One-year surveillance of pathogens in a full-scale Municipal Wastewater Treatment Plant

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BACKGROUND AND GOALS

Wastewater Treatment Plants (WWTPs) constitute a relevant reservoir and source of pathogens released into the environment.[1]

Wastewater-based surveillance of pathogens can assist in taking informed public health actions,[2] but few studies characterized thoroughly the pathogens content of WWTPs in longer time periods, which are useful for establishing indicators.[3]

The goals of this study were: (i) to characterize the wastewater profile of putative pathogens in a WWTP during a 1-year period, (ii) identify treatment effects and seasonal trends in the relative abundance of pathogens and (iii) explore the use of integrase genes as indicators of these groups.
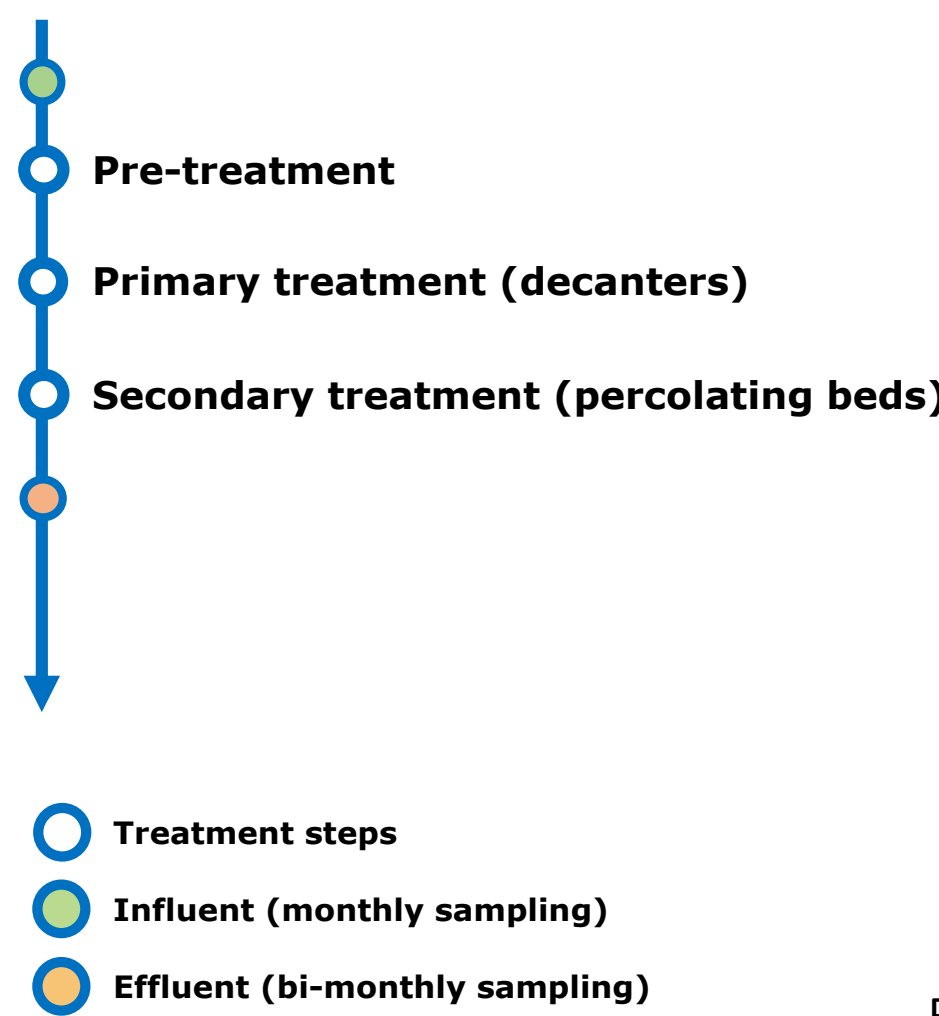
References

[1] Marutescu et al., 2023. Front Microbiol, 14: 1193907.
[2] Diamond et al., 2022. Nat Med, 28: 1992-5.
[3] Zheng et al., 2020. Environ Int, 135: 105372.
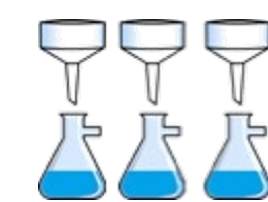
METHODS

Municipal WWTP setup

Pre-treatment
Primary treatment (decanters)
Secondary treatment (percolating beds)

Treatment steps
Treatment (monthly sampling)
Influent (monthly sampling)
Effluent (bi-monthly sampling)

1 Sampling
January to December 2020
11 influent samples
25 effluent samples

2 Water filtration
50 mL of sample
0.22 μm membrane filters

3 DNA extraction
MO BIO's PowerSoil
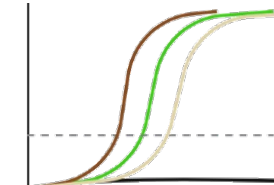DNA isolation kit

4 Metagenome sequencing
illumina
16S rRNA-encoding gene amplicon sequencing
Taxonomic affiliation using Silva database
Pathogen identification using the list of pathogenic genera from FAPROTAX and NCBI pathogens databases
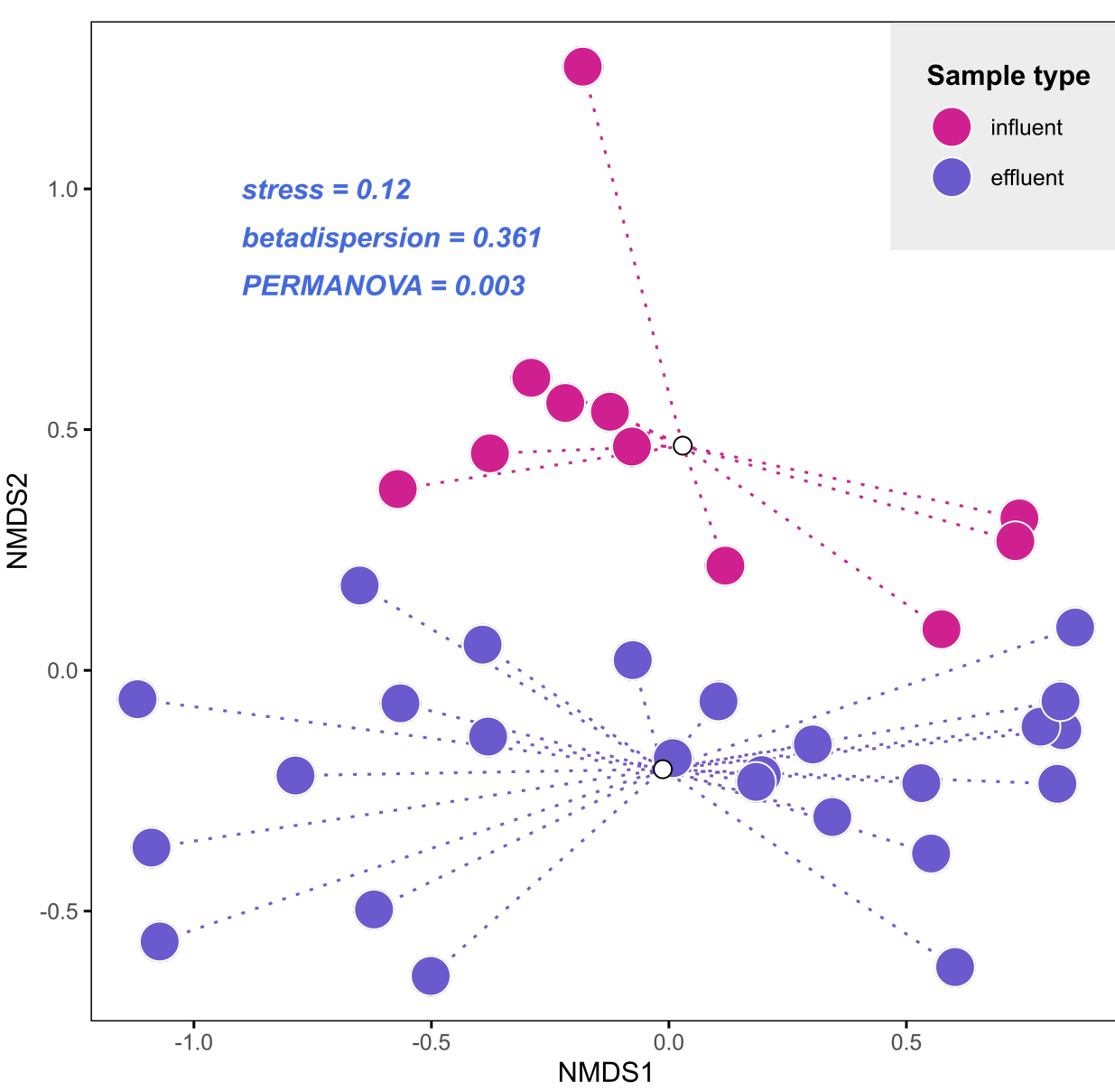
5 Quantitative PCR
4 pathogenic targets (Enterococci, Klebsiella pneumoniae, Bacteroidota and E. coli) and 3 integrase genes (intI1, intI2, intI3)

RESULTS

1. WWTP pathogen content

A | WWTP treatment impact

stress = 0.12
betadispersion = 0.361
PERMANOVA = 0.003

Sample type: influent, effluent

B| Seasonality in the effluent

stress = 0.08
betadispersion: p-value = 0.178
PERMANOVA: p-value = 0.024
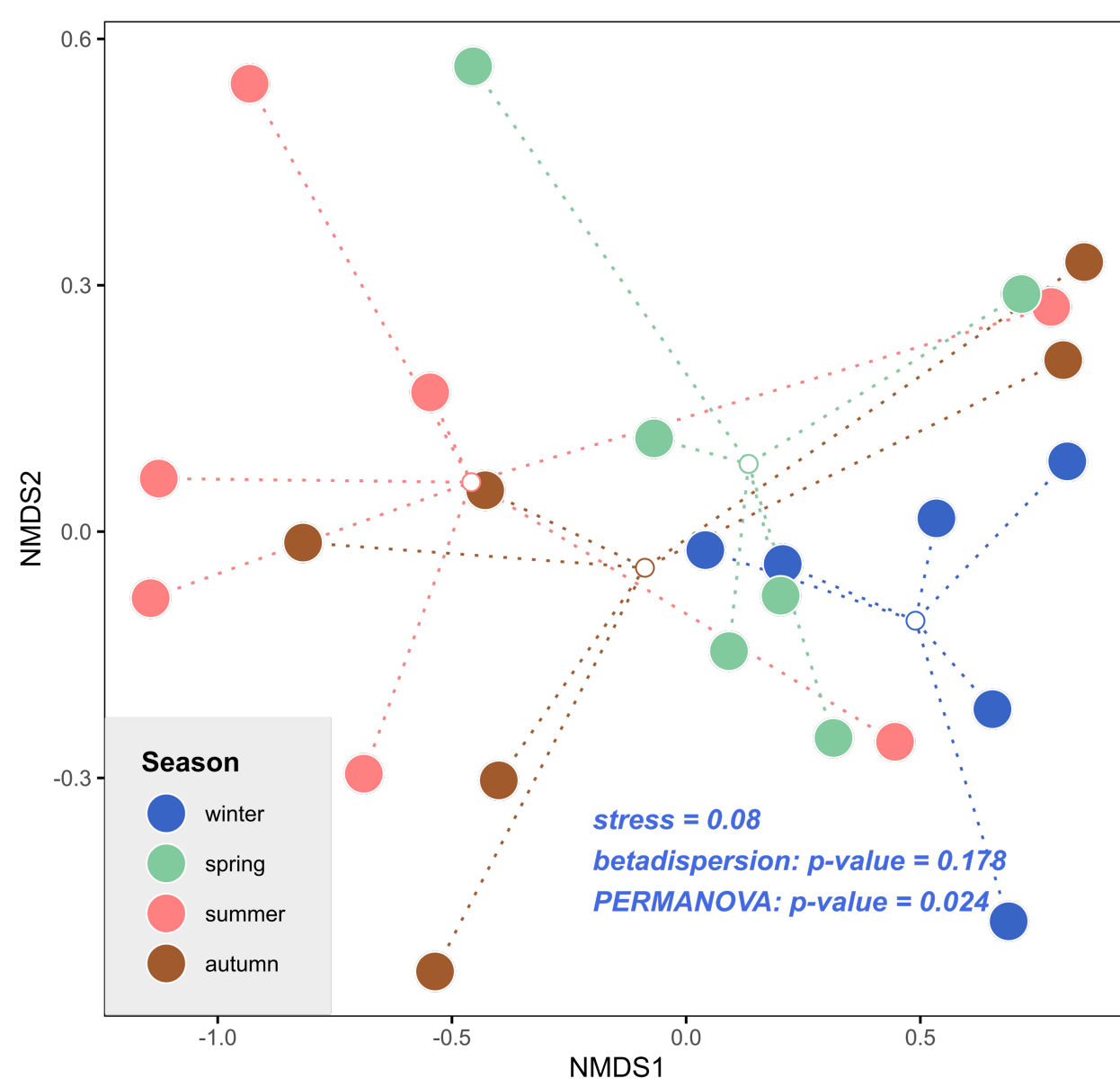
Season: winter, spring, summer, autumn

Figure 1. NMDS plots of the abundance of putative pathogenic genera between (A) influent and effluent, and (B) between seasons in the effluent. Relative abundance was obtained from Illumina sequencing data and Bray-Curtis distances were calculated from the resemblance matrixes of the putative pathogen's abundance. White circles represent the centroids of each comparing group.

▶ The pathogen content changed significantly from influent to effluent (PERMANOVA, p-value=0.003).

▶ In the effluent, pairwise PERMANOVA showed no significant differences between seasons (p-value>0.05).
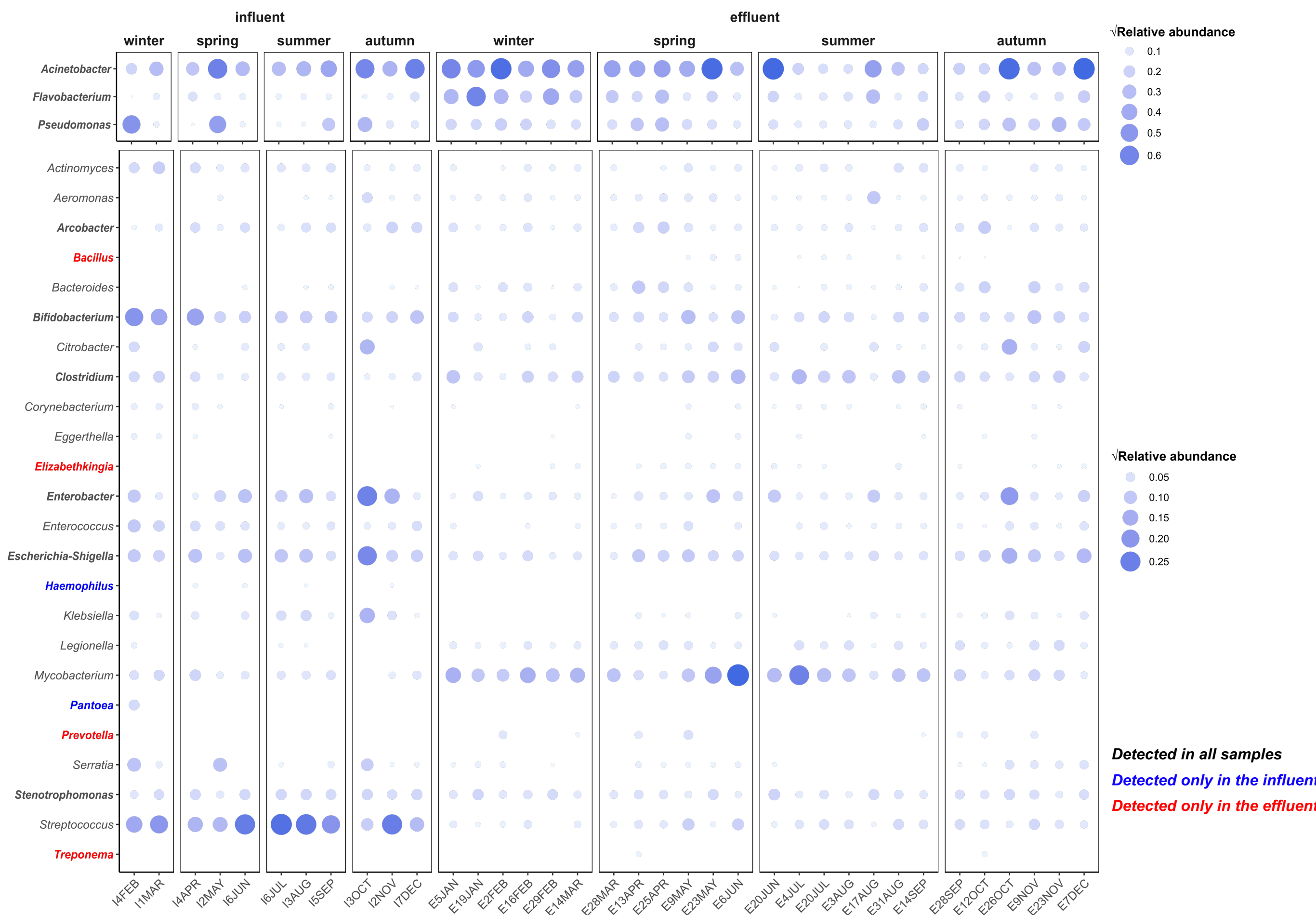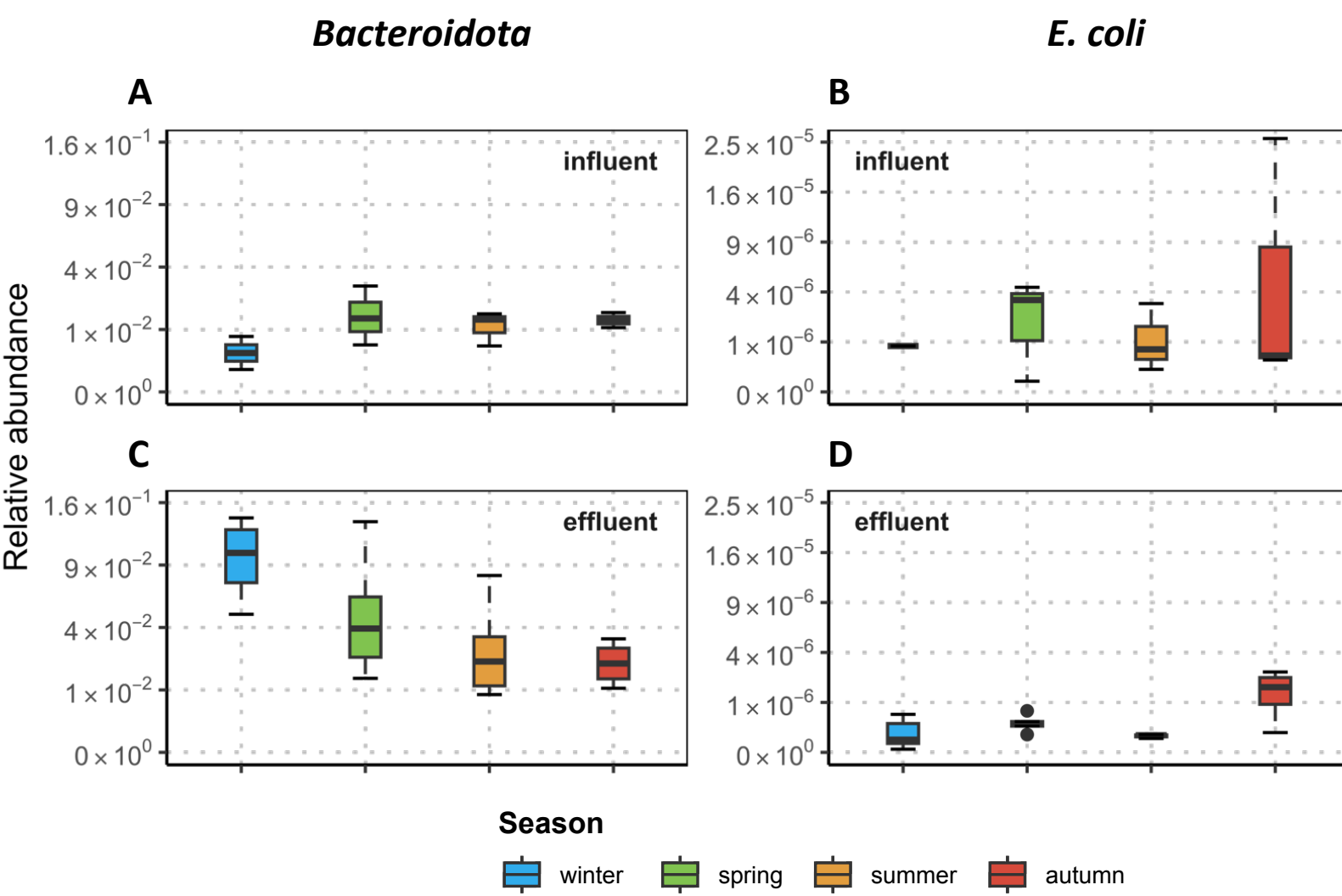
2. Putative pathogenic genera abundance

Figure 2. Bubbleplot of the relative abundance (square-rooted) of the putative genera identified in wastewater samples. Due to the large discrepancy of relative abundance between genera, the plot was separated into two distinct scales.

Detected in all samples
Detected only in the influent
Detected only in the effluent

▶ 27 putative pathogenic genera were identified in the wastewater samples, 9 of which were present in all samples (e.g., *Acinetobacter*), 2 only in the influent and 4 only in the effluent.

▶ The most abundant putative pathogenic genera were *Acinetobacter*, *Flavobacterium* and *Pseudomonas*.

Bacteroidota

E. coli

A — influent
B — influent
C — effluent
D — effluent

Season: winter, spring, summer, autumn

▶ Only *E. coli* and *Bacteroidota* were identified in the samples by qPCR. *Enterococci* and *K. pneumoniae* were below the detection limit.

▶ *Bacteroidota* was enriched in the effluent, displaying seasonal variations opposite to those observed in the influent.

▶ Quantification of pathogens by qPCR was strongly correlated with the relative abundance determined by Illumina sequencing (for *E. coli* ρ=0.92 and for *Bacteroidota* ρ=0.77).

Figure 3. Boxplot of the relative abundance of pathogenic groups detected by qPCR per season in influent and effluent samples.

3. Differential relative abundance analysis

enrichment
reduction
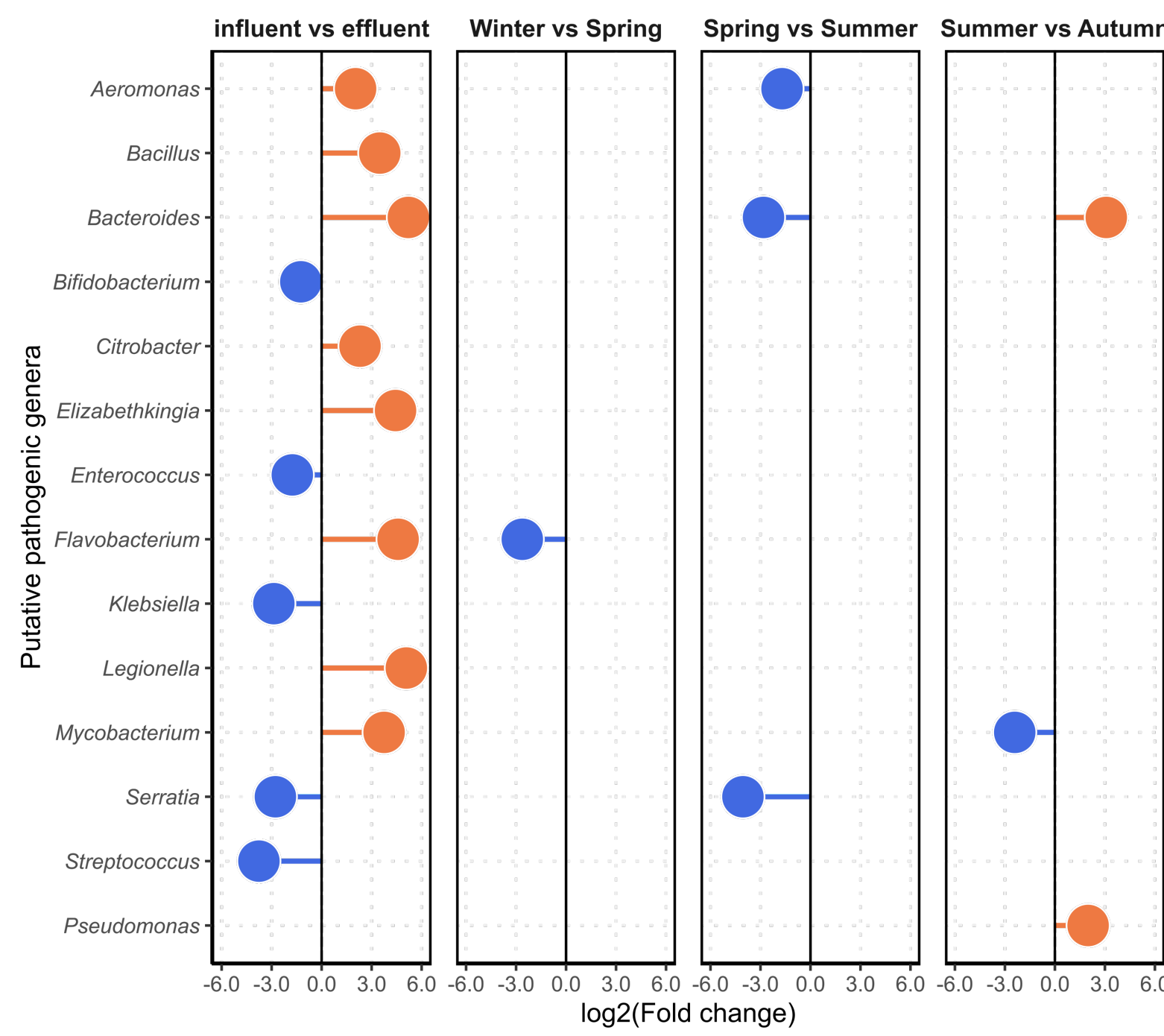
▶ The relative abundance of 5 putative pathogenic genera was significantly reduced from influent to effluent, while 8 genera were relatively enriched (DESeq2, p-value<0.05).

▶ 6 genera were enriched in the effluent samples in at least one of the seasons.

▶ qPCR quantification confirmed the relative enrichment for *Bacteroidota*'s genera in the effluent while *E. coli* relative abundance significantly decreased from influent to effluent.

Figure 4. Lollipop plot of the DESeq2-calculated log2 (Fold change) of putative pathogenic genera whose abundance was significantly different (p-adjusted values<0.05) between influent and effluent samples or between effluent samples from different seasons.

4. Potential indicators of pathogens in wastewater

Integrase gene: intI1, intI2, intI3
Negative correlation
No correlation
Positive correlation
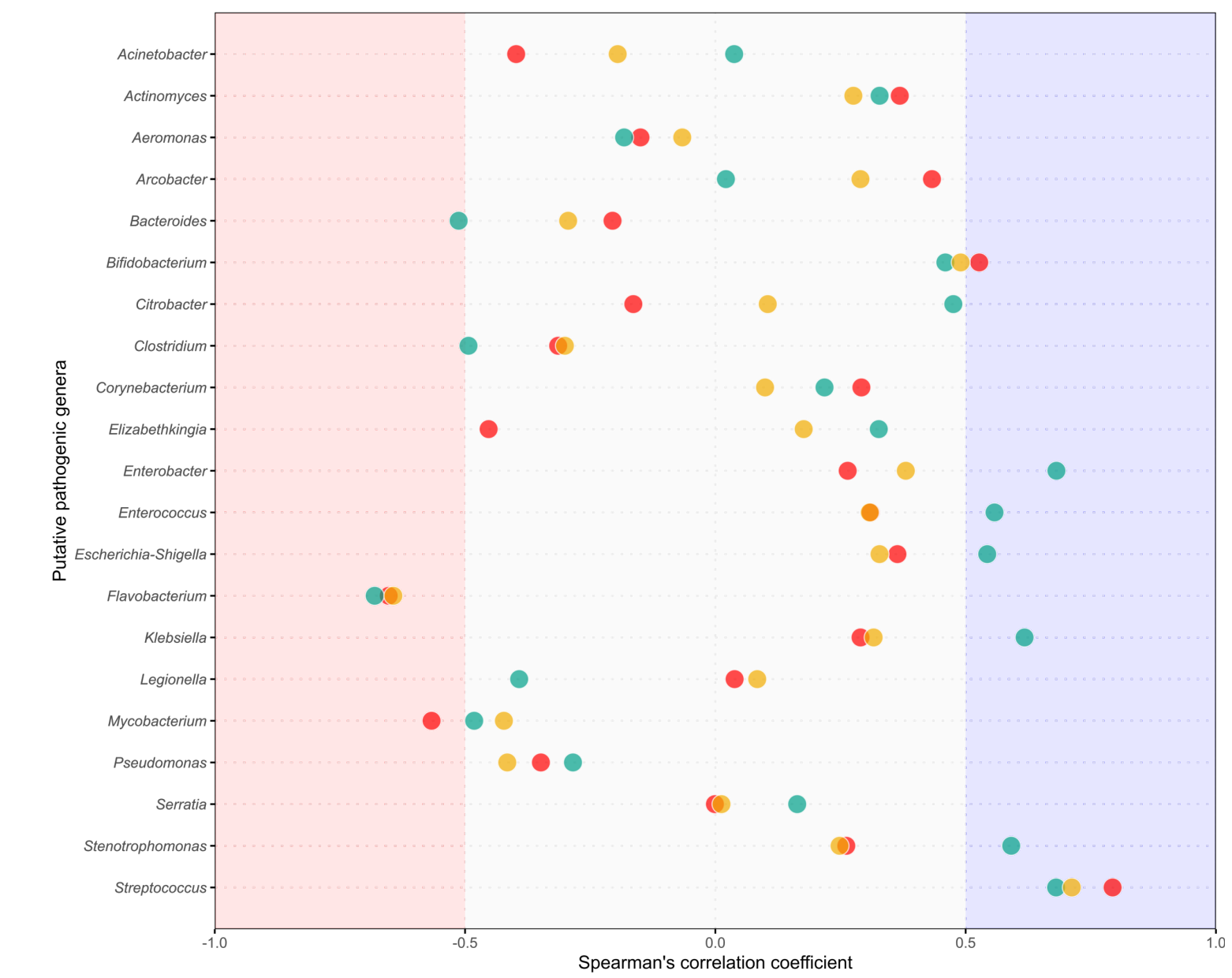
▶ The relative abundance of integrase genes was positively correlated with the abundance of a minority of the detected pathogens.

▶ The relative abundance of all *intI* genes was strongly correlated with the abundance of *Streptococcus*, reflecting the high levels of faecal contamination in the wastewater.

▶ *intI2* were the integrase genes with the highest number of positive correlations (ρ>0.5), which might be related to their lesser widespread dissemination on the bacterial communities.

Figure 5. Distribution of Spearman's correlation coefficients between each putative genera abundance and the abundance of integrase genes.

CONCLUSIONS

▶ Wastewater treatment led to shifts in the relative abundance of pathogenic genera, with significant enrichment of some and decrease of others, but the pathogen profile was stable across seasons. This indicates that WWTP's treatment should be improved.

▶ Integrase genes aren't reliable for estimating wastewaters' pathogen abundance since they correlate to only a few groups.

▶ Future studies should evaluate correlations with virulence genes, since pathogenicity traits can be strain-specific.

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