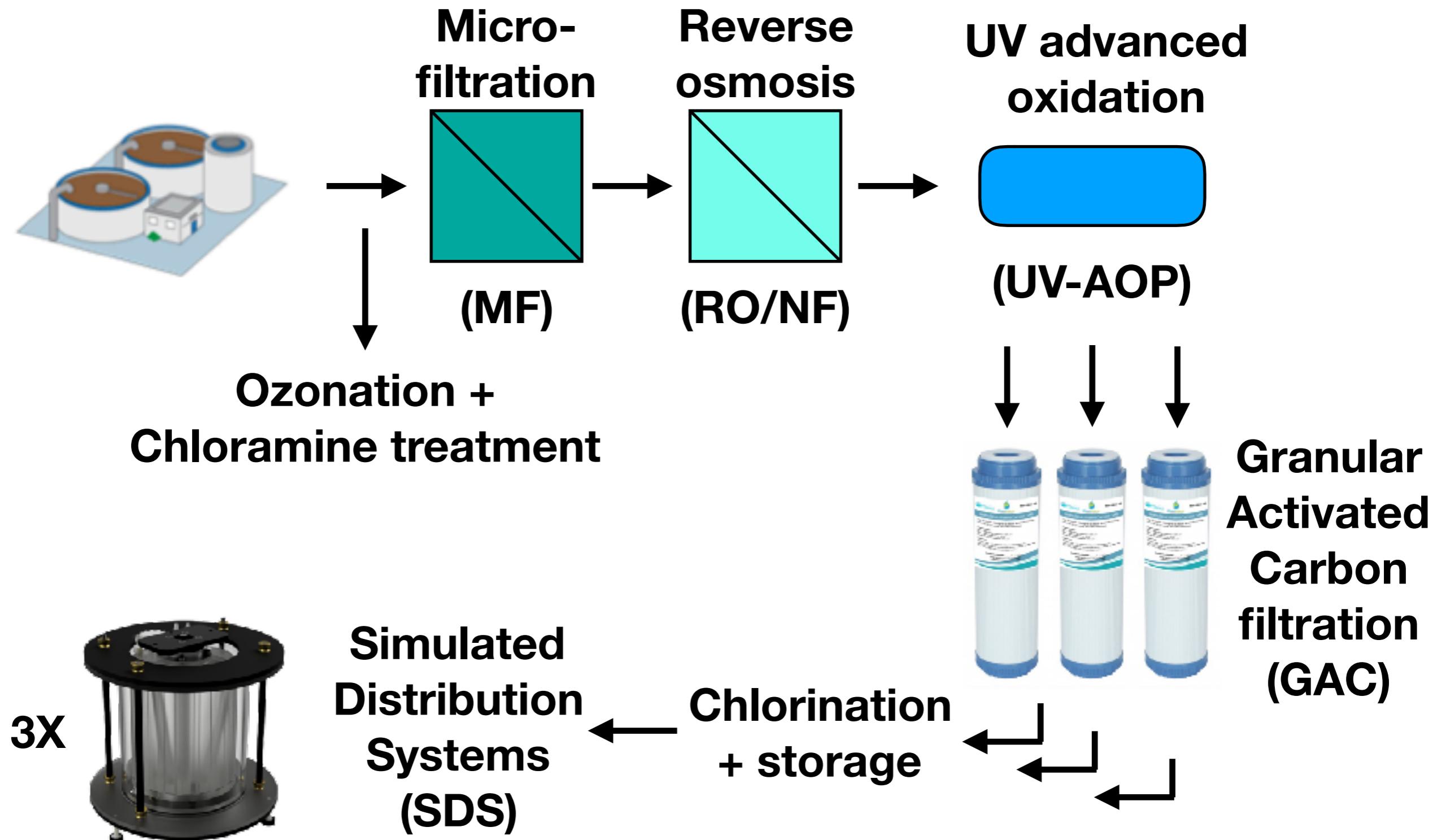
# Research site: El Paso, Texas

## Pilot-scale Advanced Treatment Facility

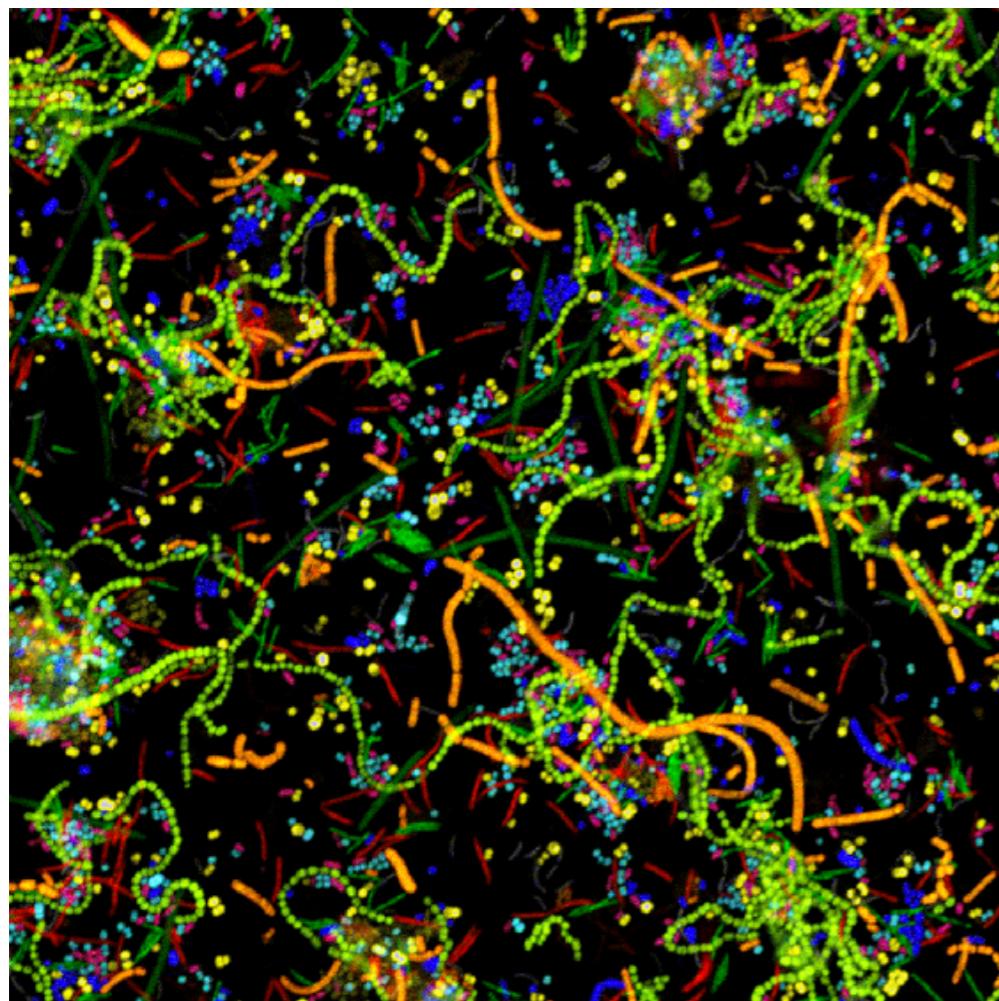


# Research site: El Paso, Texas

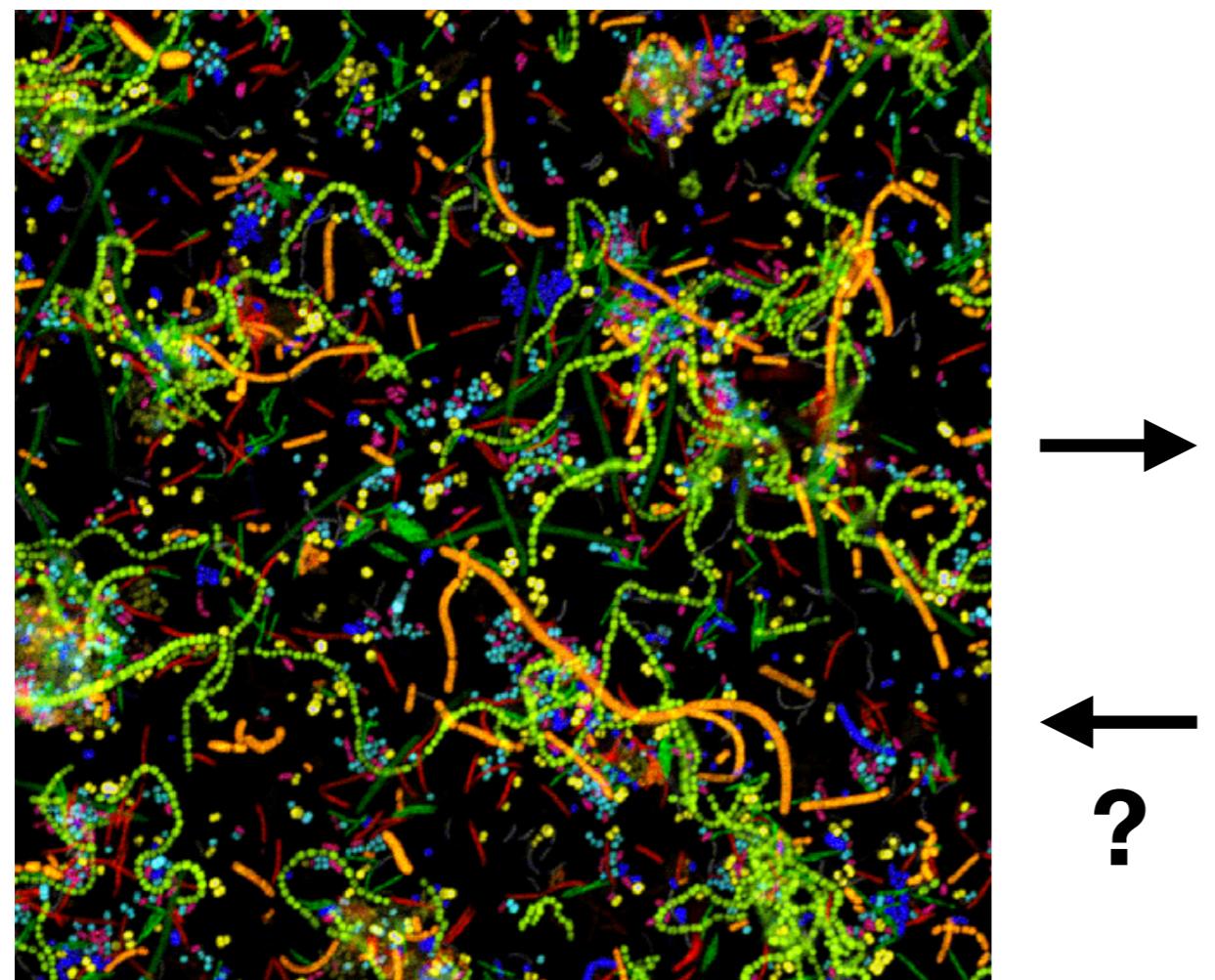
## Pilot-scale Advanced Treatment Facility



# Methods: Microbial communities via High-throughput DNA sequencing



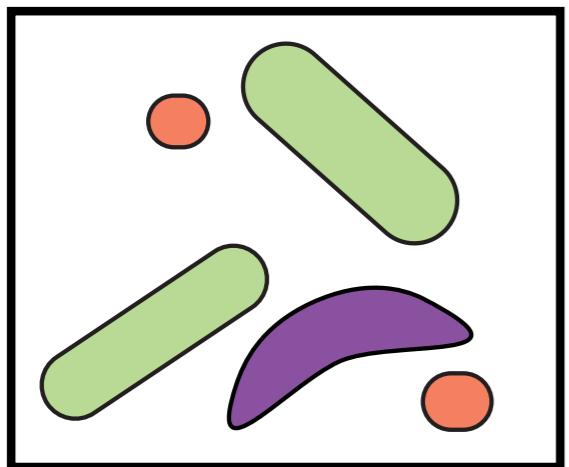
# Methods: Microbial communities via High-throughput DNA sequencing



TCATGATGTAGATGCTGCCGCCTCTTACCATGCTTGTAAATTAAATTAA  
GTGTGTGGCTGCGACTGAGAAATCTGCTGACGTTGTACGATGCTGAG  
CCCGGCAGATGTCTCACTGCGTTGACATGACGAAGAAGGGAGTACGAT  
TACTAATGCCCGGCAGATGTCTCACTGCGTTGACATGACGGGGACGGT  
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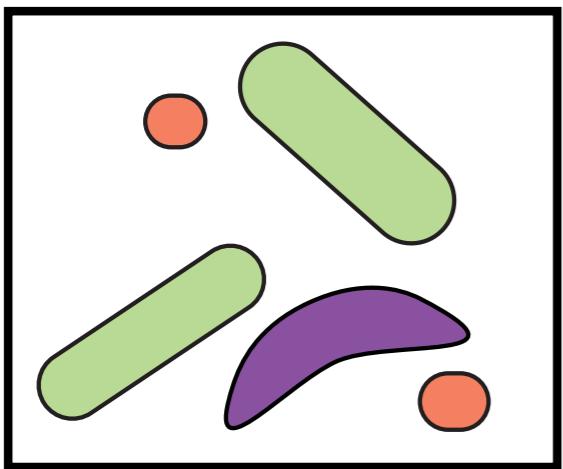
# Amplicon sequencing

## 1. Sampling

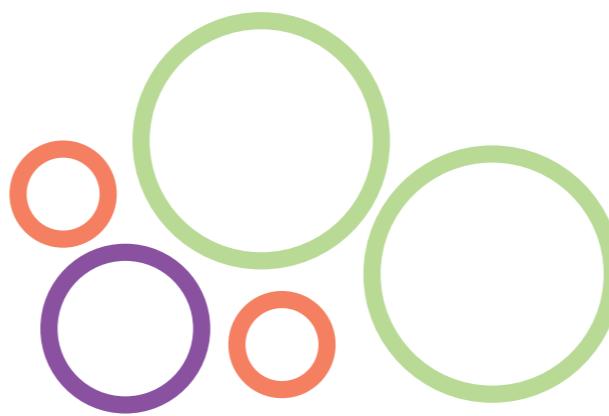


# Amplicon sequencing

1. Sampling

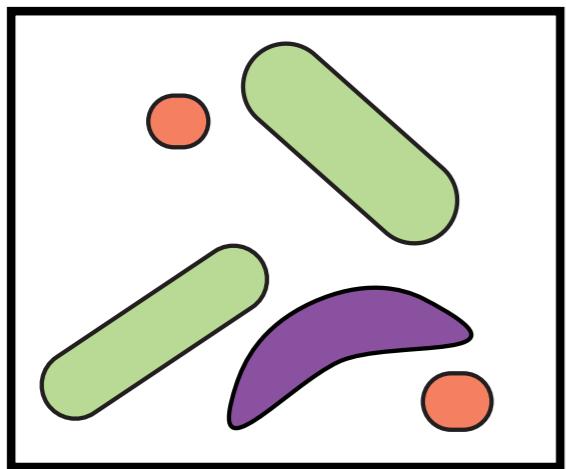


2. DNA extraction

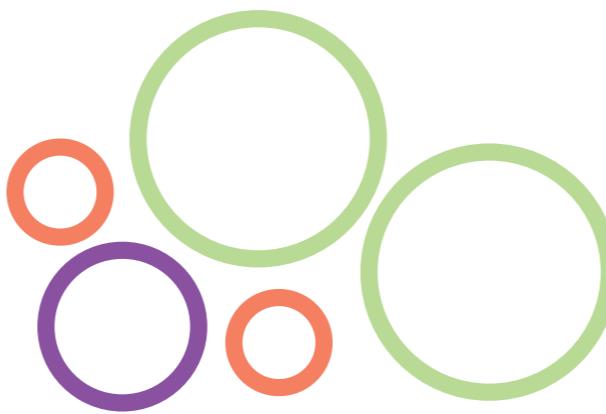


# Amplicon sequencing

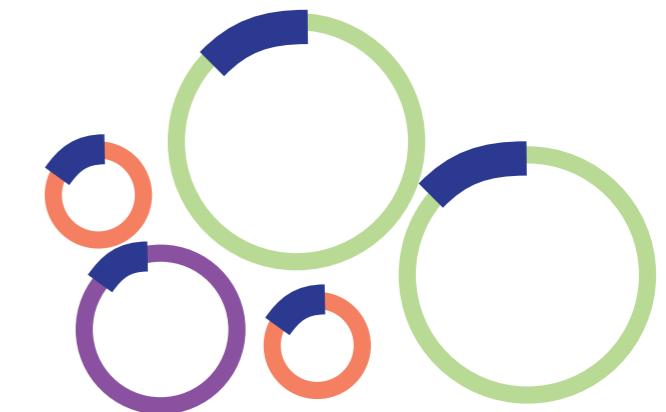
1. Sampling



2. DNA extraction



3. Amplification



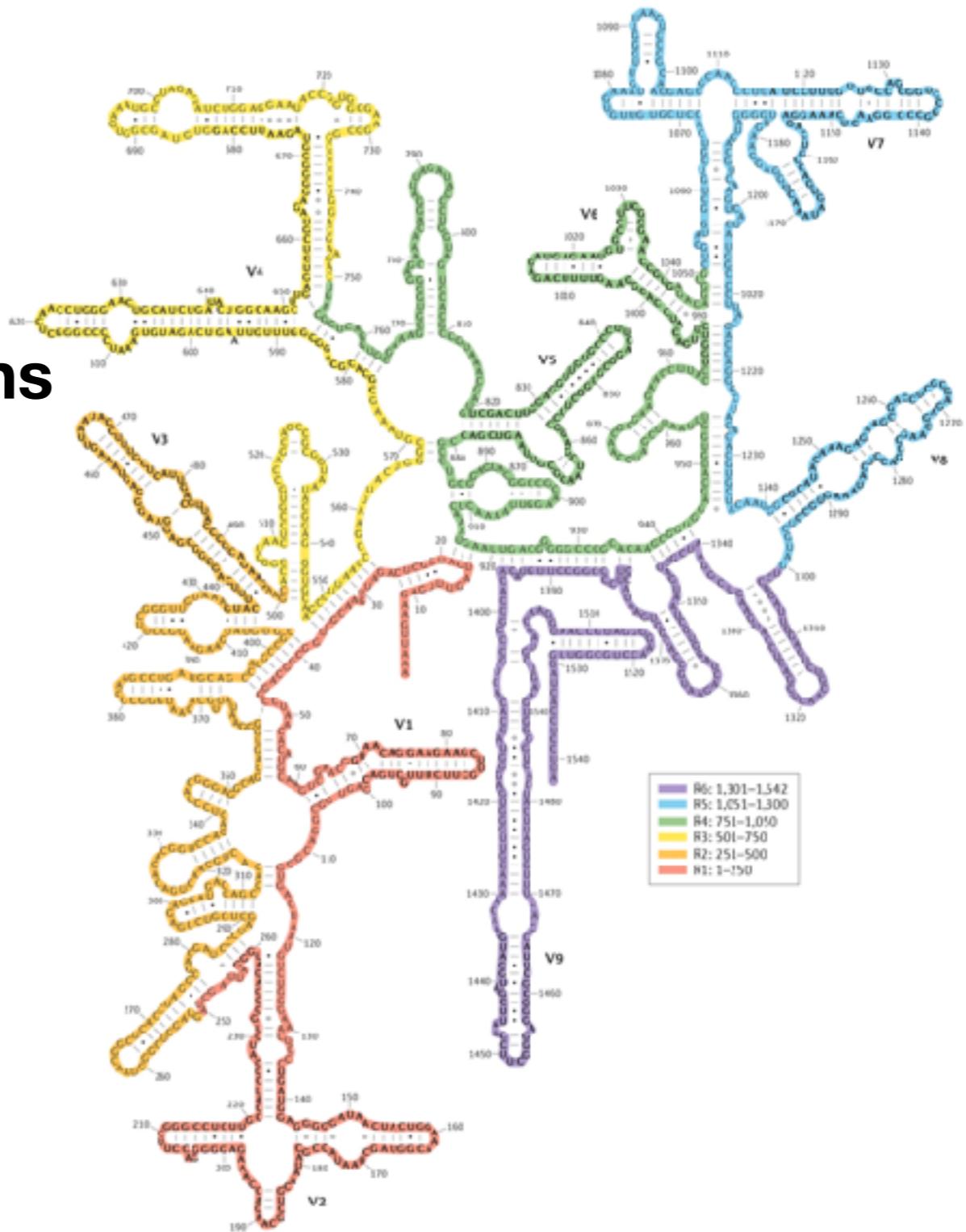
# Amplicon sequencing

16S ribosomal RNA gene:

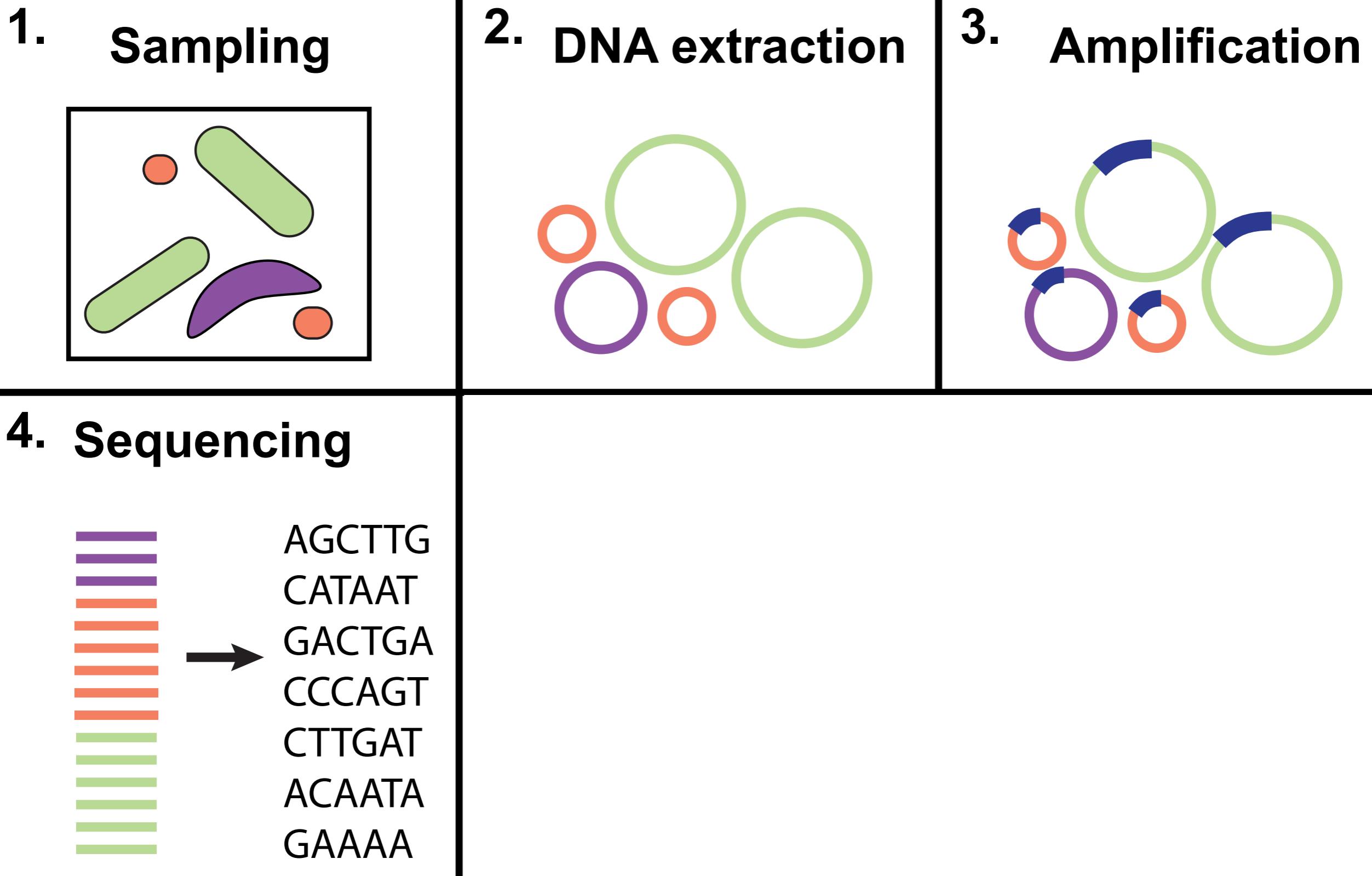
Has conserved and variable regions

Conserved → Amplify with PCR

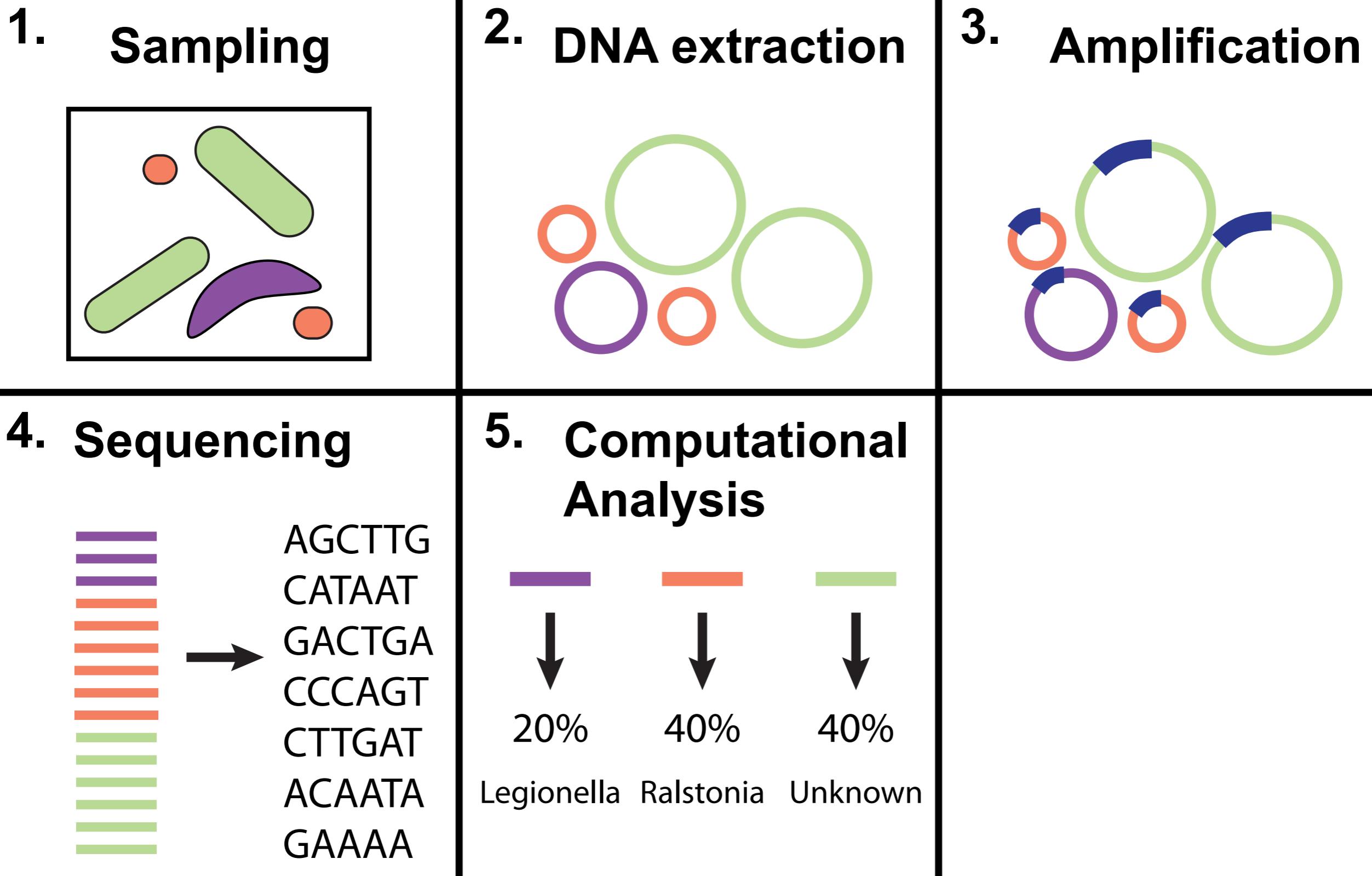
Variable → Unique “ID tag”



# Amplicon sequencing

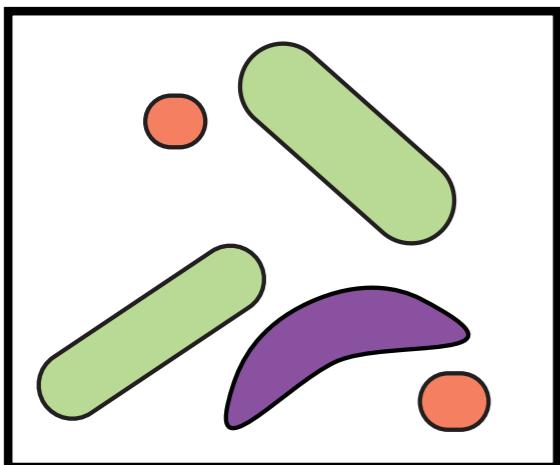


# Amplicon sequencing



# Amplicon sequencing

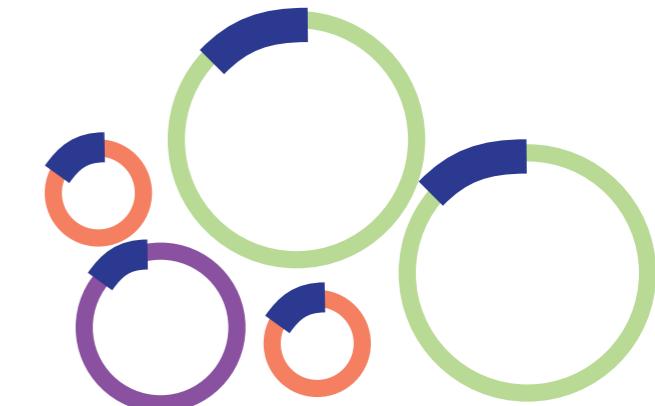
## 1. Sampling



## 2. DNA extraction



## 3. Amplification

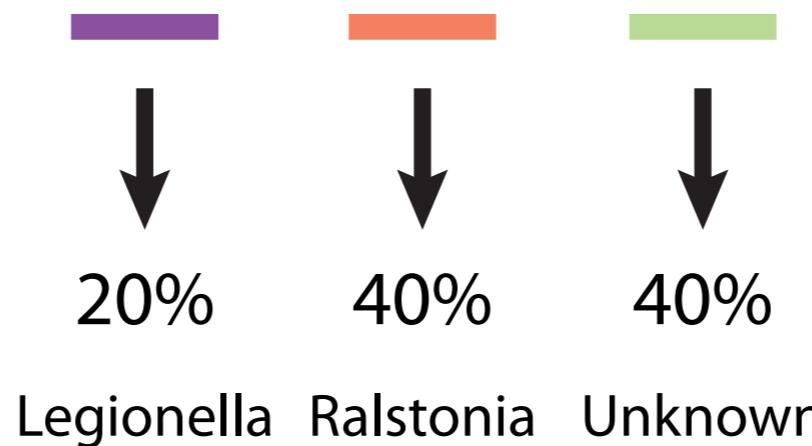


## 4. Sequencing



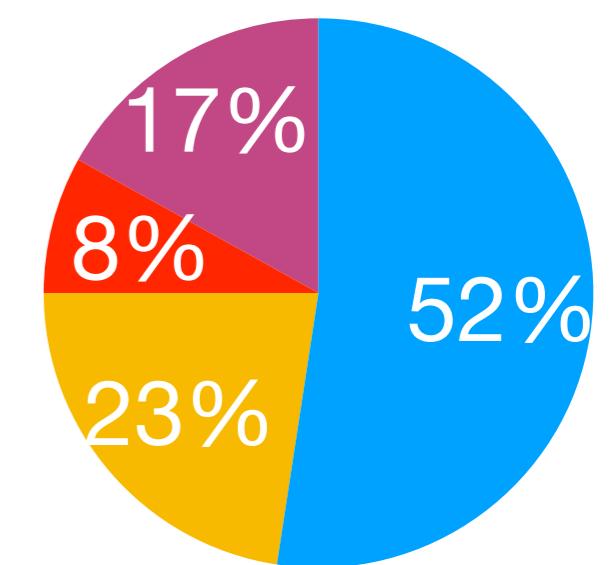
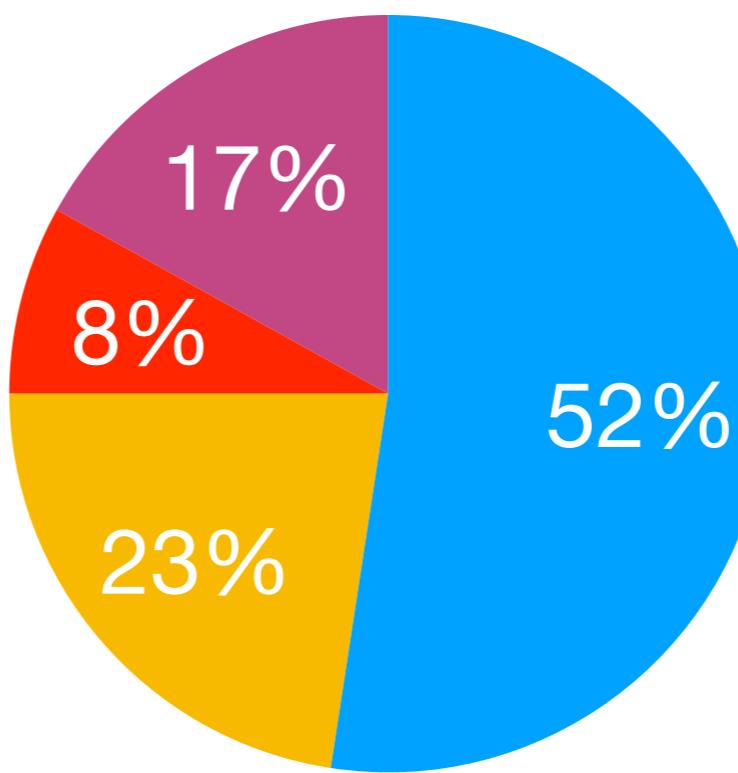
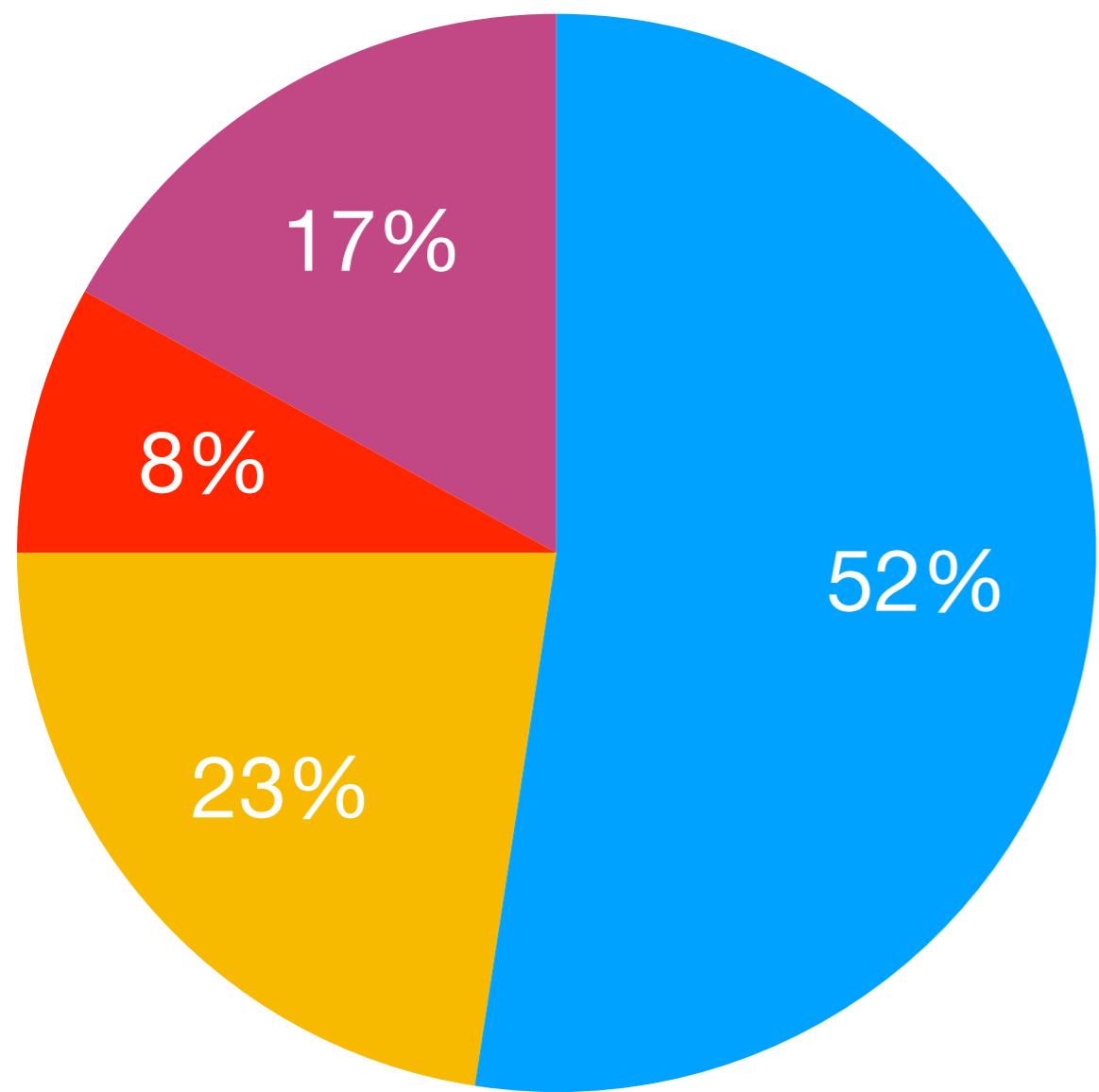
AGCTTG  
CATAAT  
GACTGA  
CCCAGT  
CTTGAT  
ACAATA  
GAAAAA

## 5. Computational Analysis



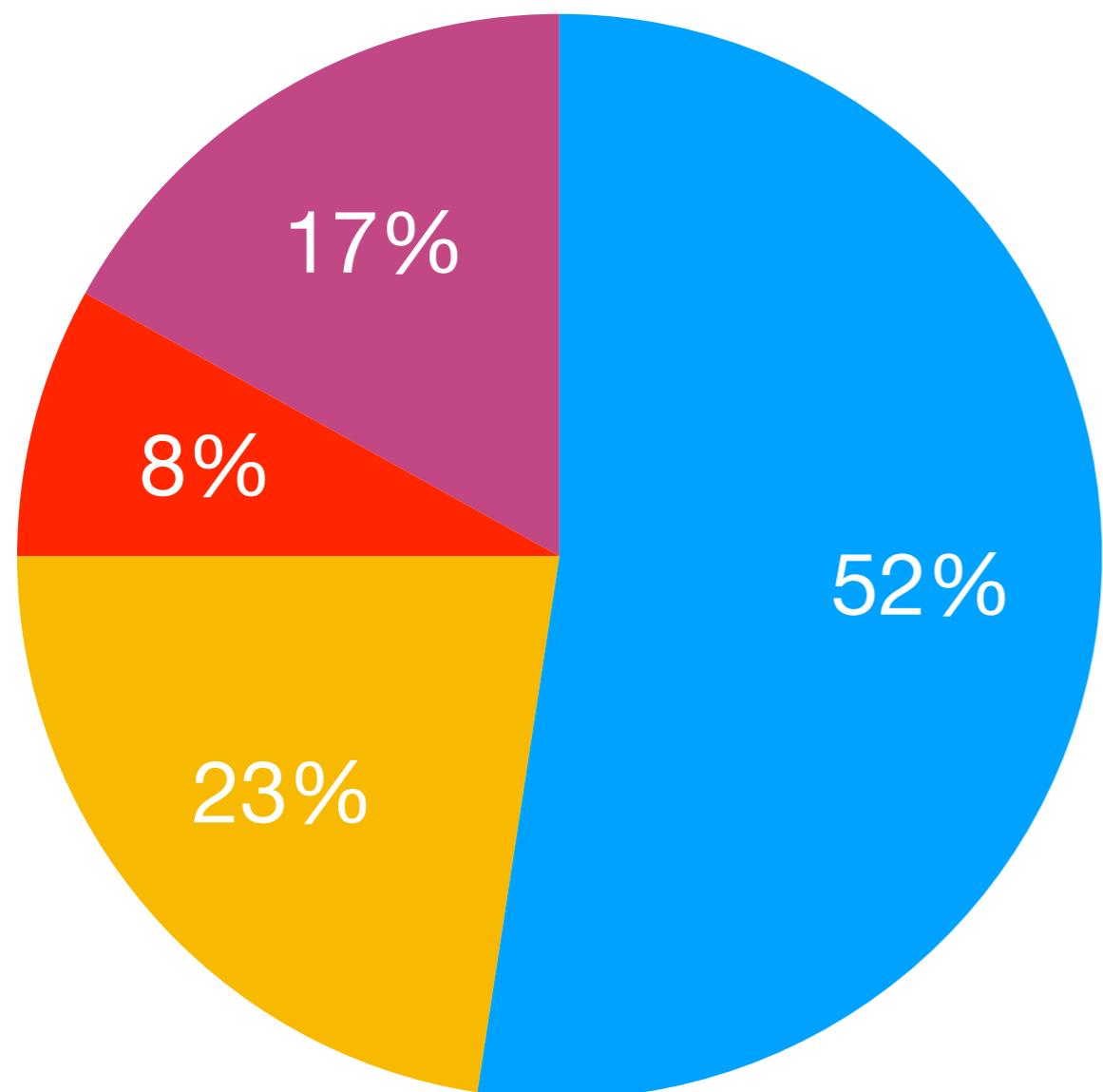
**Amplicon Sequence Variant (ASV)**  
=  
**“Barcode”**

# Relative abundance

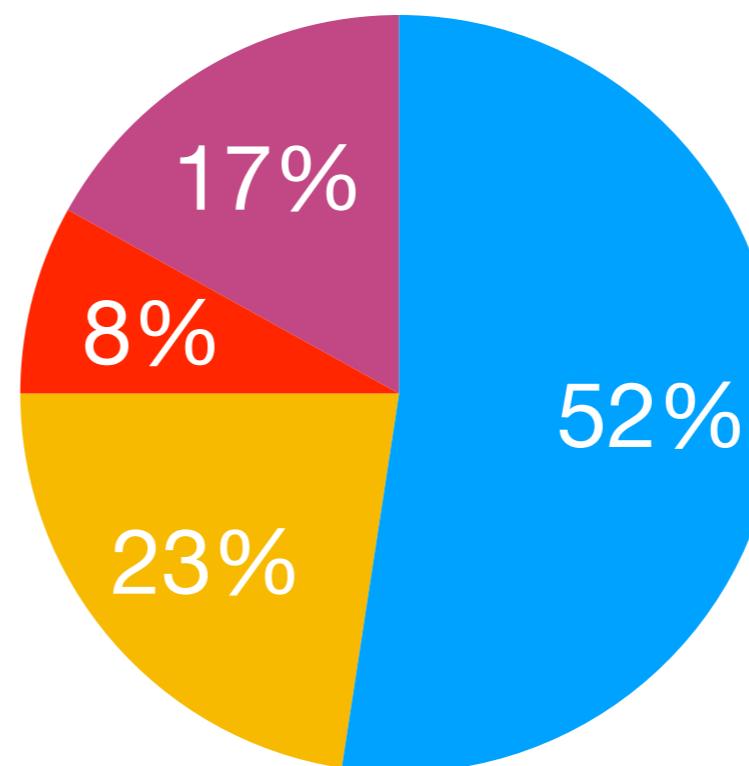


# Absolute abundance

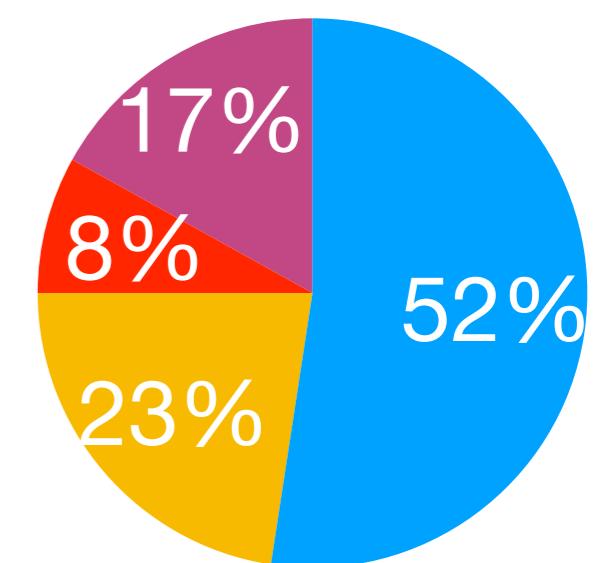
10 million cells / mL



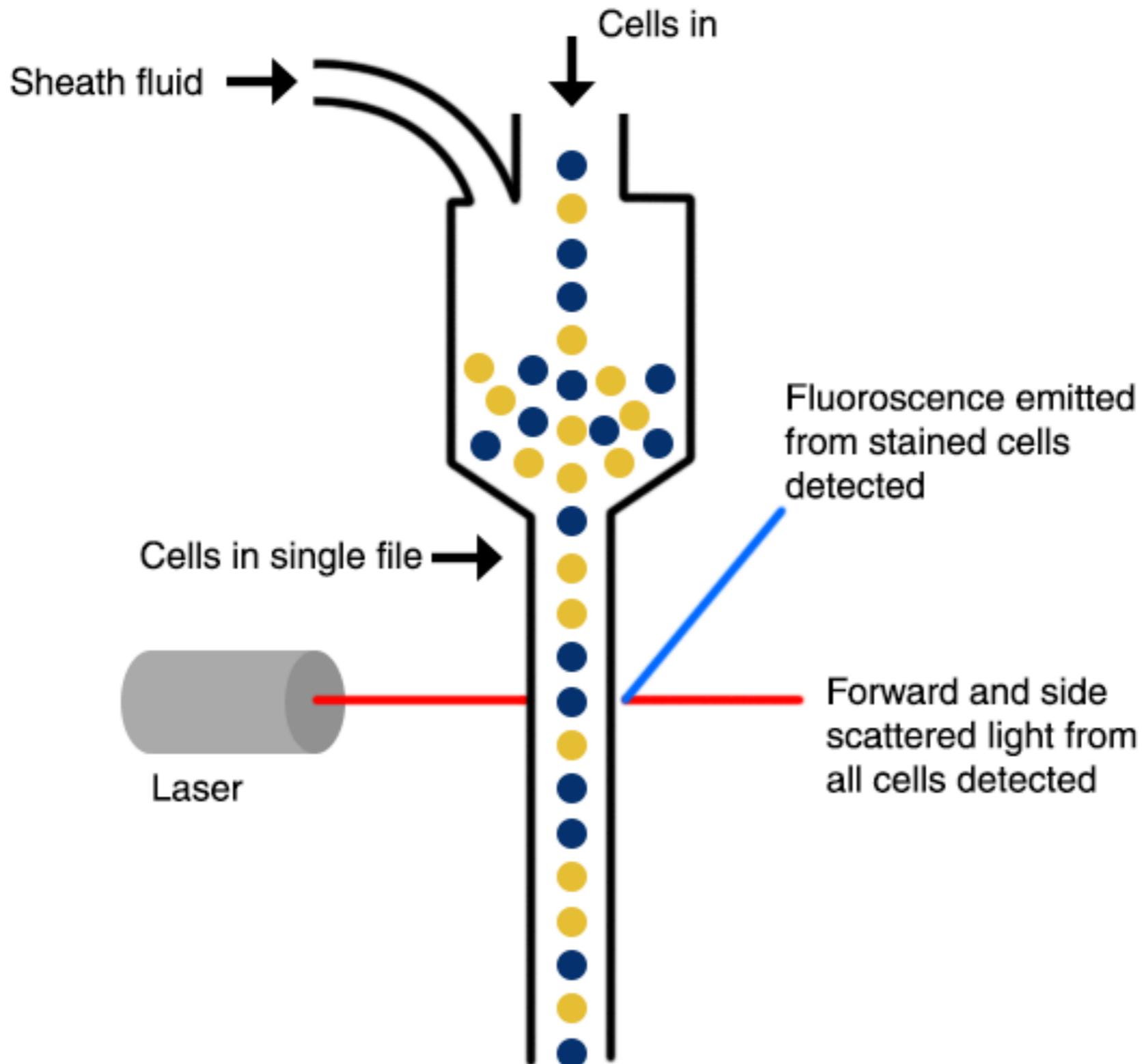
10,000 cells / mL



10 cells / mL



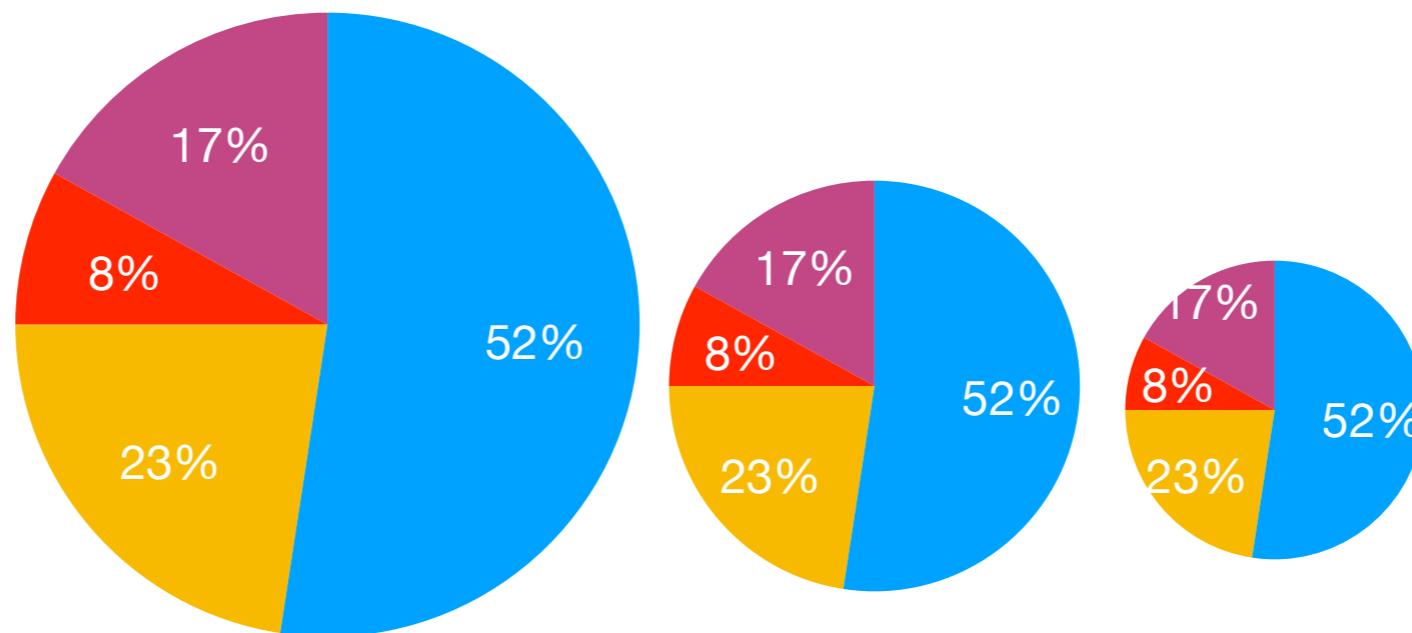
# Flow cytometry: counting bacterial cells



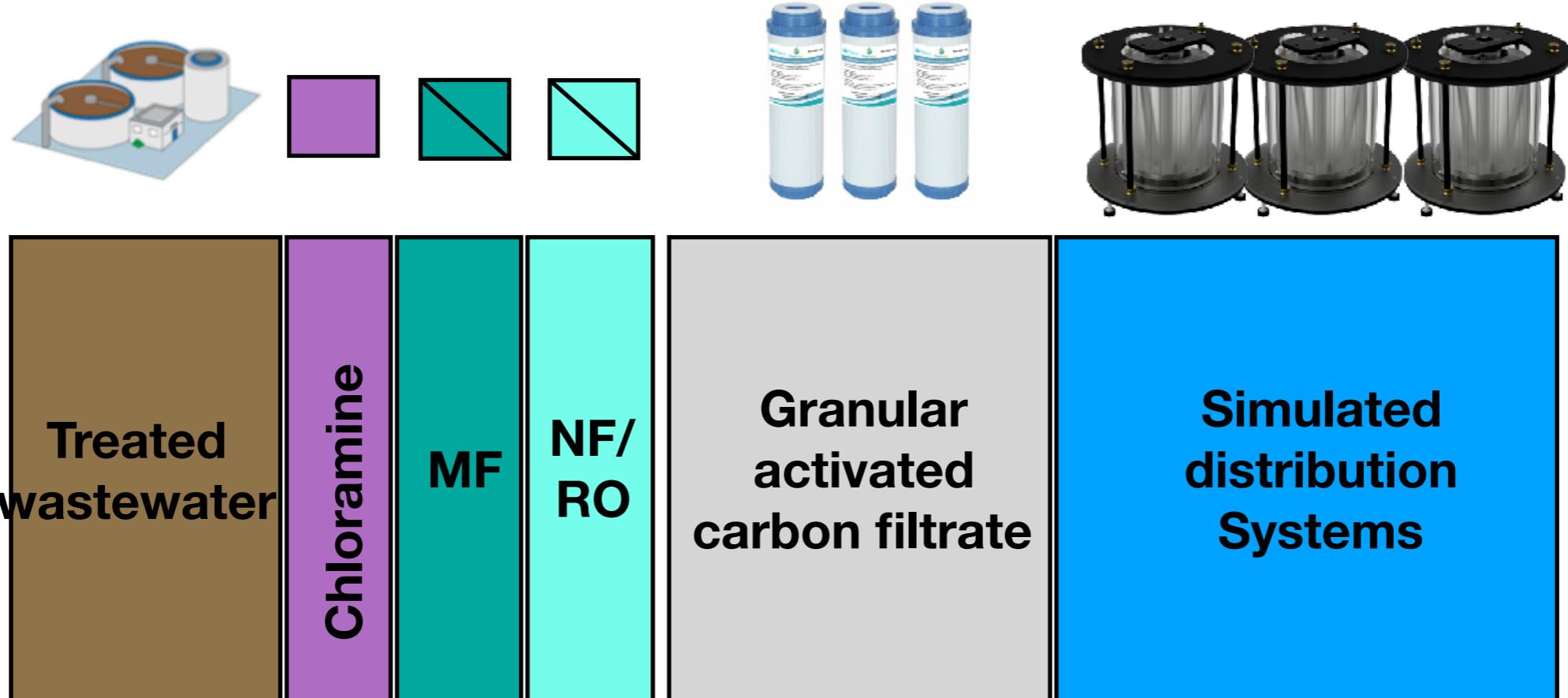
# Research questions

- 1. How does the microbial community change through treatment and simulated distribution?**

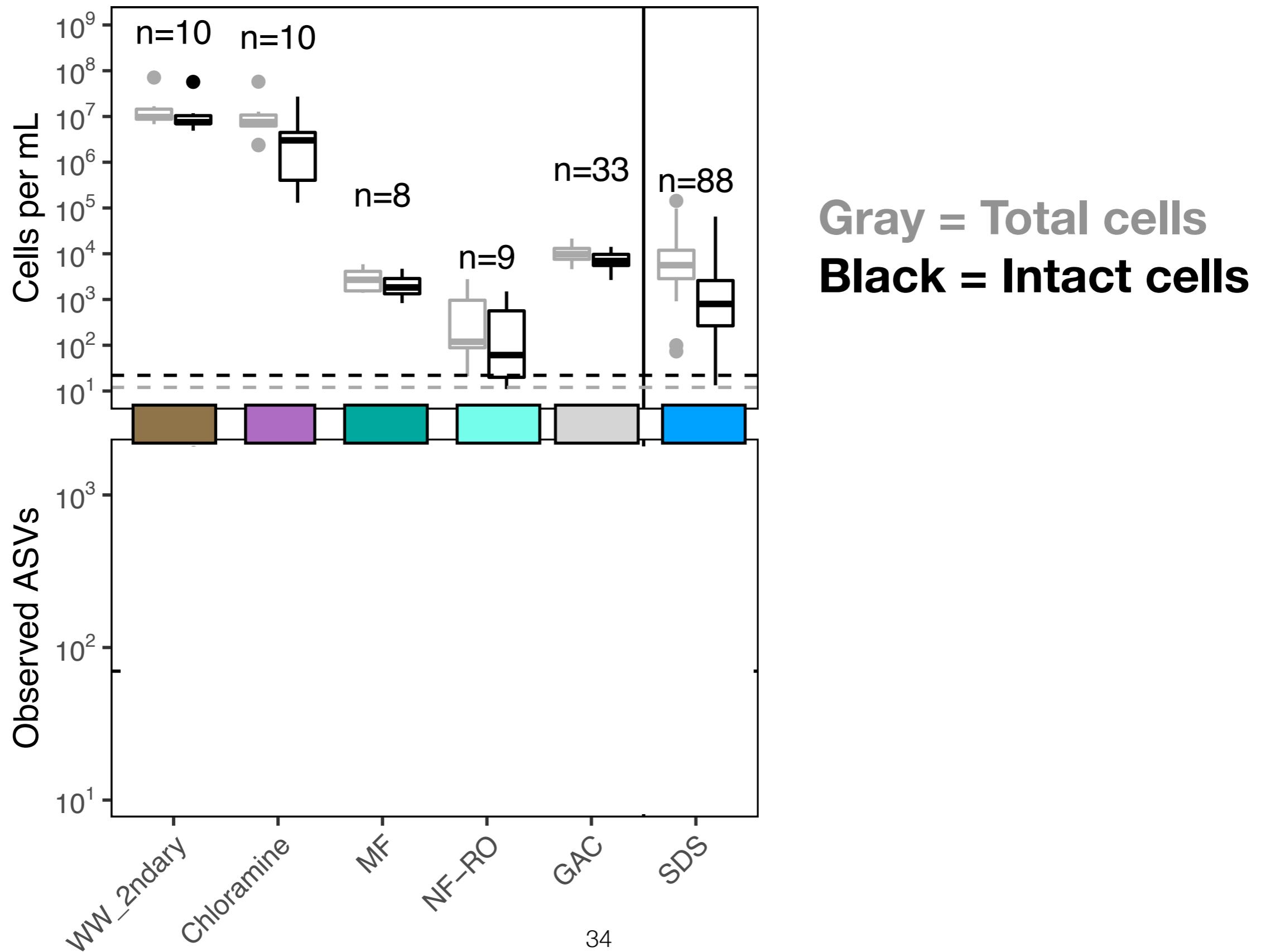
**One hypothesis:**



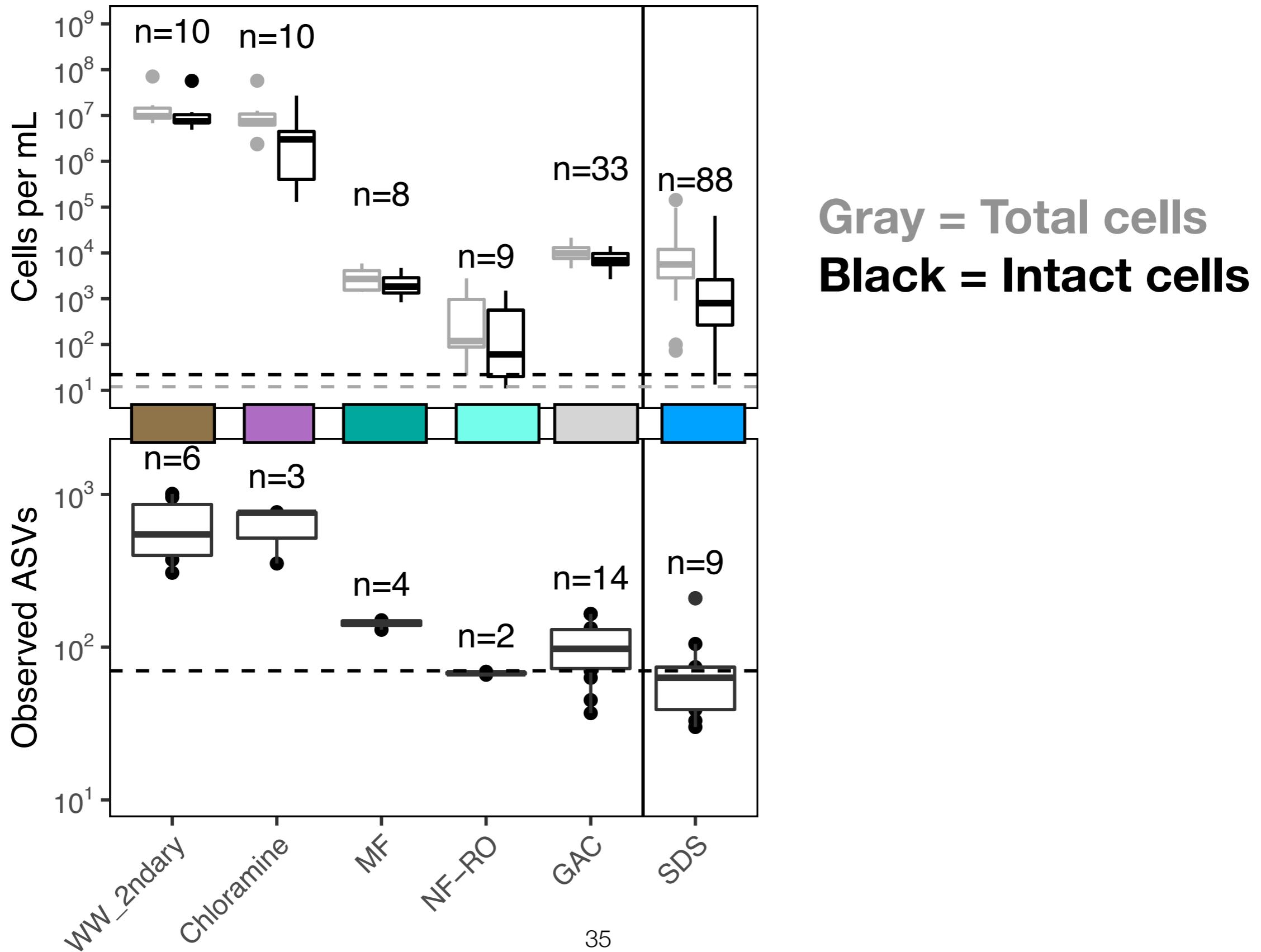
# Experimental sample points



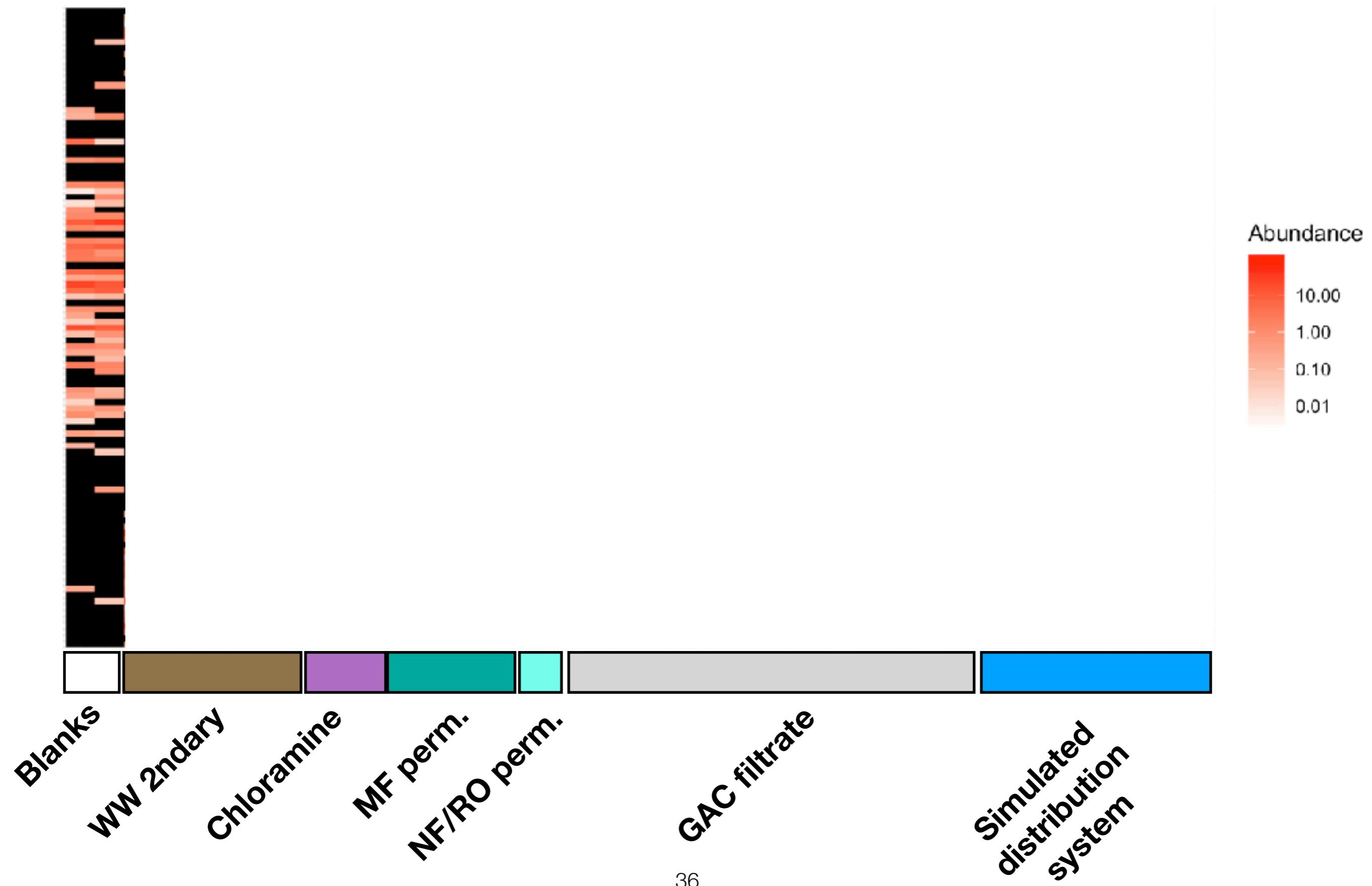
# Microbial load and microbial diversity



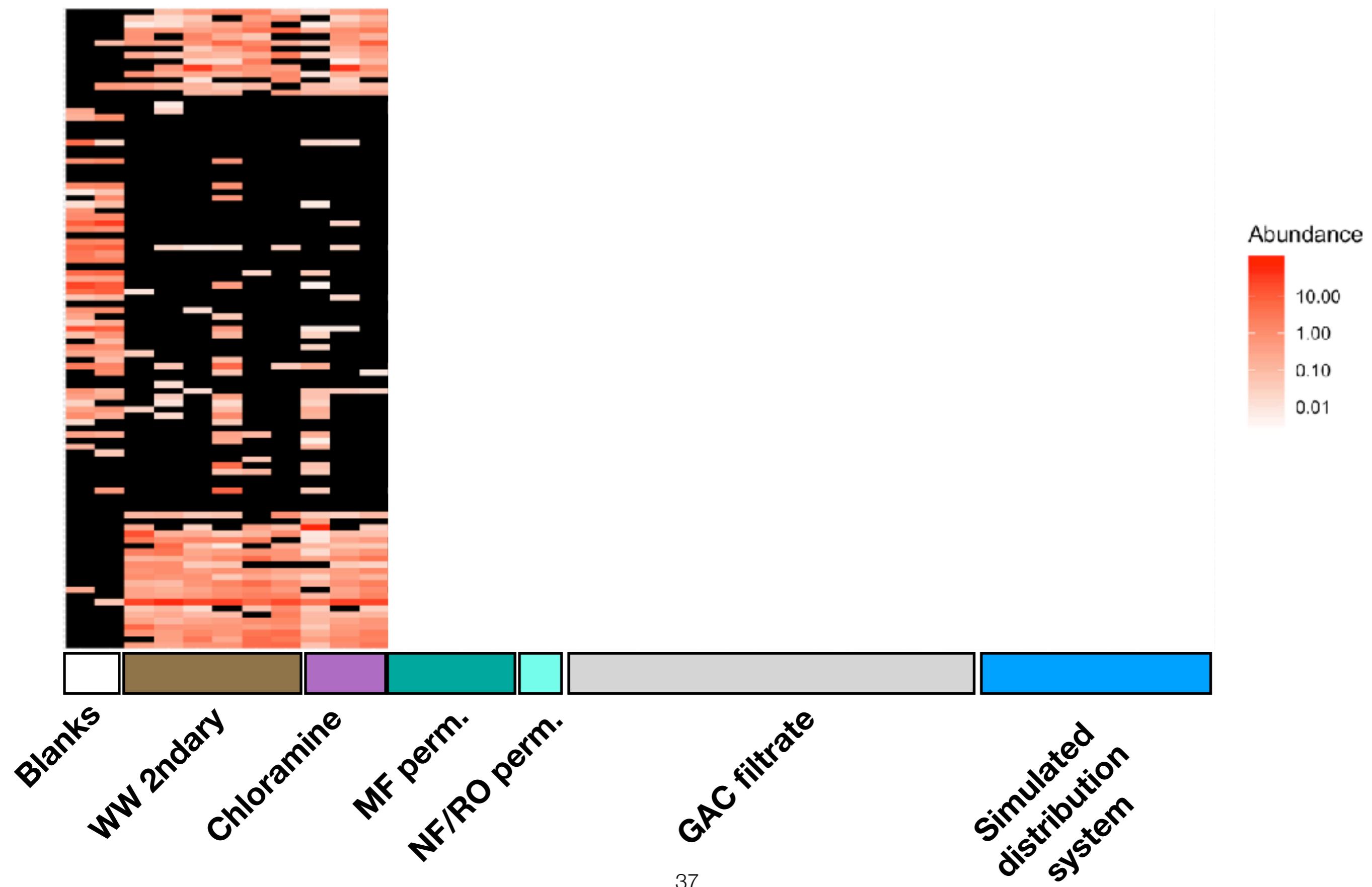
# Microbial load and microbial diversity



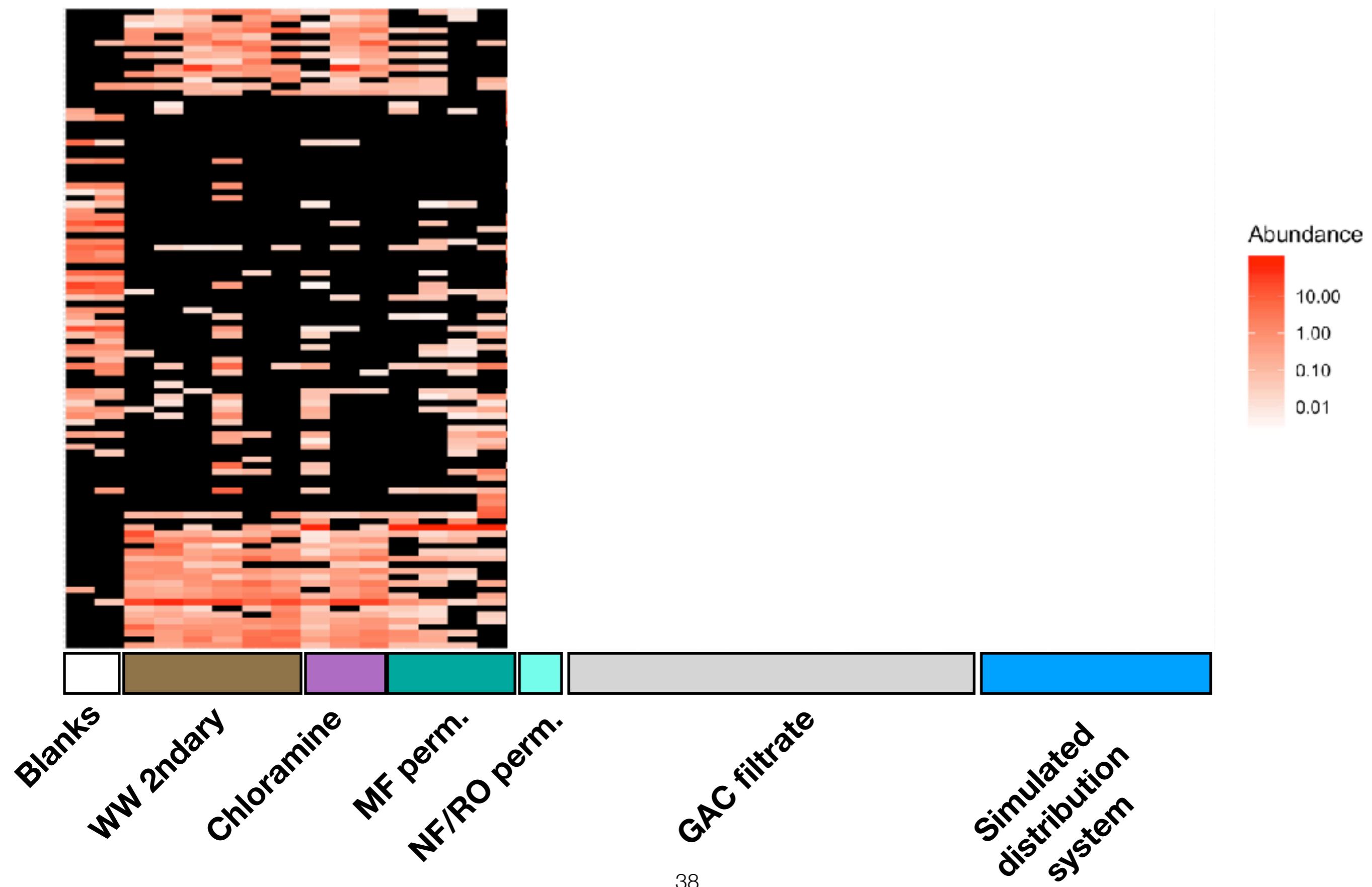
# Relative abundances of microbial community members



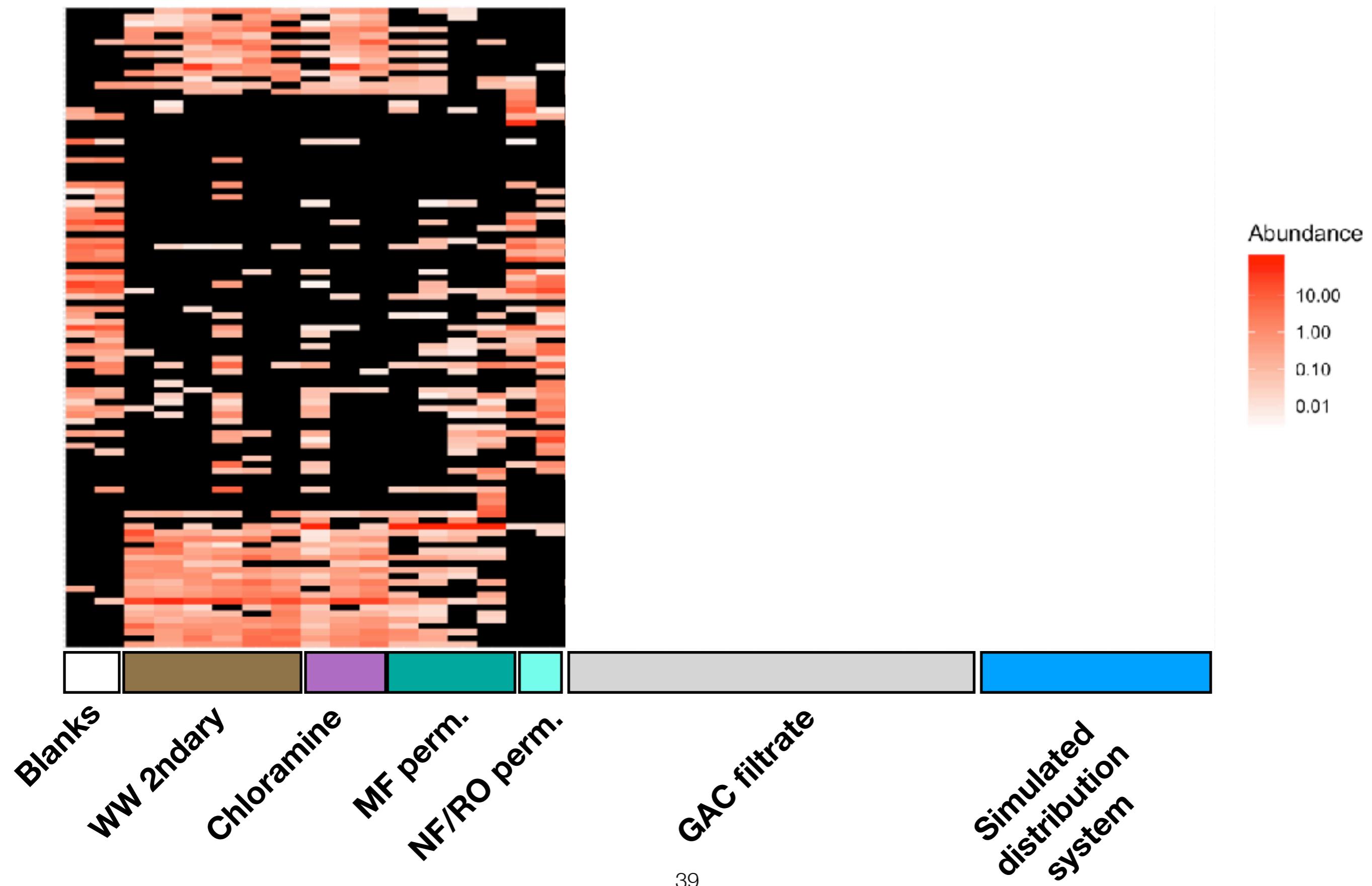
# Relative abundances of microbial community members



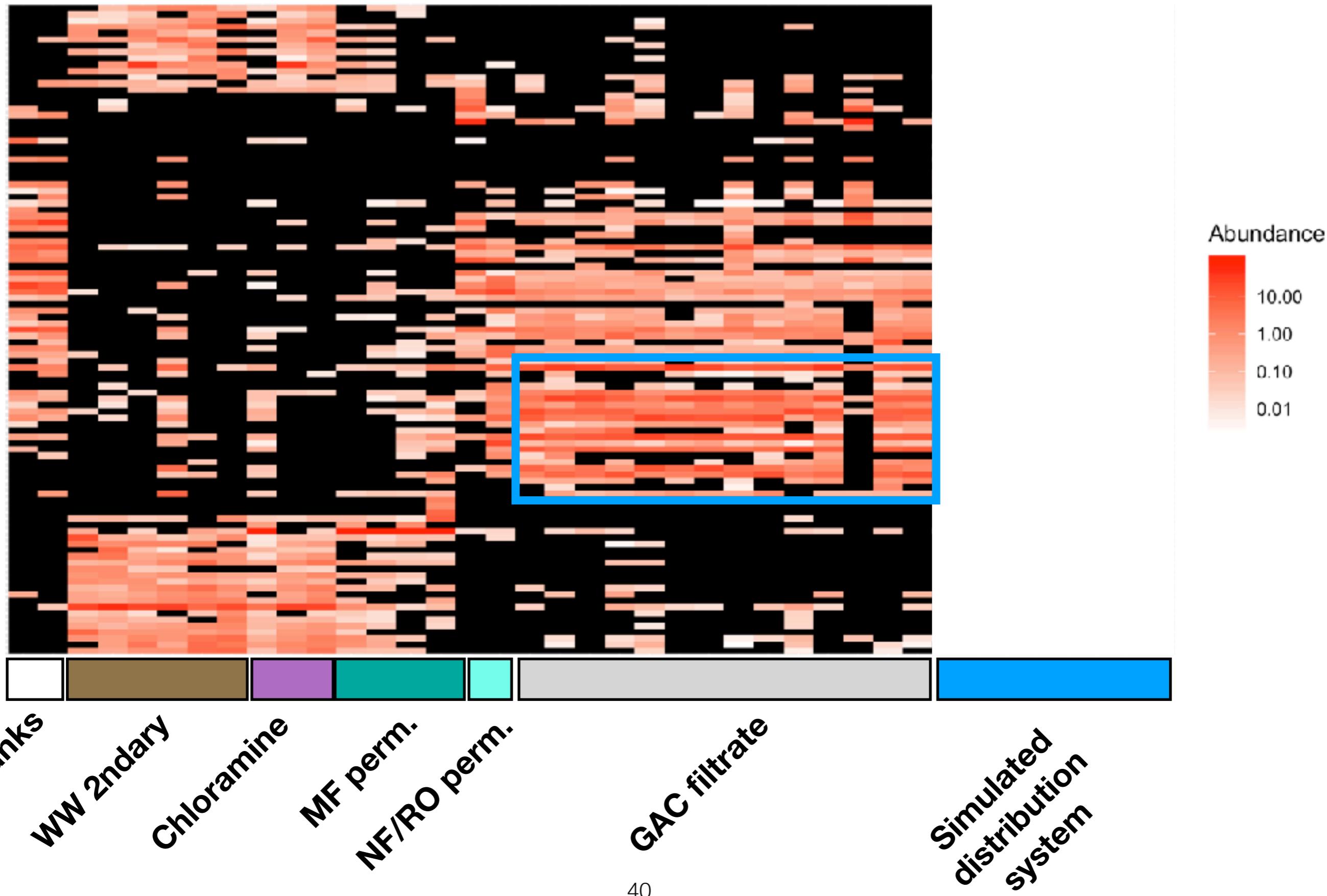
# Relative abundances of microbial community members



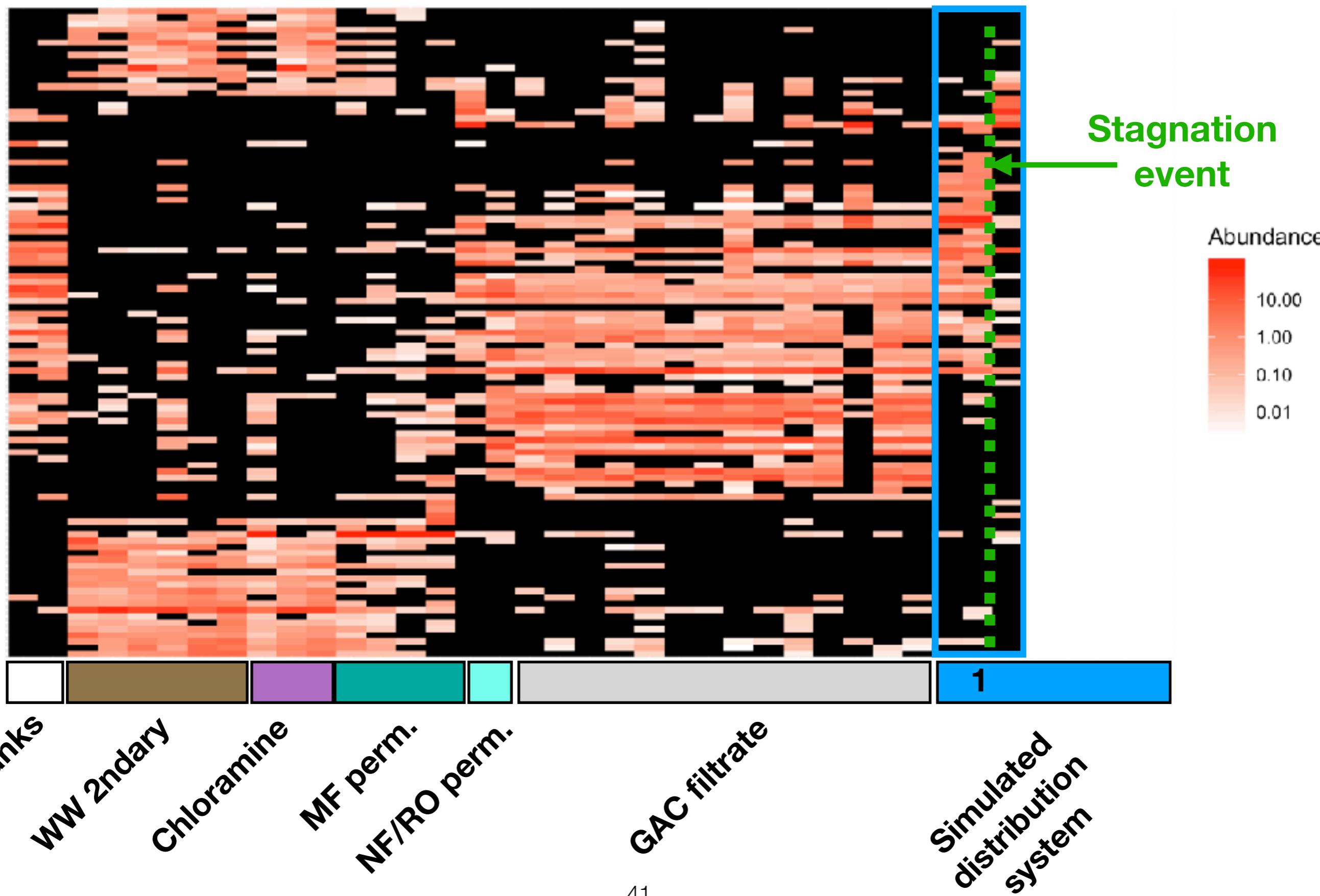
# Relative abundances of microbial community members



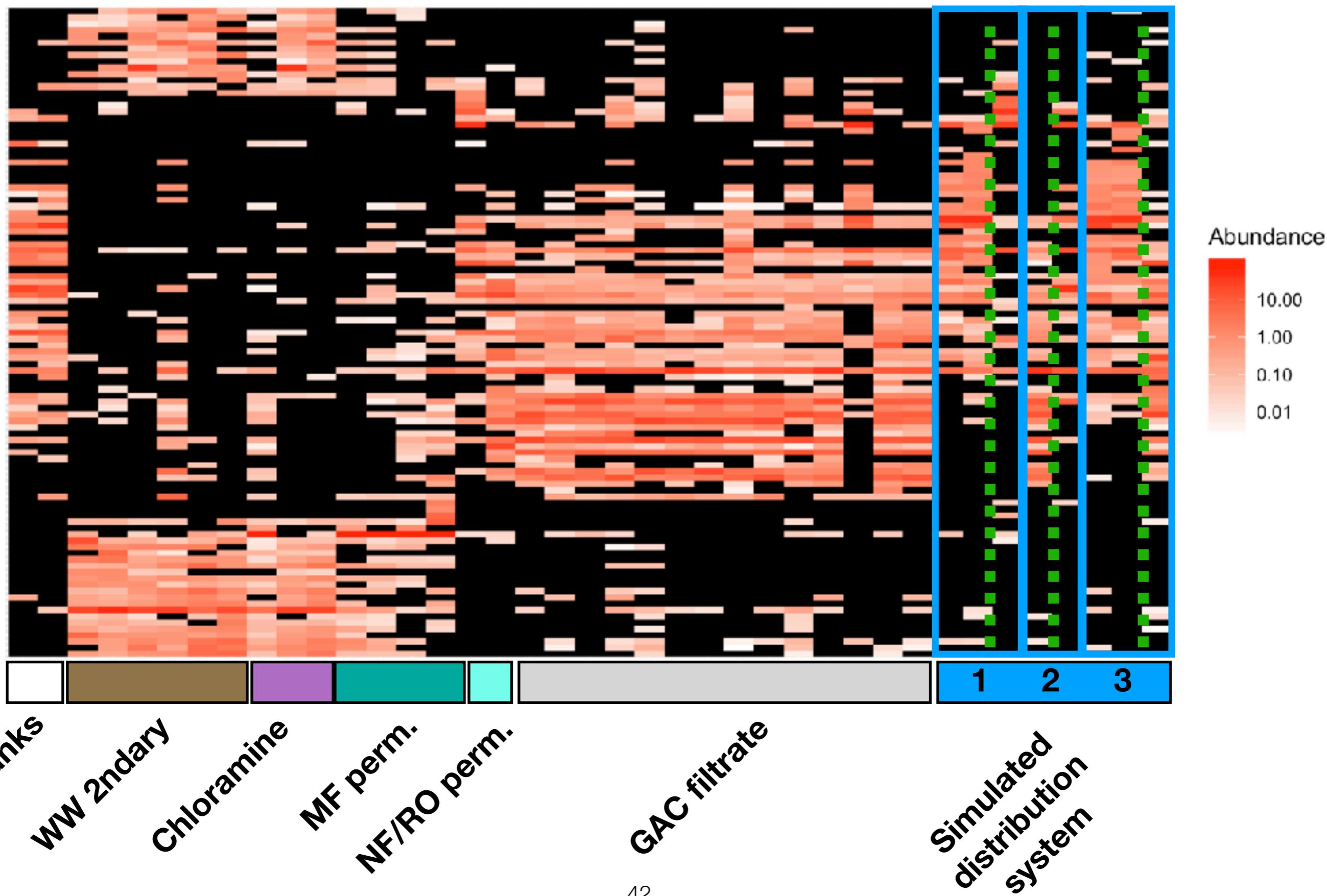
# Relative abundances of microbial community members



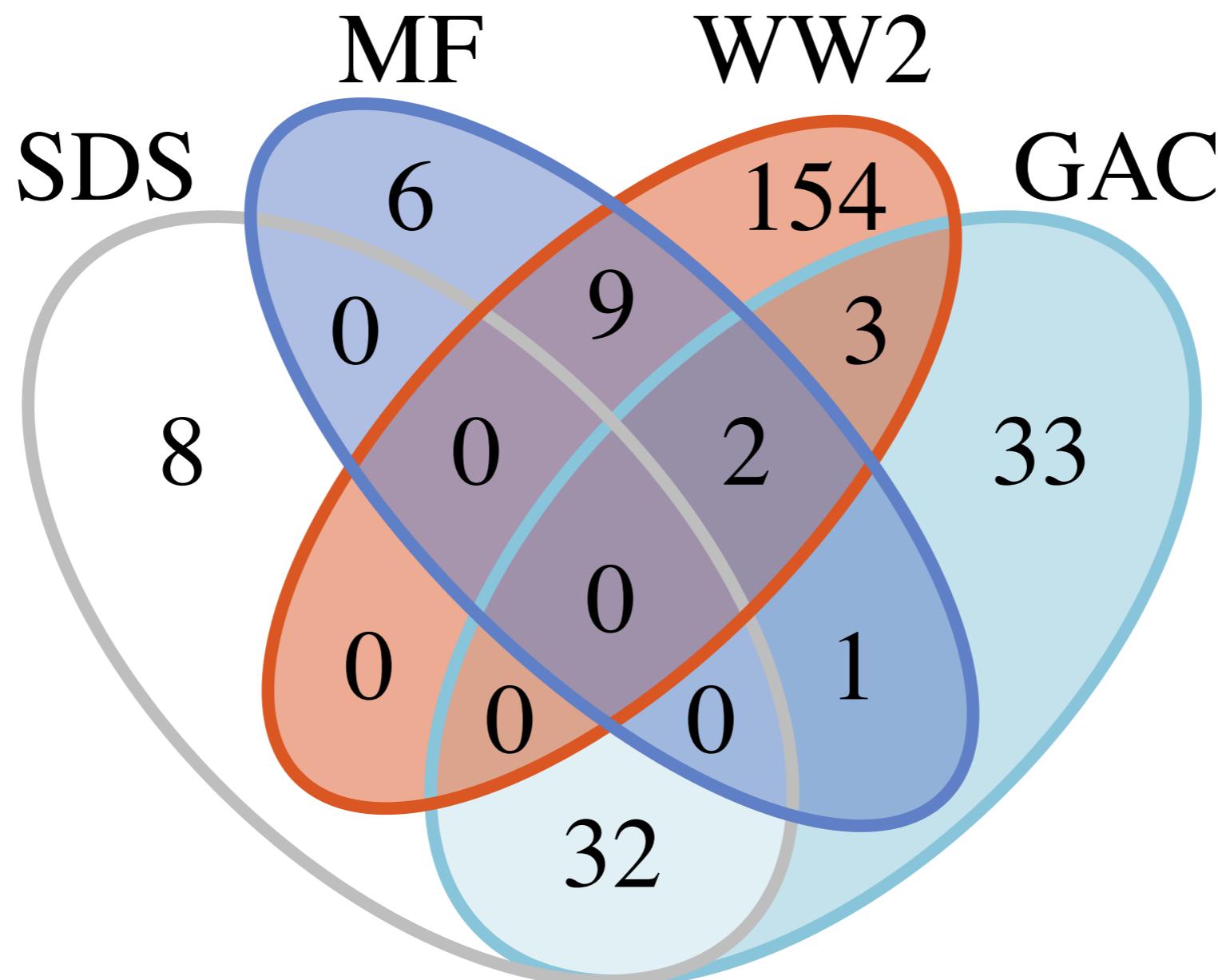
# Relative abundances of microbial community members



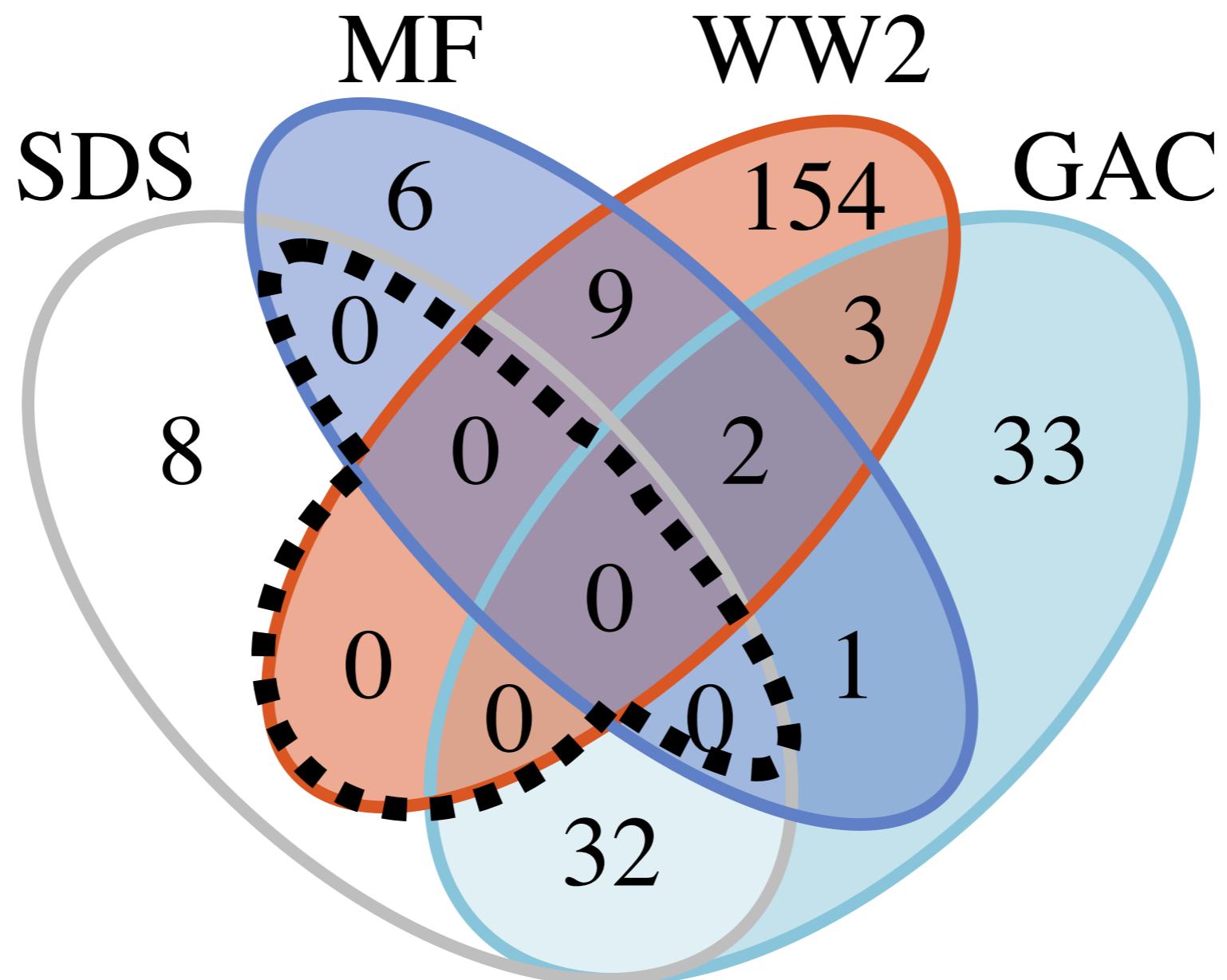
# Relative abundances of microbial community members



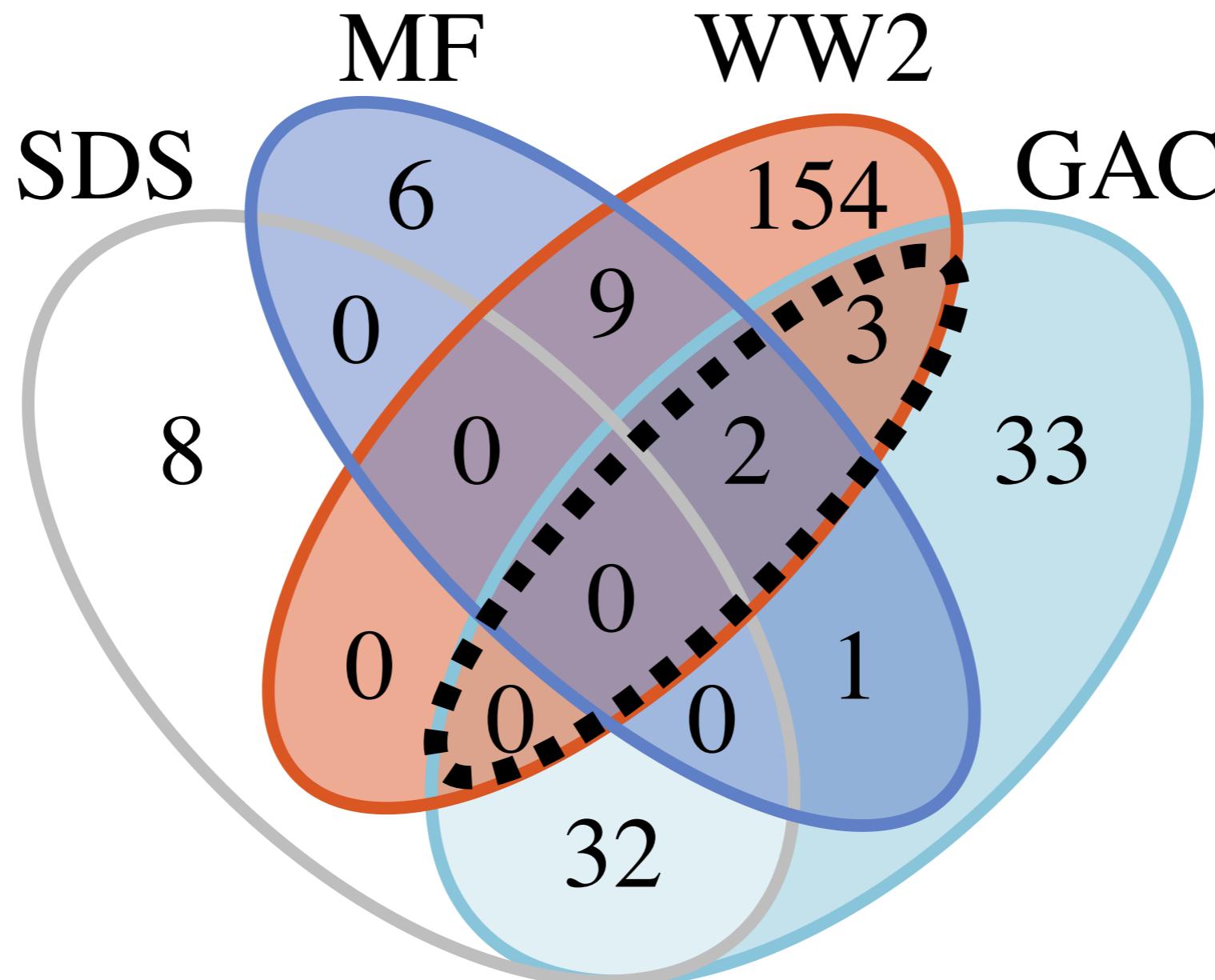
# Recurring sequences



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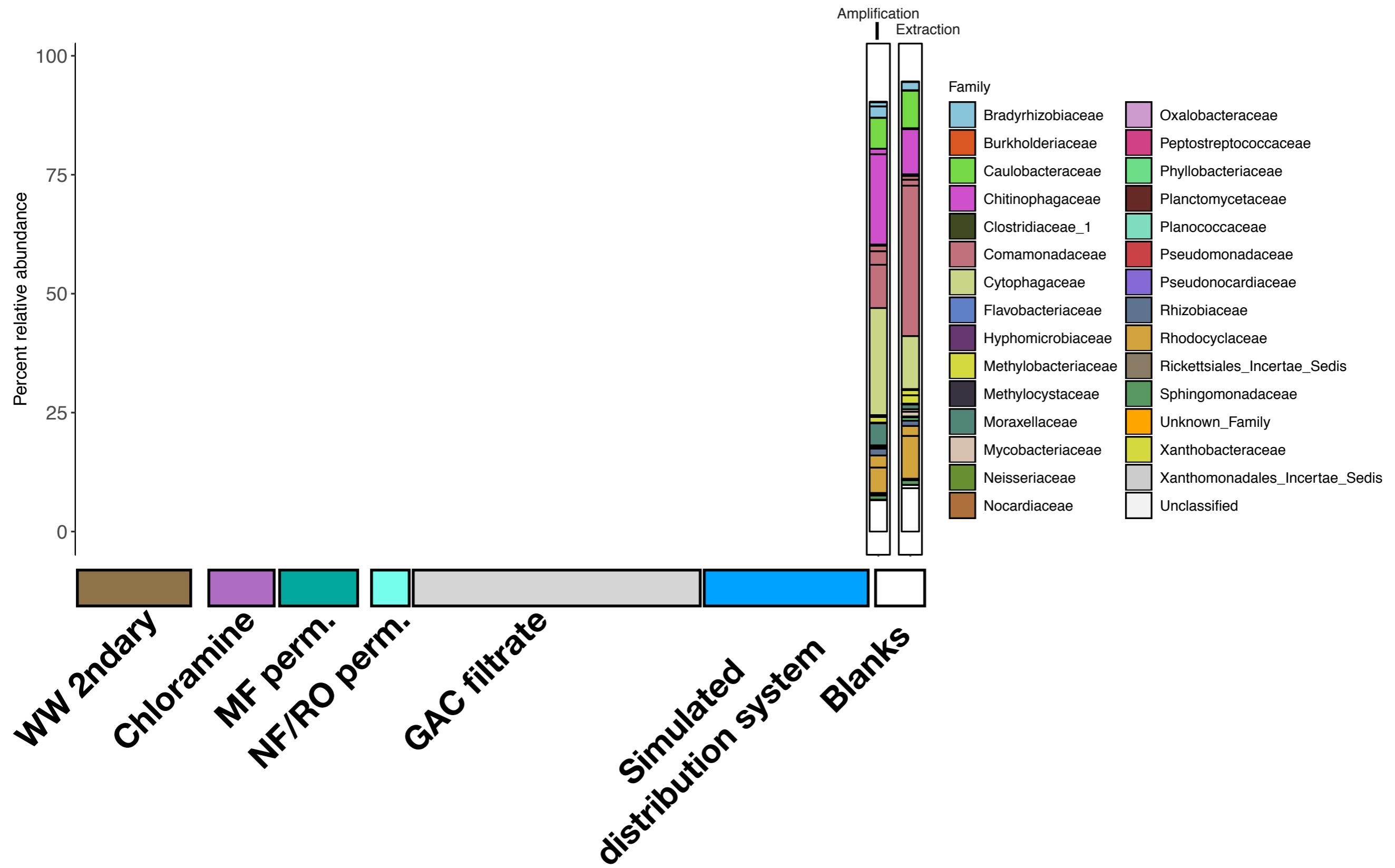


Maybe these bacteria made it through the treatment train!

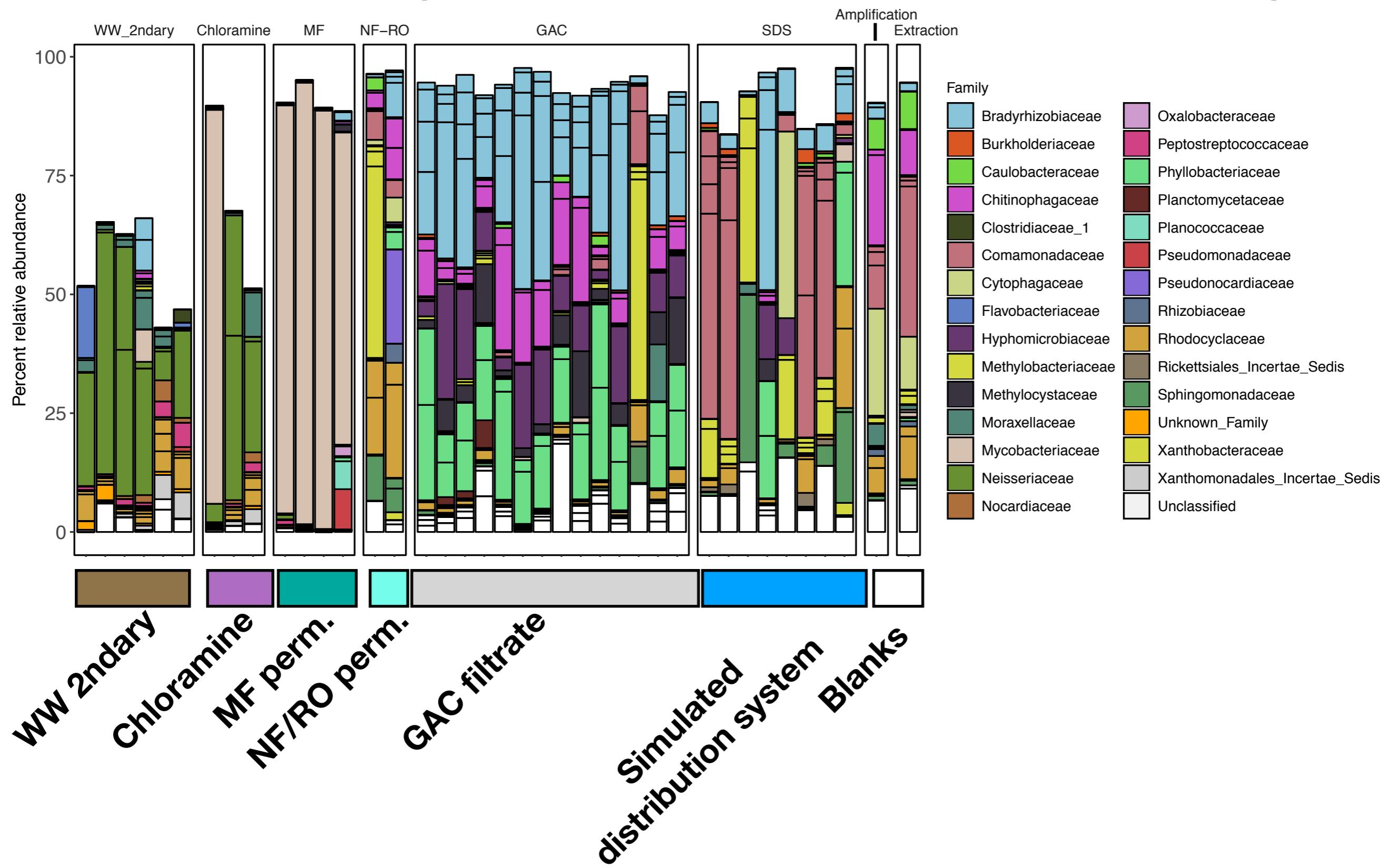
-or-

Maybe this method isn't robust enough for this analysis.

# Taxonomy of microbial community



# Taxonomy of microbial community

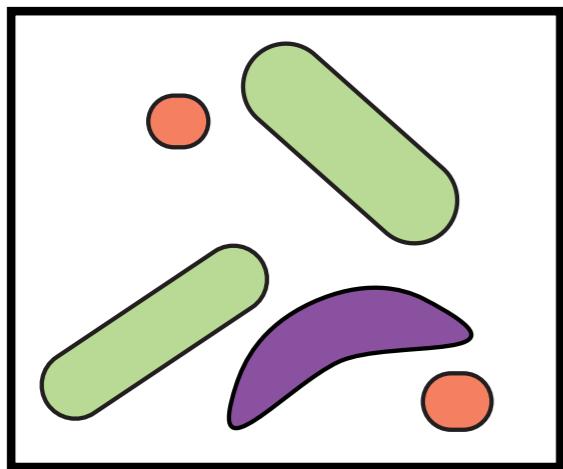


# Research questions

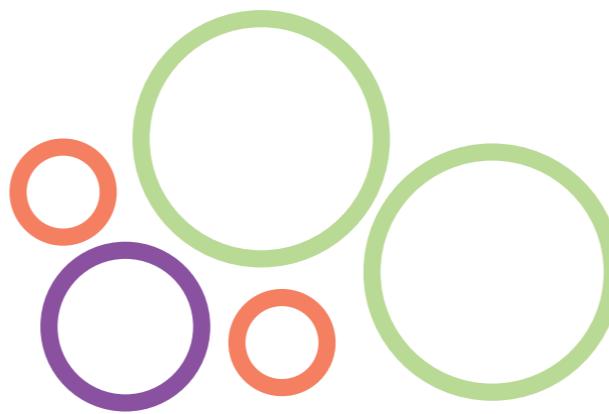
- 1. How does the microbial community change through treatment and simulated distribution?**
- 2. Before and after treatment: Who survives? Are there pathogens? Are there antibiotic resistance genes? How might bacteria survive?**

# Metagenomic sequencing

## 1. Sampling

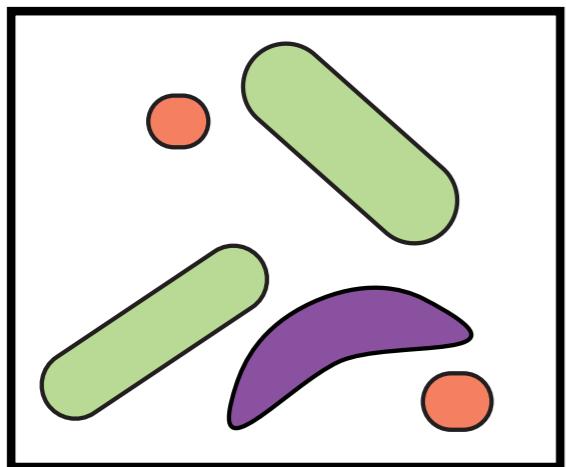


## 2. DNA extraction



# Metagenomic sequencing

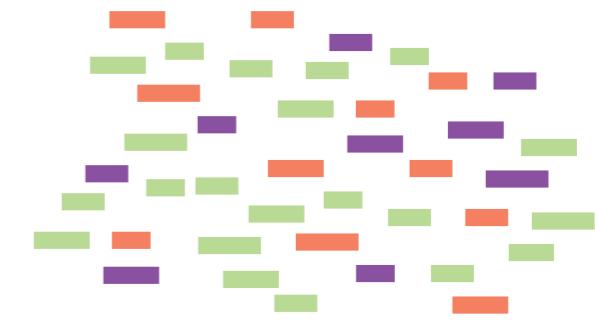
1. Sampling



2. DNA extraction

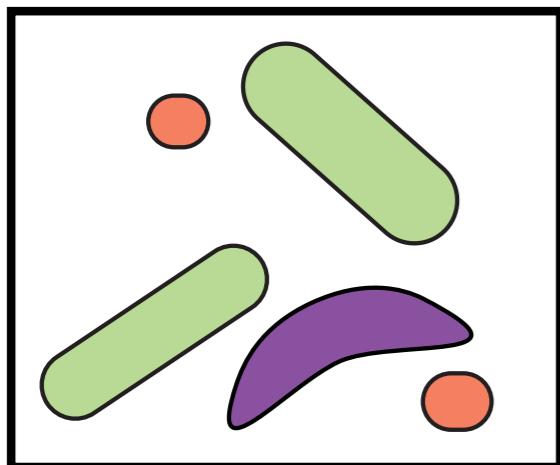


3. All DNA is sheared and sequenced

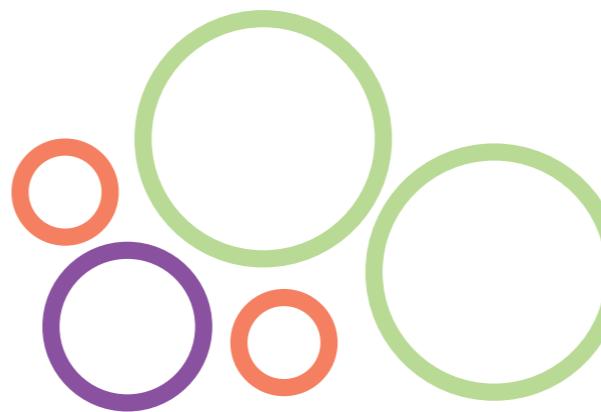


# Metagenomic sequencing

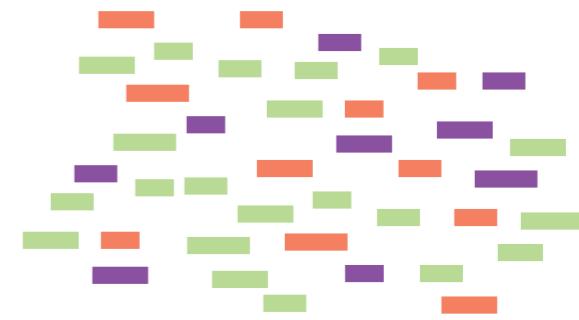
1. Sampling



2. DNA extraction



3. All DNA is sheared and sequenced

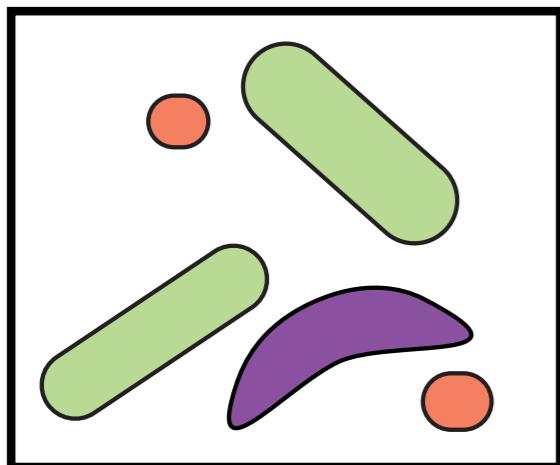


4. Assembly

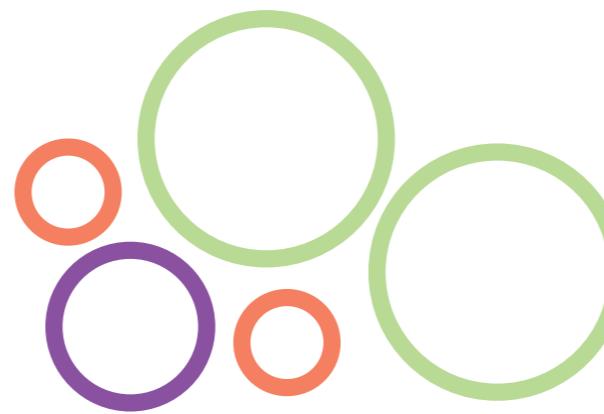


# Metagenomic sequencing

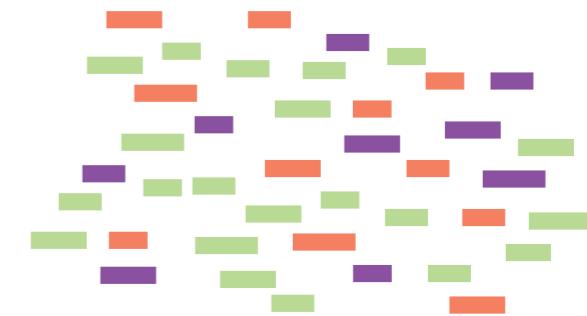
1. Sampling



2. DNA extraction



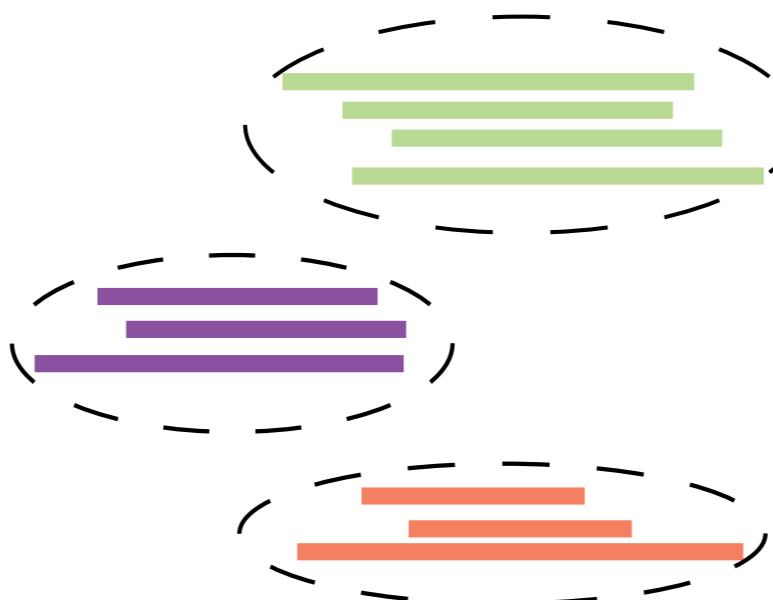
3. All DNA is sheared and sequenced



4. Assembly

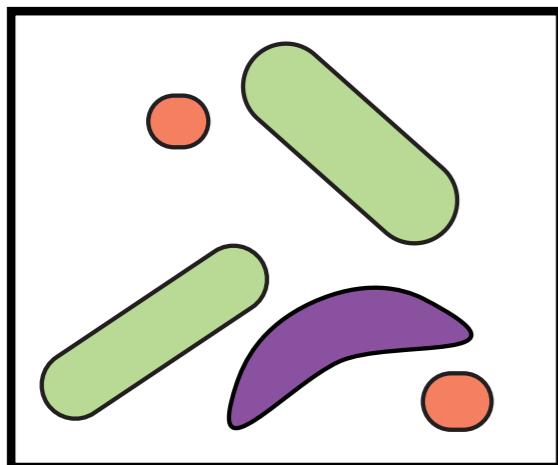


5. Binning

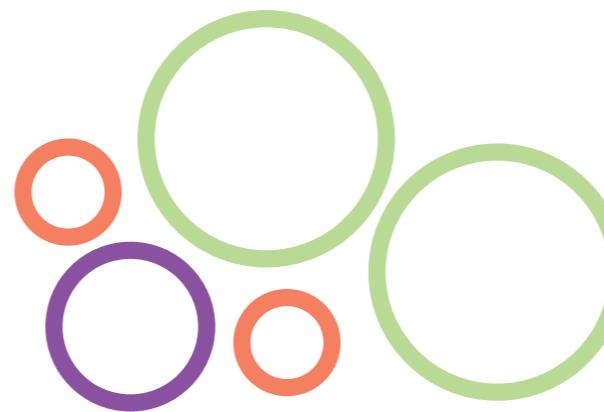


# Metagenomic sequencing

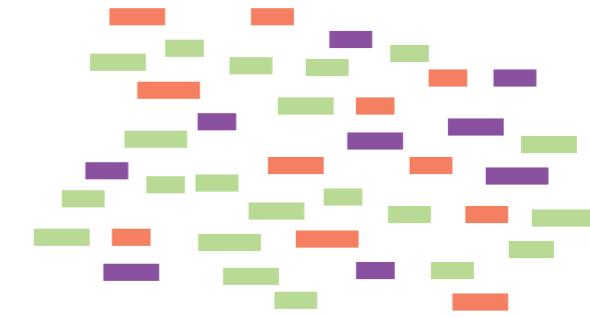
1. Sampling



2. DNA extraction



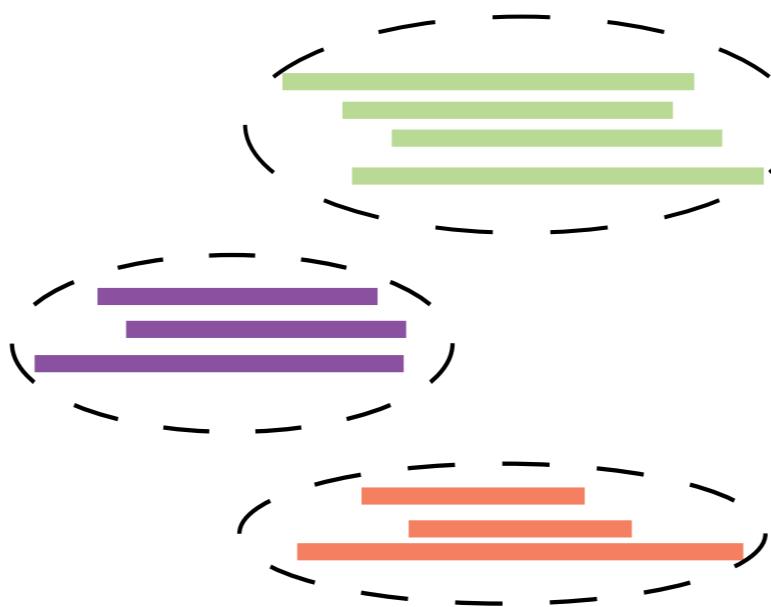
3. All DNA is sheared and sequenced



4. Assembly



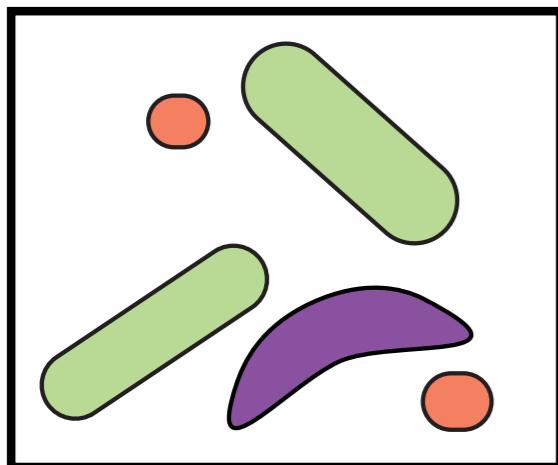
5. Binning



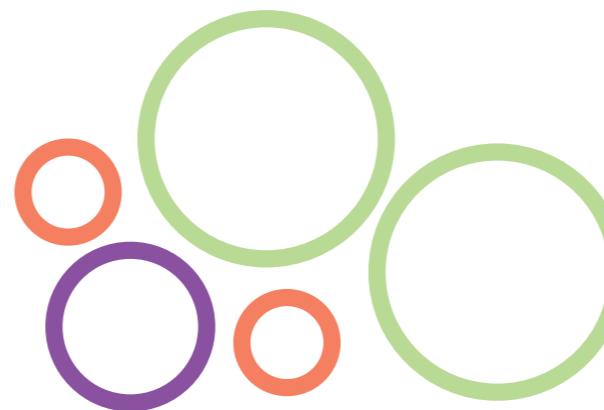
6. Annotation and metabolic prediction

# Metagenomic sequencing

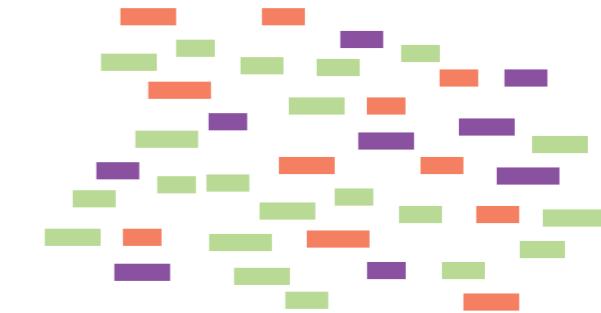
1. Sampling



2. DNA extraction



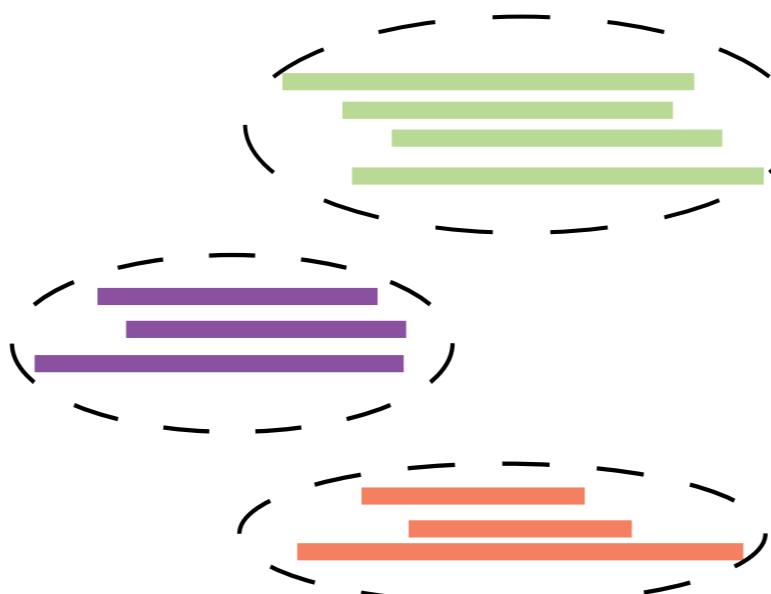
3. All DNA is sheared and sequenced



4. Assembly



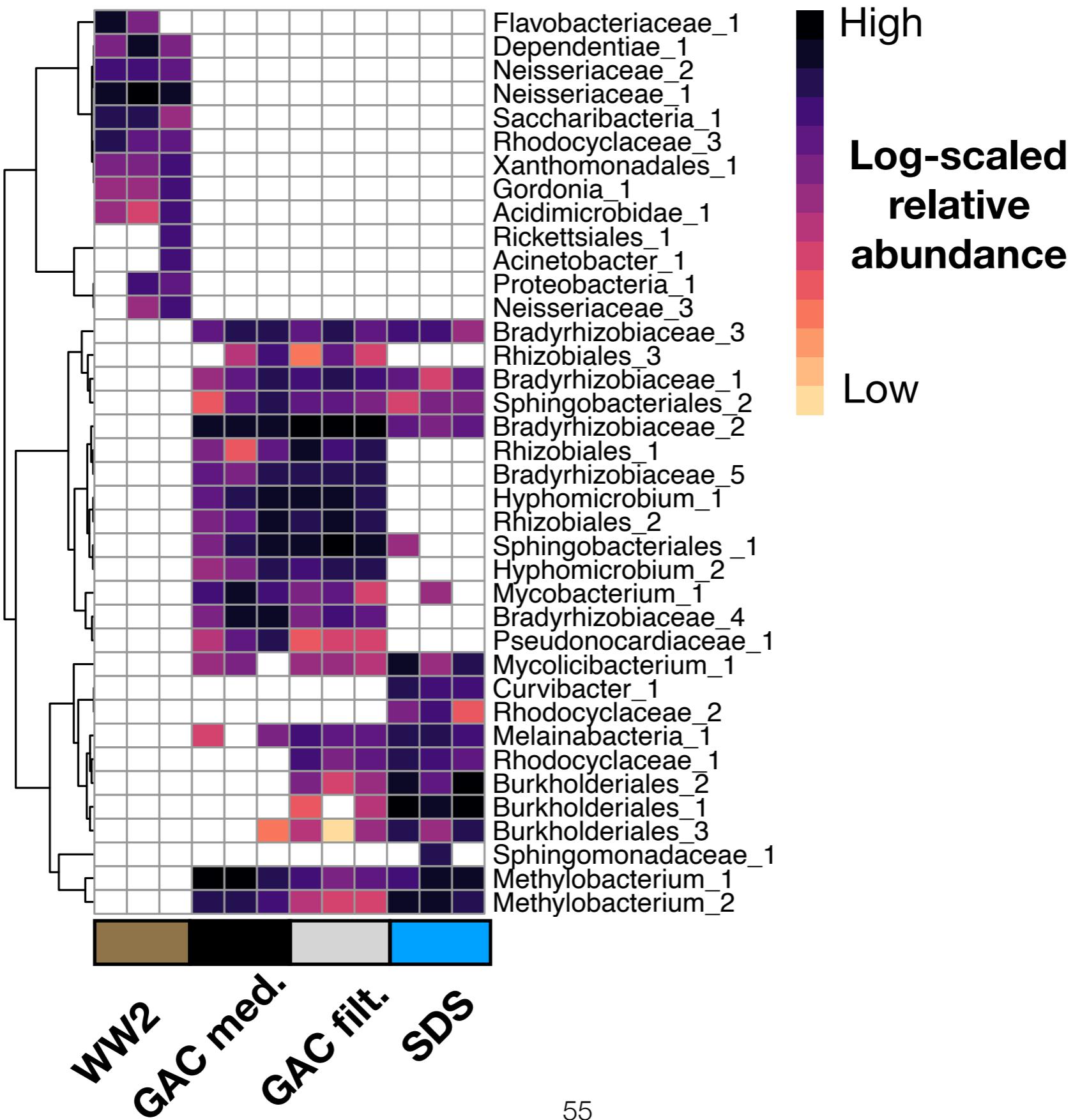
5. Binning



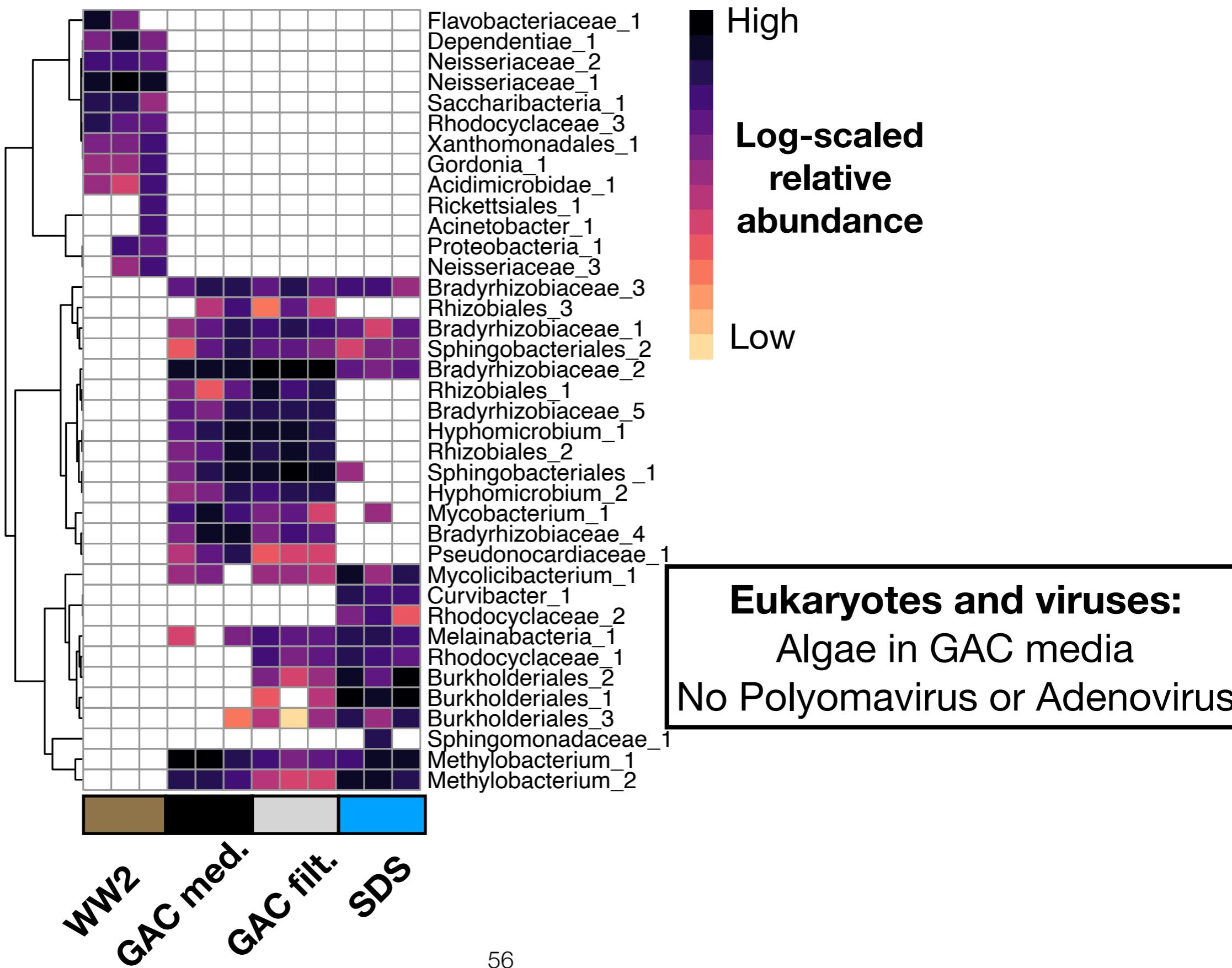
6. Annotation and metabolic prediction



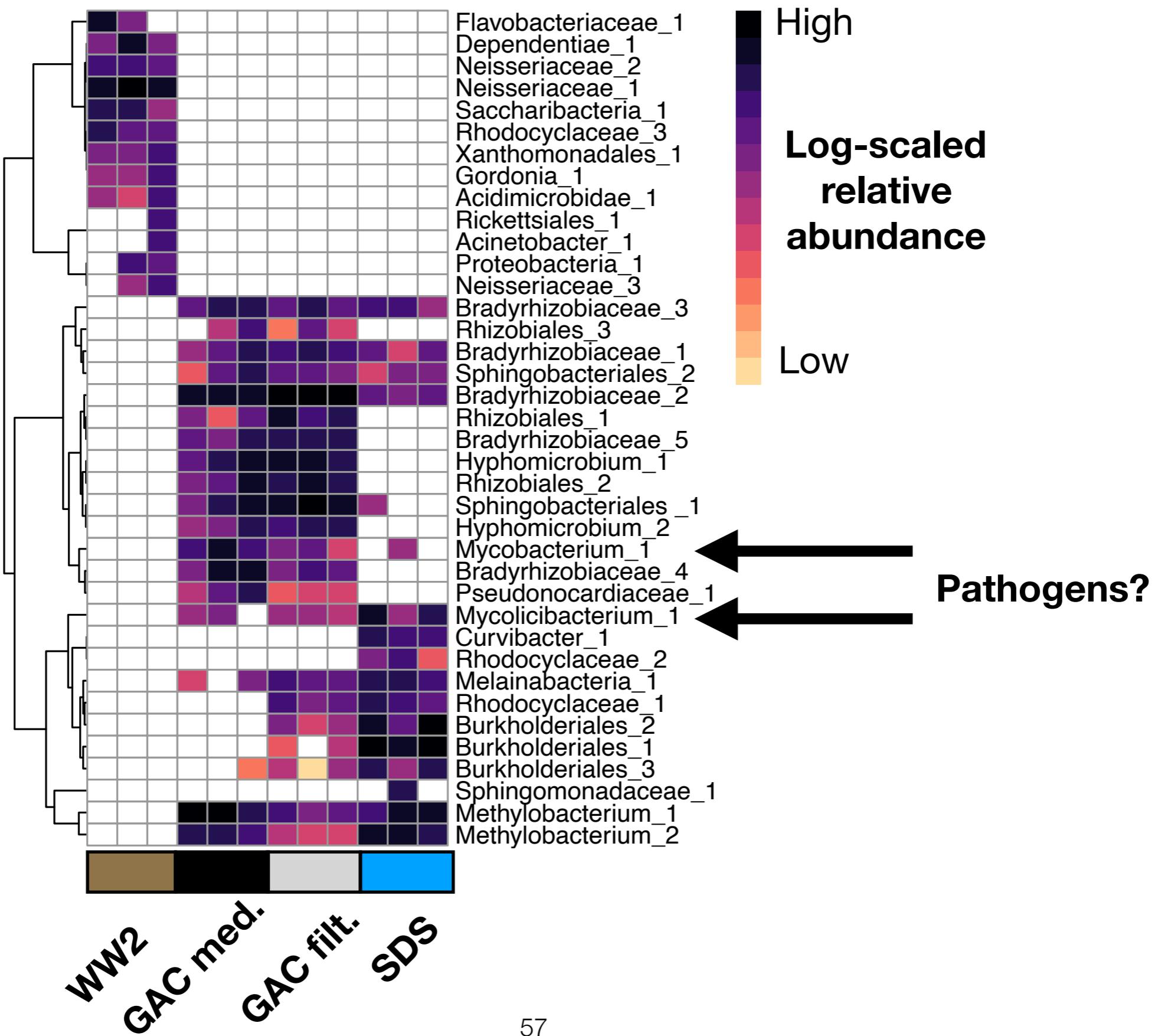
# Bacterial genomes from metagenomes



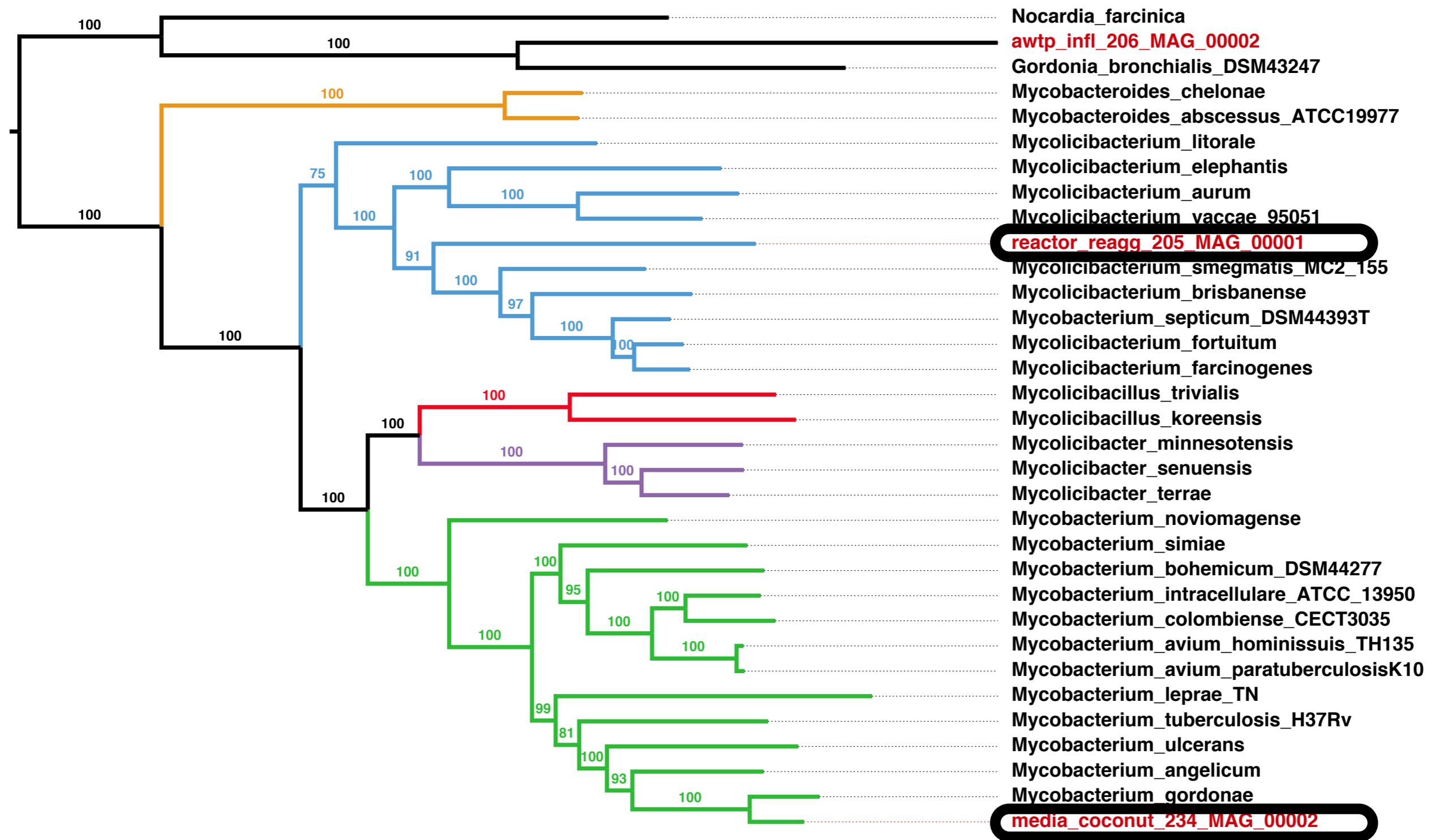
# Bacterial genomes from metagenomes



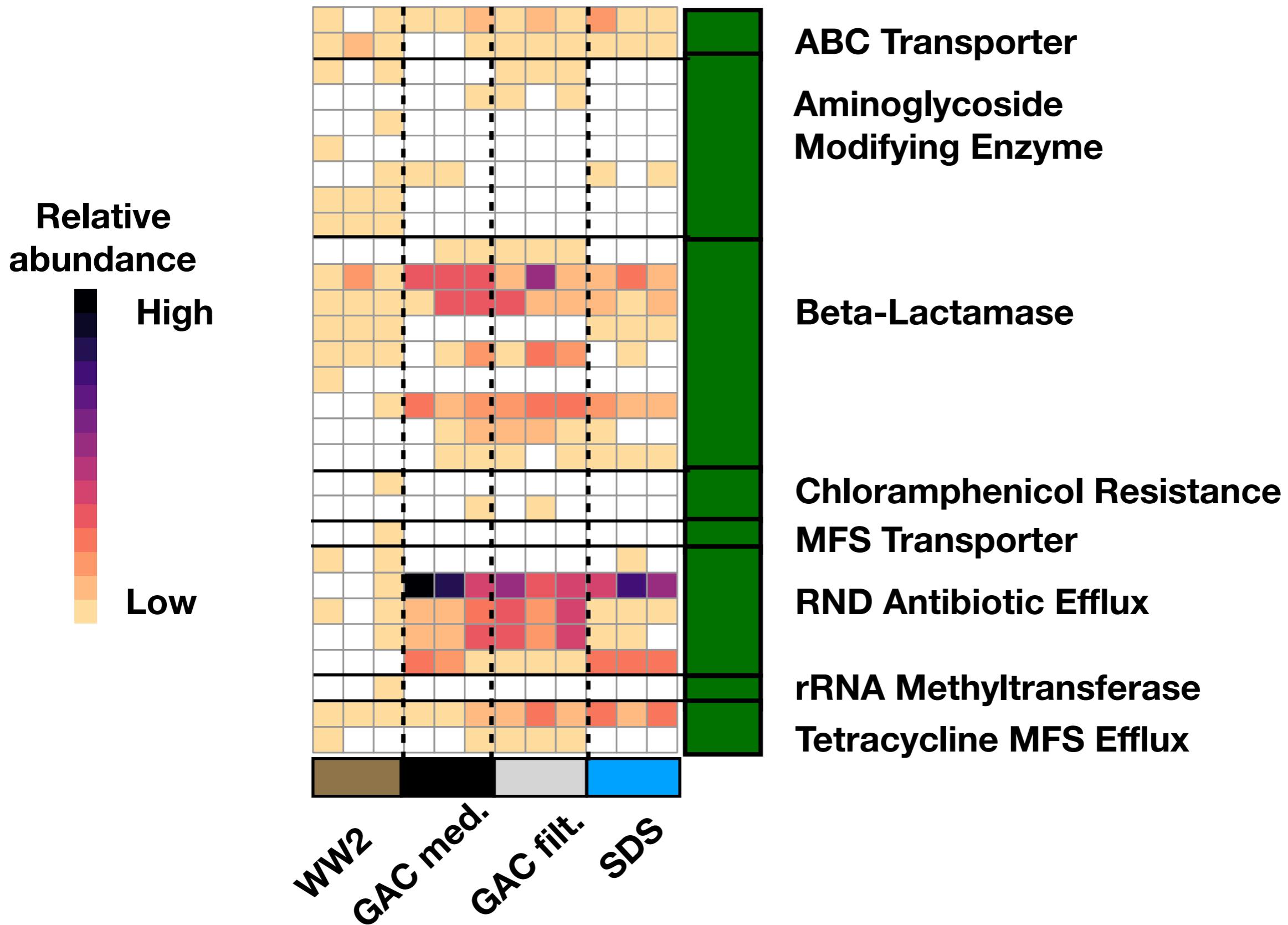
# Bacterial genomes from metagenomes



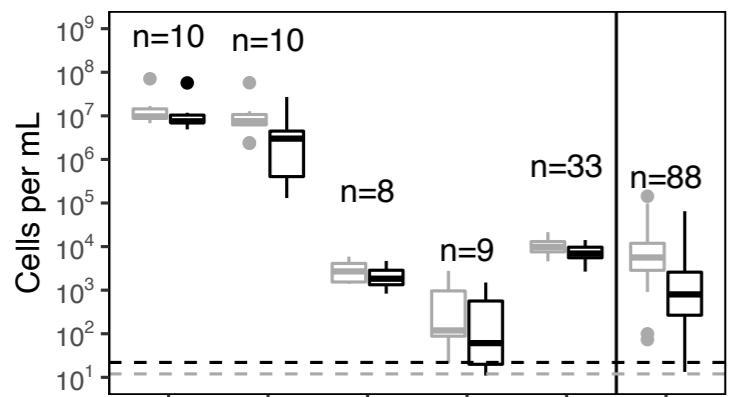
# Potential pathogen: *Mycobacterium gordonaee* relative



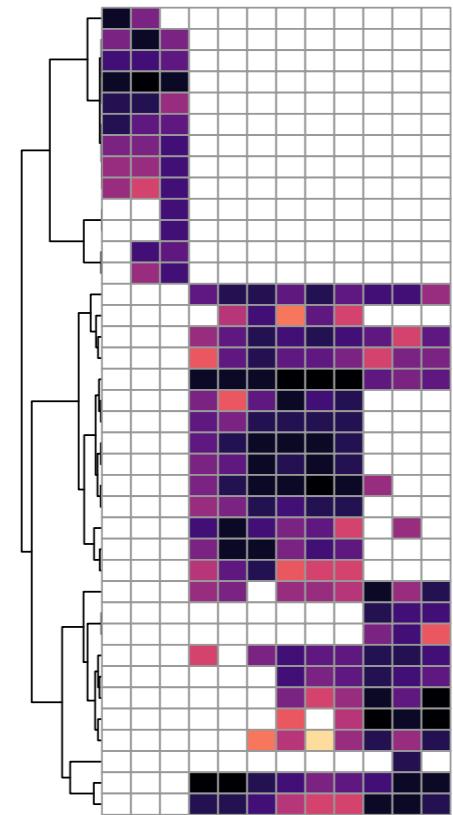
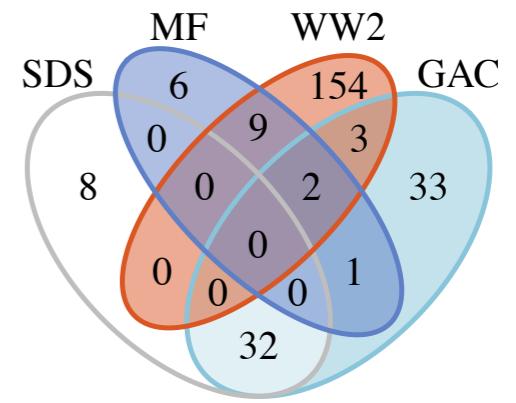
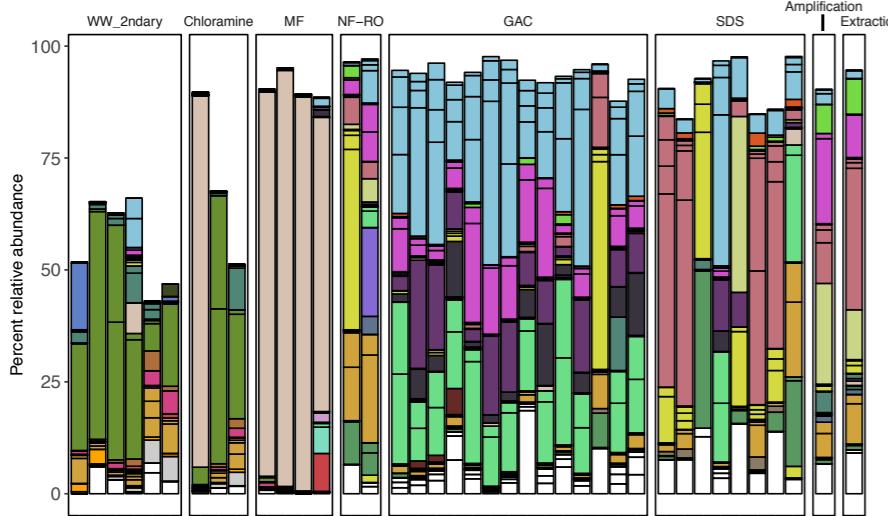
# Antibiotic resistance genes



# Conclusions



- Few bacteria persist through treatment
- Need to consider new growth after treatment
- Different bacteria before and after treatment
  - ARGs
  - Potential pathogens



# Acknowledgements

- Nelson Lab, UC Berkeley
  - Prof. Kara Nelson
  - Scott Miller
  - Lauren Kennedy
- El Paso Water
- Arcadis
- QB3 Berkeley: Functional Genomics Lab and Vincent J. Coates Sequencing facility
- Banfield Lab (computing cluster)



# Questions?

