# Municipal Activated Sludge-Derived Microplastic Microbiomes:

## *The Good, The Bad, and The Promising*

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Department of Chemistry & Environmental Science
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Austin, TX
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# What Are Microplastics?

Microplastics are plastics < 5mm

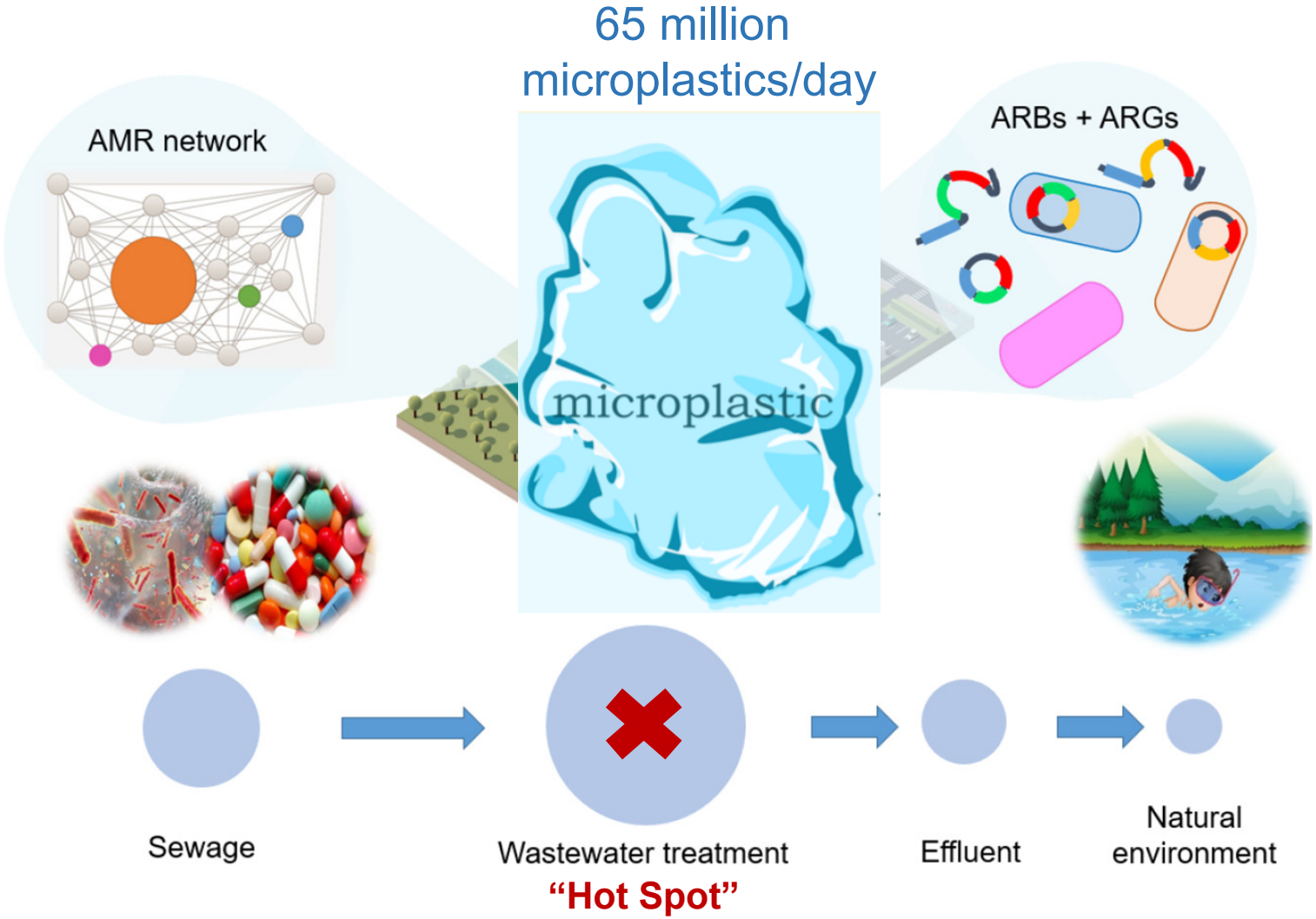
**5,000~100,000** microbeads released in a **single** use!

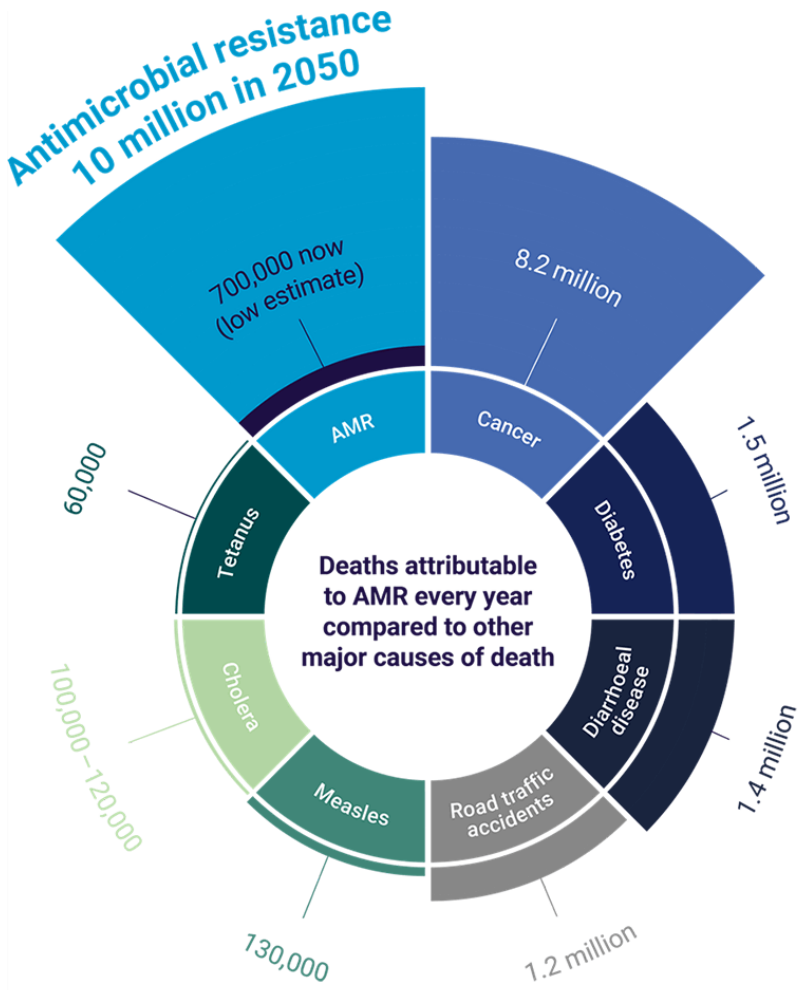
Primary microplastics - a major source of pollution


The global release: **1.5 Mtons/year** (15-30%)

Boucher, J. and Friot D. *et al.*, IUCN. (2017)

# Activated Sludge Tanks as “Hot Spots” where Microplastics, Bacteria, and Contaminants Commingle




Adapted from O’neill *et al*., Rev. Antimicrob. Resist., 2014; Nguyen *et al*., STOTEN, 2021

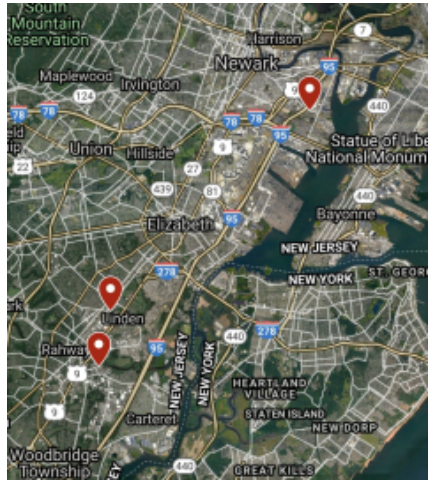
# Experimental Design

Homogenized activated sludge samples (**L, R, & P sludge**)

10 μm

resuspended in synthetic wastewater

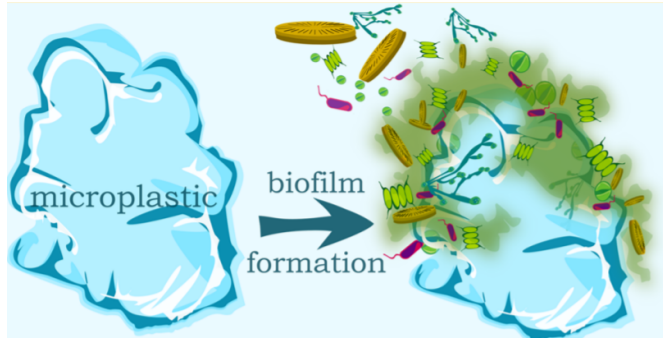
inoculated

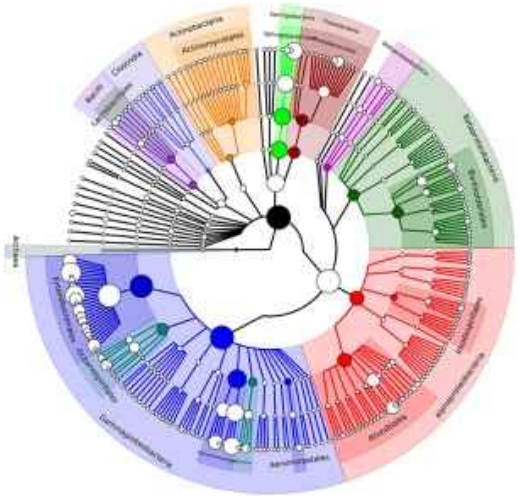



PE/PS/sand (80-105 μm)


3 days of incubation

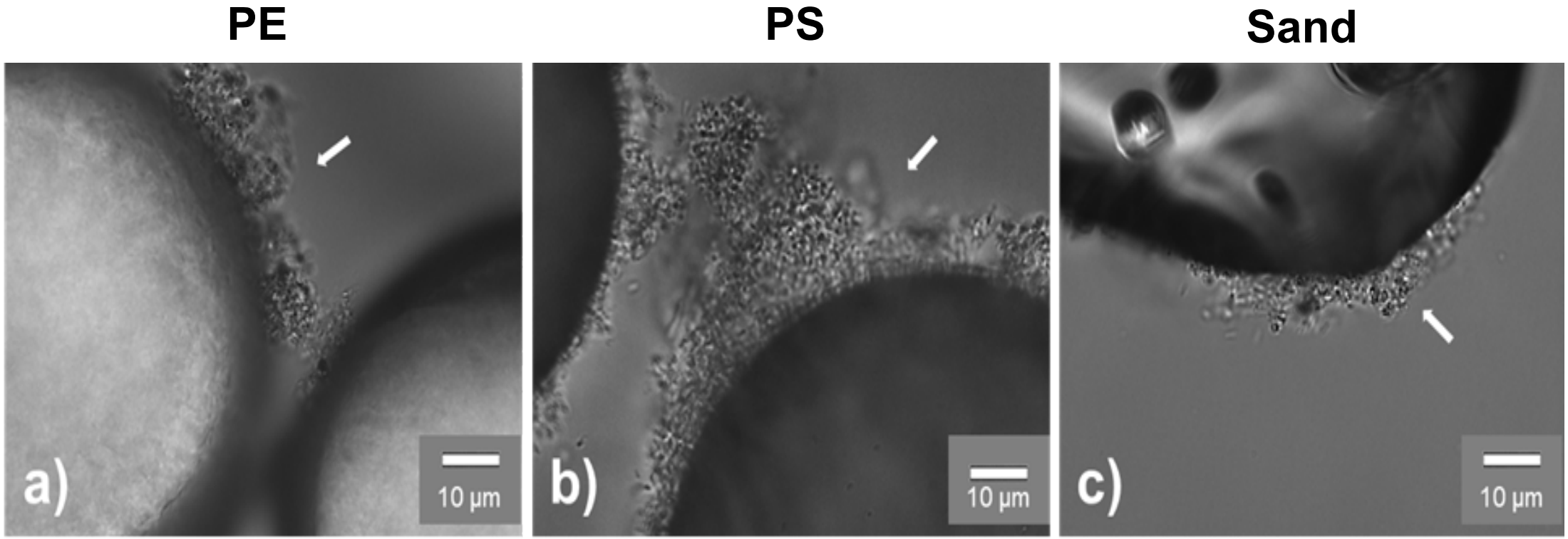
Microparticle **biofilms** were collected for DNA extraction



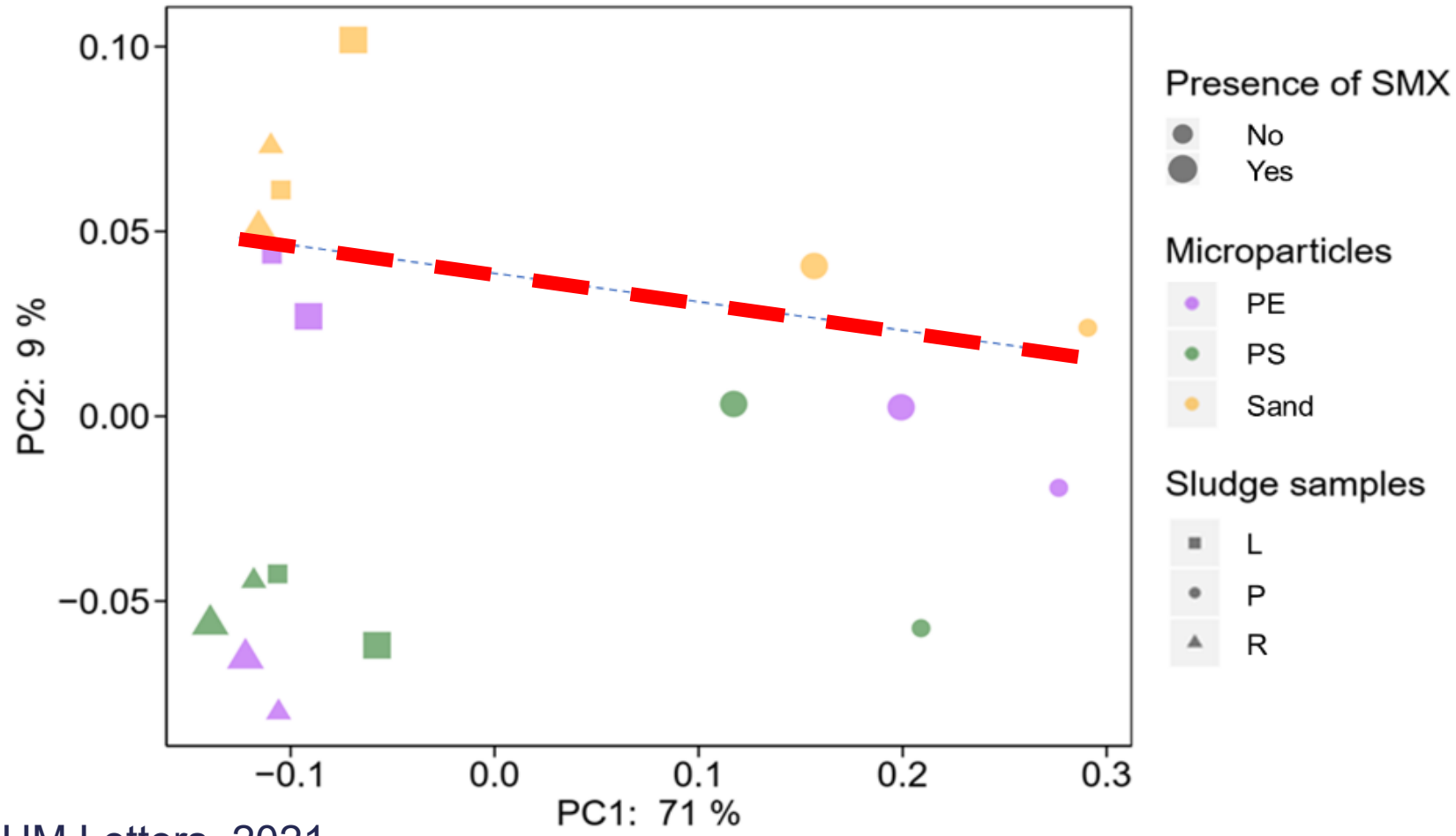
**Metagenomics Analysis**

# Biofilm Formation on Microplastics versus Sand




Pham *et al*., JHM Letters, 2021

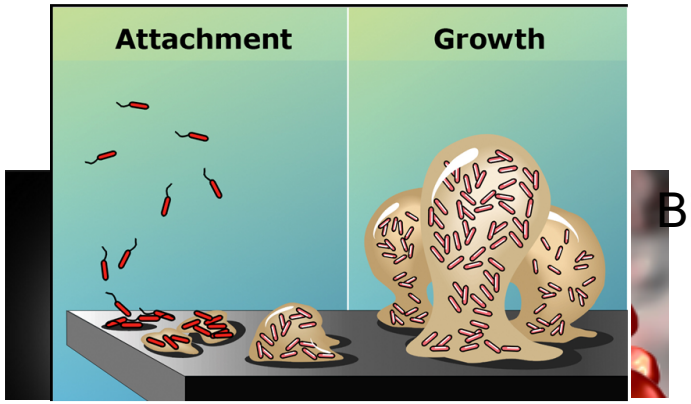
# Distinct Microbial Communities in Biofilms Attached to Microplastics versus Sand



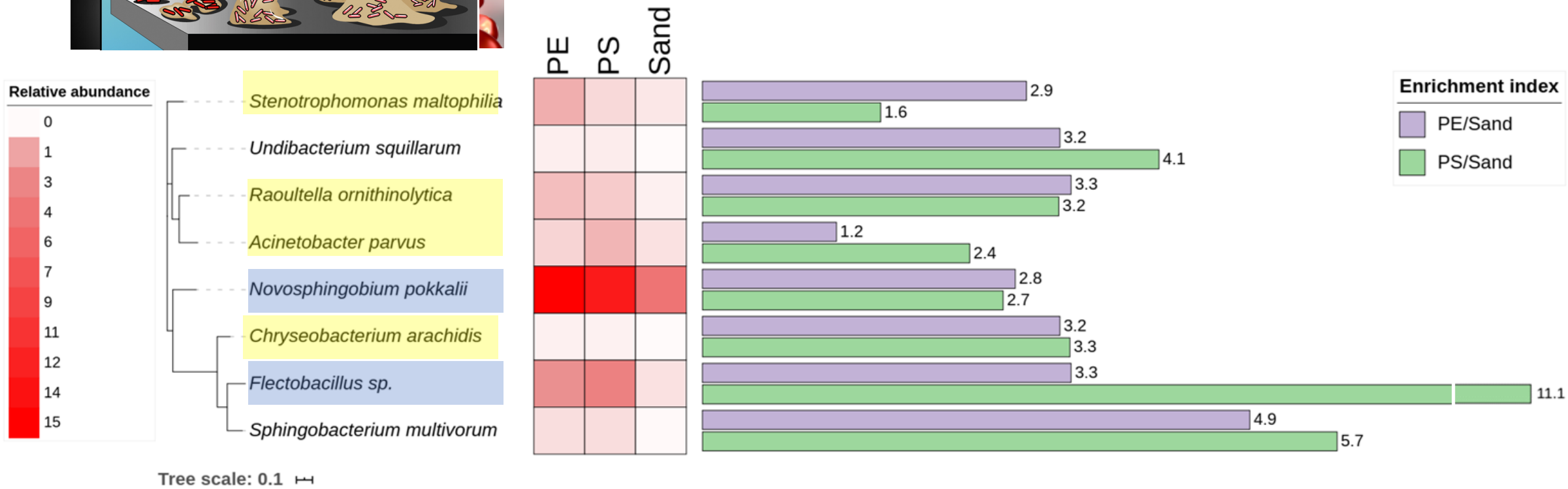

Pham *et al.*, JHM Letters, 2021

# Microplastics Enriched Eight Bacterial Species

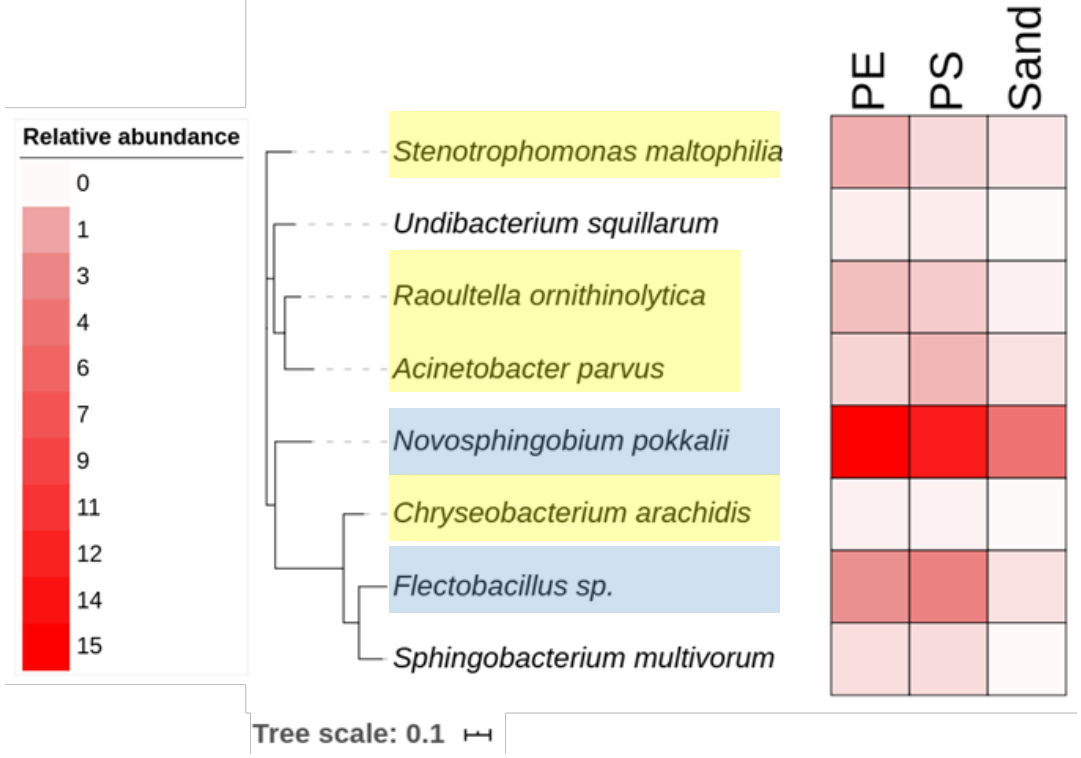
**Enrichment index** defines how many times higher that species presented on PE or PS than on sand.

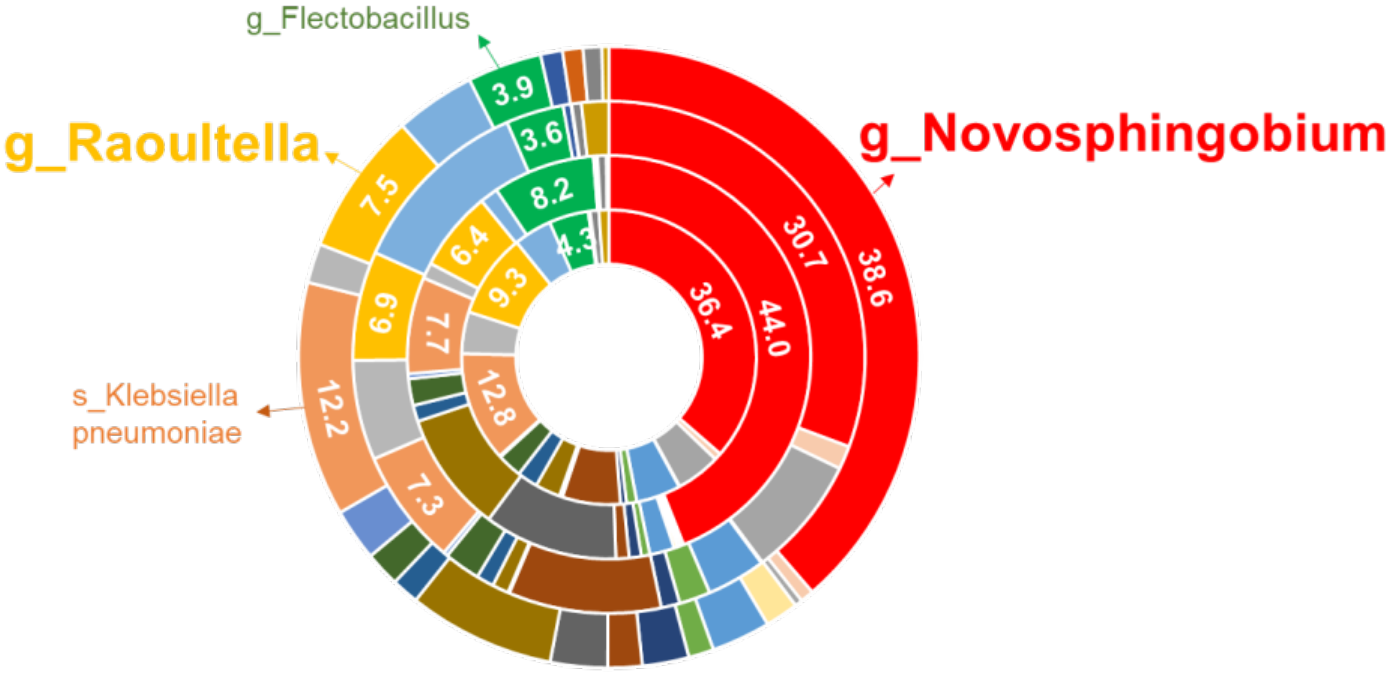



Biofilm formation



# Metagenome-Assembled Genomes (MAGs)



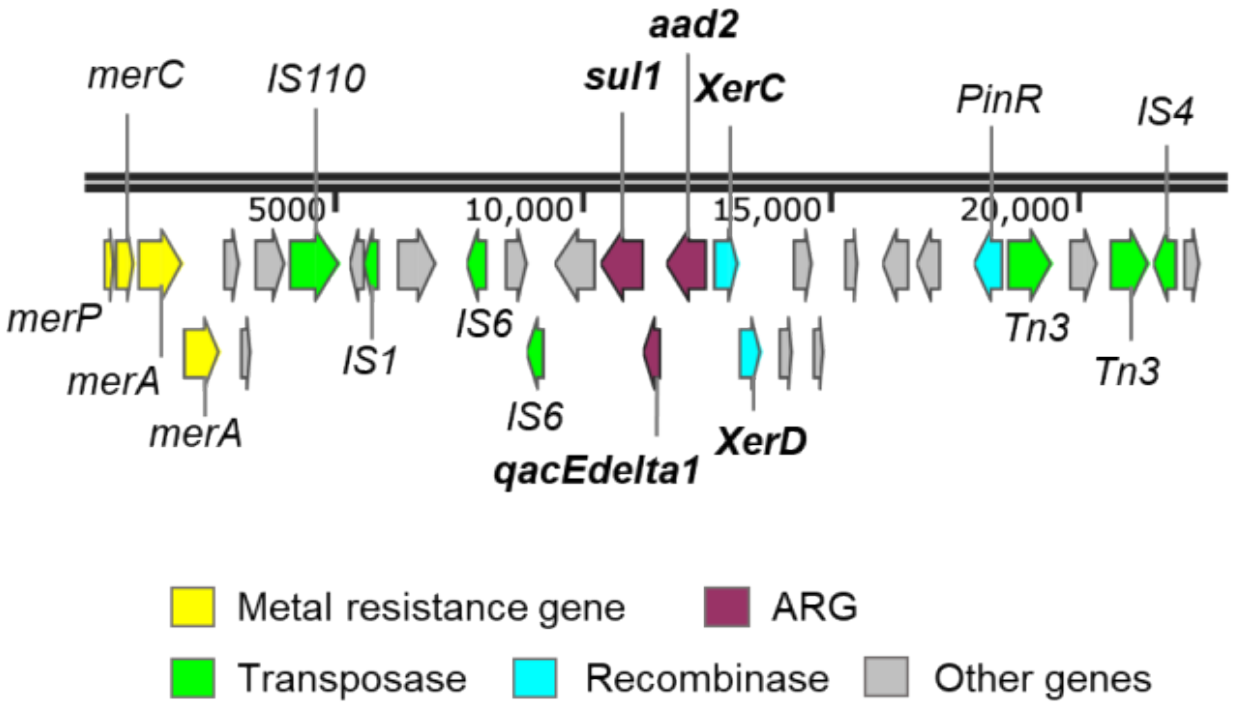




**Nanopore MinION**

Pham *et al.*, JHM Letters, 2021; Pham *et al.*, in preparation

# A *Raoultella* Genome Carries 26 ARGs and Neighboring Genes to Mobilize Them

| Antibiotic | ARG subtype | Number |
|---|---|---|
| Aminoglycoside | *kdpE, aad2* | 2 |
| Aminocoumarin | *mdtC, mdtG* | 2 |
| Beta-lactam | *OmpA, blaPLA OKP-B-7* | 3 |
| Fluoroquinolone | *mdtK* | 1 |
| Fosfomycin | *FosA6, mdtH* | 2 |
| Peptide | *bacA , yojI* | 2 |
| Phenicol | *cmlA* | 1 |
| Sulfonamide | *sul1* | 1 |
| Multidrug | *acrA, acrB, acrF, baeR, KpnE, KpnF, LptD, marA, qacEdelta1, ramA, rsmA, tolC* | 12 |

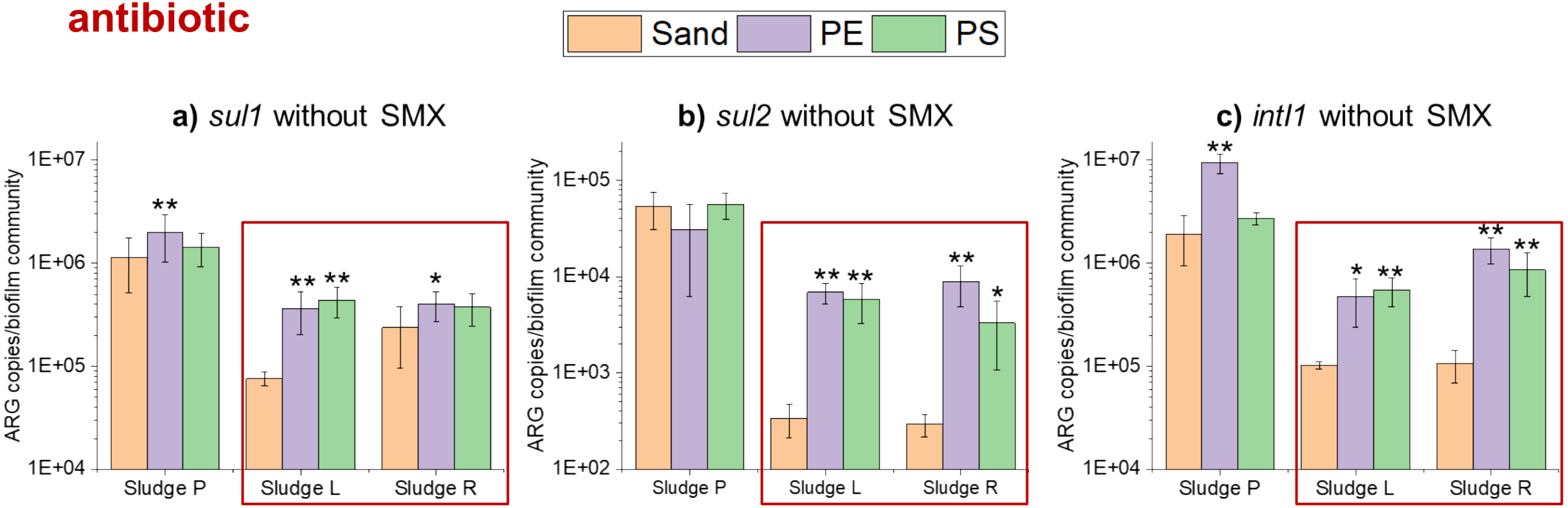



an example of a resistance island

Pham *et al*., in preparation

# Microplastics Significantly Enriched ARGs

**Without antibiotic**

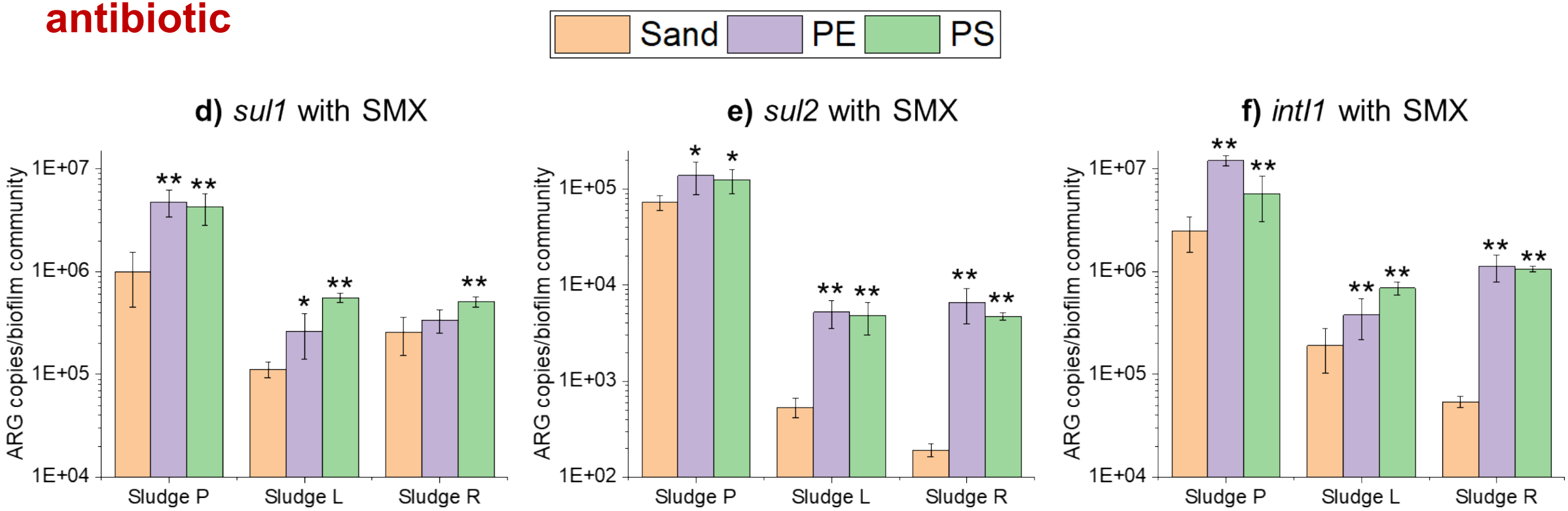



Two-way Student's t-test, $*p < 0.05$ and $**p < 0.01$.

Pham *et al.*, JHM Letters, 2021

# Microplastics Significantly Enriched ARGs

**With antibiotic**

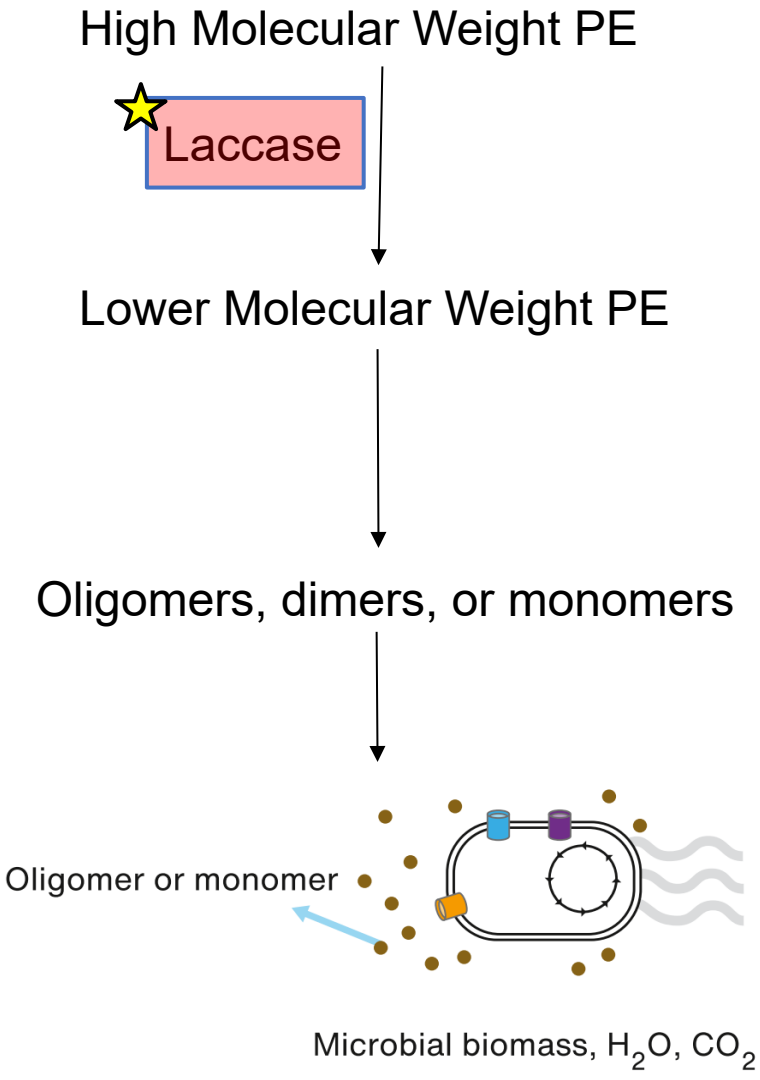



Two-way Student's t-test, $*p < 0.05$ and $**p < 0.01$.

Pham *et al.*, JHM Letters, 2021

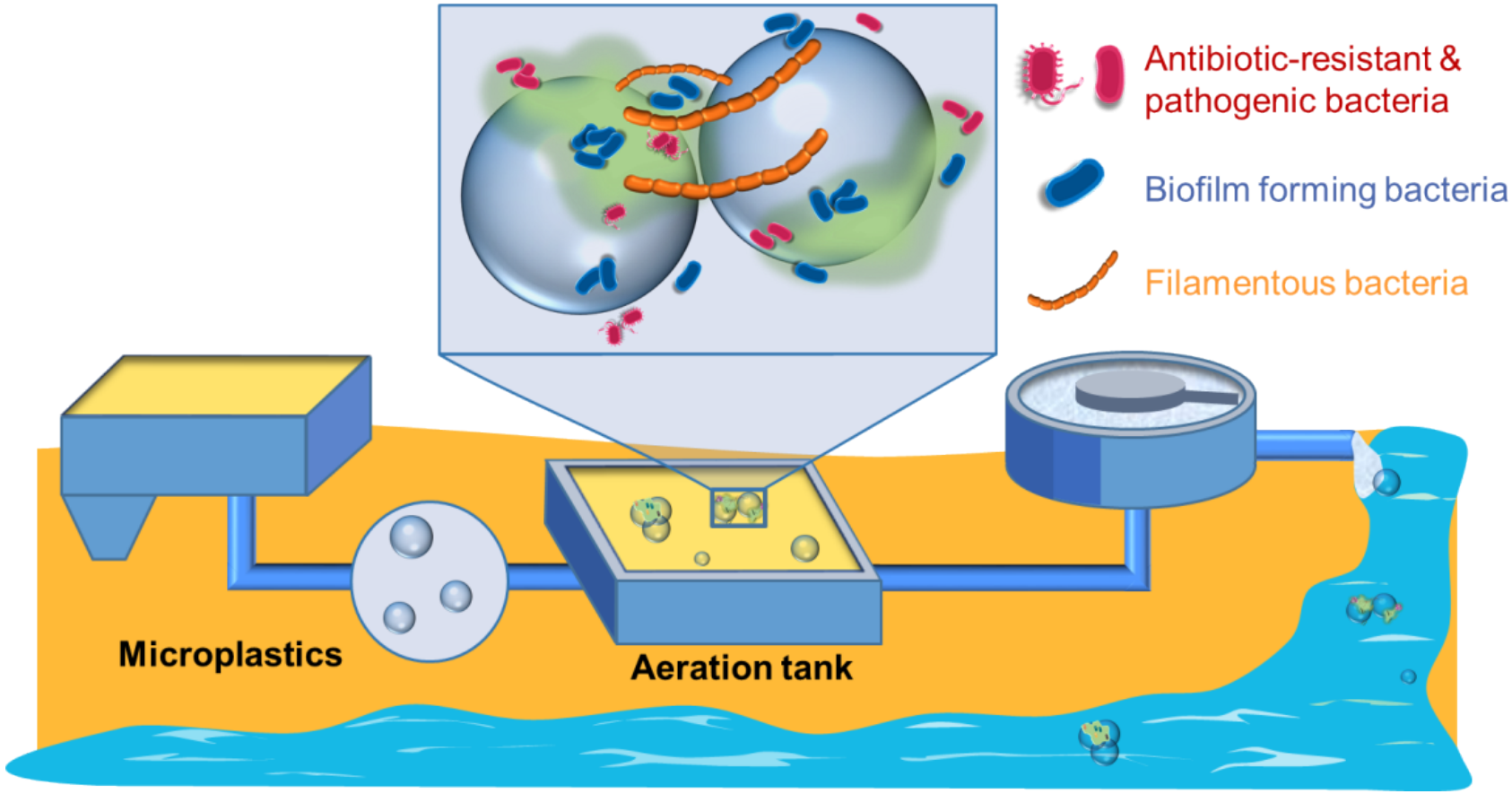
# All *Novosphingobium* Genomes Contain Putative Laccase Genes with Plastic Biodeterioration

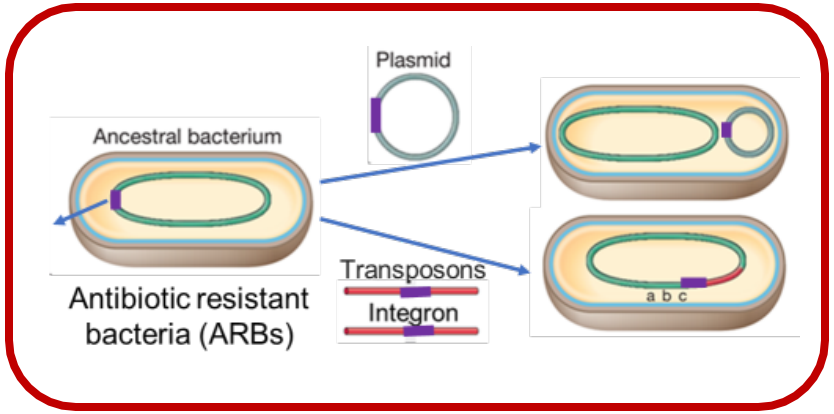
| | | Identity to gene_241689 | Length of protein (bp) |
|---|---|---|---|
| **Bin1** | | | |
| Gene_241698 | Tat signal peptides; Cu-oxidase; Cu-oxidase; Cu-oxidase_2 | 100% | 602 |
| Gene_282975 | Tat signal peptides; Cu-oxidase; Cu-oxidase; Cu-oxidase_2 | 59% | 641 |
| Gene_357237 | Tat signal peptides; Cu-oxidase; Cu-oxidase; Cu-oxidase_2 | 59% | 568 |
| **Bin19** | | | |
| Gene_21100 | Tat signal peptides; Cu-oxidase; Cu-oxidase; Cu-oxidase_2 | 59% | 628 |
| Gene_67166 | Tat signal peptides; Cu-oxidase; Cu-oxidase; Cu-oxidase_2 | 58% | 571 |
| **Bin2** | | | |
| Gene_352615 | Cu-oxidase; Cu-oxidase; Cu-oxidase_2 | 74% | 589 |
| **Bin22** | | | |
| Gene_352615 | Cu-oxidase; Cu-oxidase; Cu-oxidase_2 | 57% | 448 |
| **Bin40** | | | |
| Gene_95004 | Tat signal peptides; Cu-oxidase; Cu-oxidase; Cu-oxidase_2 | 45% | 563 |
| Gene_430096 | Tat signal peptides; Cu-oxidase; Cu-oxidase | 60% | 264 |
| Gene_430097 | Tat signal peptides; Cu-oxidase; Cu-oxidase | 59% | 379 |
| **Bin64** | | | |
| Gene_14939 | Tat signal peptides; Cu-oxidase; Cu-oxidase; Cu-oxidase_2 | 68% | 576 |

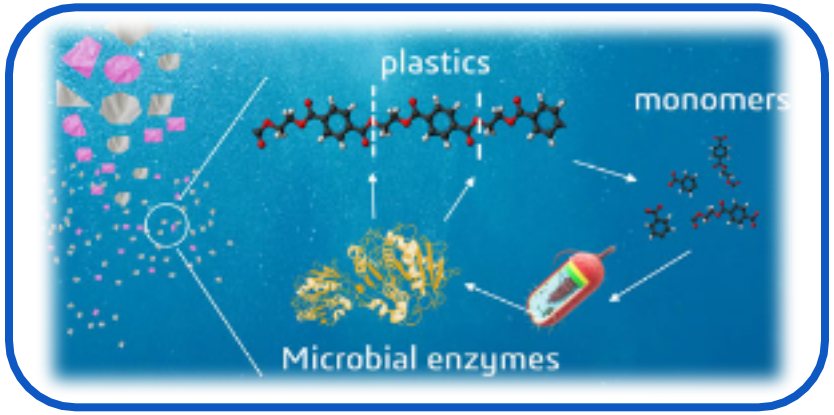



Pham *et al.*, in preparation

# Take Home Message:
# Microplastics are hubs of bacteria with antibiotic resistance and plastic biodeterioration potentials




Pham *et al*., JHM Letters, 2021; Zhang, Tech. Networks, 2021

# Welcome to our posters!

| Title | Poster Board | Time |
|---|---|---|
| Reed Straw-Derived Biochar (RESCA) for Effective Adsorption Removal of Per- and Polyfluoroalkyl Substances (PFAS) | **#25** | Wednesday 5:45 pm |
| Shifting of Target and Non-Target Per- and Polyfluorinated Alkyl Substances (PFAS) over Municipal Wastewater Treatment | **#39** | Wednesday 5:45 pm |
| A Novel Biodefluorination Pathway of Fluorotelomer Carboxylic Acids (FTCAs) by Municipal Activated Sludge | **#43** | Wednesday 5:45 pm |
| Discovery of Gram-Negative Sulfonamide Degraders from Municipal Activated Sludge | **#42** | Wednesday 5:45 pm |
| Novel Group-6 Propane Monooxygenases in Charge of 1,4-Dioxane Biodegradation in Psychrophilic Propanotrophic Consortia | **$46** | Wednesday 5:45 pm |
| Dual-Culture System Enables the Degradation of 1,4-Dioxane and Co-occurring Chlorinated Aliphatic Hydrocarbons | **#45** | Wednesday 5:45 pm |
| Propane and 1-Propanol as Auxiliary Substrate Alternatives for Effective Cometabolic Bioremediation of 1,4-Dioxane | **#50** | Wednesday 5:45 pm |

# Special Issue on “Pollution and Remediation in Soil-Groundwater Environment”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# Acknowledgements


Previous members:

- Dr. Fei Li
- Dr. Na Liu
- Dr. Daiyong Deng
- Dr. Qiong Wu
- Dr. Guifen Lyu

# We are recruiting PhD students and Postdocs!


Mengyan “Ian” Li
mengyan.li@njit.edu

- Pham, D. N.[#] and ***M. Li****. (Jun 4, 2023) Multidrug Resistant Chryseobacteria and Their Health Implications. WATERMICRO23 21st Symposium on Health-related Water Microbiology. Darwin, Australia. *(Talk)*
- Pham, D. N.[#] and ***M. Li****. (Jun 4, 2023) Microplastics as Hubs Enriching Antibiotic-Resistant Bacteria and Pathogens in Municipal Activated Sludge. WATERMICRO23 21st Symposium on Health-related Water Microbiology. Darwin, Australia. *(Poster)*
- S. Zhang, D. N. Pham[#], C. Li[#], L. Axe, and ***M. Li****. (May 29, 2023) Effective Removal of Water Contaminants Of Emerging Concern By Biologically Active Filters. IWA LET2023 Conference. Daegu, South Korea. *(Talk)*
- D. Deng[#] and ***M. Li****. (May 29, 2023) Effective Treatment of 1,4-Dioxane in Wastewater via The Bioaugmentation of *Azoarcus* sp. DD4. IWA LET2023 Conference. Daegu, South Korea. *(Poster)*

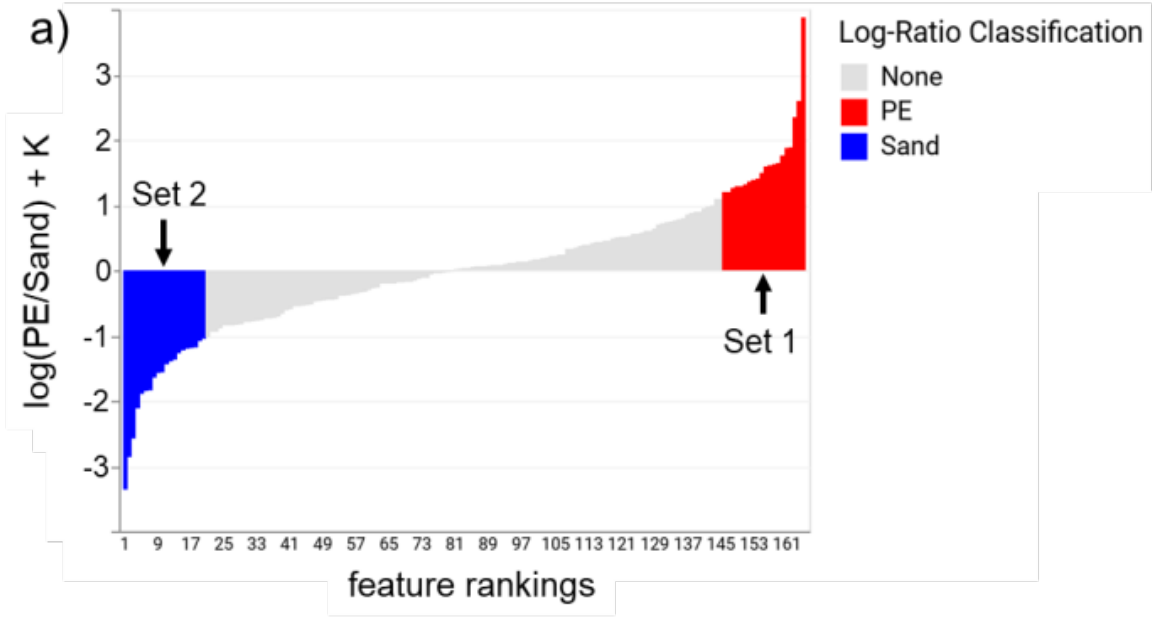
# Plastic Pollution - a Global Issue

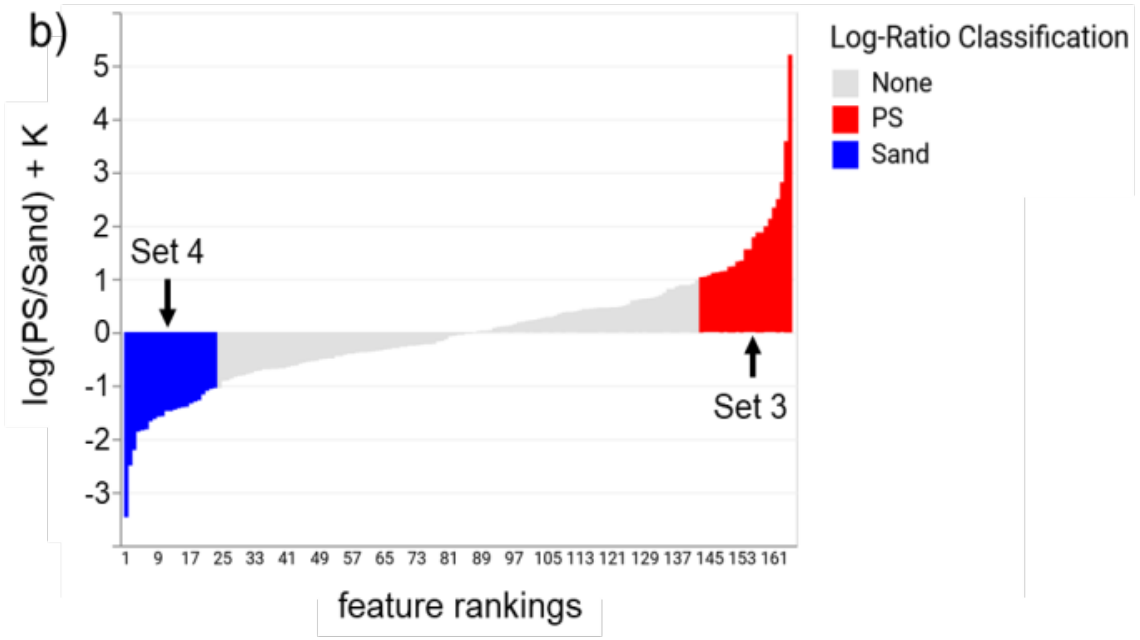
Global plastic production: **348 Mtons/year**

Plastic waste entering the ocean: **4.8 to 12.7 Mtons/year**

Boucher, J. and Friot D. *et al.*, IUCN. (2017)

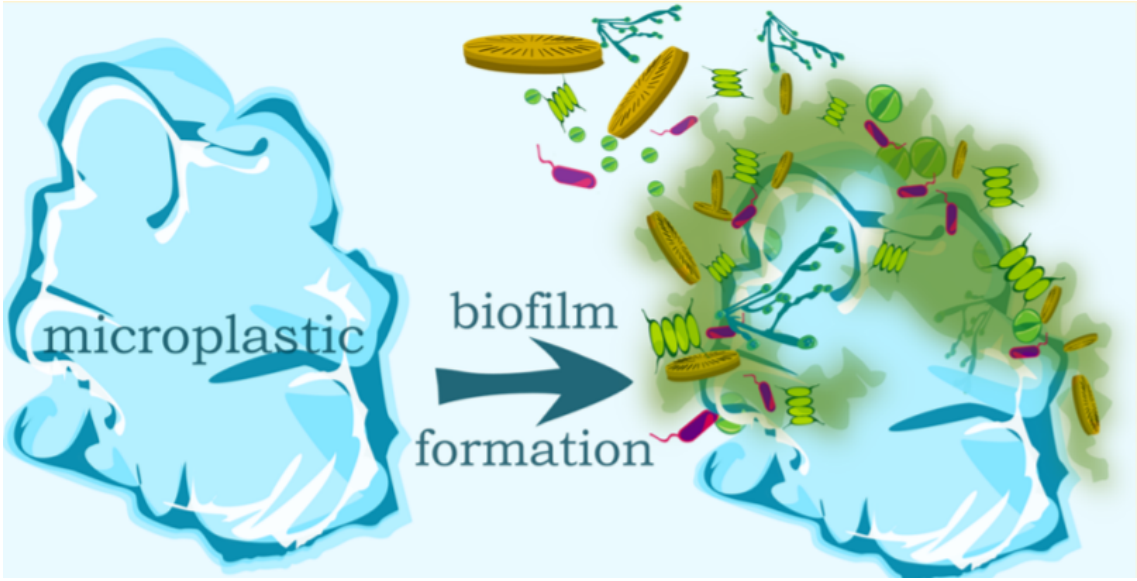
# Differential Ranking Revealed Taxa Specific to Microplastics



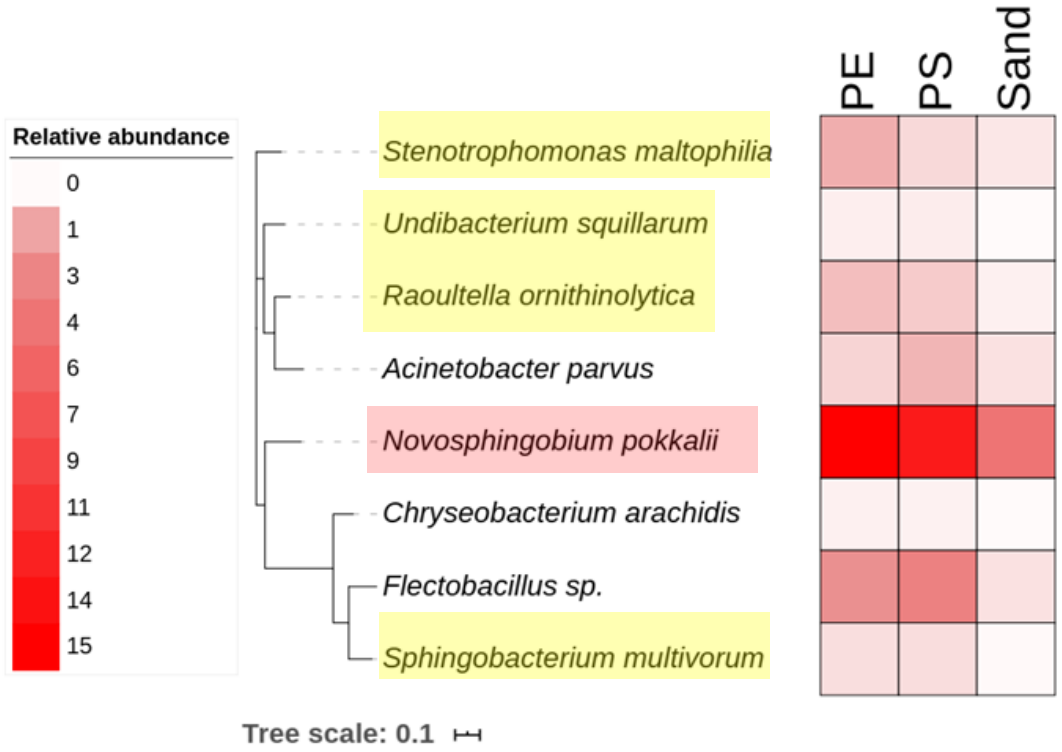
# Objective – Plastisphere Microbiomes

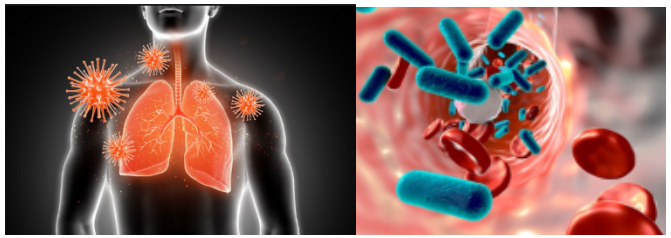
- To investigate the *antibiotic-resistant bacteria (ARB) and associated genes* in activated sludge-derived biofilms on microplastics in municipal wastewater treatment facilities.



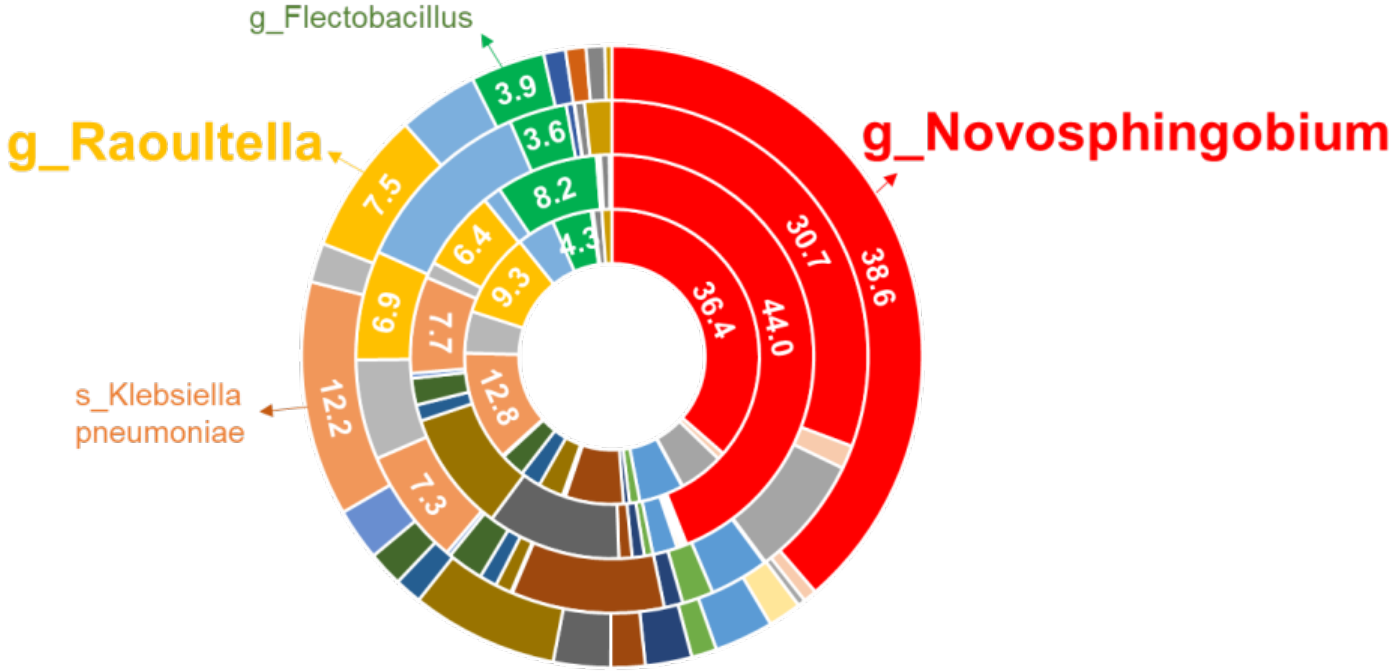

Rummel *et al*., ES&T, 2017

# Eight Bacterial Taxa Enriched on Microplastics



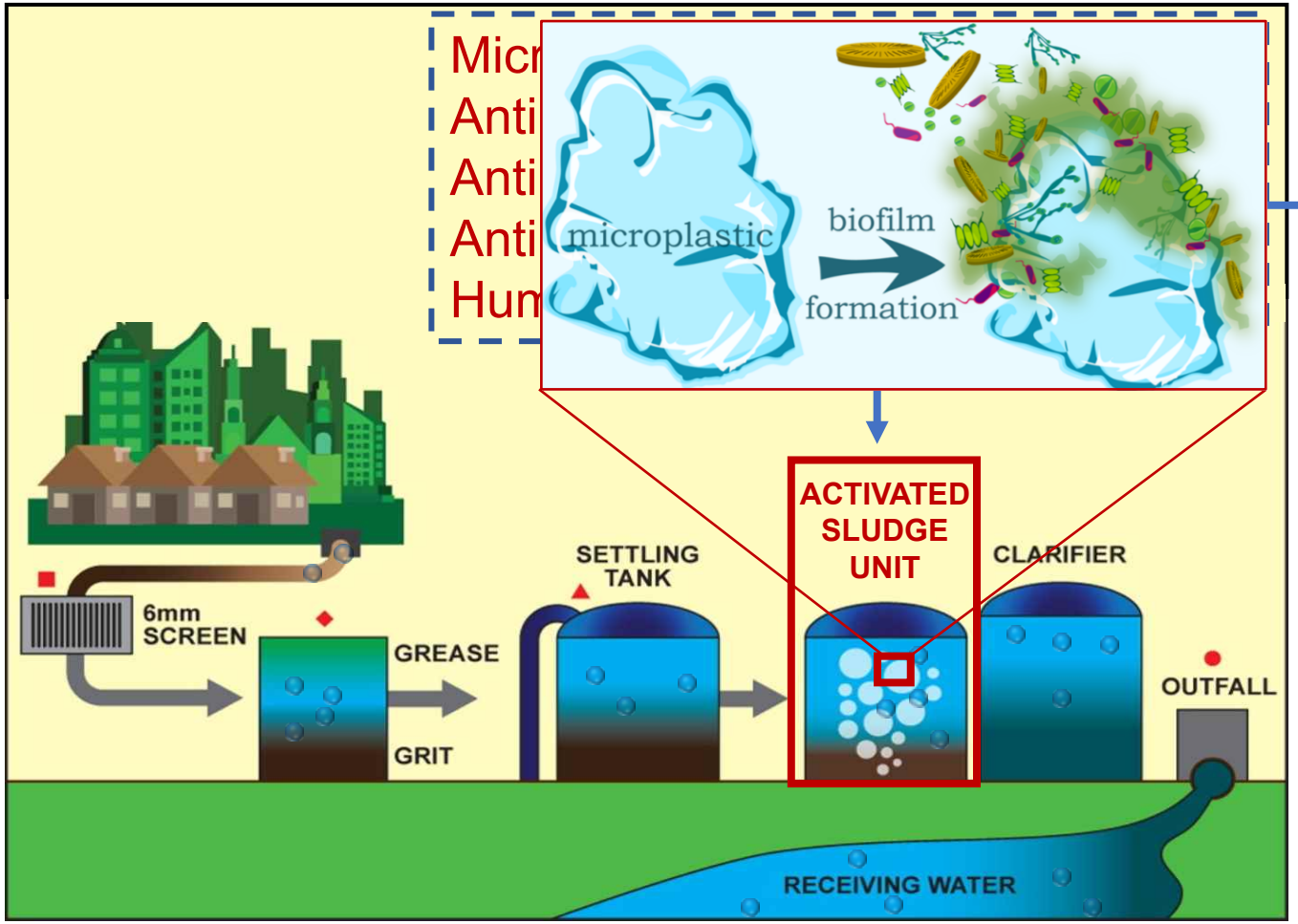



## Relative Abundance of Metagenome-Assembled Genome (MAGs)

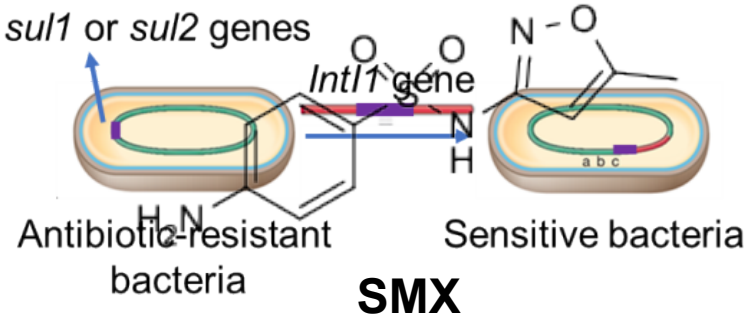



Pham *et al*., JHM Letters, 2021; Pham *et al*., in preparation

# Activated Sludge Units as Hotspots Converging Microplastics and Antibiotic Resistance



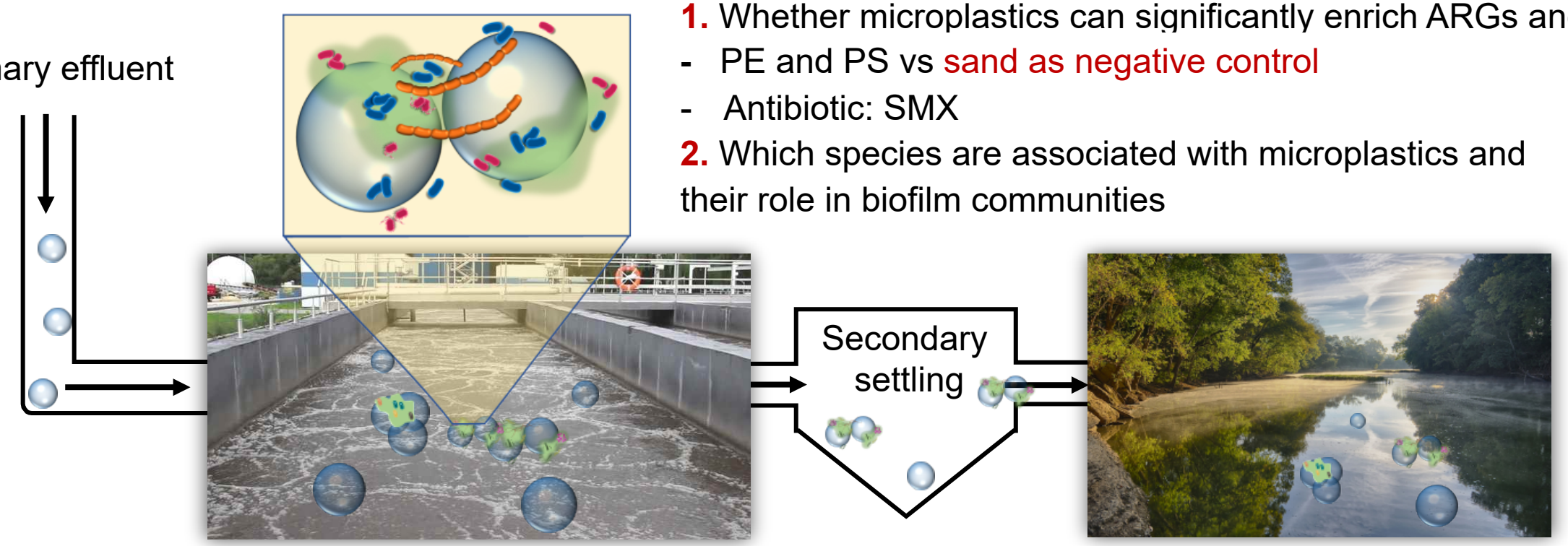

Antibiotics: Sulfamethoxazole (SMX)
SMX resistance genes: *sul1* & *sul2*
Mobile genetic elements: *IntI1*




65 million microplastics/day

Murphy *et al.*, ES&T (2016)
Rummel *et al.*, ES&T (2017)

# Objectives




**1.** Whether microplastics can significantly enrich ARGs and ARB
- PE and PS vs sand as negative control
- Antibiotic: SMX

**2.** Which species are associated with microplastics and their role in biofilm communities

**Significances**

Advance our u[illegible]icroplastics in disseminating antibiotic resistances and pathogen[illegible]stems and downstream environments

| | Maximum detection in wastewater treatment plants |
|---|---|
| Polyethylene (PE) | 51% |
| Polystyrene (PS) | 27% |

Anderson *et al.*, Mar. Pollut. Bull. (2016)

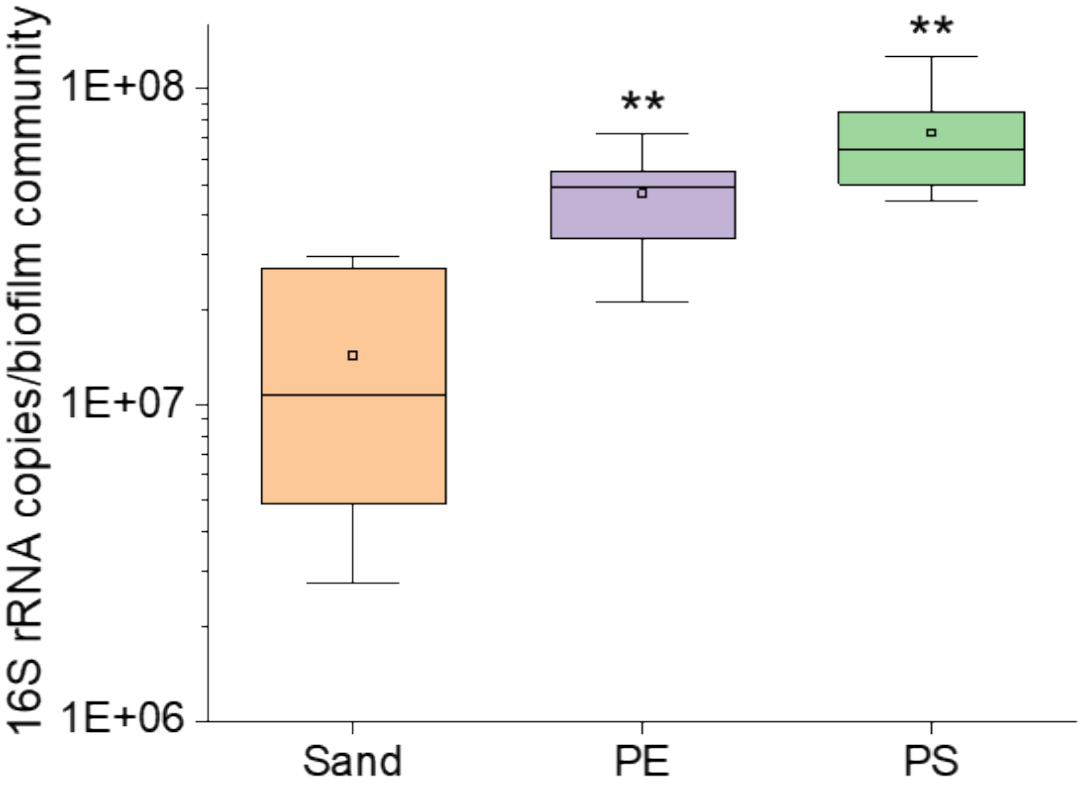
Sun *et al.*, Water Research (2019)

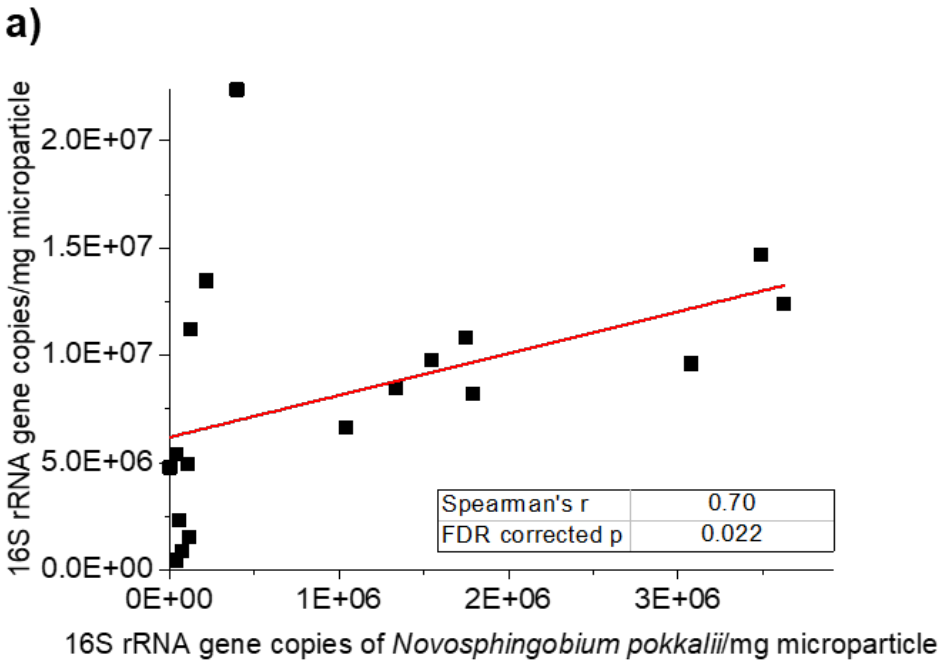
# Microplastics Promoted Biofilm Formation

1. A higher abundance of *N. pokkalii* & *Flectobacillus sp.* on microplastics than on sand

2. A higher hydrophobicity of microplastics than sand

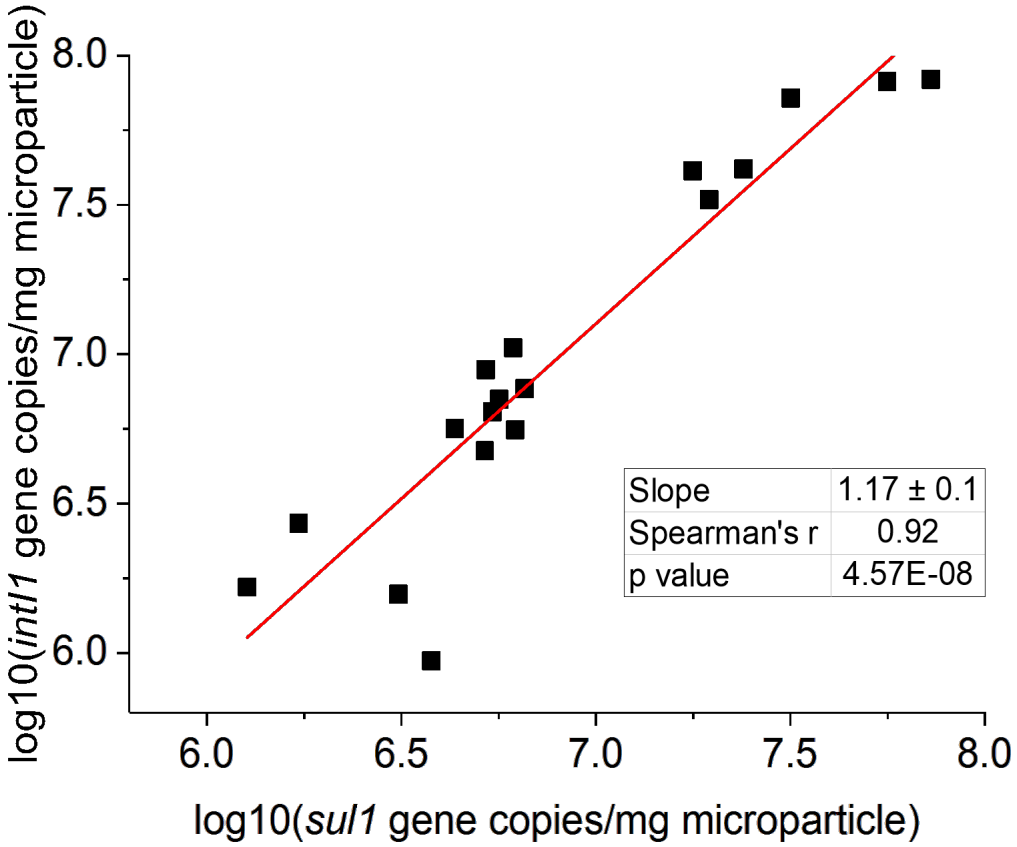
**Biomass ~**



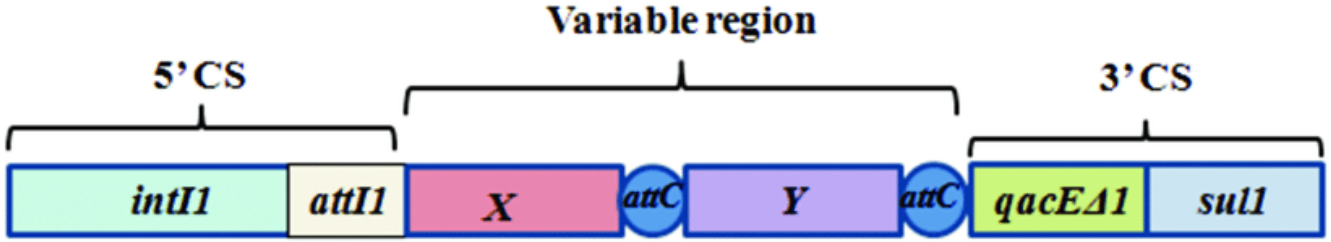




Two-way Mann–Whitney U-test (**, $p < 0.01$).

# Significant Correlation between *Sul1* and *IntI1*




## Structure of a class 1 integron



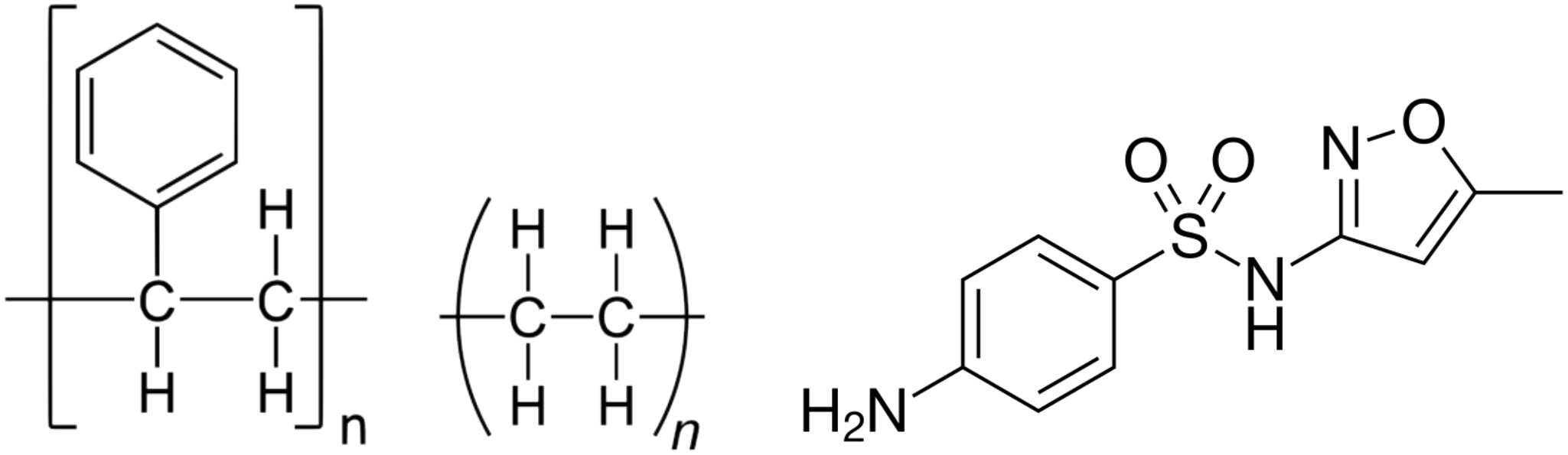

Bhardwaj *et al.*, (2015)

# Research History on Microplastic Biofilms

$$\text{Enrichment index}_{taxon_i} = \frac{N_{taxon_i}/N_{H.Huttiense}}{D_{taxon_i}/D_{H.Huttiense}}$$

$N_{taxoni}$ and $D_{taxoni}$ are average relative abundances of $taxon_i$ in PE or PS and sand biofilms, respectively.

$N_{H.\ huttiense}$ and $D_{H.\ huttiense}$ are average relative abundances of *H. huttiense* in PE or PS and sand biofilms, respectively.




Polystyrene (PS)  Polyethylene (PE)  Sulfamethoxazole (SMX)

**Figure 1**. Molecular structure of polystyrene, polyethylene, and sulfamethoxazole

# qPCR: Microplastics Significantly Enriched ARGs

**Without antibiotic**

**With antibiotic**

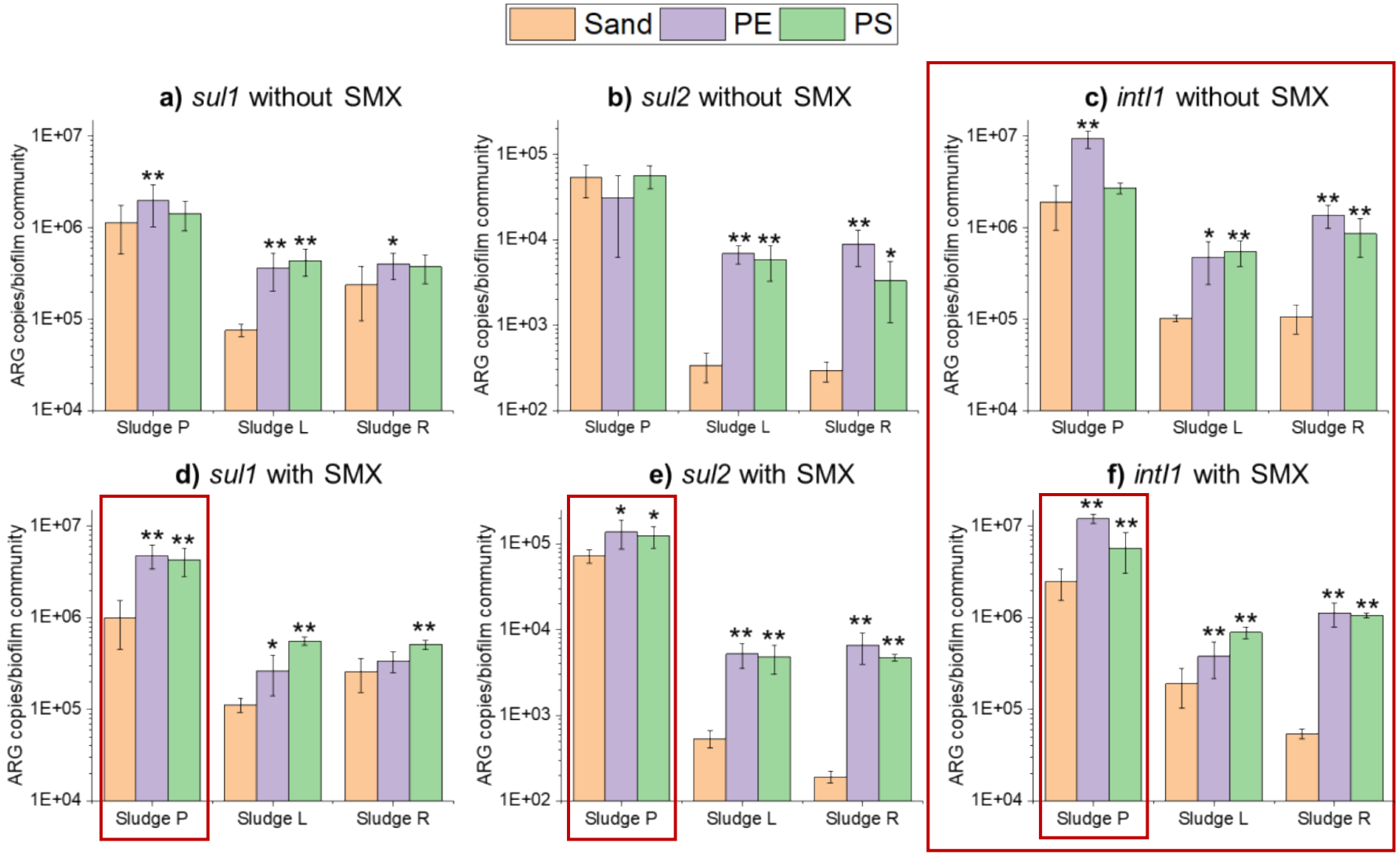



**Figure 1**. Absolute abundance of ARGs in microparticle biofilms cultivated in different activated sludge samples with or without the presence of SMX. Two-way Student's t-test, $*p < 0.05$ and $**p < 0.01$.