The microbial world within water reuse

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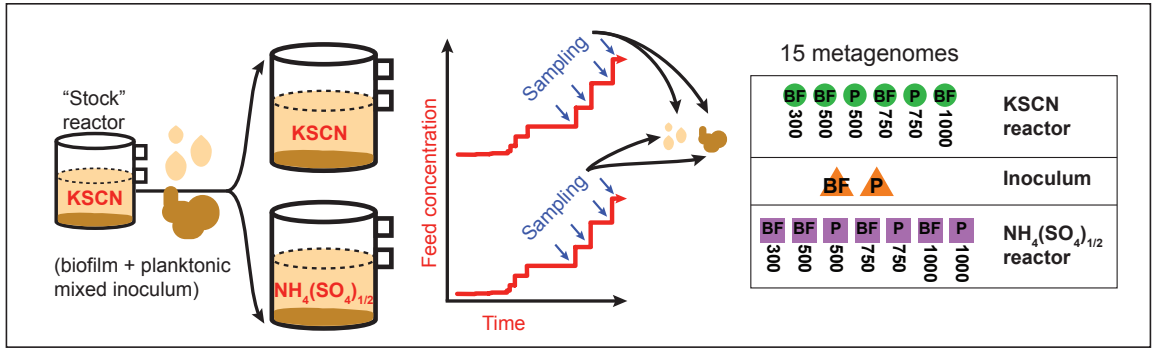
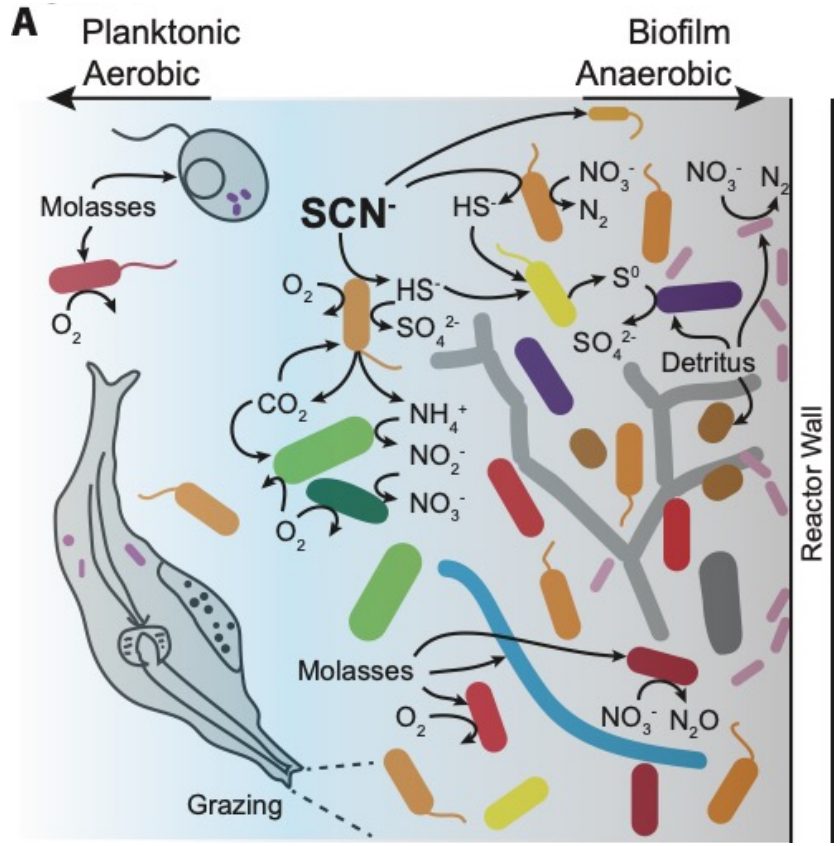
About me

- Ph.D. in Microbiology, Jill Banfield's group, UC Berkeley
  - Centre for Bioprocess Engineering Research, University of Cape Town
- Postdoctoral work Kara Nelson's group, UC Berkeley

rosekantor.github.io

Metagenomics + water engineering

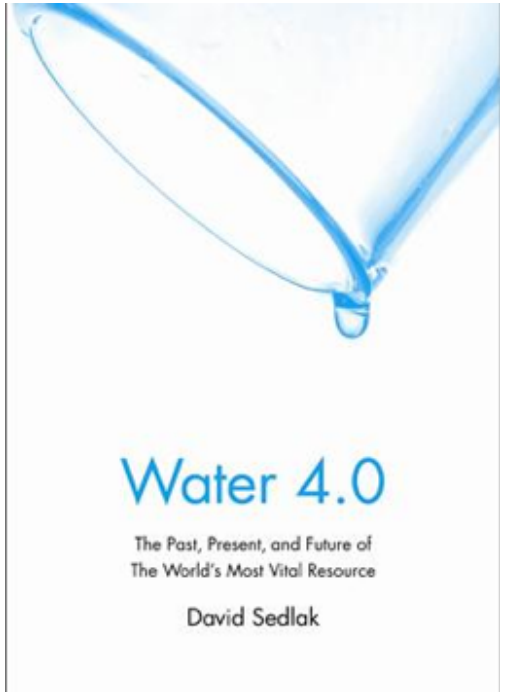




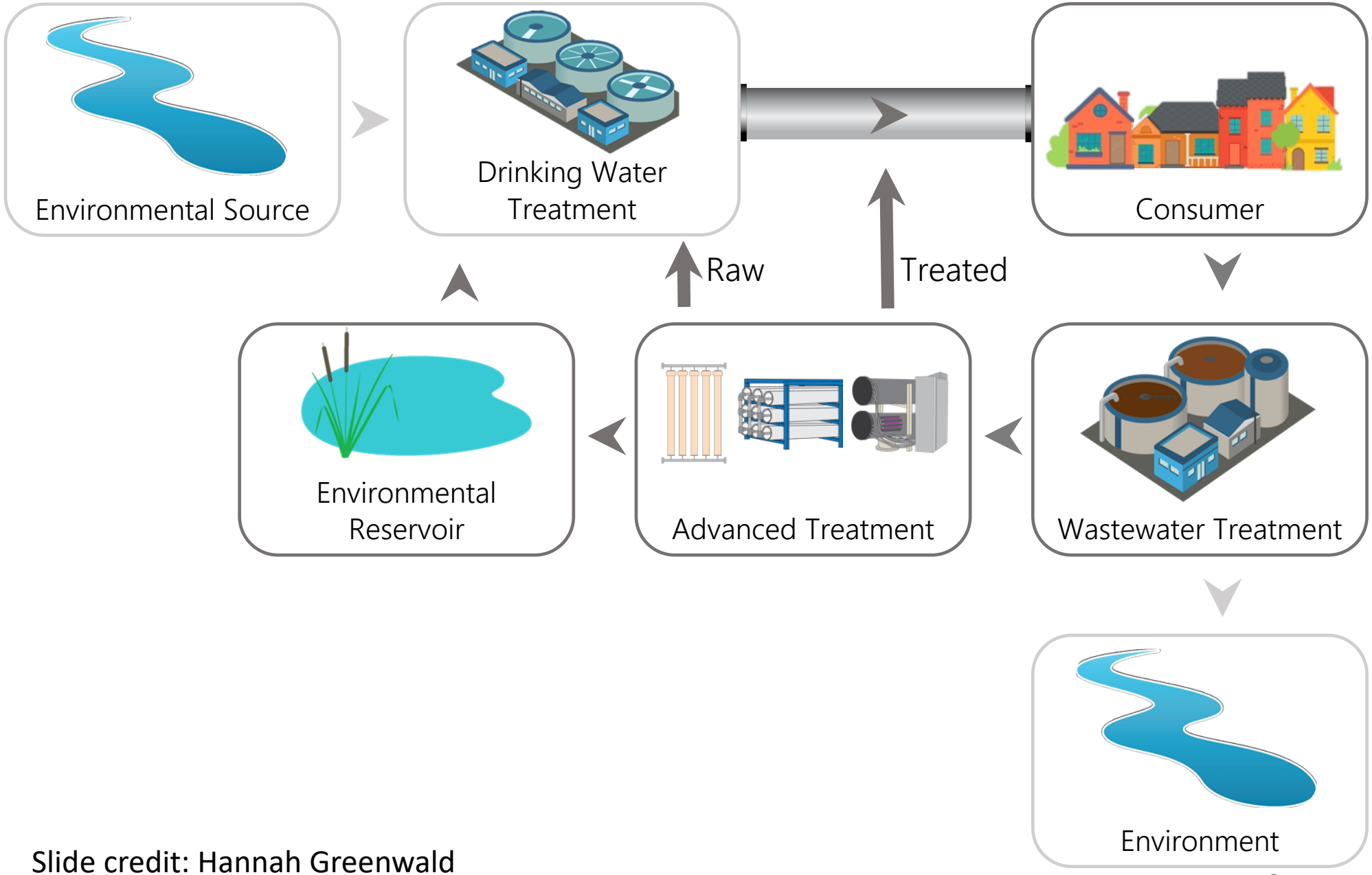
Kantor et al. (2017). ES&T.

Background: potable reuse

Drinking water



The water cycle and potable reuse



Slide credit: Hannah Greenwald

What factors are driving the current push for potable reuse?

https://watereuse.org/educate/water-reuse-101/global-connections/

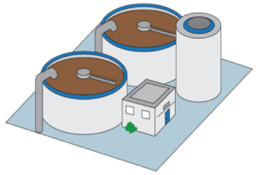
Why are we studying DPR in the Nelson Lab?

To understand how treatment processes affect microbial water quality

To learn what may happen when direct potable reuse water enters a US distribution system

Because California regulations for advanced treatment focus on viruses and protozoans but not bacteria, so they haven't been as well studied

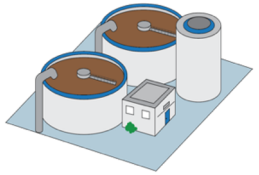
Processes in affecting microorganisms in advanced water treatment

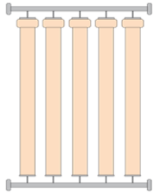


Process	**WWTP:** Wastewater treatment plant
Treatment Mechanism	Biological Physical Removal

Slide modified from Dr. Brian Pecson, Trussell Technologies by Scott Miller and Rose Kantor
Advanced treatment image credit to Emily Marron and Dr. Daniel Daft, Stanford University

Processes in affecting microorganisms in advanced water treatment





Process	**WWTP:** Wastewater treatment plant	**MF:** Micro-filtration	**RO:** Reverse Osmosis
Treatment Mechanism	Biological Physical Removal	Physical Removal	Physical Removal

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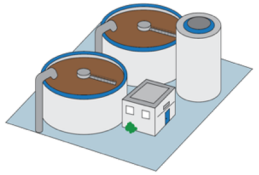
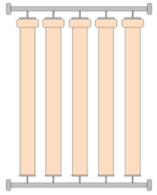
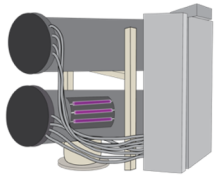
Processes in affecting microorganisms in advanced water treatment







Process	**WWTP:** Wastewater treatment plant	**MF:** Micro-filtration	**RO:** Reverse Osmosis	**UV-AOP:** Ultraviolet-advanced oxidation process
Treatment Mechanism	Biological Physical Removal	Physical Removal	Physical Removal	Oxidation Irradiation

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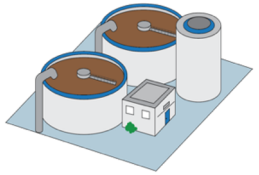
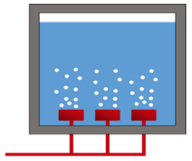
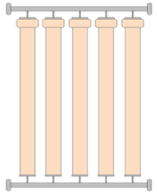
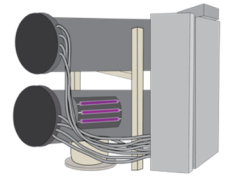
Processes in affecting microorganisms in advanced water treatment





Process	**WWTP:** Wastewater treatment plant	**Ozone**	**BAC:** Biological activated carbon	**MF:** Micro-filtration	**RO:** Reverse Osmosis	**UV-AOP:** Ultraviolet-advanced oxidation process
Treatment Mechanism	Biological Physical Removal	Oxidation	Biological Physical Removal	Physical Removal	Physical Removal	Oxidation Irradiation

Slide modified from Dr. Brian Pecson, Trussell Technologies by Scott Miller and Rose Kantor
Advanced treatment image credit to Emily Marron and Dr. Daniel Daft, Stanford University

Study questions

1. How well does advanced treatment remove bacteria?

2. How does the bacterial community change during treatment?

3. Are the same bacteria present before and after treatment?

4. What are the bacteria capable of doing?

Study design and methods

Methods for studying microbial water quality

Flow cytometry (total and intact cell counts)

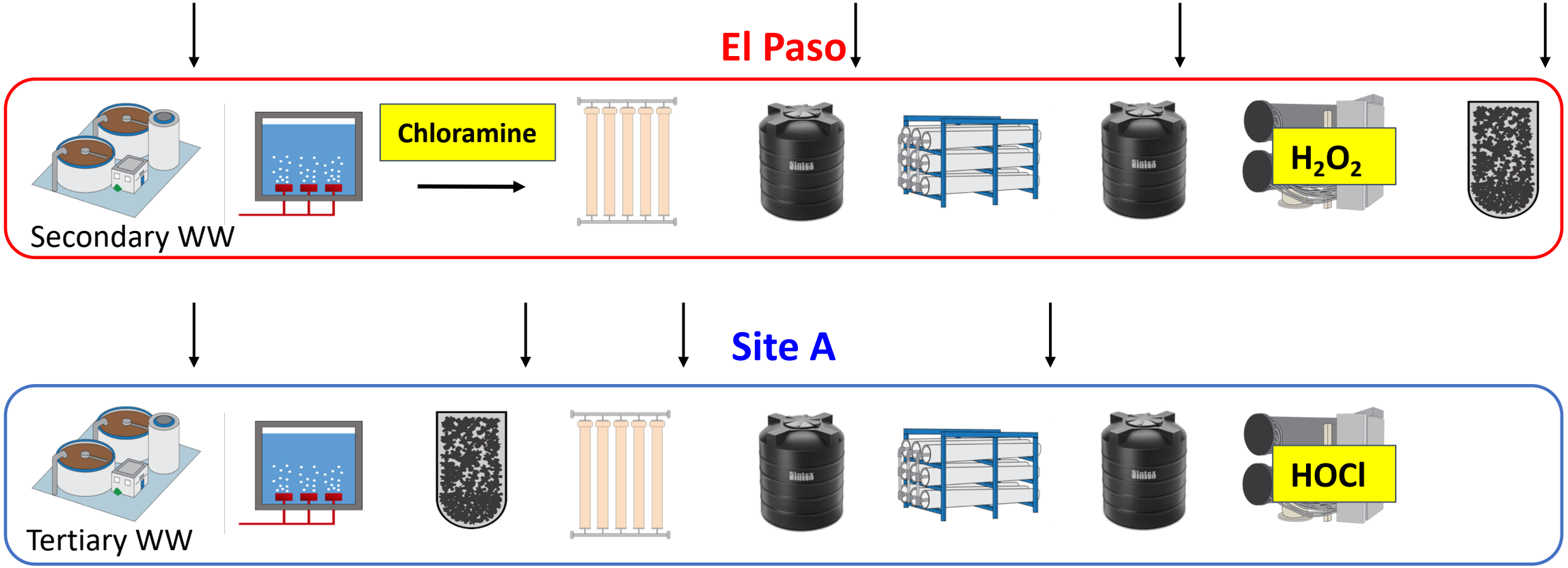
ATP concentration (intracellular and total)

amplicon sequencing (16S rRNA gene V4)

Metagenomics (whole community DNA sequencing)

qPCR (antibiotic resistance genes and pathogens)

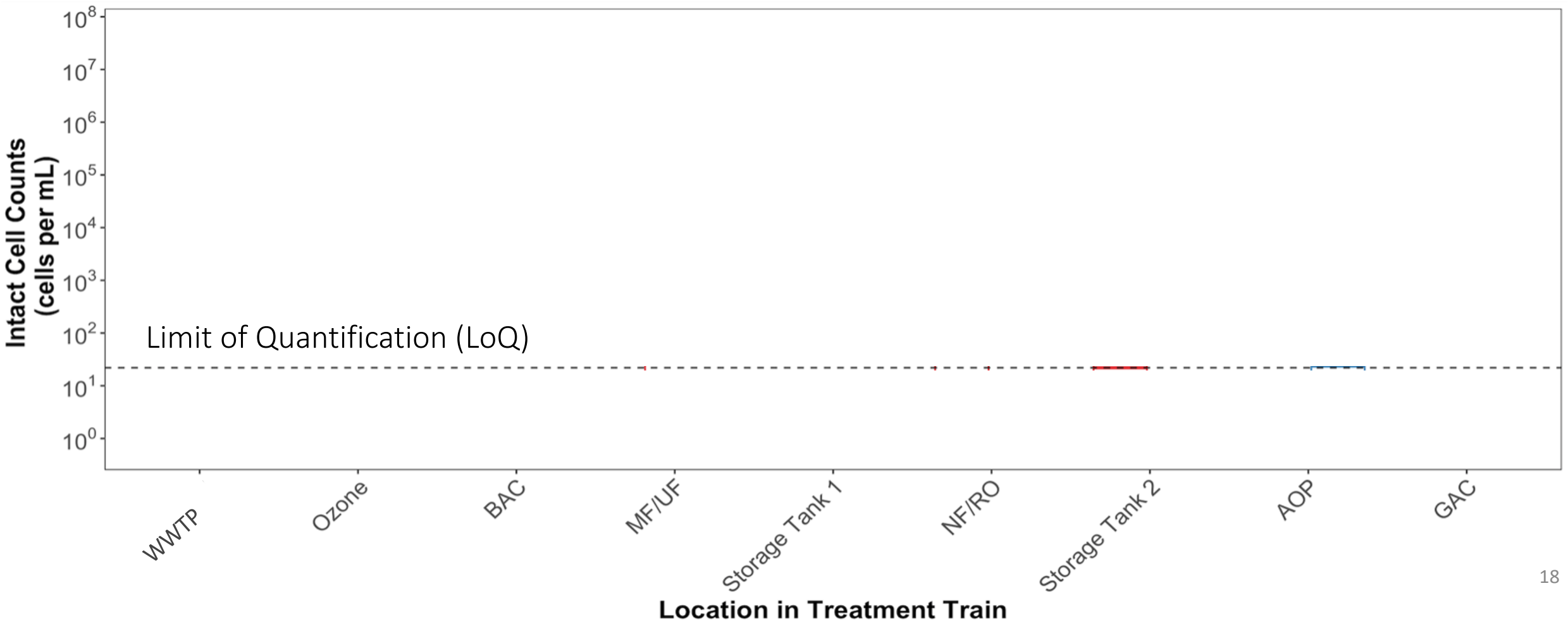
Study designs

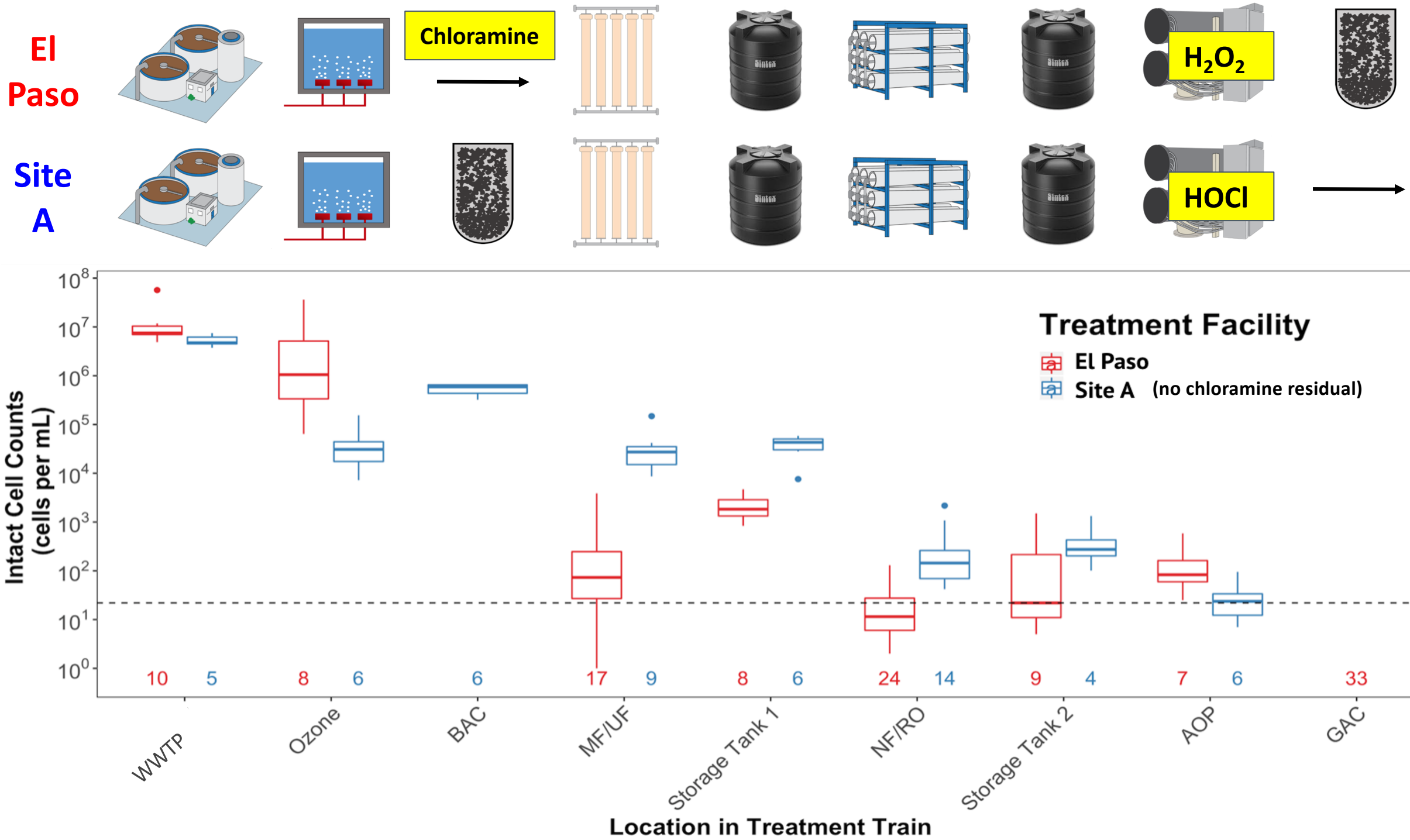


1. How well does advanced treatment remove bacteria?

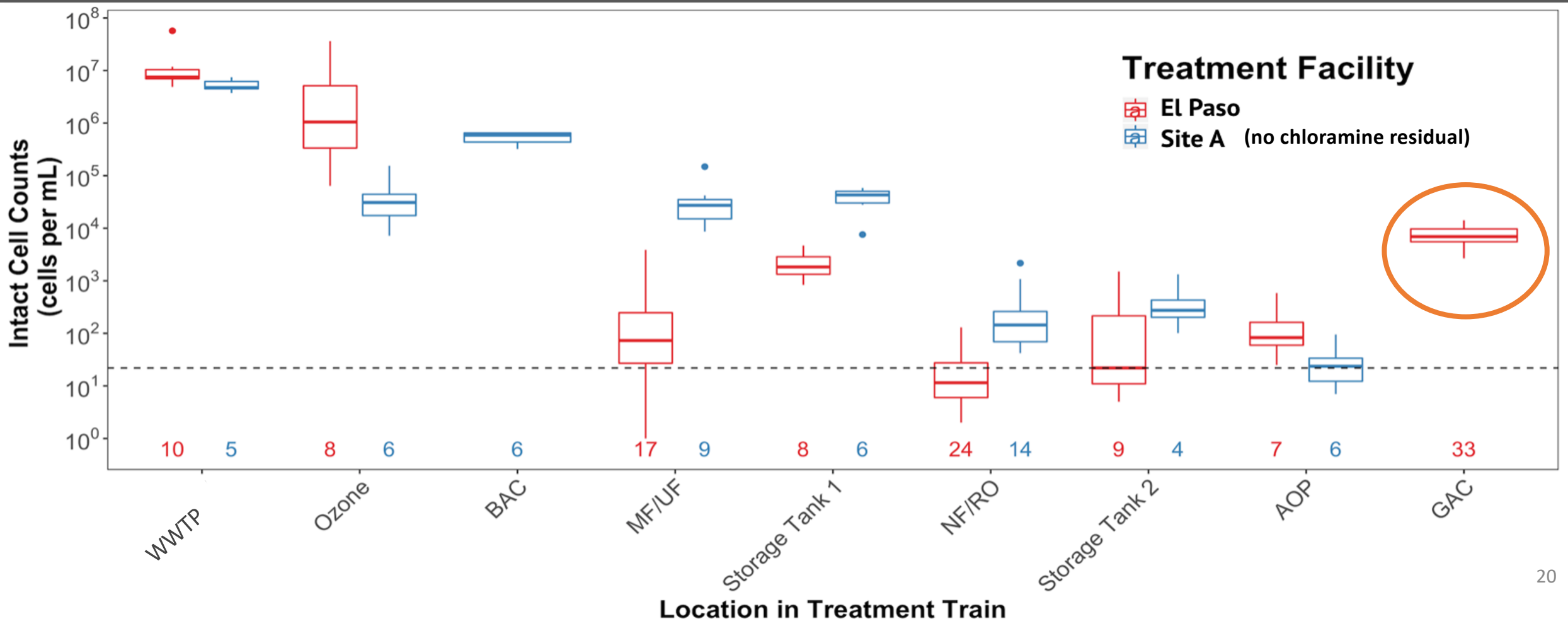
El Paso

Site A

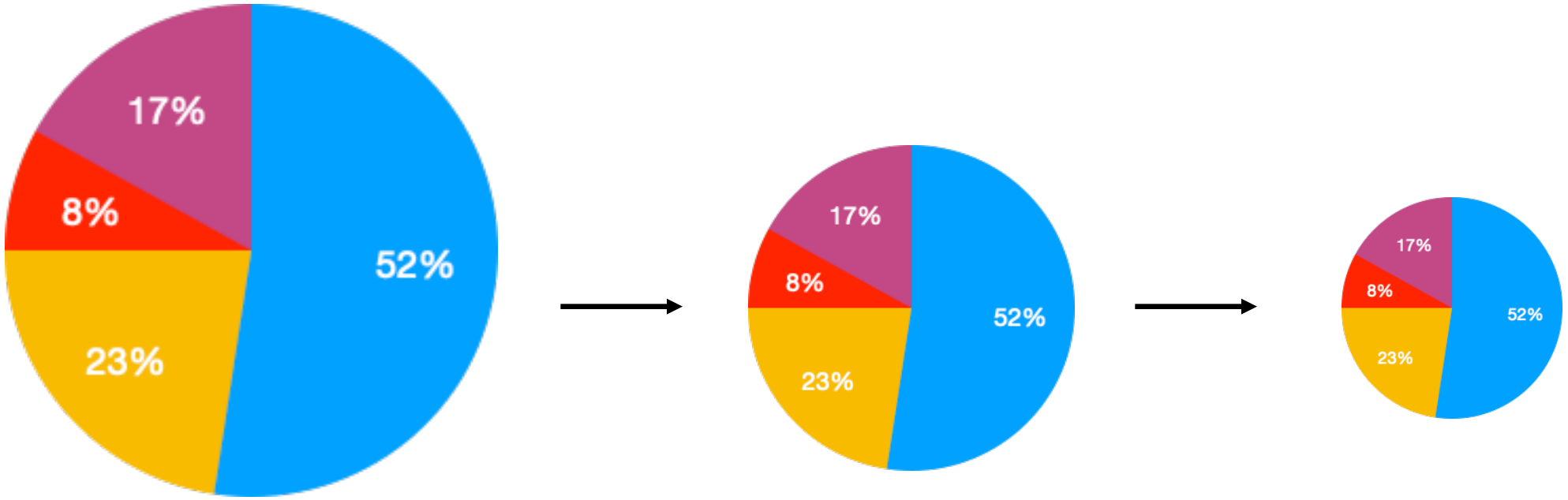



El Paso
Site A
Chloramine
H_2O_2
HOCl
Treatment Facility
El Paso
Site A (no chloramine residual)
Intact Cell Counts (cells per mL)
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
10 5 8 6 6 17 9 8 6 24 14 9 4 7 6 33
WWTP
Ozone
BAC
MF/UF
Storage Tank 1
NF/RO
Storage Tank 2
AOP
GAC
Location in Treatment Train

1. Treatment removes nearly all bacteria but there is growth after treatment

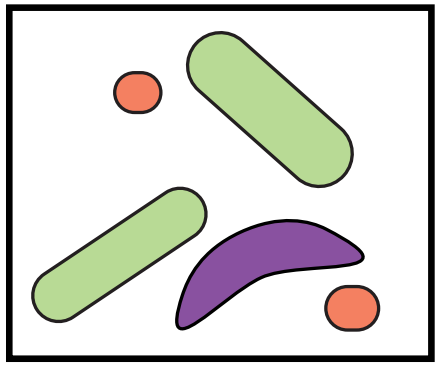


2. How does the bacterial community change during treatment?



2. 16S rRNA gene amplicon sequencing

1. Sampling



- Dead-end ultrafiltration
- Filter back-flush
- PEG flocculation

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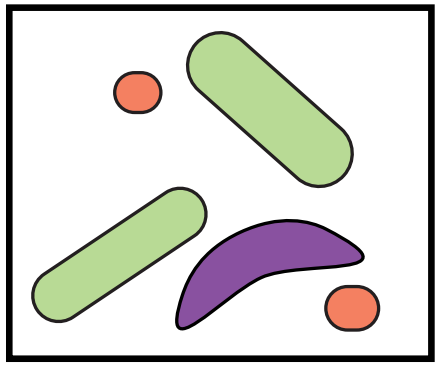
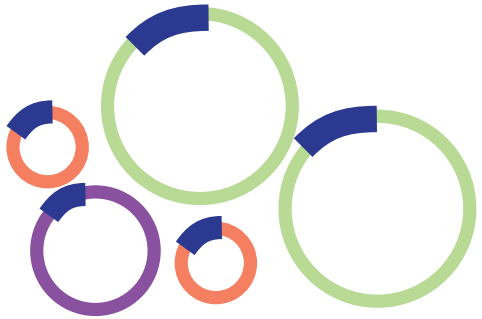


2. DNA extraction

3. Amplification



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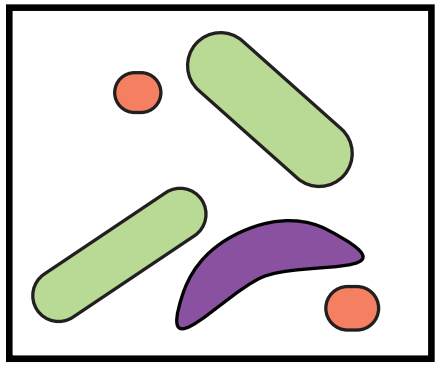

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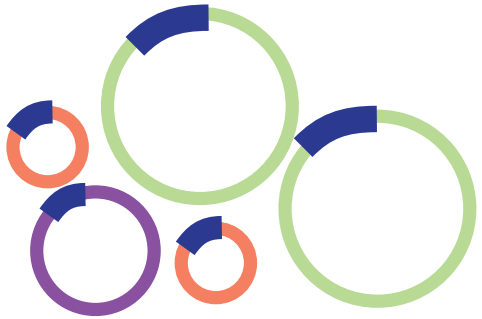

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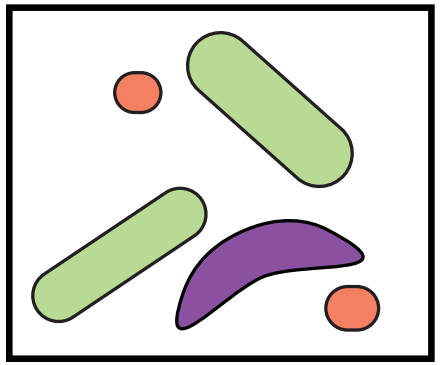

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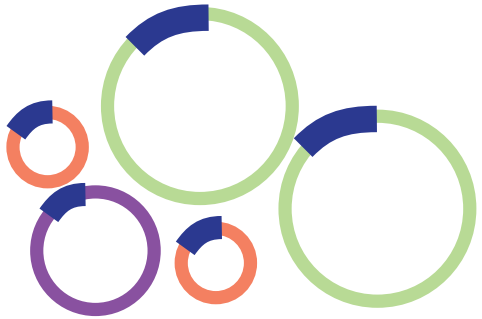
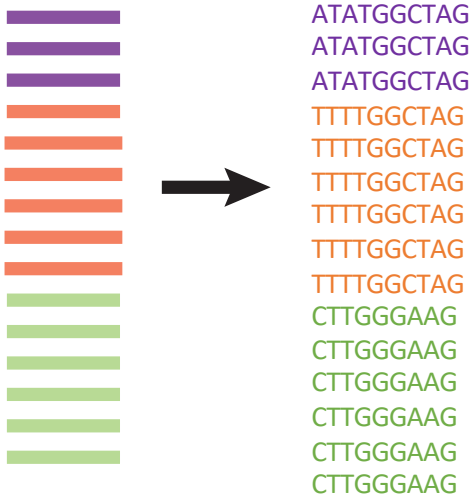


4. Sequencing

(MiSeq v3 150 bp PE)



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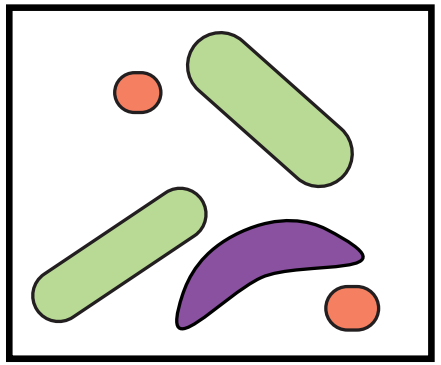

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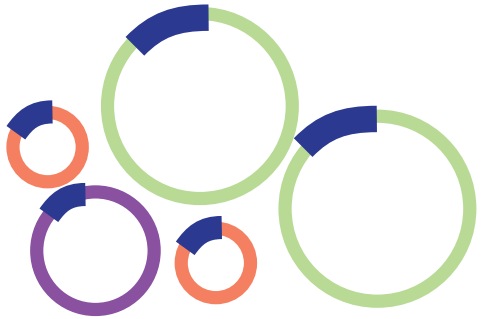

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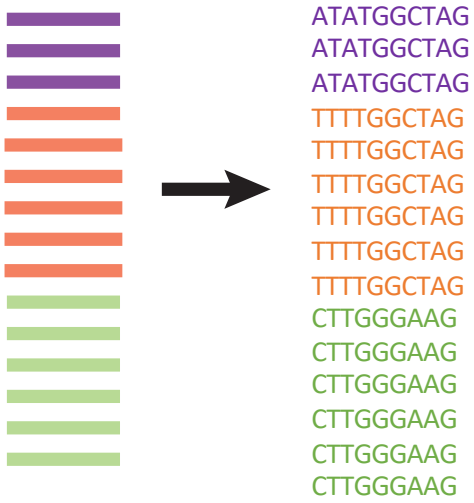
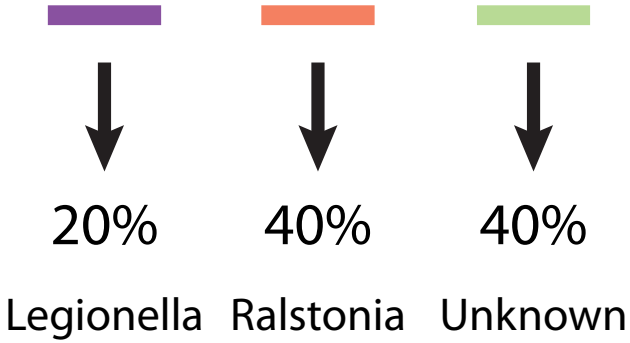


5. Computational Analysis



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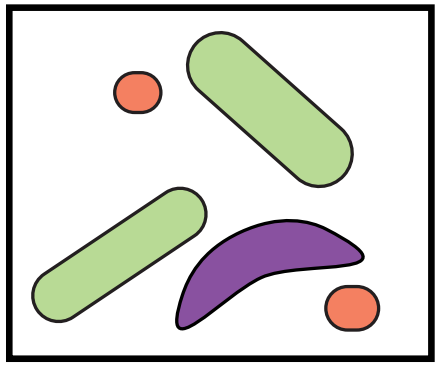

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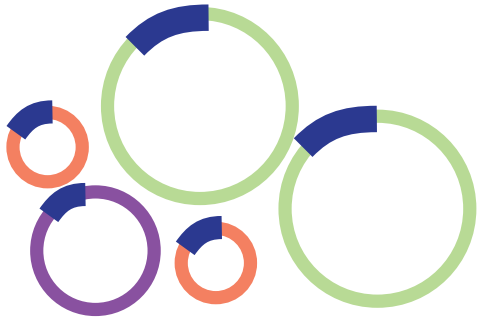

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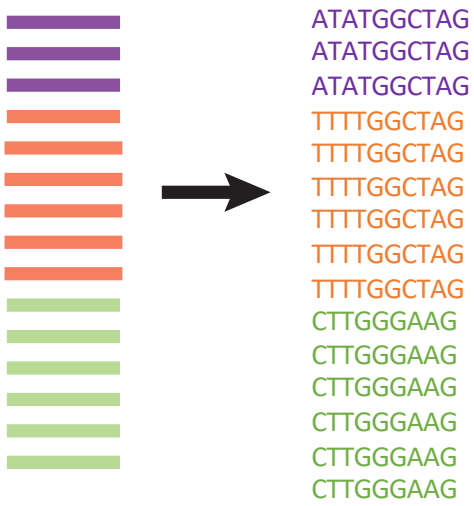

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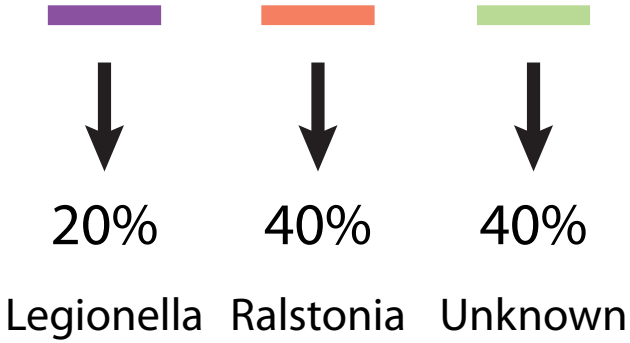
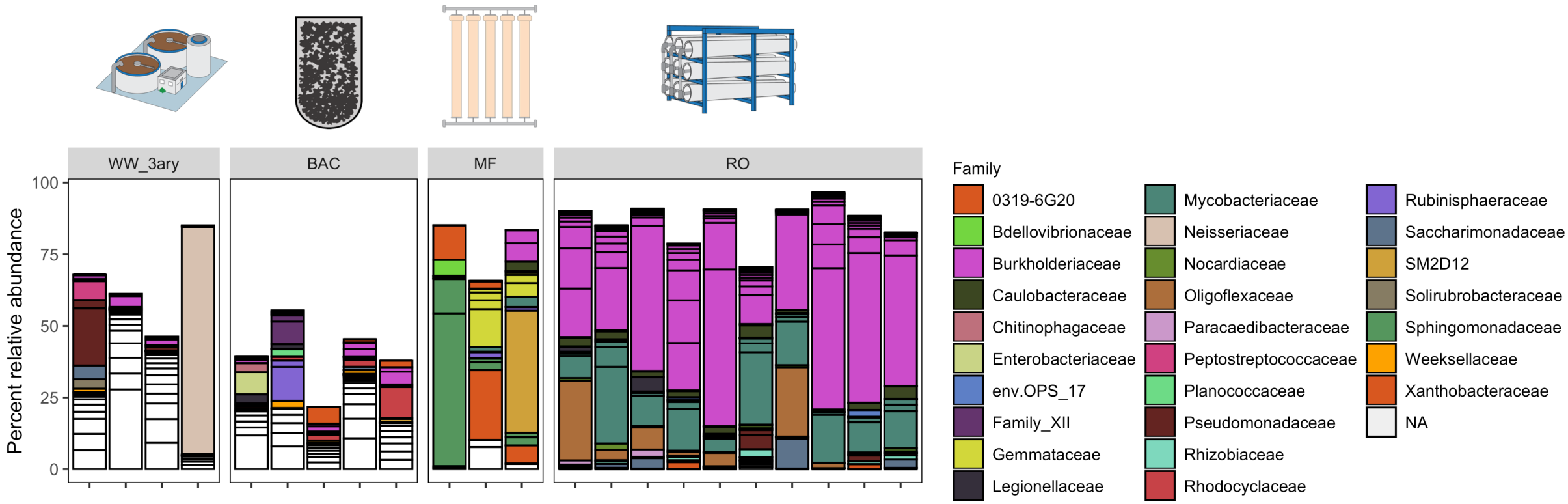


Jupyter notebook with R kernel:

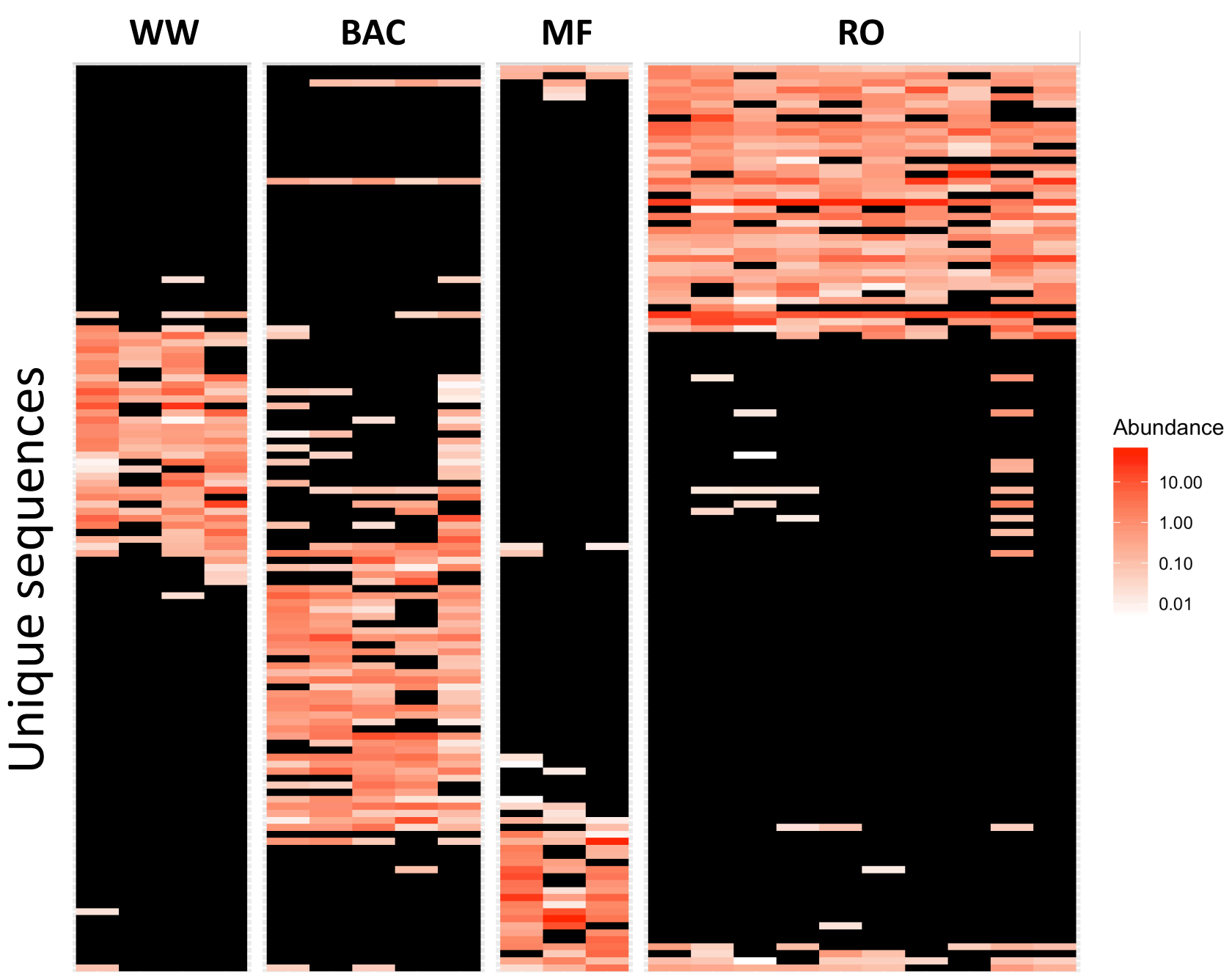
- DADA2
- DESeq2 (decontamination)
- Phyloseq

2. Community composition changes through treatment



Kantor et al. (unpublished).

2. Core community composition changes through treatment



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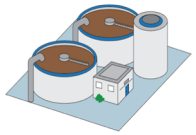
3. Are the same bacteria present before and after treatment?

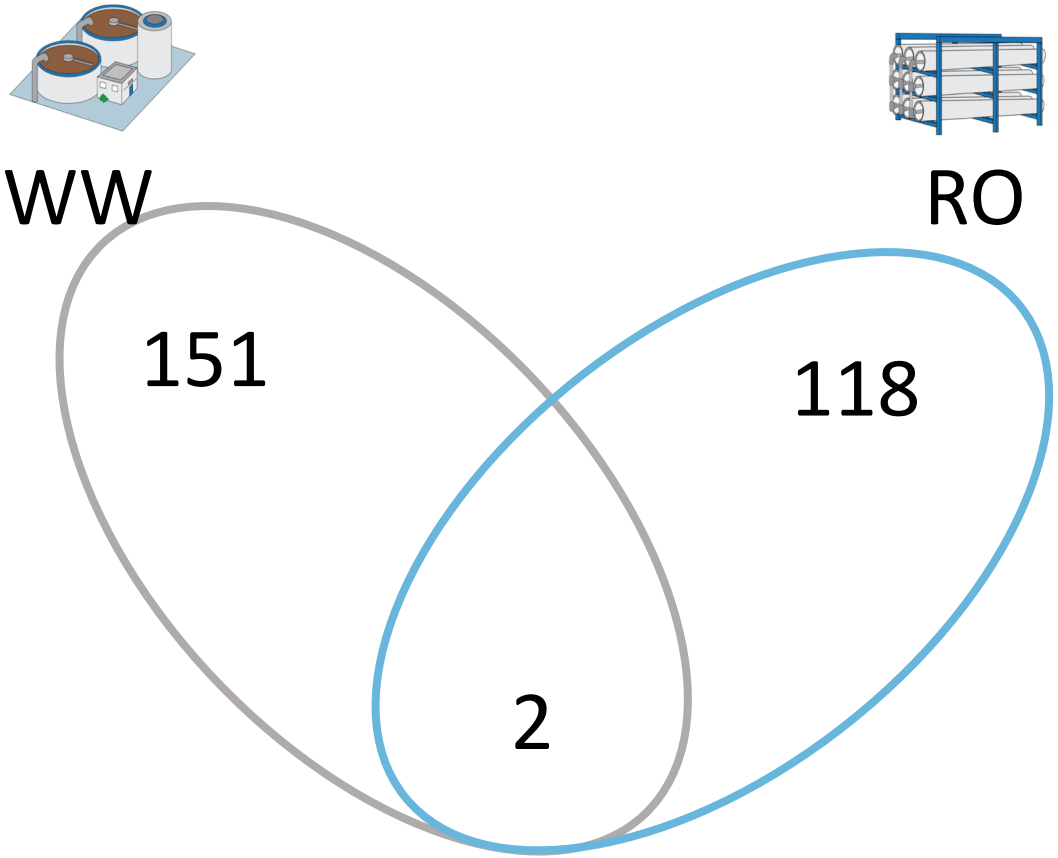


WW

153

Kantor et al. (unpublished)

3. Are the same bacteria present before and after treatment?



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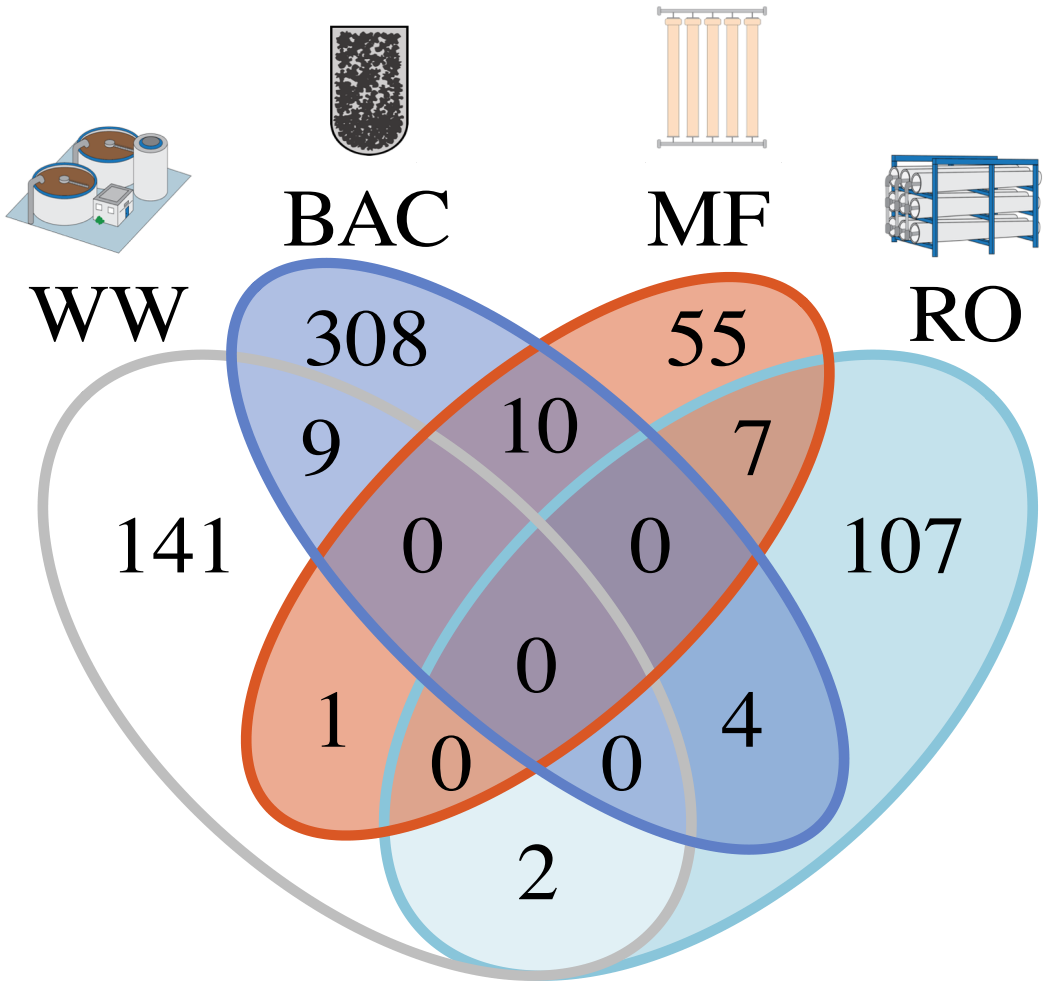


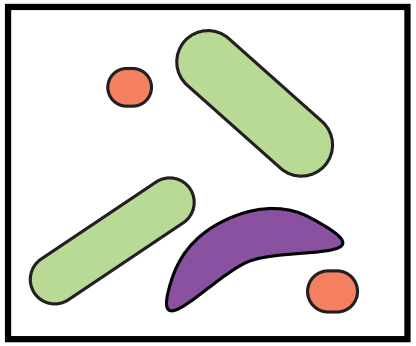
Site A core community overlaps

Kantor et al. (unpublished)

4. What are the metabolic traits of bacteria found in the treatment train?

4. Genome-resolved metagenomics

1. Sampling



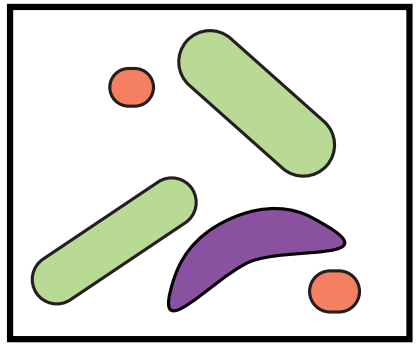
2. DNA extraction

3. Illumina sequencing

(HiSeq/NovaSeq 150 bp paired-end)

4. Genome-resolved metagenomics

1. Sampling

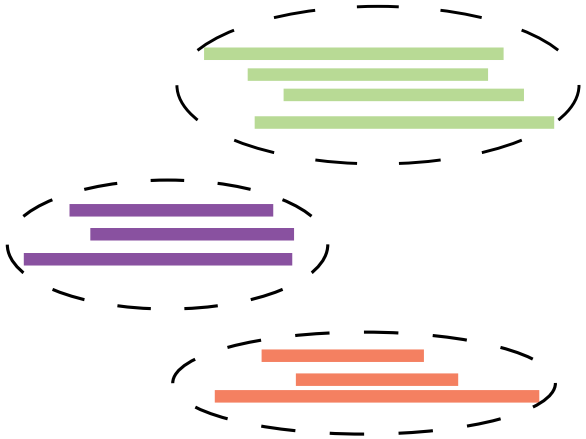


2. DNA extraction

3. Illumina sequencing (HiSeq/NovaSeq 150 bp paired-end)

4. Assembly

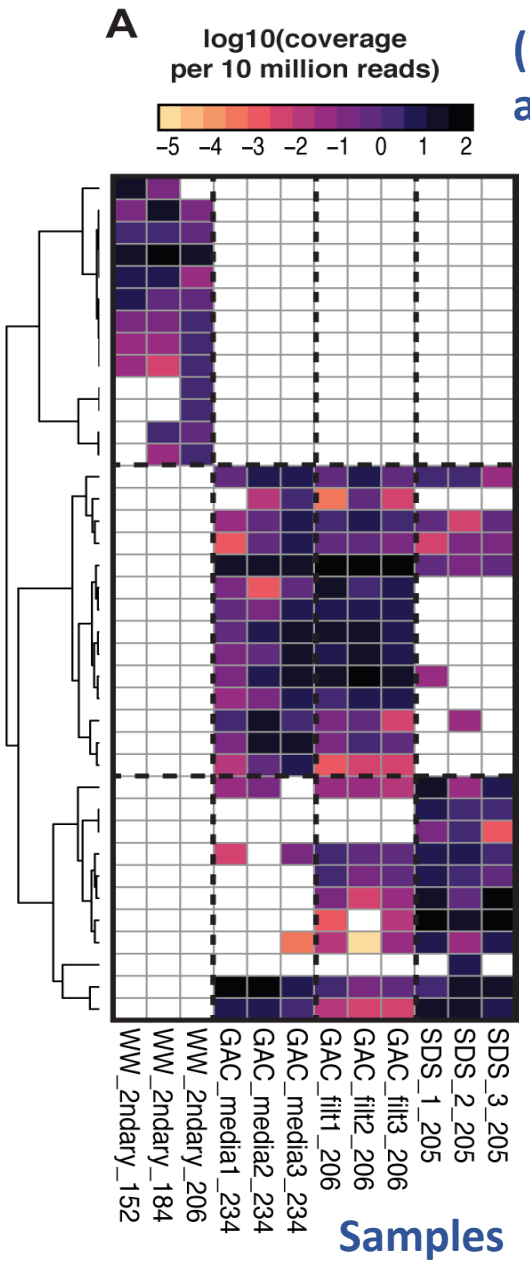
5. Binning



6. Annotation and metabolic prediction

Information gained from genome-resolved metagenomics (El Paso)

Kantor et al. (2019). Front. Micro.



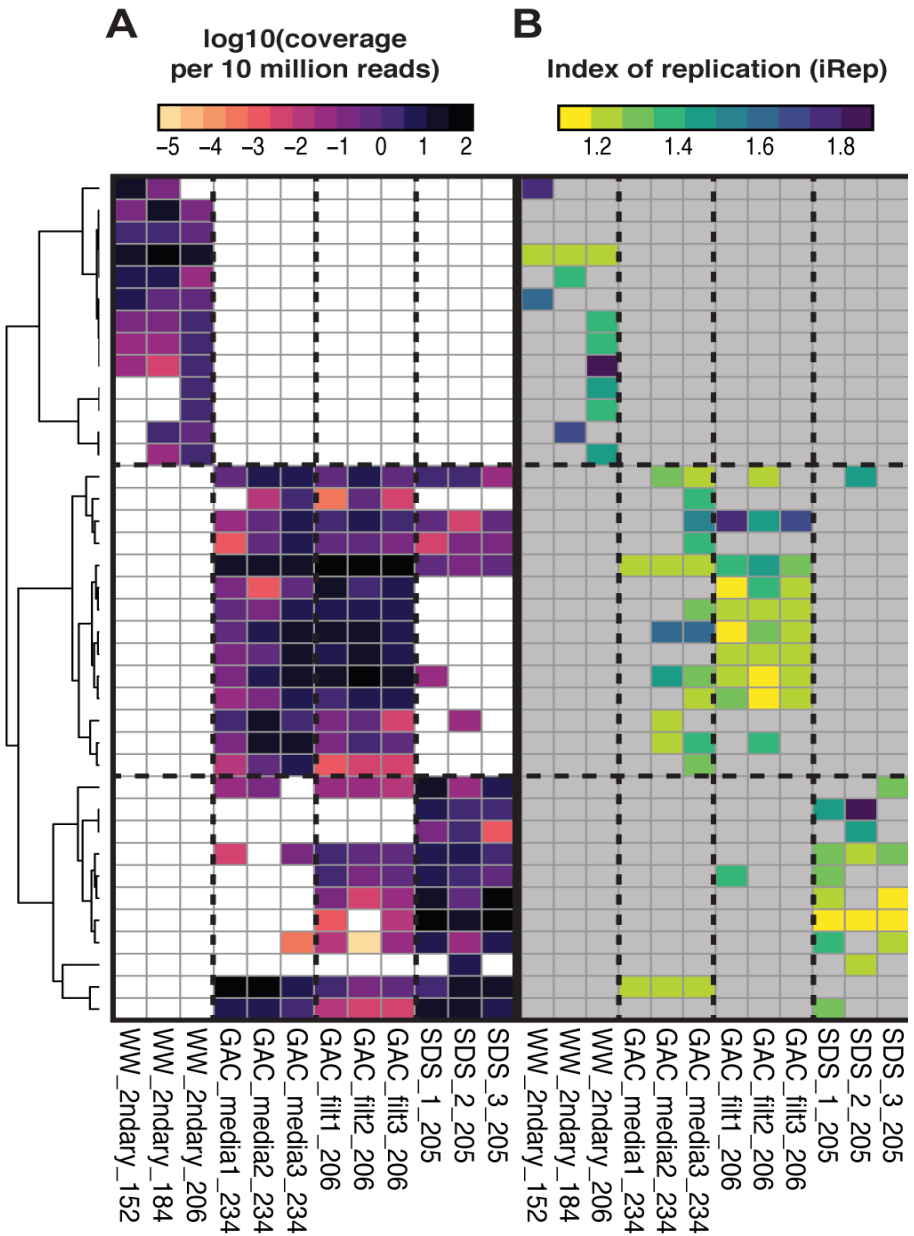
(relative abundance)

Genomes

Flavobacteriaceae_1
Dependentiae_1
Neisseriaceae_2
Neisseriaceae_1
Saccharibacteria_1
Rhodocyclaceae_3
Xanthomonadales_1
Gordonia_1
Acidimicrobidae_1
Rickettsiales_1
Acinetobacter_1
Proteobacteria_1
Neisseriaceae_3
Bradyrhizobiaceae_3
Rhizobiales_3
Bradyrhizobiaceae_1
Sphingobacteriales_2
Bradyrhizobiaceae_2
Rhizobiales_1
Bradyrhizobiaceae_5
Hyphomicrobium_1
Rhizobiales_2
Sphingobacteriales _1
Hyphomicrobium_2
Mycobacterium_1
Bradyrhizobiaceae_4
Pseudonocardiaceae_1
Mycolicibacterium_1
Curvibacter_1
Rhodocyclaceae_2
Melainabacteria_1
Rhodocyclaceae_1
Burkholderiales_2
Burkholderiales_1
Burkholderiales_3
Sphingomonadaceae_1
Methylobacterium_1
Methylobacterium_2

Information gained from genome-resolved metagenomics (El Paso)

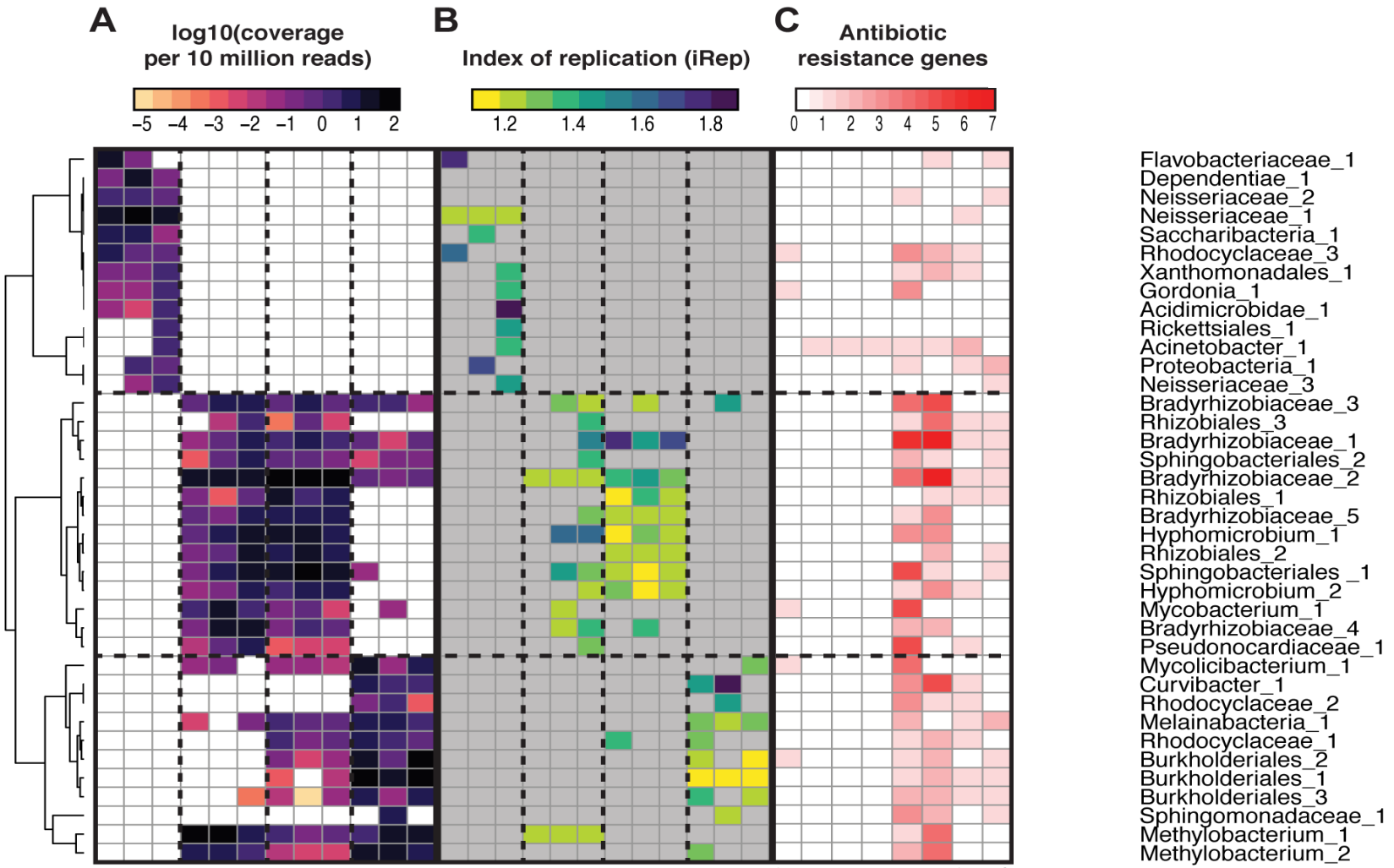
Kantor et al. (2019). Front. Micro.



Flavobacteriaceae_1
Dependentiae_1
Neisseriaceae_2
Neisseriaceae_1
Saccharibacteria_1
Rhodocyclaceae_3
Xanthomonadales_1
Gordonia_1
Acidimicrobidae_1
Rickettsiales_1
Acinetobacter_1
Proteobacteria_1
Neisseriaceae_3
Bradyrhizobiaceae_3
Rhizobiales_3
Bradyrhizobiaceae_1
Sphingobacteriales_2
Bradyrhizobiaceae_2
Rhizobiales_1
Bradyrhizobiaceae_5
Hyphomicrobium_1
Rhizobiales_2
Sphingobacteriales _1
Hyphomicrobium_2
Mycobacterium_1
Bradyrhizobiaceae_4
Pseudonocardiaceae_1
Mycolicibacterium_1
Curvibacter_1
Rhodocyclaceae_2
Melainabacteria_1
Rhodocyclaceae_1
Burkholderiales_2
Burkholderiales_1
Burkholderiales_3
Sphingomonadaceae_1
Methylobacterium_1
Methylobacterium_2

Information gained from genome-resolved metagenomics (El Paso)

Kantor et al. (2019). Front. Micro.



Flavobacteriaceae_1
Dependentiae_1
Neisseriaceae_2
Neisseriaceae_1
Saccharibacteria_1
Rhodocyclaceae_3
Xanthomonadales_1
Gordonia_1
Acidimicrobidae_1
Rickettsiales_1
Acinetobacter_1
Proteobacteria_1
Neisseriaceae_3
Bradyrhizobiaceae_3
Rhizobiales_3
Bradyrhizobiaceae_1
Sphingobacteriales_2
Bradyrhizobiaceae_2
Rhizobiales_1
Bradyrhizobiaceae_5
Hyphomicrobium_1
Rhizobiales_2
Sphingobacteriales _1
Hyphomicrobium_2
Mycobacterium_1
Bradyrhizobiaceae_4
Pseudonocardiaceae_1
Mycolicibacterium_1
Curvibacter_1
Rhodocyclaceae_2
Melainabacteria_1
Rhodocyclaceae_1
Burkholderiales_2
Burkholderiales_1
Burkholderiales_3
Sphingomonadaceae_1
Methylobacterium_1
Methylobacterium_2

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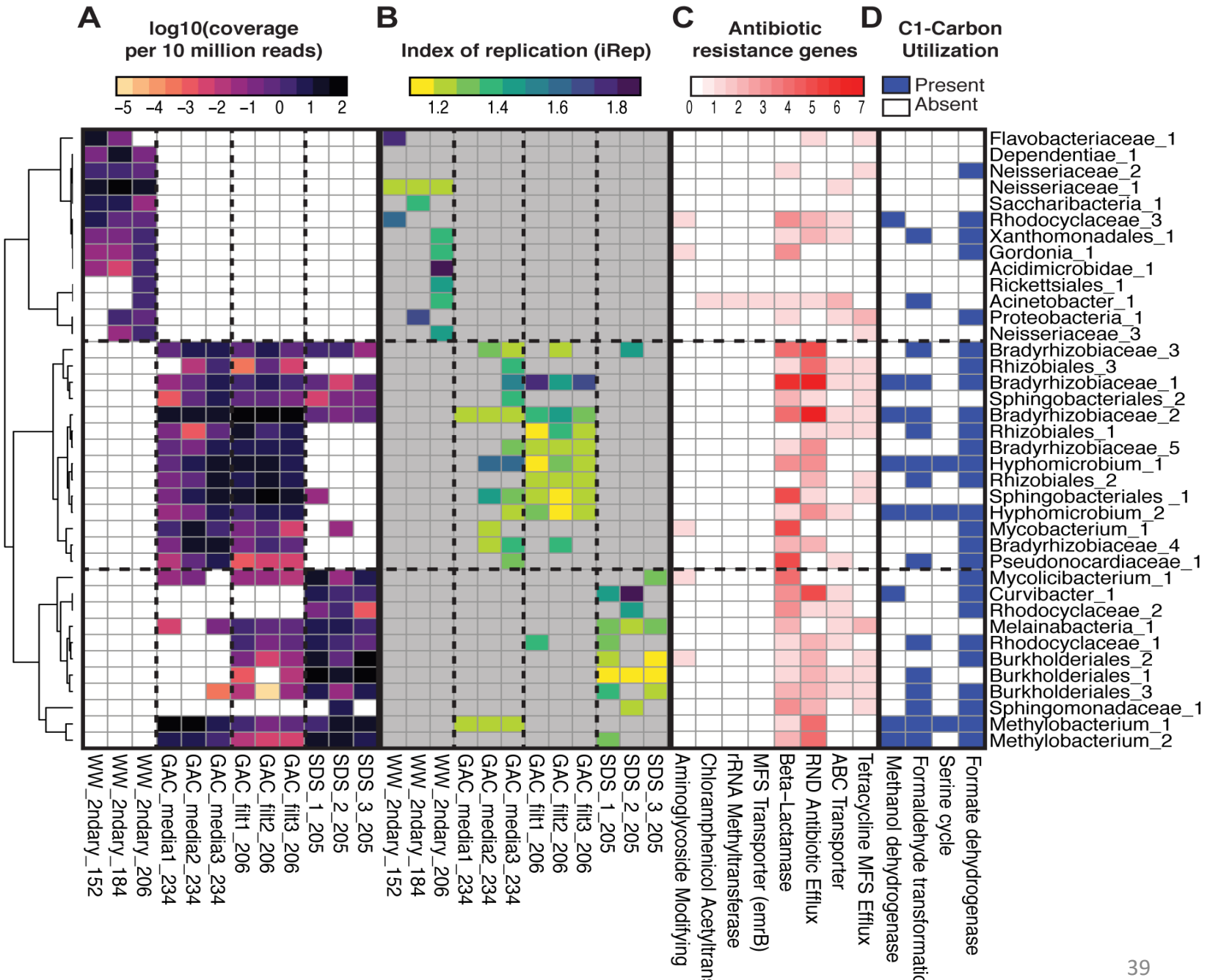
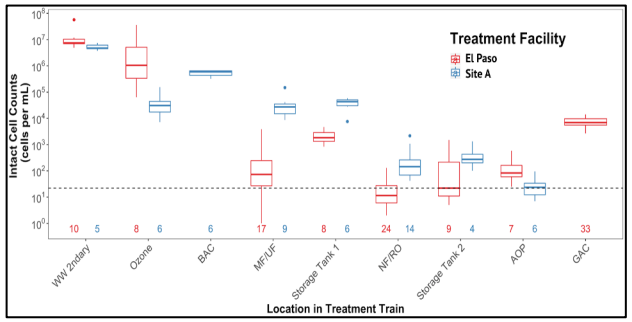
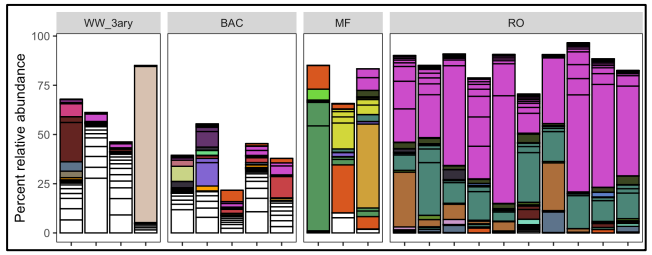


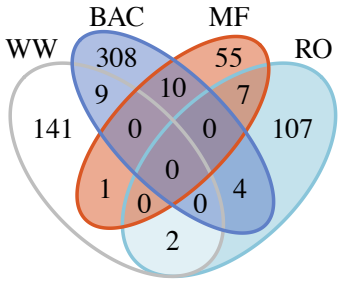
Conclusions



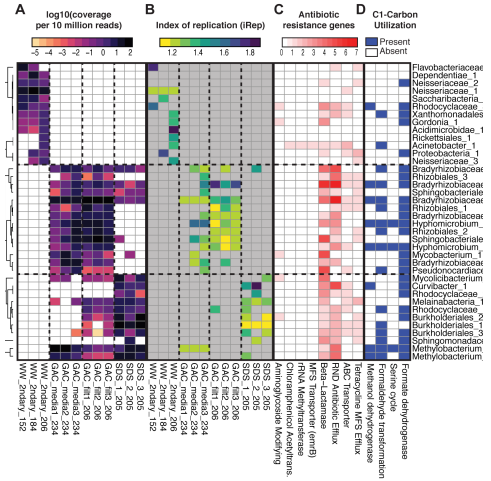
1. Bacteria are nearly completely removed by treatment, growth afterward



2. Community composition changes with each treatment process



3. Different bacteria are present before and after treatment



4. Growth rates, antibiotic resistance, C1-carbon metabolism

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- QB3 Vincent J. Coates Genome Sequencing Lab, UC Berkeley
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- El Paso Water
- Arcadis