

**INTERNATIONAL SYMPOSIUM**  
**Biological Treatment & Potable Reuse**

# Profiling Microbial Communities in Advanced Treatment Trains with High-Throughput Sequencing

Rose Kantor Ph.D., Scott Miller Ph.D., Lauren Kennedy,  
Prof. Kara Nelson



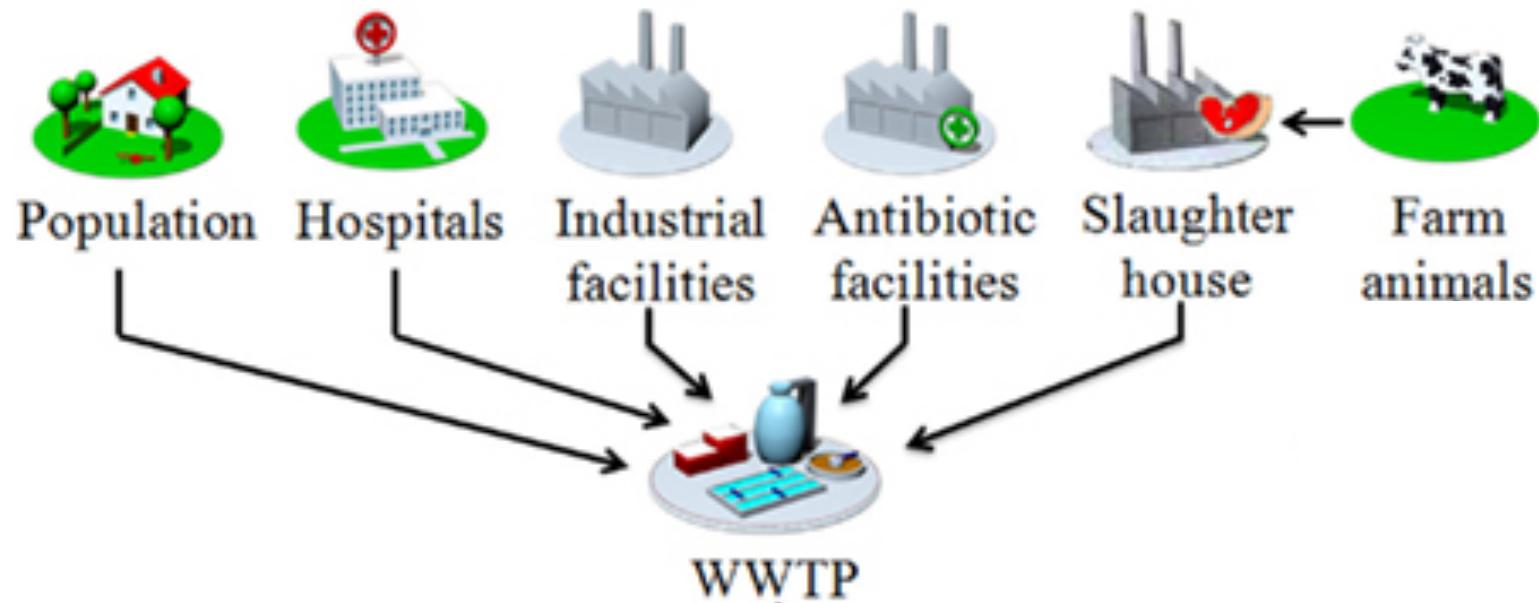
University of California, Berkeley  
ReNUWIt NSF-ERC



# Outline

- Background on antibiotic resistance and removal by advanced treatment
- Our recent findings on removal of bacteria and resistance genes from two AWTPs

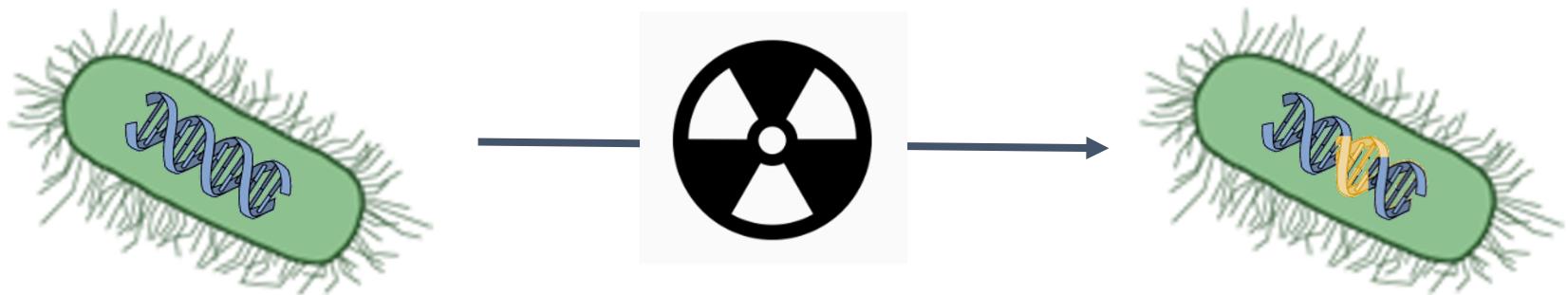
## Background: Wastewater as a hotspot for antibiotic resistance



- High density of human-associated bacteria
- High concentrations of antibiotics that select for resistance

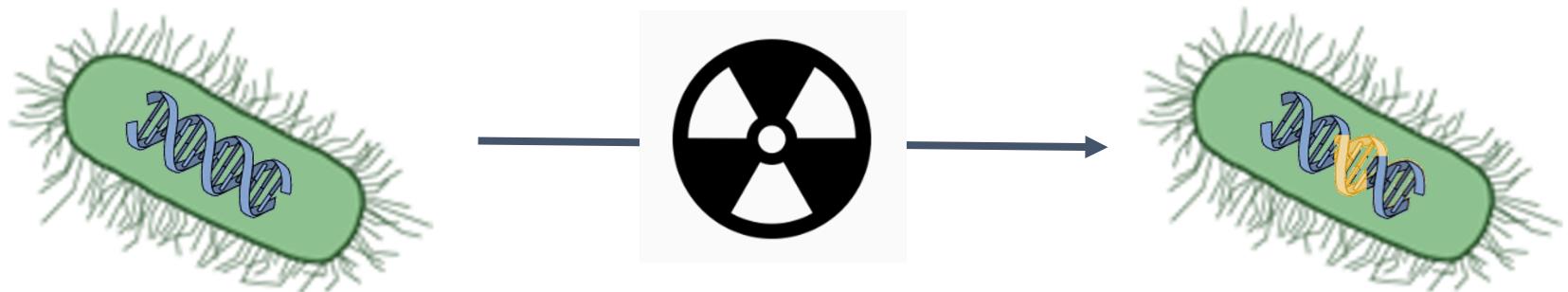
Background: How does antibiotic resistance arise?

## 1) Genetic mutation

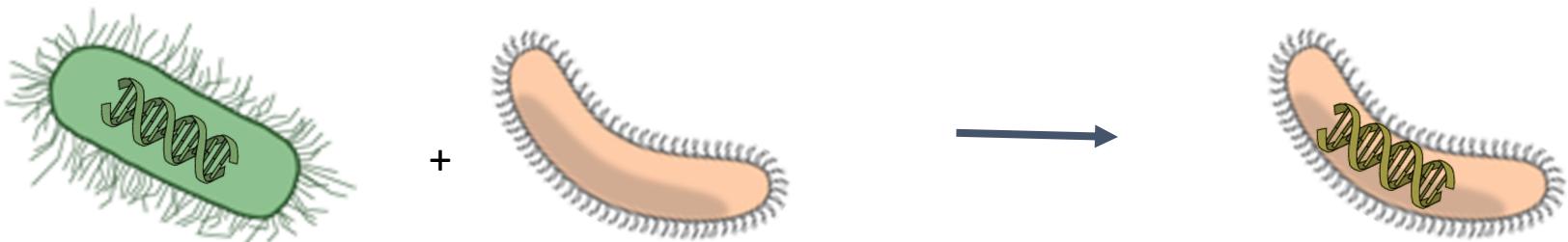


Background: How does antibiotic resistance arise?

## 1) Genetic mutation



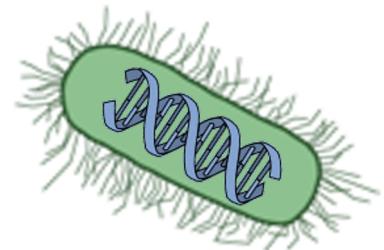
## 2) Horizontal gene transfer



Background: Removal during advanced treatment

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ARB = Antibiotic Resistant Bacteria



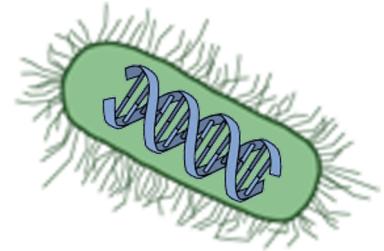
ARG = Antibiotic Resistance Genes



Background: Removal during advanced treatment

## ARB = Antibiotic Resistant Bacteria

Treat these the same as removal of all bacteria

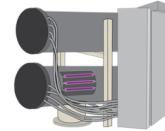
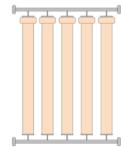
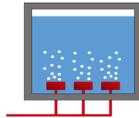


## ARG = Antibiotic Resistance Genes

Treat these the same as removal of  
organic chemical constituents



# Background: Removal during advanced treatment processes



Process	WWTP	Ozone	BAC	MF	RO	UV-AOP
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Process	WWTP	Ozone	BAC	MF	RO	UV-AOP
Treatment Mechanism	Biological Physical Removal	Oxidation	Biological Physical Removal	Physical Removal	Physical Removal	Oxidation Irradiation

# Background: Removal during advanced treatment processes



Process	WWTP	Ozone	BAC	MF	RO	UV-AOP
Bacteria	+	+		+	+	+
Treatment Mechanism	Biological Physical Removal		Biological Physical Removal	Physical Removal	Physical Removal	Oxidation Irradiation

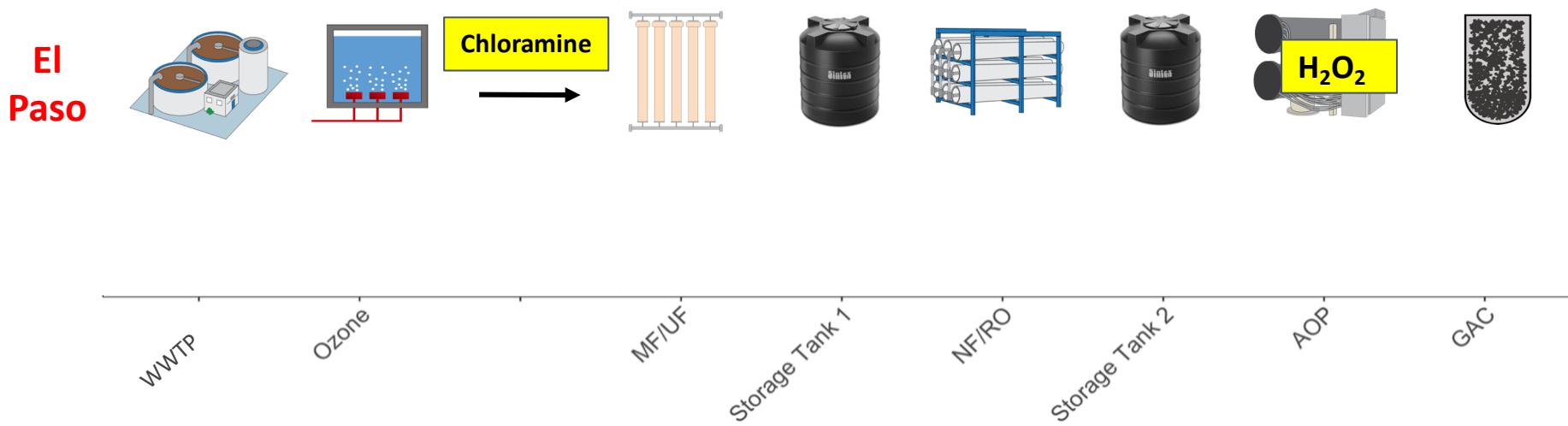
# Background: Removal during advanced treatment processes



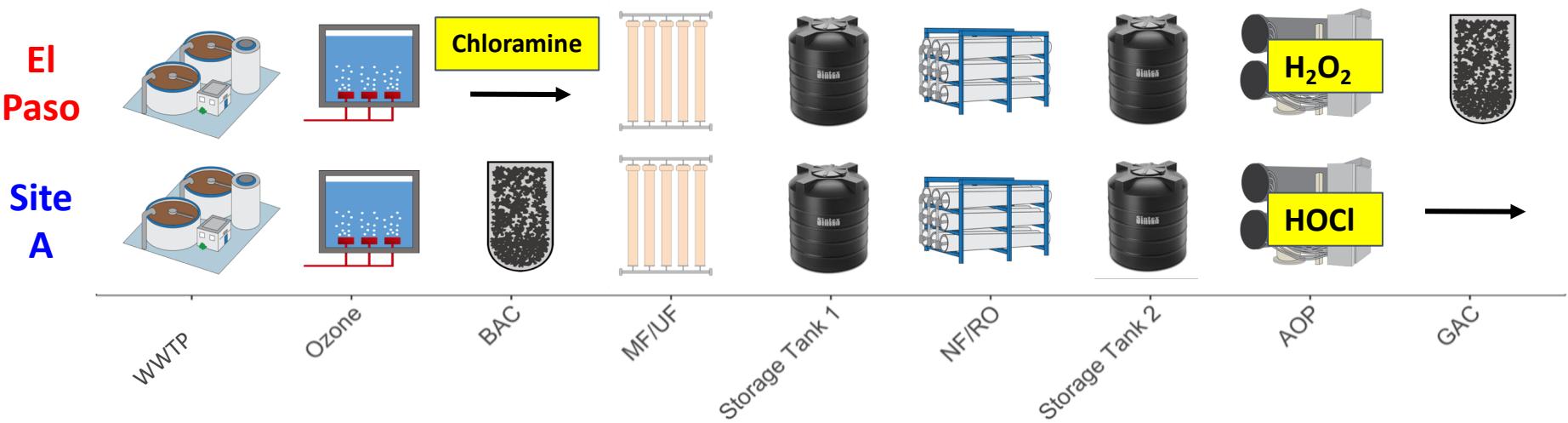
Process	WWTP	Ozone	BAC	MF	RO	UV-AOP
<b>Bacteria</b>	+	+		+	+	+
<b>Free DNA</b>	+	+	+		+	+
<b>Treatment Mechanism</b>	Biological Physical Removal	Oxidation	Biological Physical Removal	Physical Removal	Physical Removal	Oxidation Irradiation

Study sites: Two pilot treatment trains sampled

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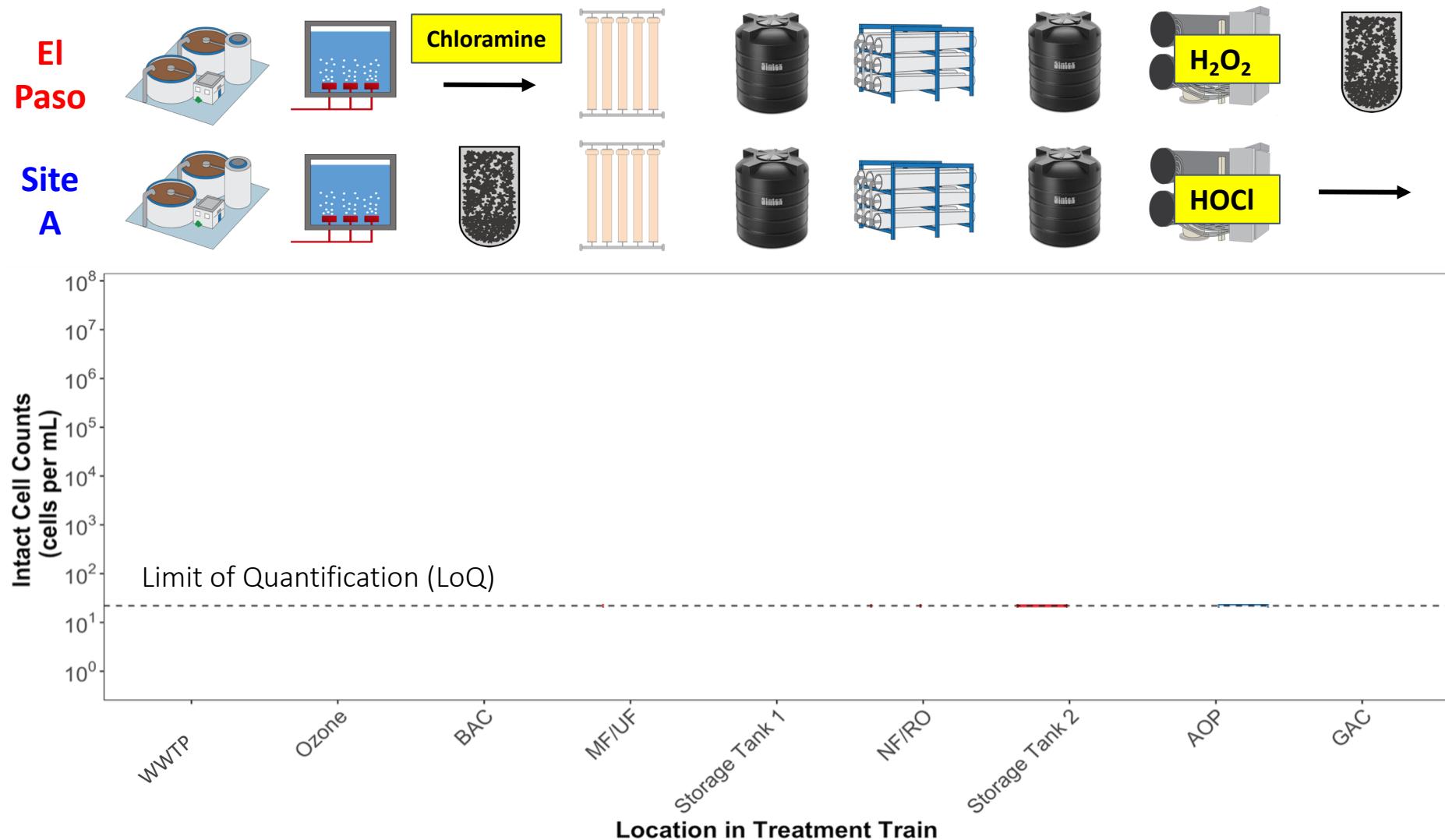
# Study sites: Two pilot treatment trains sampled



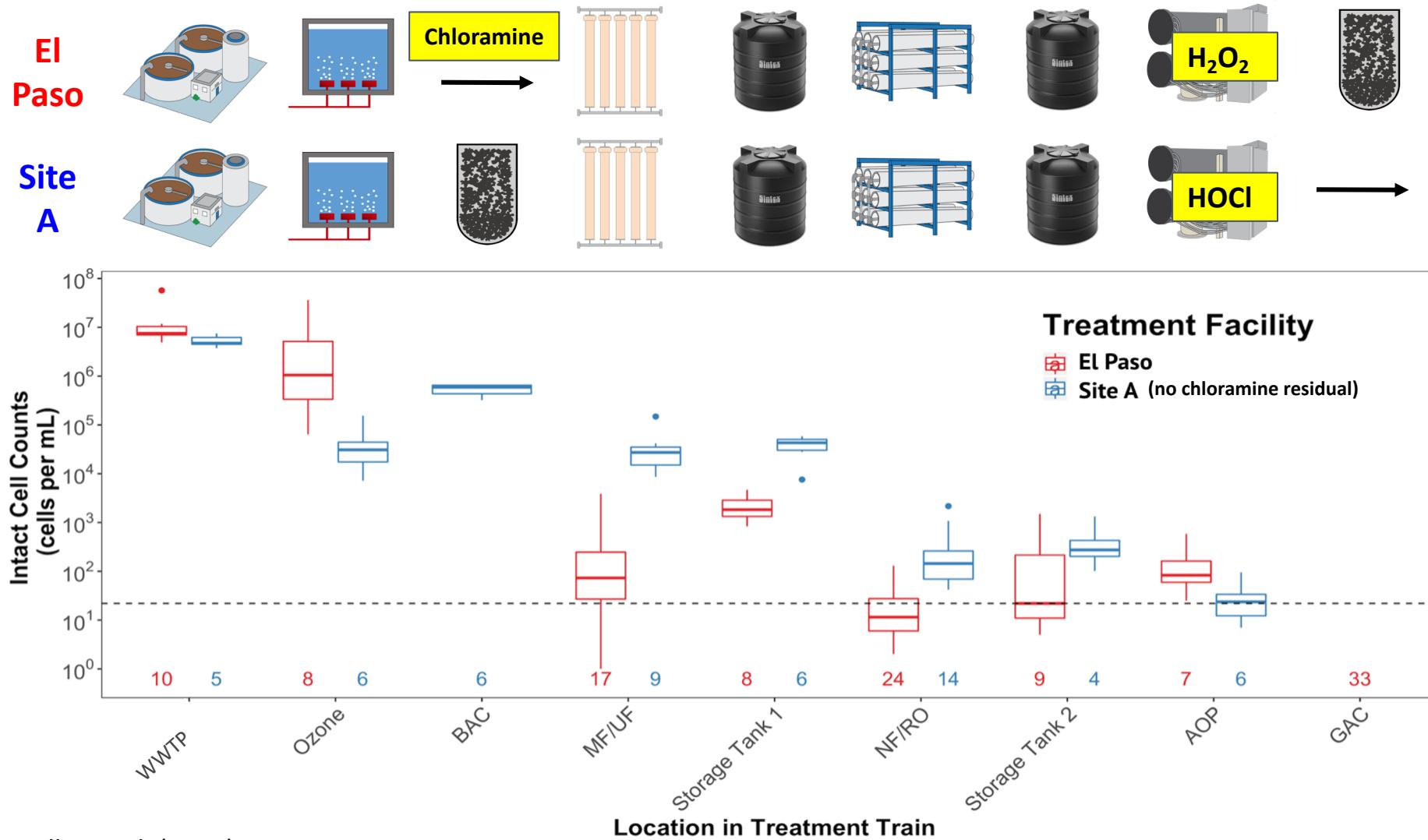
1. How well does advanced treatment remove bacteria?

**Method: Flow cytometry**

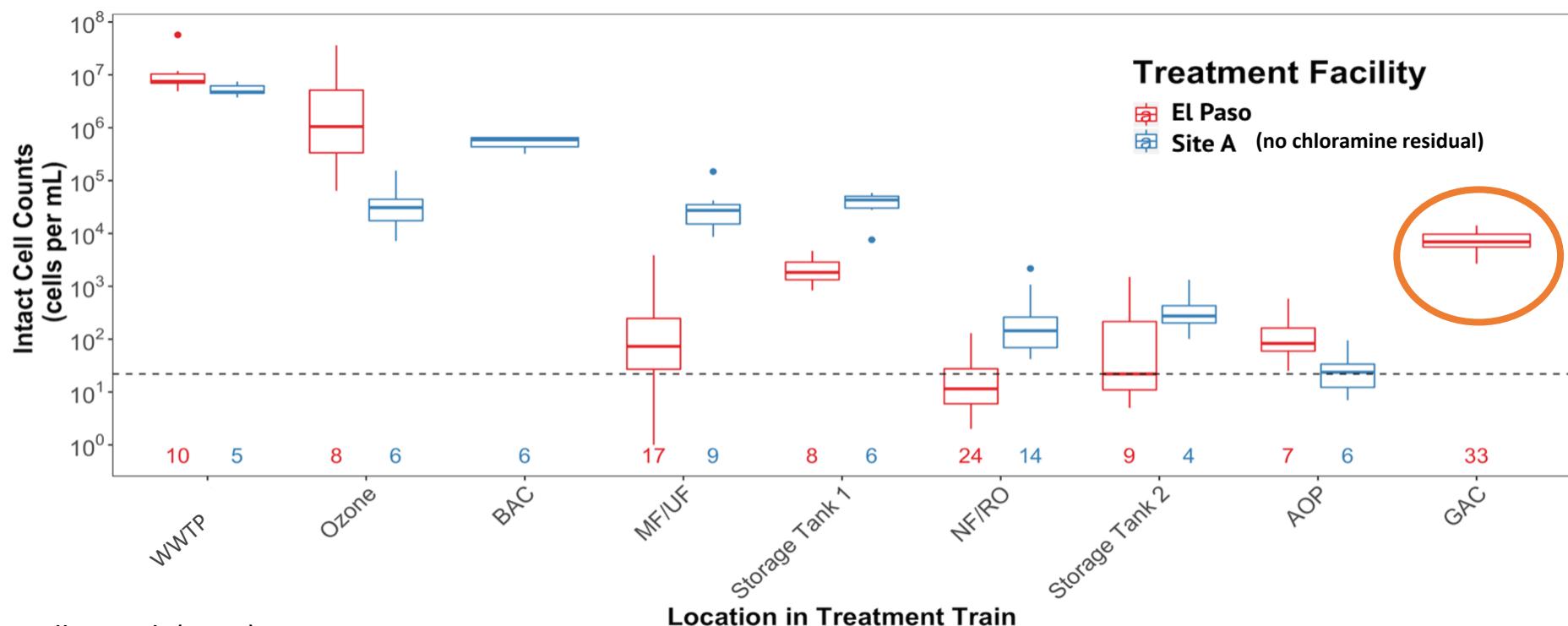
# 1. How well does advanced treatment remove bacteria?



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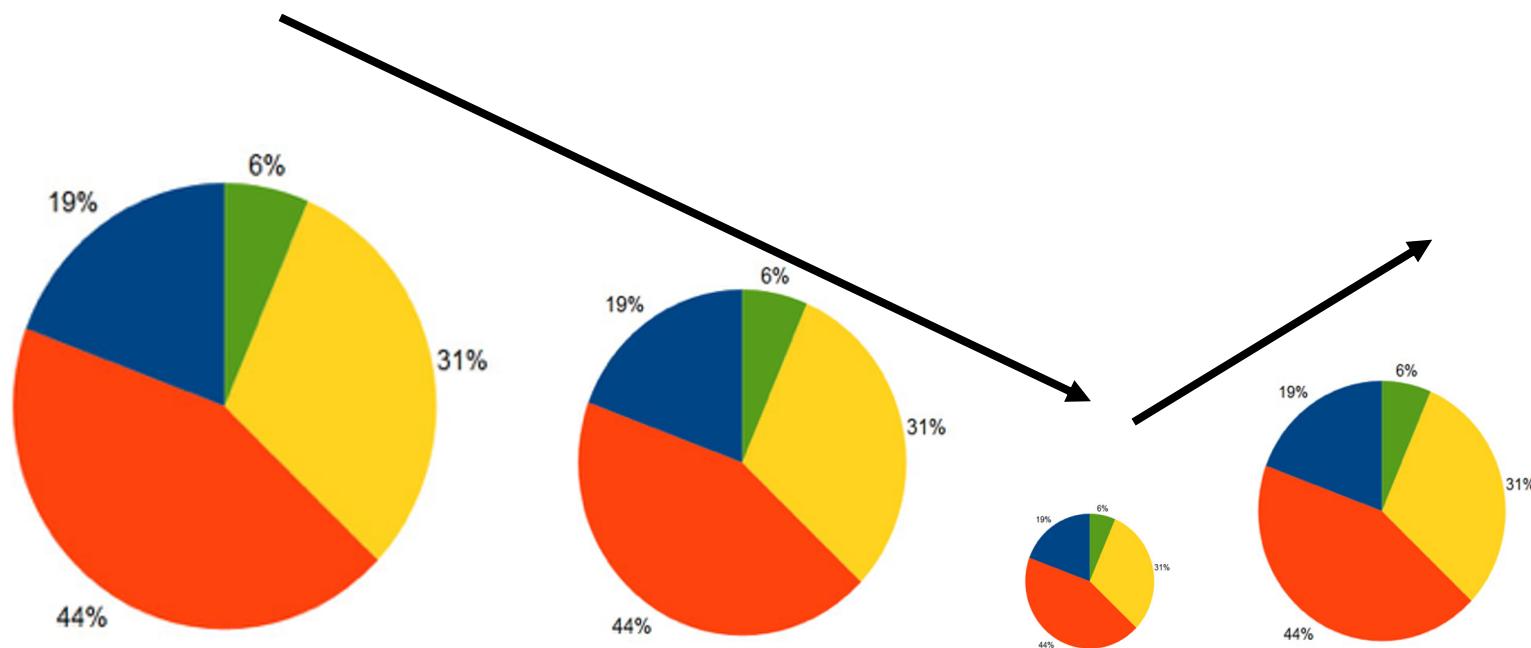


1. Treatment removes nearly all bacteria but there is growth after treatment.



## 2. Are the SAME bacteria present before and after treatment?

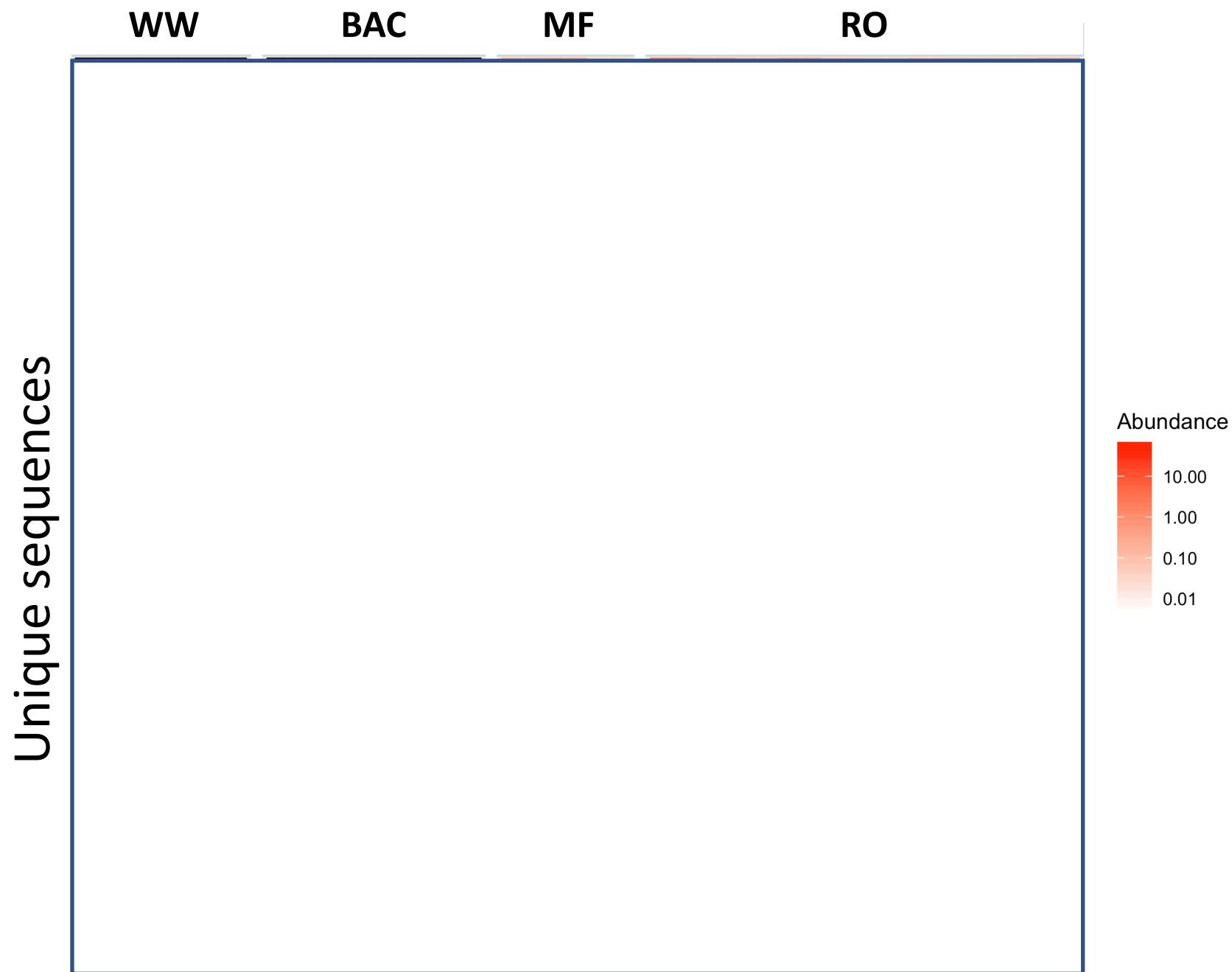
- Null hypothesis: Successive removal (and growth) of all species in equal proportions through treatment

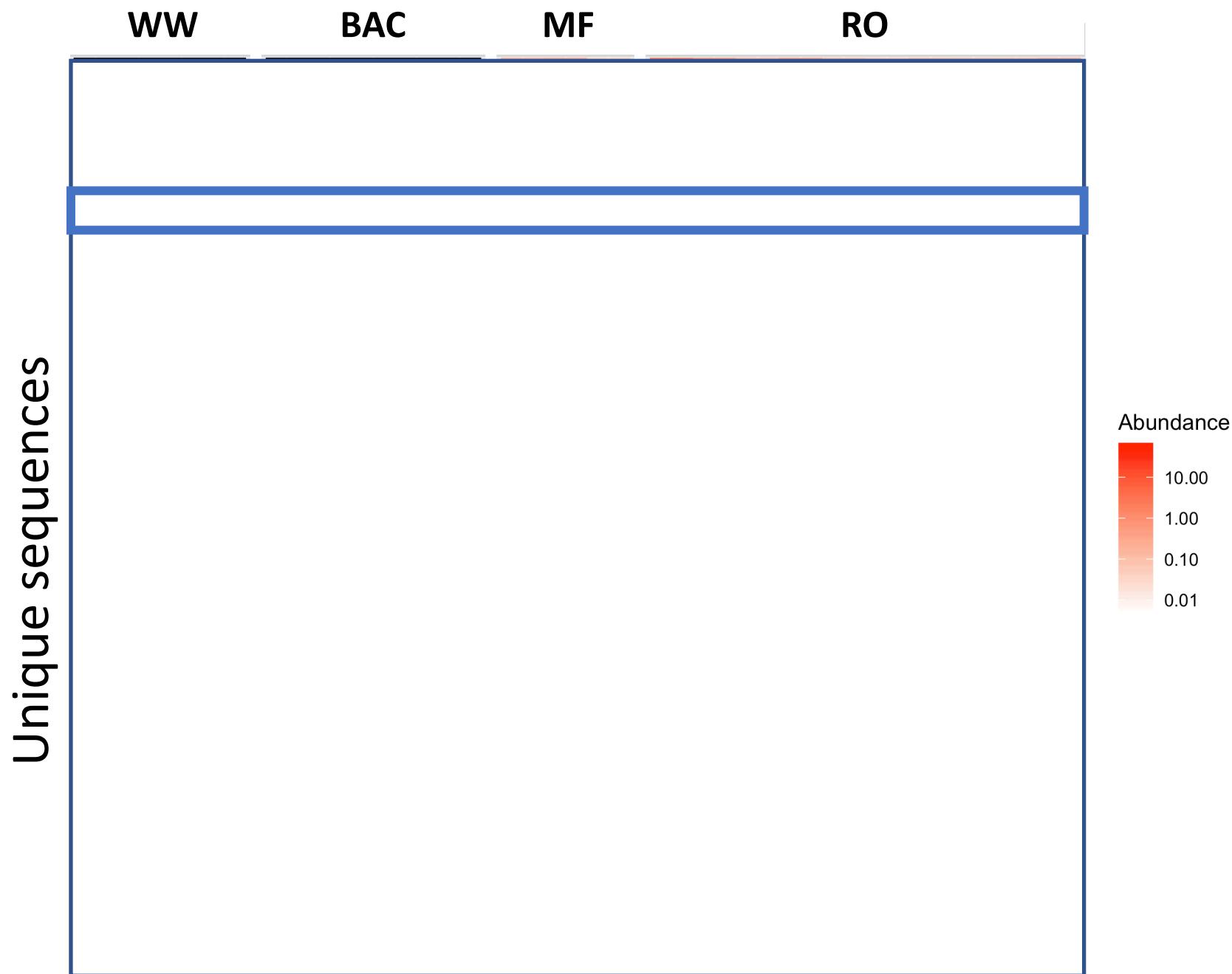


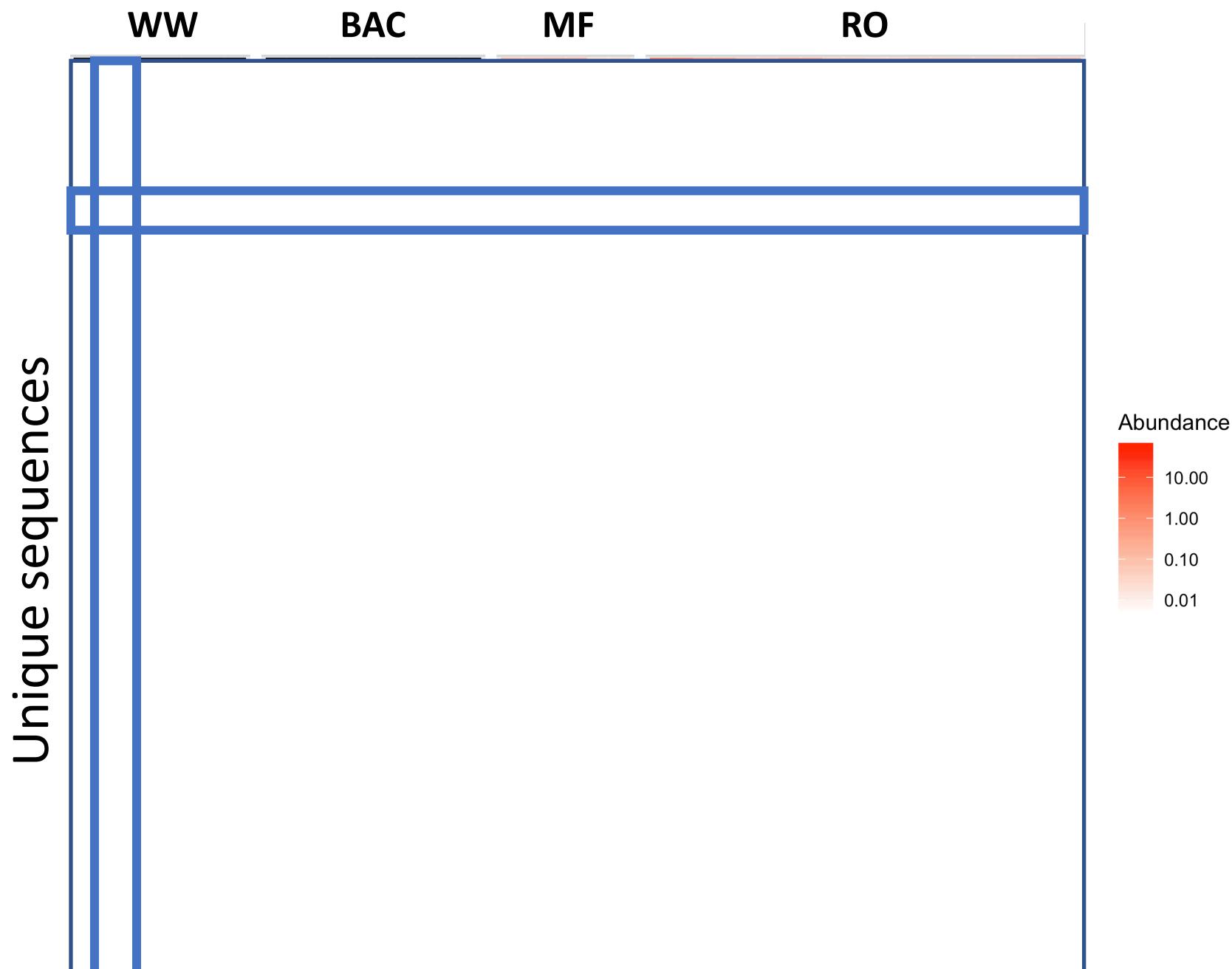
2. Are the SAME bacteria present before and after treatment?

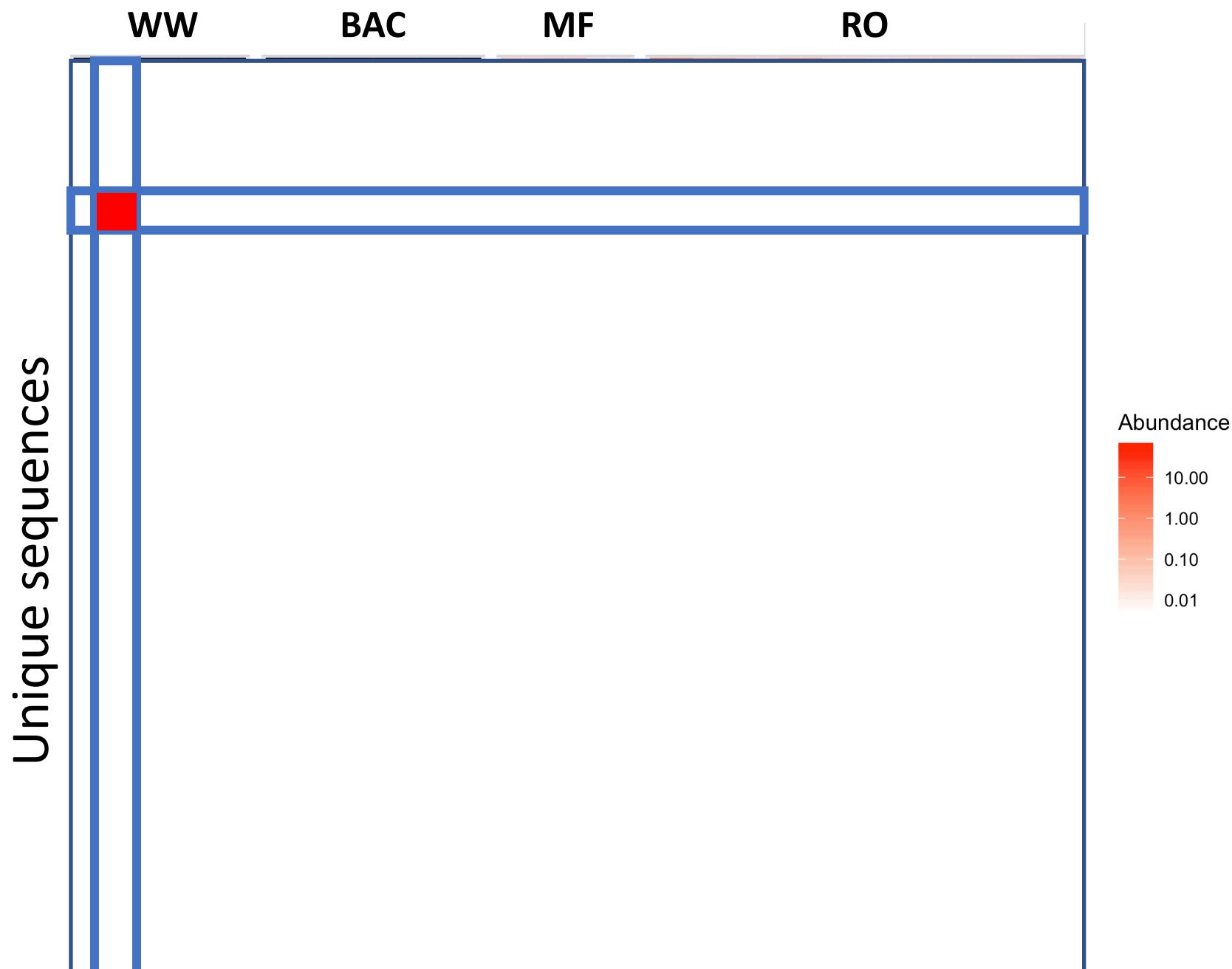
## **Method: amplicon sequencing**

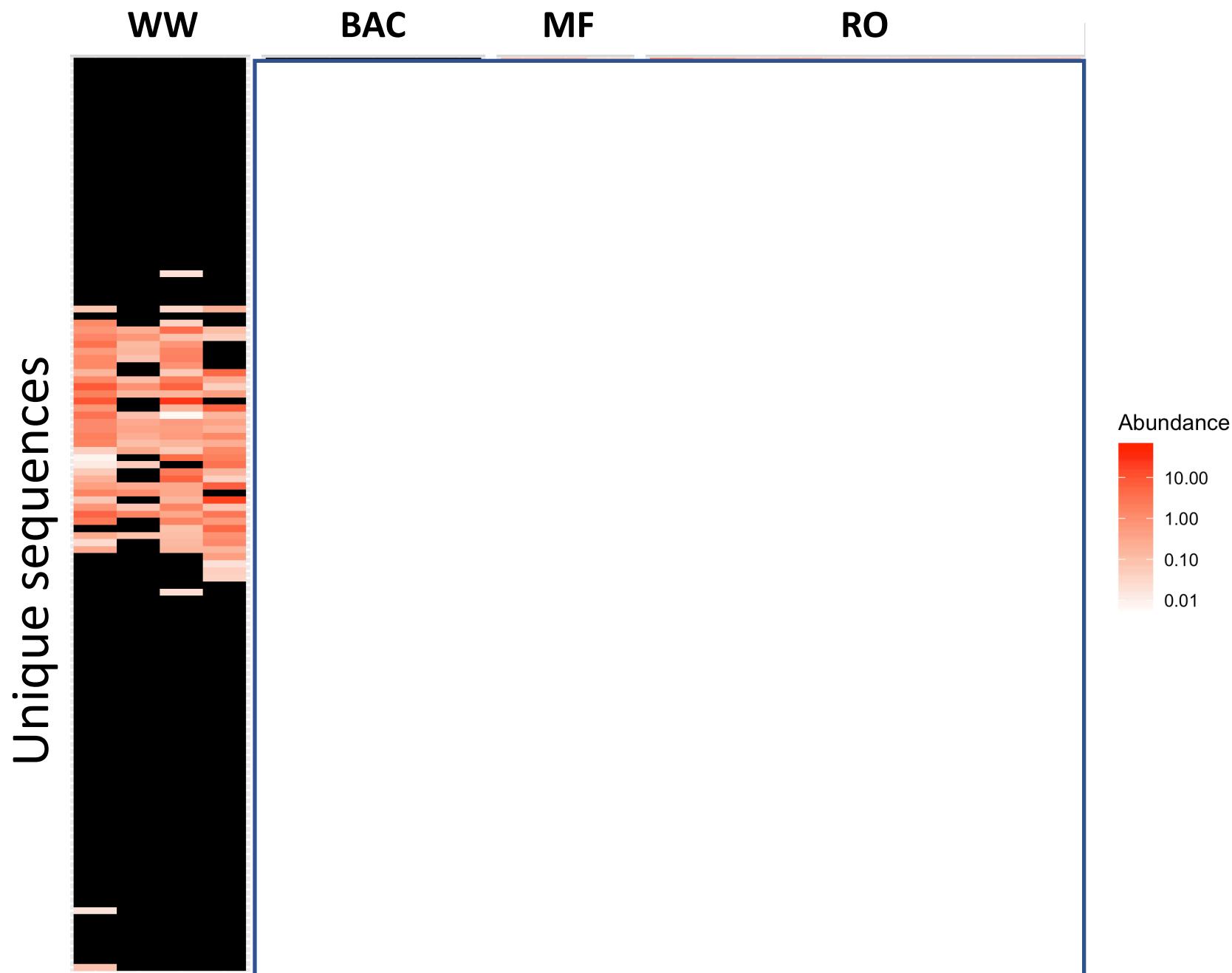
(High-throughput DNA sequencing of  
the 16S rRNA, gene V4 region)



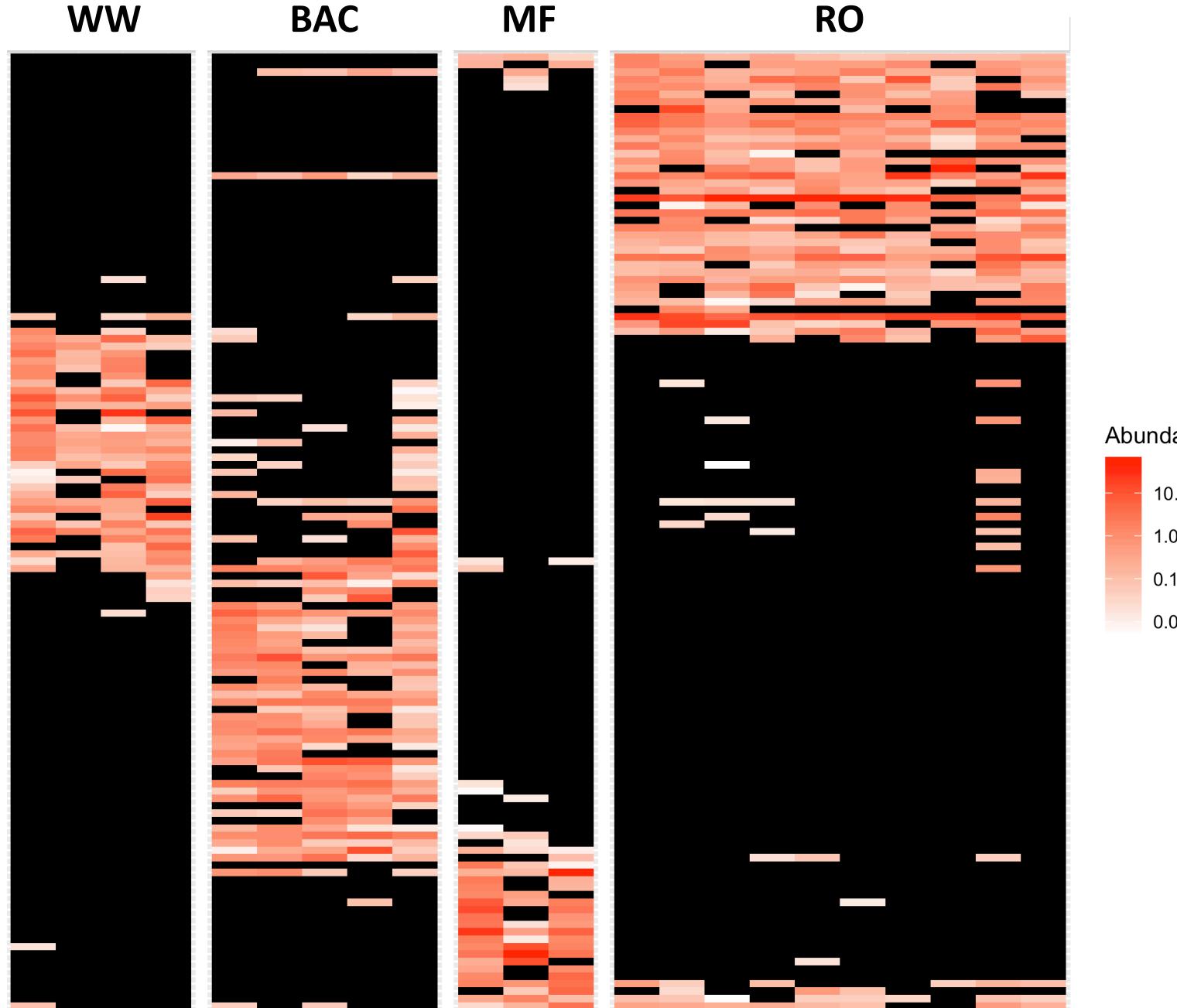




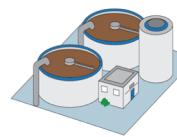




Unique sequences



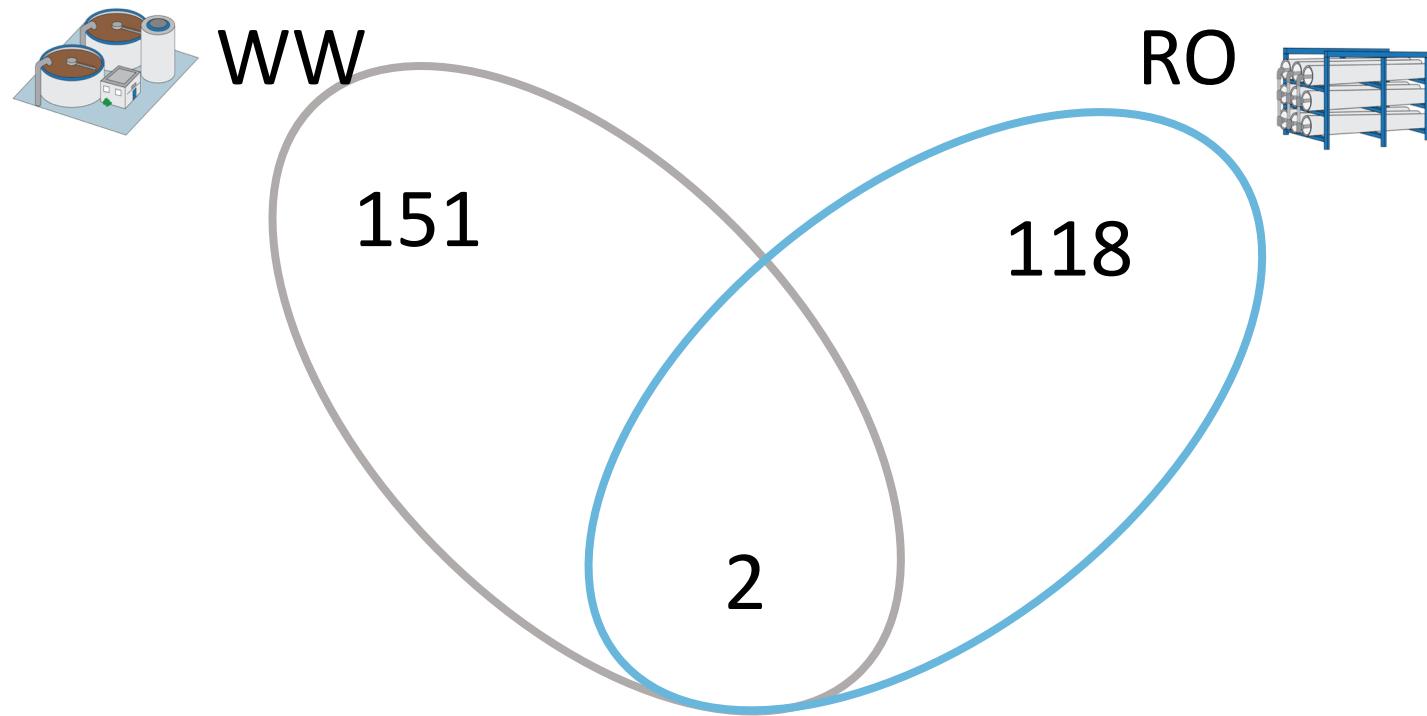
## 2. Are the SAME bacteria present before and after treatment?



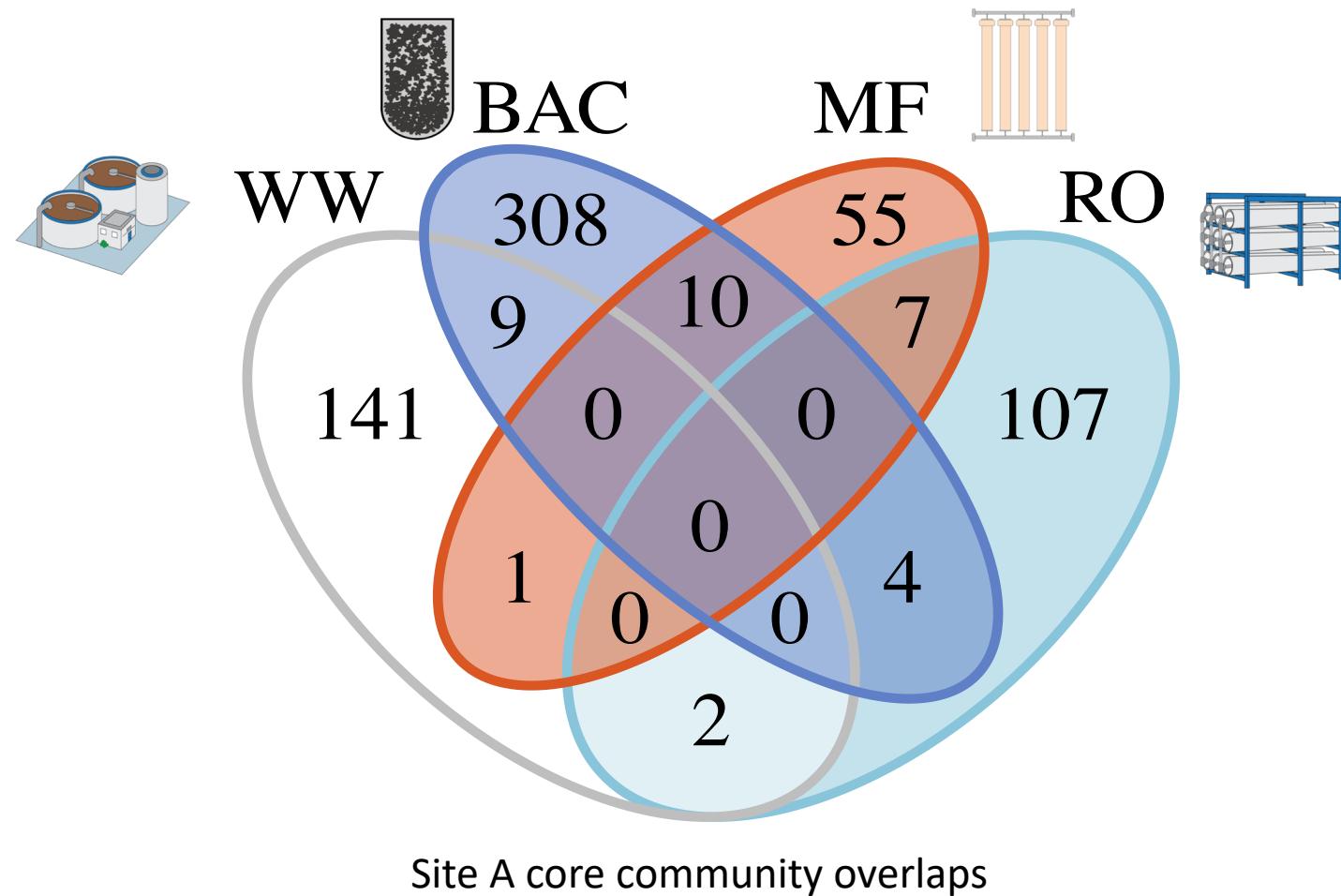
WW

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## 2. Are the SAME bacteria present before and after treatment?



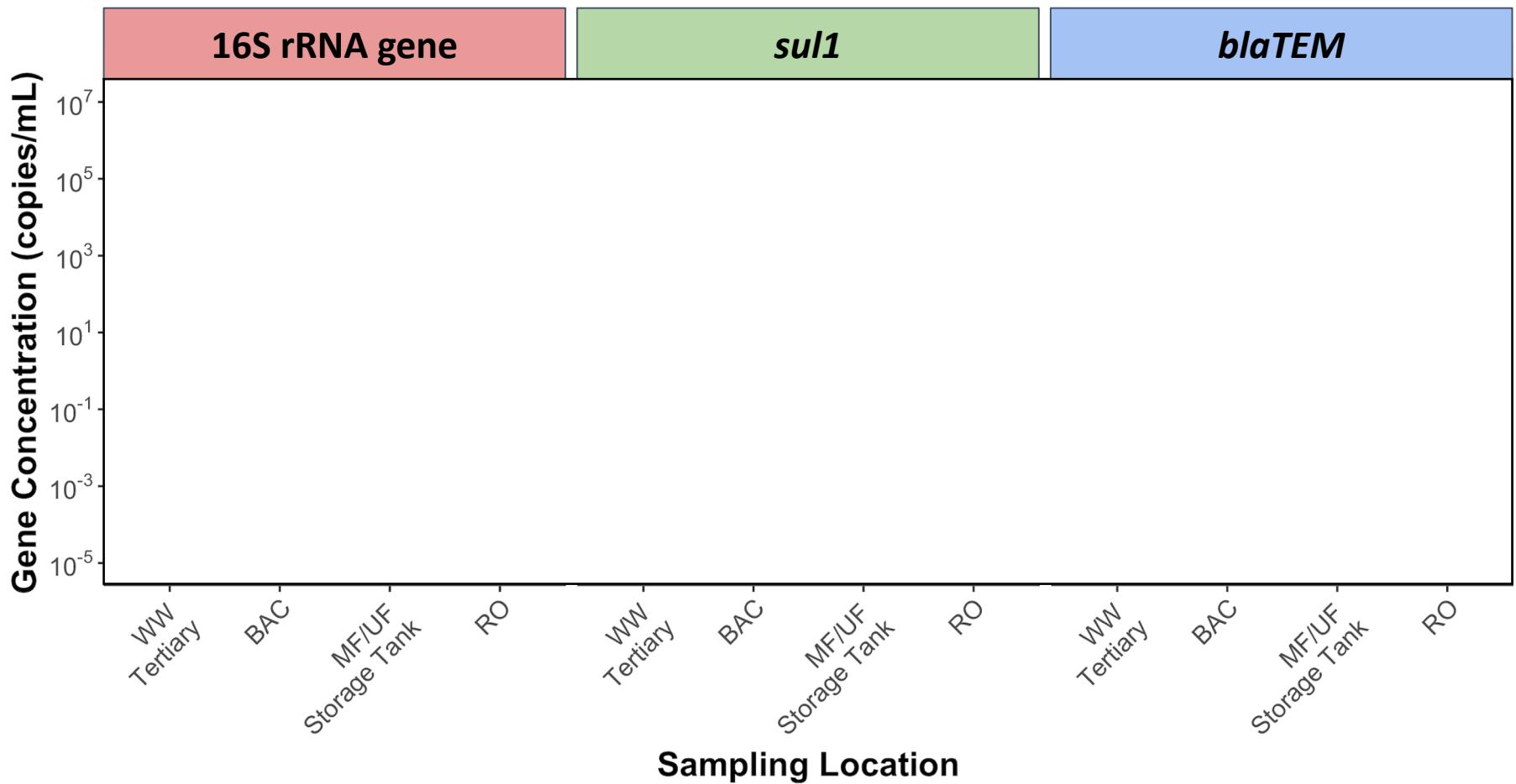
## 2. Very few of the SAME bacteria are present before and after treatment



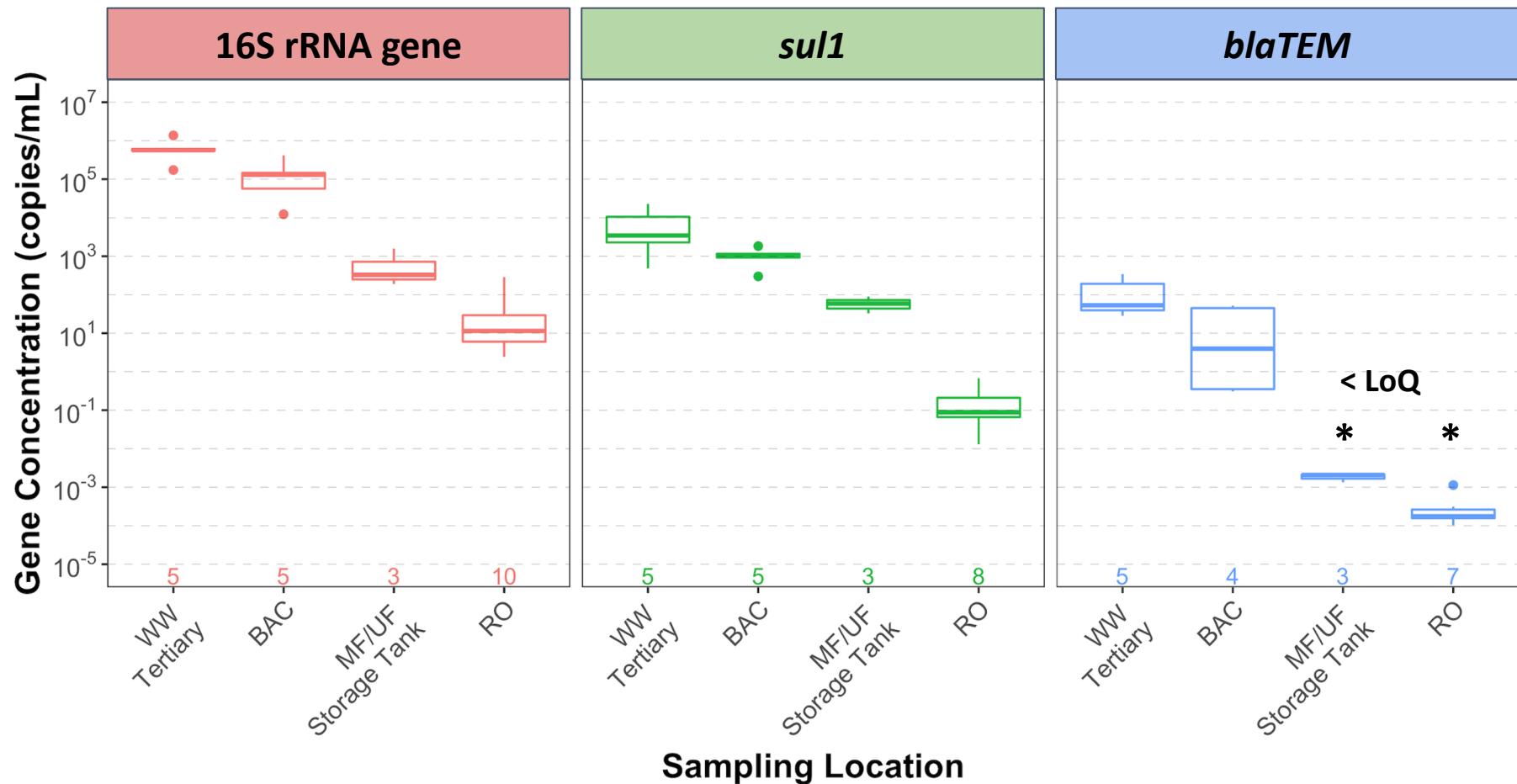
### 3. How well does advanced treatment remove ARGs? (Site A)

**Method: quantitative PCR**

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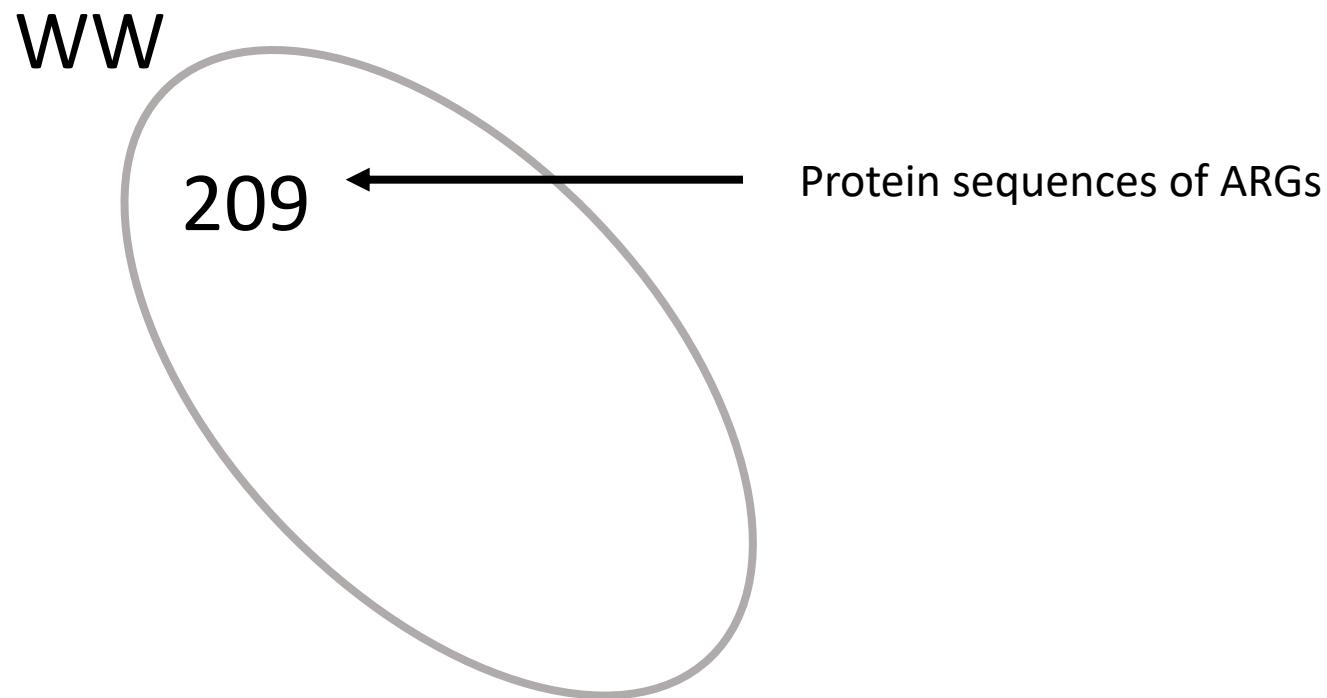
### 3. Advanced treatment removes ARGs (Site A).



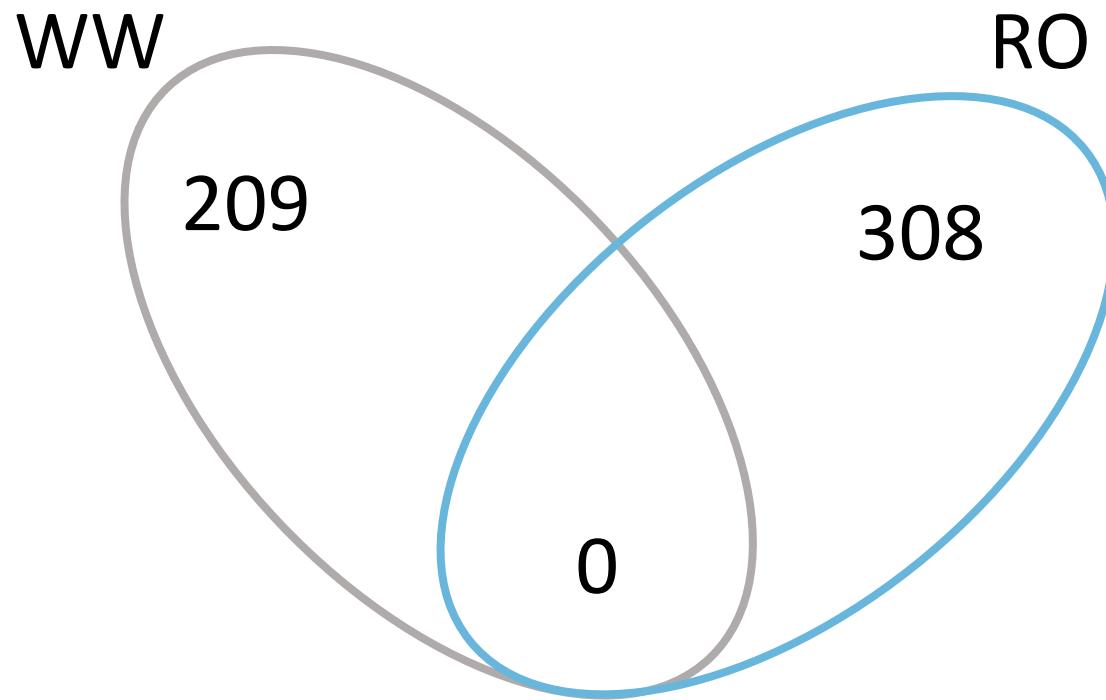
4. Are the SAME ARGs present before and after treatment?

**Method: metagenomics**  
(Whole community genome  
shotgun sequencing)

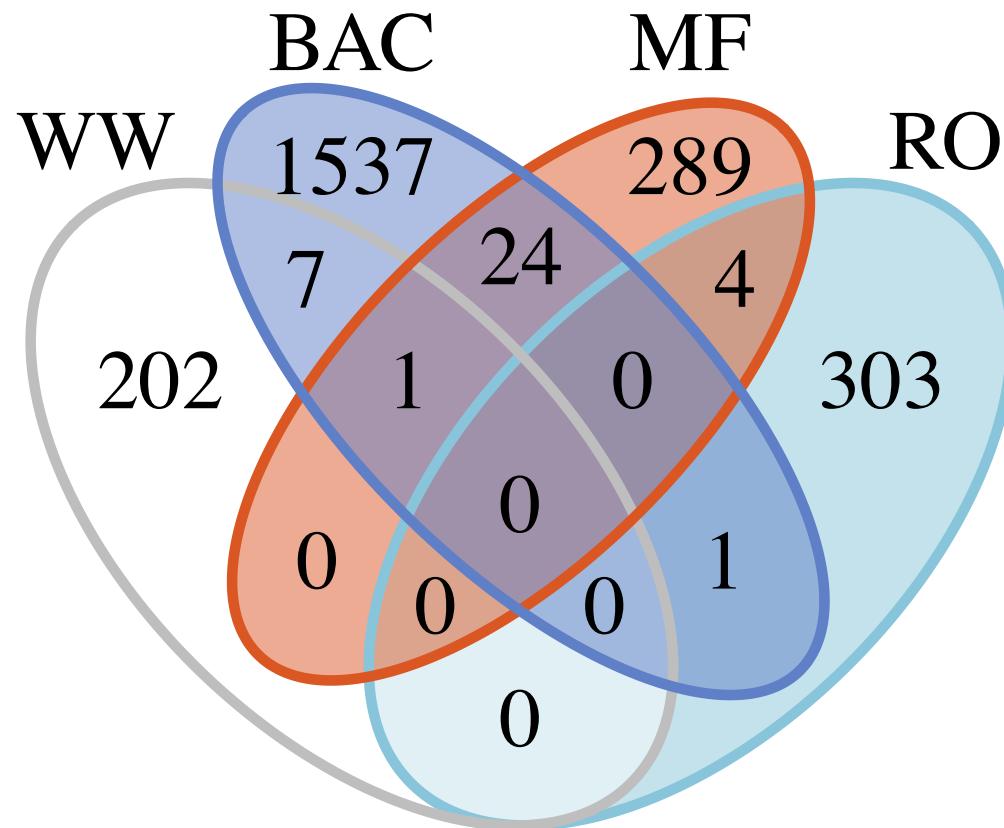
#### 4. Are the SAME ARGs present before and after treatment?



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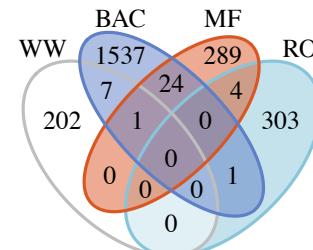
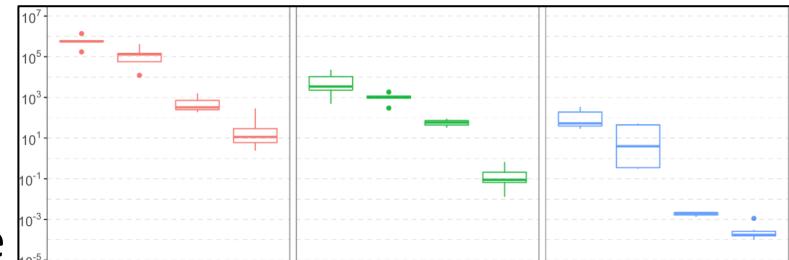
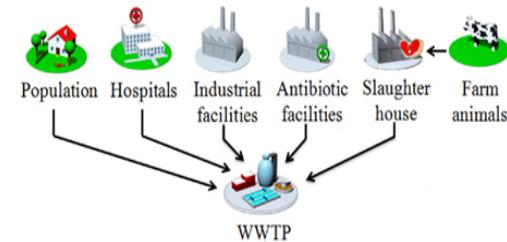


#### 4. Different ARGs are present before and after treatment.



# Summary

- Wastewater is a “hotspot” for ARGs
- Methods: flow cytometry, qPCR, DNA sequencing
- Removal of bacteria and DNA through treatment:
  1. How well does treatment remove bacteria? **Very well.**
  2. Are the SAME bacteria present before and after treatment? **No/not many.**
  3. How well does treatment remove ARGs? **Very well.**
  4. Are the SAME ARGs present before and after treatment? **No/TBD.**



# Acknowledgements

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Questions?



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