

# Comparative analysis of the microbial communities in wastewater and natural water reservoirs

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[https://github.com/inna-tsymb/metagenomics/tree/main/lecture\\_02](https://github.com/inna-tsymb/metagenomics/tree/main/lecture_02)

## 1. Introduction

The increase in urbanization is creating unprecedented pressure on water ecosystems. Wastewater and activated sludge from sewage treatment plants are unique technogenic niches that permanently interact with the natural environment via the release of treated water. Understanding the microbial community of wastewater is critical for evaluating of environmental risks.

**Objective:** To determine if there are fundamental differences in the structure and diversity of microbial communities between anthropogenic sources (wastewater, sludge) and natural aquatic ecosystems (ponds).

**Hypothesis:** Expected that natural environments would have significantly higher biodiversity compared to anthropogenic systems due to lower pollution pressure and more stable ecological conditions.

## 2. Methodology

Metagenomic data from five studies were used for analysis, and were divided into two comparative groups:

- **Anthropogenic group** – characterized by high technogenic impact and includes samples of hospital wastewater (Rowe et al., 2017), activated sludge (Chu et al., 2017), and municipal wastewater (Schulz et al., 2017).

- **Environmental group** – characterized by less technogenic impact and includes pond water (Chopyk et al., 2020) and river water (Lekunberri et al., 2018).

Due to the sample imbalance in Schultz et al. study – 141 sample (>50% of the data) – stratified random sampling was performed to avoid statistical bias (20 samples was included). So, the total sample size after balancing was 113 samples.

Statistical analysis were performed:

- **Alpha diversity:** calculated with Shannon index, statistical significance was tested with Mann-Whitney test.

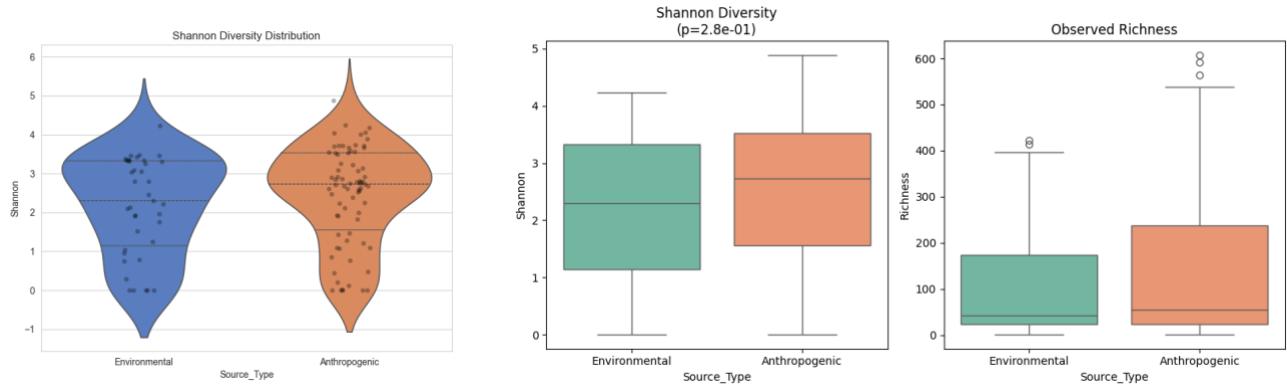
- **Beta diversity:** principal component analysis (PCA) and hierarchical clustering were used to visualize community structure.

- **Differential analysis:** marker species were identified by comparing mean relative abundance.

## 3. Results

### 3.1. Alpha diversity: wastewater paradox

In contrast to the hypothesis, statistical analysis showed no significant difference in biodiversity levels between groups ( $p=0.28$ ). Moreover, average Shannon index for anthropogenic samples (2.44) was slightly higher than natural samples (2.18).

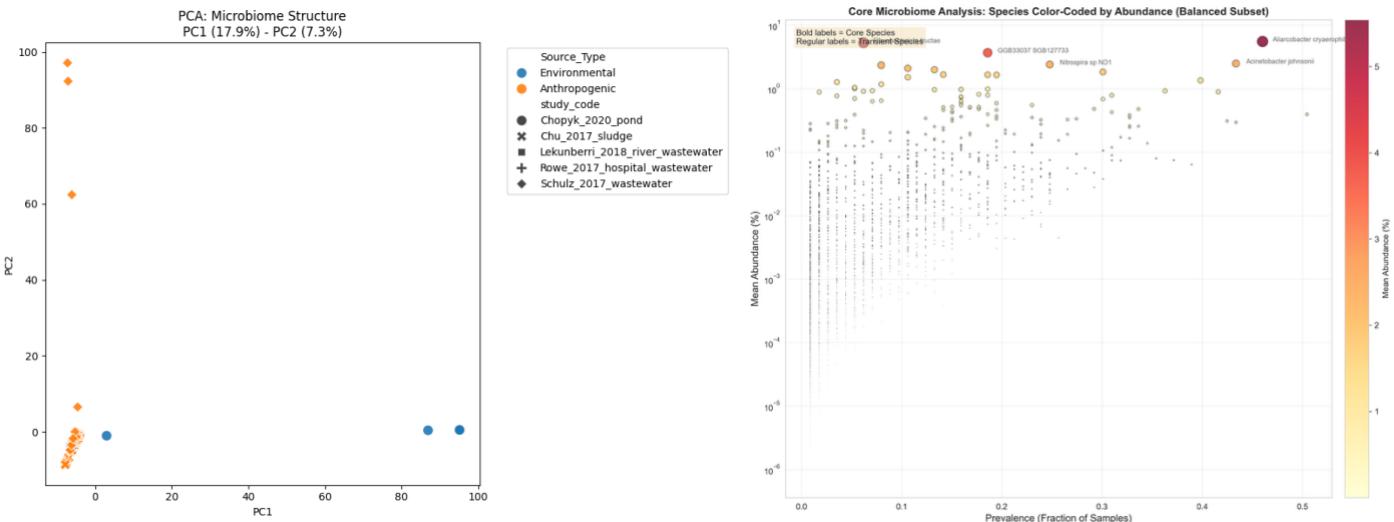


**Fig.1.** Comparison of alpha diversity (Shannon index) between the studied groups – anthropogenic and environmental samples.

Wastewater isn't dead environment – it's an ecosystem rich in organic matter and nutrients, able to support complexity of microbial community comparable to natural water.

### 3.2. Beta diversity and community structure

Principal component analysis showed a clear differentiation between samples, and its indicates that despite the same number of species (alpha diversity) their identity differs radically. Anthropogenic and natural systems function as parallel worlds with different species.



**Fig.2.** PCA shows clear spatial segregation of samples. Core microbiome shows species present in >50% of samples with an average abundance of >1%.

### 3.3. Marker species

Differential analysis allowed to identify key species for each environment:

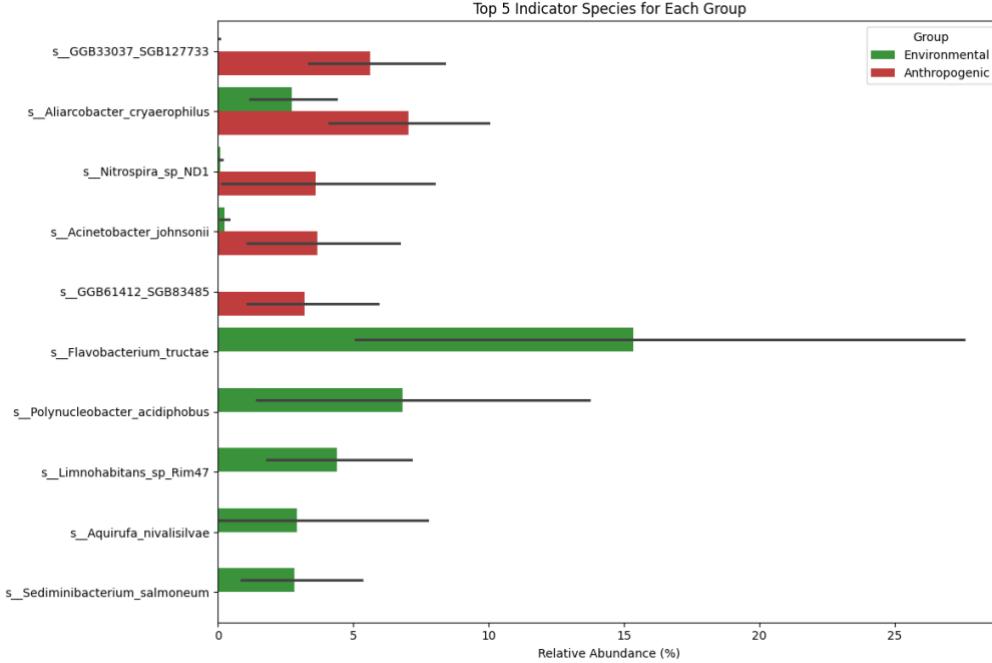
#### A. Anthropogenic indicators (Technogenic impact)

1. *Aliarcobacter cryaerophilus* (7.0%): dominant species, known pathogen associated with fecal contamination.
2. *Nitrospira* sp. (3.6%): nitrifying bacterium, critical for technological process of nitrogen removal.
3. *Acinetobacter johnsonii* (3.7%): indicator of antibiotic resistance, typical for sludge.
4. *SGB127733* (5.6%): Unknown to science (Species Genome Bin), but makes up a significant part of the wastewater biomass – microbial dark matter.

#### B. Natural indicators (Ecological specialists)

1. *Flavobacterium tructae* (15.3%): typical fishes pathogen, indicator of the presence of ichthyofauna.

## 2. *Polynucleobacter acidiphobus* (6.8%): obligate symbiont of freshwater plankton, marker of clean water.



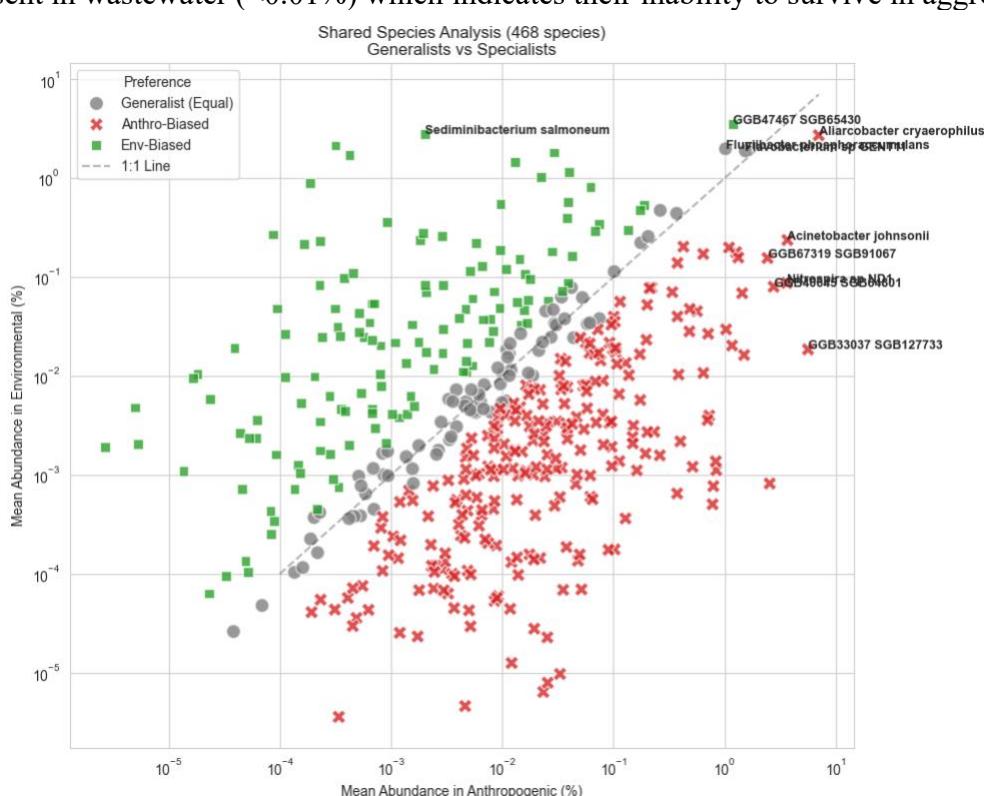
**Fig.3.** Top-5 differential marker species for each group.

### 3.4. Shared species and assessment of the leakage effect

To determine the degree of impact of wastewater on natural ecosystems was conducted analysis of species translocation. And the analysis showed an alarming asymmetry in species exchange:

- **Pathogenic translocation:** *Aliarcobacter cryaerophilus*, abundant in wastewater at level 7.03%, was also found in natural water bodies at level of 2.72%. this is direct evidence of untreated or poorly treated wastewater into rivers.

- **Ecological barrier:** in contrast, native species of natural waters (like *Reyranella aquatilis*) are practically absent in wastewater (<0.01%) which indicates their inability to survive in aggressive environment.



**Fig.4.** Ecological specialization of common species (Generalists vs Specialists).

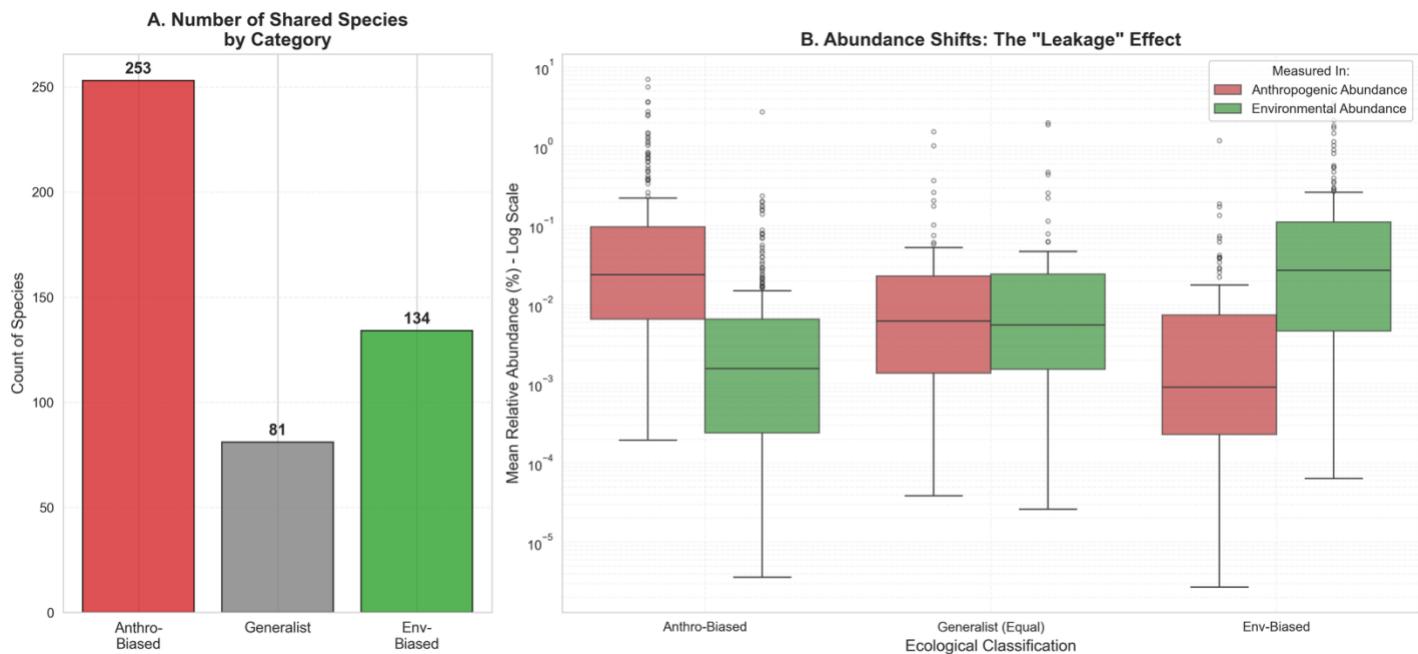
Based on the ratio of abundance all common species was classified into three categories:

- Anthro-Biased: species whose numbers in wastewaters exceed natural levels by more than 2 times (red zone), which includes *Aeromonas media* and *Aeromonas caviae* – potential pathogens for fish and humans.
- Env-Biased: species like *Reyranella aquatilis* and *Propionispira arboris*, are native for clean waters.
- Generalists: a small group of bacyerias, like *Pseudomonas* strains, which equal success in both environments.

### 3.5. Assessment of the permeability of ecological barriers: The phenomenon of “one-way traffic”

Sewage bacterias demonstrate high invasive capacity in successful colonization of natural water reservoirs. This phenomenon of microbial pollution creates risk for natural waters to become a secondary reservoirs of pathogens and antibiotic resistance genes.

In the Anthro-Biased group a leakage effect was observed: species dominated in wastewater are also presented in natural reservoirs in significant numbers, confirming antropogenic pollution. In the Env-Biased group the opposite pattern was observed: natural species rapidly reduce thei number counts when exposed to technogenic environment.



**Fig.5.** Distribution of ecological niches of shared species and the translocation effect. A. Quantitative distribution of species by category. B. Comparison of species abundance in different environments.

## 4. Discussion

### 4.1. The biodiversity paradox

Results refuted initial hypothesis that anthropogenic pollution reduces biodiversity. The fact that there was no statistically significant difference in Shannon indexes between wastewater and natural water indicates that wastewater plants functioning as highly productive technogenic bioreactors.

The high diversity in wastewater can be explained by effect of hyperutrophication. The continuous flow of organic carbon, nitrogen, and phosphorous creates ideal condition for coexistence of wide range of microorganisms: from human intestinal anaerobes to specific activated sludge bacteria. Unlike natural waters, which usually poor in nutrients, wastewater provides resources for a complex artificial trophic network.

### 4.2. Structural reorganization

Analysis of beta diversity confirmed the concept of “same complexity different functions”. The natural microbiome in rivers and ponds based on natural symbiotic relationships. And the dominance of *Flavobacterium tructae* and *Polynucleobacter* indicates a close relationships between bacterias and fishes or algae. Anthropogenic microbiome formed under the pressure of technological purification processes. The high proportion of *Nitrospira* reflects the selective selection of species capable of surviving in aerotanks.

#### **4.3. Leakage effect and risks to public health**

The presence of sewage zootopic pathogen *Aliarcobacter cryaerophilus* at level of 2.7% in river samples is direct evidence of the ineffectiveness of existing environmental barriers. This can be classified as biological pollution, and this microorganisms capable of:

- Reproducing in suitable conditions (eg during warm season)
- Form biofilms, protecting them from UV and predators
- Accumulate in bottom debris, creating long-term reservoirs of infection

#### **4.4. Rivers as reservoirs of antibiotic resistance**

Translocation of *Acinetobacter johnsonii* is critical in the context of One Health concept. *A.johnsonii* is known for ability to carry plasmids with multidrug resistance genes, and even small number of those bacteria (here ~0.24%) in rivers creates conditions for horizontal gene transfer to resident microflora. Thus, wastewater turns natural waters to ground for superbugs which can return to humans through food chains or water use.

#### **4.5. Dark matter of the microbiome**

Discovery of uncultivated species SGB127733 which represent for more than 5% of wastewater biomass, highlight the limitations of knowledges about anthropogenic ecosystems. Due to fact that one of the most common species in our drains still has no official name and described functions indicate huge hidden metabolic potential that can be both – exploited in biotechnology or pose a hidden danger.

### **5. Limitations**

Despite the results, this study has a number of limitations:

- Heterogeneity of metadata and lack of physicochemical parameters.
- Sample imbalance and potential batch effect.
- Lack of temporal dynamics.
- Limitation of reference databases.
- Geographic fragmentation.

### **6. Conclusions**

Study demonstrates the fundamental erosion of ecological barriers between urban environments and natural ecosystems. Obtained results force to revise the traditional view waste treatment facilities as isolated filters, and function as powerful technogenic incubators that actively exports own uniquely formed microbiome into natural environments. This process has three critical outcomes:

- Ecological invasion: river microbiomes are being anthropogenized, where natural species being replaced or diluted by wastewater generalists
- One Health risk: rivers are become dynamic reservoirs for zoonotic pathogens and antibiotic resistance genes, creating hidden epidemiological risk that cannot be detected by standard methods

- Paradigm shift in monitoring: detection of significant proportion of microbial dark matter in wastewater proves that traditional sanitary indicators like *E.coli* are outdated. Modern environmental management requires the implementation of metagenomic surveillance for a complex biosafety assessment of water resources.

## Literature

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