



Microbial community and antimicrobial resistance niche differentiation in a multistage, surface flow constructed wetland

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ABSTRACT

Free-living (FL) and particulate-associated (PA) communities are distinct bacterioplankton lifestyles with different mobility and dissemination routes. Understanding spatio-temporal dynamics of PA and FL fractions will allow improvement to wastewater treatment processes including pathogen and AMR bacteria removal. In this study, PA, FL and sediment community composition and antimicrobial resistance gene (ARG; *tetW*, *ermB*, *sul1*, *intI1*) dynamics were investigated in a full-scale municipal wastewater free-water surface polishing constructed wetland. Taxonomic composition of PA and FL microbial communities shifted towards less diverse communities (Shannon, Chao1) at the CW effluent but retained a distinct fraction-specific composition. Wastewater treatment plant derived PA communities introduced the bulk of AMR load (70 %) into the CW. However, the FL fraction was responsible for exporting over 60 % of the effluent AMR load given its high mobility and the effective immobilization (1–3 log removal) of PA communities. Strong correlations ($r^2 > 0.8$, $p < 0.05$) were observed between the FL fraction, *tetW* and *ermB* dynamics, and amplicon sequence variants (ASVs) of potentially pathogenic taxa, including *Bacteroides*, Enterobacteriaceae, Aeromonadaceae, and Lachnospiraceae. This study reveals niche differentiation of microbial communities and associated AMR in CWs and shows that free-living bacteria are a primary escape route of pathogenic and ARG load from CWs under low-flow hydraulic conditions.

1. Introduction

Aquatic environments support a variety of microbial lifestyles and niches, including particle-associated (PA) and free-living (FL) bacterial communities. PA bacteria exploit nutrients found in organic and inorganic aggregates and use these aggregates for shelter against harsh conditions protection from predators, and as transport vectors into new habitats (Bydalek et al., 2023; Malham et al., 2014; Oberbeckmann et al., 2015; Thit et al., 2022). PA communities display higher metabolic activity and diversity than FL communities, but both interact through the exchange of nutrients, metabolites, and genes (Jang et al., 2022; Liu et al., 2020; Parveen et al., 2013). In oligotrophic environments, suspended solids serve as nutrient hotspots, attracting FL bacteria to interact and settle, thereby increasing diversity in established PA

biofilms (Heins et al., 2021). Many PA bacteria can adapt to FL lifestyles when particulate nutrient sources are depleted (Grossart, 2010; Jain and Krishnan, 2017).

Wastewater effluent is a primary pathway for human pathogens and antimicrobial resistance (AMR) to enter the environment (Czekalski et al., 2014; Karkman et al., 2018). In wastewater treatment plants (WWTPs), secondary treatment processes such as sedimentation, filtration, coagulation, and activated sludge effectively retain particulate matter, including PA bacteria. However, while total suspended solids (TSS) and turbidity are routine indicators of WWTP performance, efficient particle removal may not guarantee pathogen and AMR exclusion in the effluent (Mulling et al., 2013). Specifically, the FL fraction, which is less efficiently removed in WWTPs, harbors pathogens and AMR hosts (Yu et al., 2021). Targeted removal of pathogens and AMR bacteria and

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antimicrobial resistance genes (ARGs) in WWTPs can be implemented through disinfection and other tertiary treatment stages (Choi et al., 2020; Nnadozie et al., 2017), but research reveals increasing resistance to disinfection agents and enhanced selection for AMR bacteria (Adefisoye and Olaniran, 2022; Guo and Kong, 2019). Disinfection processes have been notably hindered by the protective effect of particles, thereby granting the PA fraction a survival advantage (Dietrich et al., 2003, 2007). However, recent studies indicate that FL bacteria can also survive disinfection processes just as well as PA bacteria (Huang et al., 2018). This is important since the FL community typically constitutes a smaller portion of effluent biomass (Liu et al., 2016), it has been reported to contain a significantly higher relative abundance of pathogens and AMR genes than the PA community (Huang et al., 2018; Odjadjare and Okoh, 2010; Yu et al., 2020). This highlights the need for wastewater treatment processes to account for the distinct dynamics between PA and FL bacteria. Adopting such an approach could enhance existing and develop new pathogen and AMR-targeted treatment processes (Maganha de Almeida Kumlien et al., 2021; Rice et al., 2020). However, there is limited literature addressing niche-specific microbial community dynamics in WWTPs, particularly in those utilizing non-conventional wastewater treatment methods such as constructed wetlands (CWs).

Constructed wetlands (CWs) are nature-based solutions widely used in wastewater management schemes, serving as either alternatives or support for conventional wastewater treatment plants (Vymazal, 2011). Although the primary treatment objective is not necessarily to remove biological contamination, CWs employ various processes that mimic the functioning of natural wetlands, effectively co-removing pathogens and AMR bacteria (Alexandros and Akrotos, 2016). Consequently, pathogen and AMR removal in CWs can match conventional WWTPs' performance of 1–3 log reduction (Hazra et al., 2022). However, CWs' performance is more variable and weather-dependent, (Stein and Hook, 2005; Zhu et al., 2021). Understanding the microbial community composition and its response to environmental and operational conditions has been crucial for optimizing CWs' performance in addressing chemical pollution targets (Fu et al., 2020; Lv et al., 2017; Verduso Garibay et al., 2022a). Previous research on PA and FL community fractions has enhanced our understanding of biochemical cycles in various aquatic ecosystems, including eutrophic lakes (Hu et al., 2020), oligotrophic surface oceans (Ren et al., 2022), and estuary systems (Liu et al., 2020). These studies have also provided vital information on pathogen dissemination routes in the environment (He et al., 2022; Bian et al., 2021). Meanwhile, the PA and FL communities within CWs remain largely uncharacterized.

This study aims to explore the microbial community composition in CWs by examining both free-living (FL) and particle-attached (PA) community fractions. The objectives are to reveal, for the first time (1) the spatial succession dynamics of fraction-specific microbial communities across a CW and (2) the fate of associated antimicrobial resistance. The research was conducted at a full-scale free-water surface CW (FWS CW) operating as a polishing step for an upstream conventional wastewater treatment plant (WWTP).

Unlike subsurface flow constructed wetlands, FWS CWs lack a marked filtering capacity. In these wetlands, the dispersion of PA bacteria is restricted only by particle settling velocities and entrapment within the vegetation cover. In FWS CWs, PA and FL bacteria can be immobilized through adsorption on sediments or plant tissues. A distinctive feature of FWS CWs is the exposure of PA and FL bacteria to photoinactivation and the ensuing oxidative stress caused by solar radiation in open water zones (Wenk et al., 2019). Likewise, the population and composition of FL and PA bacterioplankton fractions could be influenced by the bacterivorous activities of, for instance, protozoa (Decamp and Warren, 1998). PA and FL fractions in planktonic communities can facilitate AMR dissemination within FWS CWs. However, no study has determined a specific mechanism for targeting AMR in FWS CWs effectively. Data suggest minimal annual AMR removal efficiency (>1 log) in FWS CWs, though seasonal effectiveness (1–3 log removal)

under certain conditions shows potential for AMR mitigation (Anderson et al., 2013; Fang et al., 2017; Lan et al., 2019; Lamori et al., 2019; Bydalek et al., 2023a). Like other CW systems, FWS CWs risk becoming AMR reservoirs due to antibiotic and heavy metal accumulation in sediments, which promotes AMR in sediment microbial communities (Fang et al., 2017). Sediment disturbance from hydrodynamics or animal interactions can release AMR into the water, raising AMR levels in both PA and FL fractions (Sabri et al., 2021).

To gain a comprehensive understanding of the internal dynamics of PA and FL bacteria, sediment samples were also collected to examine the interaction and exchange between sediment and planktonic microbial communities. The PA and FL community composition was then correlated with the niche-specific fate of ARGs to identify potential AMR host bacteria and their dissemination patterns.

2. Methods

2.1. Study site

The study was conducted at the Cromhall Constructed Wetland (CW), situated in Cromhall, South-West England. The CW system under investigation is approximately 8000 m² surface flow type CW, serving as a polishing step for the upstream Cromhall Sewage Treatment Works (2100 PE; 1.4 ML/d). The maximum operational discharge for Cromhall CW is 30 L/s. During the study period of July to September 2021, the average daily inflow was 8.0 L/s (fig. S1). Wastewater inflow quality, including total suspended solids (TSS) and turbidity measurements for this sampling period, are described in a previous study (Bydalek et al., 2023) and are shown in Tables S1, S2.

The Cromhall CW features a modular design, consisting of 12 separate rectangular treatment cells (Fig. 1). The first treatment cell (TC1) functions as a buffering and settling pond, equalizing inflow variability with a cell depth of 1.5 m and minimal vegetation cover (<10 %). Following TC1 are three treatment cells (TC2-TC4) with dense vegetation cover (>90 %) and shallow water depth (0.3–0.4 m), primarily vegetated with *Schoenoplectus lacustris* and *Typha augustifolia*. The effluent from TC4 is equally divided into two parallel treatment lines (A and B), each comprising four treatment cells (TC5-TC8) in series. Downstream treatment cells TC5-TC8 have a depth of approximately 0.6 m and variable plant coverage (30–80 %), with open water zones. The plant cover in TC5-TC8 includes both emergent (*Schoenoplectus lacustris*, *Typha augustifolia*, *Glyceria maxima*, or *Carex acutiformis*) and floating (*Nymphaea alba*, *Apium nodiflorum*, *Lemna minor*) water plants. The Cromhall CW was operational for 15 months at the time of our study. The plant cover was yet to be fully developed and had not achieved its intended coverage and structure. The insufficient baffling effect of the vegetation was evident through visible preferential flow paths and short circuiting, which could potentially compromise the designed hydraulic residence time.

2.2. Sampling

Water samples were collected from four different sampling points: the inflow point to the CW (Inflow), the effluent from treatment cell 1 (TC1), the effluent from treatment cell 4 (TC4), and the combined final effluent (TC8) (Fig. 1). To obtain representative outflow samples, equal volumes of sub-samples were collected from both TC8A and TC8B effluent points and later combined. A total of five sampling events took place over two months (7th July - 12th September 2022). The timing of sampling events was strategically chosen to coincide with base flow conditions, thereby avoiding rainy and high-flow events (Fig. S1). The average water temperature during these sampling events was 20.4 ± 1.5 °C. At each sampling event, each sampling site was sub-sampled (1 L) 4–5 times during daytime (8am–6pm) and the collected water was stored in a cooling box. Collected water samples were transported to the laboratory and processed on the same day. Respective sub-samples were

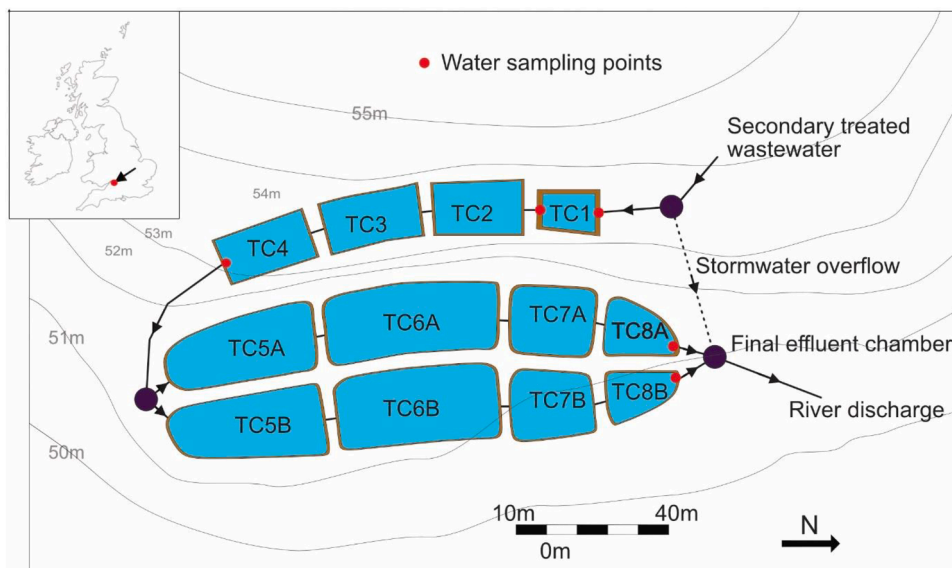


Fig. 1. Schematic view of Cromhall Constructed Wetland. Sediment samples were collected at the TC1, TC2, TC4, TC8A and TC8B from the sampling transects containing 6 subsampling points. Sludge samples were collected at TC2.

pooled together to obtain a quasi-daily composite sample (hereafter referred to as "sample"). Detailed water sampling and pooling method is presented in SI (Fig. S2A). Over the course of the two-month study, five daily composite samples were collected at each sampling point.

2.3. PA and FL community sequential filtration fractionation

Composite samples were vacuum-filtered using sterilized 47 mm diameter, 3 μm pore size mixed cellulose ester (MCE) membranes (MF-Millipore) to collect particle-associated (PA) microorganisms. The collected filtrate was then passed through sterile 47 mm diameter, 0.22 μm pore size nitrocellulose (NC) membranes (Sartorius) to capture free-living (FL) bacteria. The volume of filtered water was noted for subsequent biomass and AMR gene concentration calculations. All filter membranes were stored at $-20\text{ }^{\circ}\text{C}$ for later analysis.

Filter-size distinction into PA and FL fractions is widely practiced, including in wastewater (Yu et al., 2020; Yu et al., 2021). However, this method may introduce biases in classifying PA and FL bacteria (Padilla et al., 2015; Xie et al., 2020). Mechanical stress from filtration can disrupt particle aggregates, inadvertently releasing PA bacteria into the FL fraction. Additionally, filtering large volumes can lead to membrane clogging, causing FL bacteria to be trapped in the PA fraction. These issues can be reduced by limiting the water volume to prevent filter blockage and using low-pressure gravity filtration. Sedimentation, an alternative method, avoids the problem of particle disintegration by eliminating the mechanical stress associated with filtration or centrifugation. However, sedimentation introduces its own biases, as bio-aggregates buoyant from extracellular polymeric substances and colloidal particles might be misclassified as part of the FL fraction (Heins et al., 2021).

In this study, we utilized vacuum filtration, adjusting the water sample volume to prevent filter membrane saturation. There are no standardized protocols for evaluating the efficiency of filter size separation methods in distinguishing between PA and FL bacterial communities. Preliminary tests filtering 5 L samples through a 3 μm filter over 1 hour identified 0.5 L as the optimal filtration volume. This volume maintained a steady flow, using less than 10 % of the filter capacity for low-turbidity TC4 samples and 20 % for high-turbidity Inflow samples.

After identifying 0.5 L as the optimal filtration volume, further preliminary experiments were conducted to assess whether larger pore sizes, less susceptible to clogging, would impact separation accuracy. We

filtered three daily composite water samples, collected on different days from the Inflow point, through 3 μm , 5 μm , and 10 μm membranes to isolate PA bacteria. This was followed by secondary filtration through 0.22 μm to capture free-living FL bacteria.

The analysis of triplicate datasets revealed that the filter pore size did not significantly impact the Shannon diversity index of FL and PA communities (Kruskal-Wallis, $p > 0.05$) or the taxonomic composition (PERMANOVA, $p > 0.05$) for the tested 0.5 L water samples. Based on these findings, we determined that using a 3 μm filter for filtering 0.5 L of water offers a reliable separation between PA and FL communities. This method was therefore adopted for our study.

2.4. Sediment and sludge sample collection

Sediment samples were collected in transects across treatment cells 1, 2, 4, 8A, and 8B (Fig. 1) once, midway through the study due to restricted access. Sediment collection released suspended solids into the water column, affecting the water quality of the final effluent, which must comply with regulations. Sediments in CWs maintain stable seasonal compositions making temporal variability during the two-month study period negligible (Choi et al., 2022; Elhaj Baddar and Xu, 2022; Jia et al., 2017). Sediment sampling involved six subsampling points per transect, later combined into one composite sample per cell, detailed SI (Fig. S2B). In the deeper treatment cell 1 (settling basin), sub-samples were collected about 1 meter from the edge, focusing only on the top 5 cm of the sediment core. Concurrently, the sludge layer in TC2 was sampled to examine its potential impact on the microbial community composition of both sedimentary and planktonic environments, especially near sludge zones. TC2 was unique in harboring sludge debris from the upstream WWTP (Fig. S9), with no sludge observed in other parts of the CW system. Due to logistical reasons, water samples were not collected alongside sediment and sludge samples at TC2.

2.5. Sample processing

2.5.1. DNA extraction

Under aseptic conditions, water sample membranes were carefully cut into small pieces and transferred into lysing matrix E tubes (MP Biomedicals, Irvine, CA, USA). Similarly, E tubes were filled with 0.5–1.0 g of sediment and sludge. Before proceeding with downstream processing, tubes containing sediment samples were filled with 978 mL

of sodium phosphate buffer (according to the downstream DNA extraction protocol) and shaken on a wrist action shaker at maximum speed (to break-up the 'sticky' sediments; Buongiorno et al., 2017) for 1 h to enhance bacterial detachment from the clay substrate. The downstream DNA extraction followed the manufacturer's protocol (FastDNA SPIN Kit for Soil; MP Biomedicals, USA) using a dedicated bead-beating device (Fast Prep; MP Biomedicals, USA). The bead-beating step was performed twice for 30 s each, at a speed of 5.5 m/s (Webster et al., 2003).

2.5.2. 16S rRNA gene profiling and qPCR

DNA extracted from water, sediment and sludge samples was processed for 16S rRNA sequencing to uncover microbial community composition. Sequencing was conducted using single-step amplification of the V4 region of the 16S rRNA, following the dual-indexing methods by Kozich et al. (2013). Each PCR reaction consisted of 17 μ L of Accu-prime Pfx Supermix, 1 μ L of template DNA, and 2 μ L of each paired set of index primers. PCR conditions included an initial 2 min at 95 °C, 30 cycles of 20 s at 95 °C, 15 s at 55 °C, and 5 min at 72 °C, followed by a final step of 10 min at 72 °C. PCR products were purified using SPRI magnetic beads (Beckman Coulter, IN, USA) and quality-checked via an automated electrophoresis tool (4200 TapeStation, Agilent, CA, USA).

The 16S rRNA library was quantified using a Qubit dsDNA high-sensitivity assay (Qubit 4, Invitrogen, UK) and normalized based on the lowest concentration in the pool. Sequencing was performed on the Illumina MiSeq platform at the Cardiff University Genomic Research Hub, using 2 \times 250 bp paired-end flow cells and reagent cartridges. Sequencing included three out of five composite samples per sampling site, collected on 14/07/2022, 24/08/2022, and 12/09/2022.

Abundance quantification of AMR genes (*sul1*, *int1*, *tetW*, *ermB*) and bacterial biomass (16S rRNA) was carried out via qPCR in water samples only. The detection limit of the machine (Stratagen Mx3000P, Agilent, CA, USA) was 10 gene copies (GC)/mL; hence, values below the detection limit were rounded down to 0 in calculations. We selected to analyze the *tetW*, *ermB*, and *sul1* AMR genes due to their conferred resistance to commonly employed broad-spectrum antibiotics such as tetracycline, erythromycin, and sulfonamide, respectively. These antibiotics are regularly detected in municipal wastewater and represent a diverse array of AMR mechanisms (Hendriksen et al., 2019; Reygaert, 2018). The *int1* gene acts as an indicator of the potential for antibiotic resistance gene transfer and dissemination among bacterial populations (Gillings et al., 2015). Primers and qPCR conditions used in the study are presented in Supplementary Materials Table S3. Based on the filtered volume of samples, the quantified abundance of genes was converted into concentrations expressed as gene copy numbers (GC) per 1 mL.

2.6. Data processing and statistics

The QIIME 2 (Quantitative Insights Into Microbial Ecology 2) pipeline was utilized for demultiplexing (DADA2 pipeline), quality filtering, taxonomy assignment (Greengenes 16S rDNA sequences database (DeSantis et al., 2006)) and phylogenetic tree creation (q2-phylogeny plugin with MAFFT and FastTree2 programs) of sequencing reads of water, sediment and sludge samples. QIIME2 artifacts were processed in phyloseq package (v 1.38.0) in R software (v 4.1.3) (McMurdie and Holmes, 2013; R Core Team, 2013). Taxonomic filtering excluded non-bacterial and unknown sequences like Archaea, chloroplasts or mitochondria, and amplicon sequence variants (ASVs) with an abundance of less than 10 reads per sample. Samples were rarefied to 21,000 reads to normalize sequencing depth across samples. Rarefaction led to the exclusion of one sample with a low number of reads (<10,000) (Fig. S3). Processed samples showed coverage of min. 99.9 % of the initial sample sequence profile. One replicate of PA fraction collected at TC4 had to be excluded from the analysis due to contamination of the filter during sample handling. Alpha diversity of the microbial population across all samples was assessed via Chao1 richness (estimate of

number of species present in the community) and the Shannon (Sh) community diversity index (heterogeneity of the community based on diversity and evenness). These metrics were calculated using the phyloseq package (v 1.38.0) in R software (v 4.1.3) and the q2-diversity plugin in the QIIME2 pipeline. Taxa bar plots for community composition visualization were generated using the ggplot R package (v 3.3.6). Spatial and fraction similarities between FL, PA, and sediment communities were assessed via UniFrac distances based on ASV abundance using QIIME2 and the phyloseq pipeline. QIIME2 pipeline output rarefied and filtered feature table served as input for PICRUSt2 (v2.5.2) pipeline to predict functions and metabolic pathways of FL and PA bacteria based on MetaCyc database. The distance matrix was visualized on principal coordinate analysis plots and network plots.

Potential AMR hosts were identified via Pearson correlation between the relative abundance of ASVs and AMR genes. The cut-off for strong correlation was $r^2 > 80\%$ and $p < 0.05$. T-tests or Wilcoxon rank-sum test were applied to assess data variability between samples based on the normality and variance of the dataset. To determine the changes in the abundance of antimicrobial resistance (AMR) genes, the log absolute abundance difference between the respective inflow and outflow sampling points was calculated.

$$\log \text{ ARG removal} = \log(\text{ARG}_n) - \log(\text{ARG}_{n+1}) \quad (1)$$

Where ARG_n – absolute abundance of AMR gene at the upstream sampling point, $\text{ARG}_{(n+1)}$ – absolute abundance of AMR gene at the next downstream sampling point.

3. Results and discussion

3.1. Planktonic and sediment microbial community alpha diversity

Particle-associated (PA) planktonic communities exhibited higher species richness (Chao1) and community diversity (Shannon) than the free-living (FL) bacterial fraction (Fig. 2). This is consistent with findings in various aquatic environments and is attributed to the diverse spectrum of microhabitats within particle structures (Hu et al., 2020; Liu et al., 2020; Rieck et al., 2015). The highest microbial diversity was observed at TC4, presumably due to it being a transitional zone between the wastewater-impacted inflow zone of the CW (TC1-TC4) and the downstream treatment cells (TC5-TC8). In addition, within the shallow (0.3 m depth) and densely vegetated TC4 cell, diversity might be positively affected by baffling and exchange of planktonic communities with periphyton and plant-associated epiphytic biofilms (Khouja et al., 2020; Menon et al., 2013). Measurements of suspended solids and turbidity (Table S2) showed that >80 % particulate matter input and associated PA bacteria from the WWTP were deposited within TC2-TC4. The decreasing diversity of TC5-TC8, reflects the increasingly oligotrophic conditions downstream towards the effluent of the CW, which supports a more specialized microbial community, comparable to natural wetlands (Button et al., 2015; Li et al., 2008; Mulling, 2013; Zhi et al., 2015). The average Shannon index of sediment microbial communities was 5.3 ± 0.1 (Table S4), which was lower than the PA at the CW inflow zone (Shannon = 5.7 ± 0.2) but higher than the PA at the CW effluent (TC8; Shannon = 4.3 ± 0.1). Sediment communities in both natural and constructed wetlands are typically more diverse than planktonic communities and often play a key role in nutrient cycling (Sun et al., 2019; Truu et al., 2009).

Water samples from the four sampling points (inflow, TC1, TC4, and TC8) contained, on average, 2800 ± 300 unique taxa (ASVs). Notably, the PA and FL fractions hosted a similar proportion of fraction-unique taxa (*t*-test, $p > 0.05$). These fraction-unique taxa made up an average of $16 \pm 4\%$ and $27 \pm 3\%$ of the FL and PA community population abundance, respectively.

The composition of taxa in PA and FL planktonic communities varies depending on environmental conditions, such as weather and nutrient fluxes (Hoke et al., 2020; Roth Rosenberg et al., 2021). High

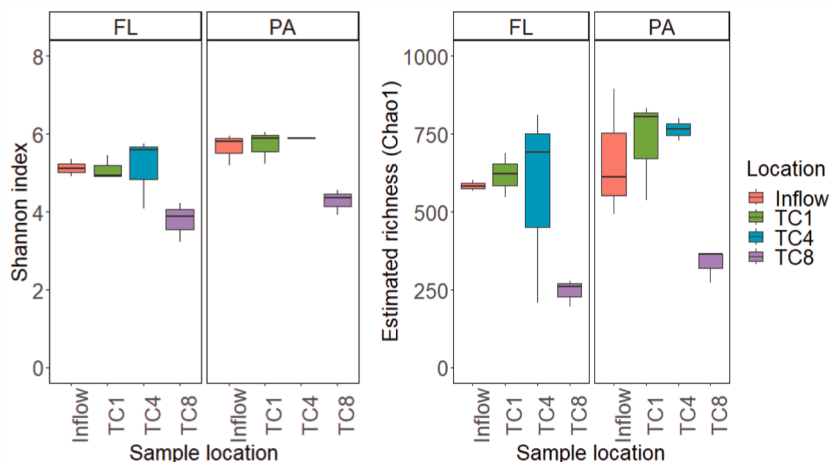


Fig. 2. Diversity and richness indices (Shannon and Chao1) of particle-associated (PA) and free-living (FL) bacteria communities in water samples collected at different stages of the constructed wetland (CW): Inflow, TC1, TC4 and TC8. Note there were only 2 replicates of TC4 PA sample due to contamination incident; remaining samples are represented by triplicates.

phylogenetic overlap between PA and FL communities of greater than 90 % has been observed in highly eutrophic freshwater bodies (Hu et al., 2020; Li et al., 2018; Tang et al., 2017), while taxonomic specialization between FL and PA is more prevalent in oligotrophic conditions (Blažina et al., 2009; Jain and Krishnan, 2017; Milici et al., 2017).

3.2. Taxonomic composition of the planktonic and sediment communities

Planktonic community: The planktonic community composition at the phylum and family level is shown in Figs. 3A and S5, respectively. The PA community of TC1 near the inflow of the CW was dominated by Proteobacteria (40 %), Bacteroidetes (15 %), Firmicutes (10.5 %), and

Chloroflexi (9.5 %), represented by Campylobacteraceae (6 %), Flavobacteriaceae (3.1 %), Veillonellaceae (3.3 %), and Kouleothrixaceae (1.9 %). This composition reflects the diverse metabolic conditions of the upstream WWTP oxidation ditch (Xu et al., 2017), including typical activated sludge bacterial species, for example biofilm-associated filamentous Chloroflexi (i.e., A4b family), extracellular polymeric substance (EPS) degrading Kouleothrixaceae and biofilm-forming Veillonellaceae (Astorga-Eló et al., 2020; Yang et al., 2022), as well as nitrogen-cycling Pirellulaceae (2.5 %).

The inflow FL community exhibited a similar abundance of Proteobacteria (40 %), Bacteroidetes (12 %), and Firmicutes (8 %) when compared to the PA community, with only a minor Chloroflexi

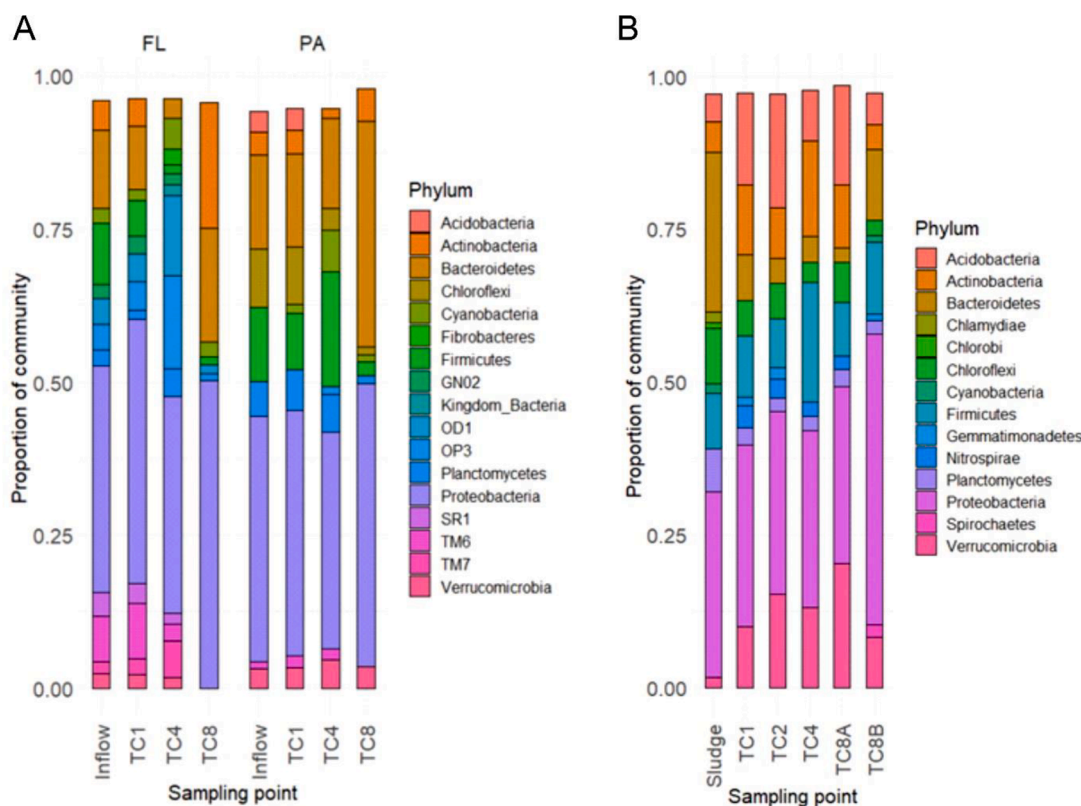


Fig. 3. Phylum-level taxonomic composition of (A) PA and FL bacteria community and (B) sediments and sludge bacteria community. Only Phyla representing more than 1 % of the community composition are presented.

component (<1 %). In contrast to the PA community, FL communities displayed a presence (3–8 %) of candidate phyla OD1, TM6, OP3, and SR1, which mostly comprise parasitic/symbiotic bacteria associated with anoxic environments (Castelle et al., 2017; Glöckner et al., 2010; Kantor et al., 2013).

The PA community of TC4 was dominated by Proteobacteria (35.4 %), Firmicutes (18.8 %), and Bacteroidetes (14.7 %), represented by Comamonadaceae and Pseudomonadaceae (4.4 %), Lachnospiraceae (7.0 %), and Flavobacteriaceae (5.6 %), respectively. This taxonomic composition reflects continued WWTP discharge influence at this stage of the CW, particularly given the high abundance of human-fecal indicating Lachnospiraceae (Feng et al., 2018). Presence of typical phyla found in sediment bacterial communities such as Verrucomicrobia (4.7 %) indicate PA community sediment interaction (Op den Camp et al., 2009). Additionally, TC4 was colonized by Cyanobacteria (6.9 %), particularly biofilm-filamentous Phormidiaceae (4.4 %) capable of creating bacterial mats as observed occasionally during this study. The TC4 FL community primarily consisted of Proteobacteria (35 %) and phyla OD1 (13.1 %), OP3 (15.2 %), and Cyanobacteria (5 %) but also cellulose-degrading Fibrobacteres (2.5 %), which may be attributed to the dense plant vegetation cover and cyanobacterial mats (Rampadarath et al., 2017). A significant presence of the motile, nitrogen fixing YS2 species (at 4.3 %) was noted (Di Rienzi et al., 2013).

At the CW effluent (TC8) the PA community was the least diverse, with nearly 85 % of the phyla composition shared between Proteobacteria and Bacteroidetes. The dominant family taxa were Flavobacteriaceae (28.7 %) and Campylobacteraceae (10.9 %). However, there were also Helicobacteraceae (3.9 %), primarily consisting of the Sulfurimonas genus (3.4 %), which is typical for sulfidic wetland habitats (Han and Perner, 2015). The FL community was mainly composed of Proteobacteria (50.4 %), Bacteroidetes (18.5 %), and a high abundance of Actinobacteria (20.6 %) of the Microbacteriaceae family. The latter included the plant pathogenic genus Clavibacter (2.5 %) and the planktonic ultramicrobacteria genus Rhodoluna (11 %). Elevated presence of Bacteroidetes and Actinobacteria suggests increasingly oligotrophic conditions in the effluent zone as both of those phyla specialize in breakdown of complex organic matter including refractory compounds which are typically present in the late stages of a polishing CW system after exhaustion of the initial pool of biodegradable compounds found in the WWTP discharge (Button et al., 2015). This observation is further supported by the notable presence of the Planktoluna genus (2.4 %; family Microbacteriaceae) which serves as an indicator for the low impact of wastewater contamination in the environment (McClary-Gutierrez et al., 2021).

Sediment and Sludge Community: The sediment and sludge community composition is shown in Figs. 3b and S6, respectively. At the phylum level, sediments did not display clear spatial heterogeneity as observed in the water samples. The CW sediment community showed an even distribution of Proteobacteria (29.4 %), Acidobacteria (14.5 %), Verrucomicrobia (14.6 %), Actinobacteria (11.4 %), and Firmicutes (11.6 %) across all samples. The sediment community composition exhibited taxa associated with oligotrophic conditions, such as the DA101 genus (11.2 %) and the chemoorganotrophic Gaiellaceae family (3.2 %), indicating a low C:N ratio in the sediments (Hermans et al., 2017; Shen et al., 2017). Sediment habitats attracted methylotrophic Hyphomicrobium (1.2 %) and phototrophic Rhodoplans (2.8 %) (both belonging to the Hyphomicrobiaceae family; 5.4 %) further suggesting poor availability of labile carbon in oligotrophic environment. The discovery of overrepresentation of oligotrophic organisms in the CW system which has been operational for 18 months at the time of the study, suggests that the CW sediments are still in the initial phase of microbial succession and possibly haven't reached full operational capacity of a matured CW system. CW sediment maturation depends on nutrient input, organic matter availability and hydraulic management of the system (Liu et al., 2015; Pinney et al., 2000). Typical maturation for a model CW mesocosm can take at least 100 days, while full-scale systems can require 12

months or more (Abou-Kandil et al., 2021; Aguado et al., 2022; Ramond et al., 2012). For polishing CWs exposed to relatively low inflow nutrient input, such as the one studied here, reaching sediment maturation can take several years (Kasak et al., 2018; Weaver et al., 2012).

Despite the overall uniformity of the microbial community in sludge and sediments, distinct local conditions contributed to the development of different communities. For instance, the sediment community composition in treatment cell TC8B displayed notable differences compared to other sampling sites. This was attributed to recurring hydraulic blockages in this part of the CW, resulting in periodical water stagnation, that created different habitat conditions for microorganism (Ishida et al., 2006; Verduso Garibay et al., 2022) and algal blooms (Fig. S8). TC8B had increased Proteobacteria (47.7 %) and Bacteroidetes (11.5 %) populations, with overrepresentation of heterotrophic Comamonadaceae (2.3 %), Rhodobacteraceae (7.8 %) and Flavobacteriaceae (3.2 %) known for co-occurrence and mutualistic relationship with microalgae (Morillas-España et al., 2021; Niu et al., 2011). Anaerobic sulfate-reducing genus Desulfobulbus (2.0 %), and the cyanobacterial Planktothrix (0.5 %) and Paulinella (0.3 %) genera were also present at one order of magnitude higher relative abundance than elsewhere in the sediments.

Similarly, sludge samples collected at the treatment cell 2 (TC2; Fig. S7) had a different community composition from both PA, FL and sediment bacterial communities. This sludge community primarily consisted of Proteobacteria (30.0 %), Bacteroidetes (26.1 %), Chloroflexi (9.1 %), and Firmicutes (9.1 %), with particularly high populations of Chitinophagaceae (7.2 %), Peptostreptococcaceae (2.8 %), and Saprospiraceae (6.9 %). Based on information on these groups, this microbial community would excel in breaking down complex organic compounds, both in aerobic and anaerobic environments, such as EPS or cellulose, which may be deposited alongside wastewater particulate matter (Hou et al., 2021; McIlroy & Nielsen, 2014; Robledo-Mahón et al., 2020). Interestingly the sludge layer revealed presence of Caldilineaceae bacteria (2.1 %) which belongs to phosphorus accumulating organisms responsible for biological phosphorus removal in WWTPs (Cao et al., 2021). Sludge accumulation in the inflow zone of FWS CWs, as studied here, is a known issue for CW operation however, its influence on the overall performance of CWs remains incompletely understood. In subsurface flow CWs, sludge layer deposition can have dual effects – it can be advantageous by forming a biologically active layer that enhances treatment processes, or detrimental by restricting the system's hydraulic conductivity, thereby impeding overall performance (Paing et al., 2015; Wang et al., 2021). Here, sludge deposition could have a positive effect as it forced a subsurface flow what could add additional particulate immobilization within the TC2-TC4 zone which showed to be a key particulate deposition zone in the system (Table S2). Nonetheless, the impact of peak flow rain events, which can potentially dislodge and disseminate the sludge layer throughout the CW, on its long-term performance remains uncertain.

3.3. Spatial distribution and succession of PA and FL bacteria, and their interaction with the CW environment

Spatial dynamics and succession patterns of bacterial communities and sediment interactions are visualized using Principal Coordinate Analysis (PCoA) and network analysis (Fig. 4A and 4B; respectively). The PCoA plot, based on the UniFrac dissimilarity matrix, reveals that the FL and PA communities had similar spatial dynamics and formed two distinct groups: the inflow zone (Inflow and TC1) and the wetland zone (TC4 and TC8). PA data separation indicates distinctively separate communities. For FL data, the 95 % confidence intervals of both groups overlap, which implies migration of WWTP-associated FL communities throughout the CW system. Additionally, an overlap between FL and PA occurs within the wetland zone indicating active community migration between FL and PA fractions possibly due to an increasingly limited nutrient pool triggering shifts of bacterioplankton lifestyle, like that

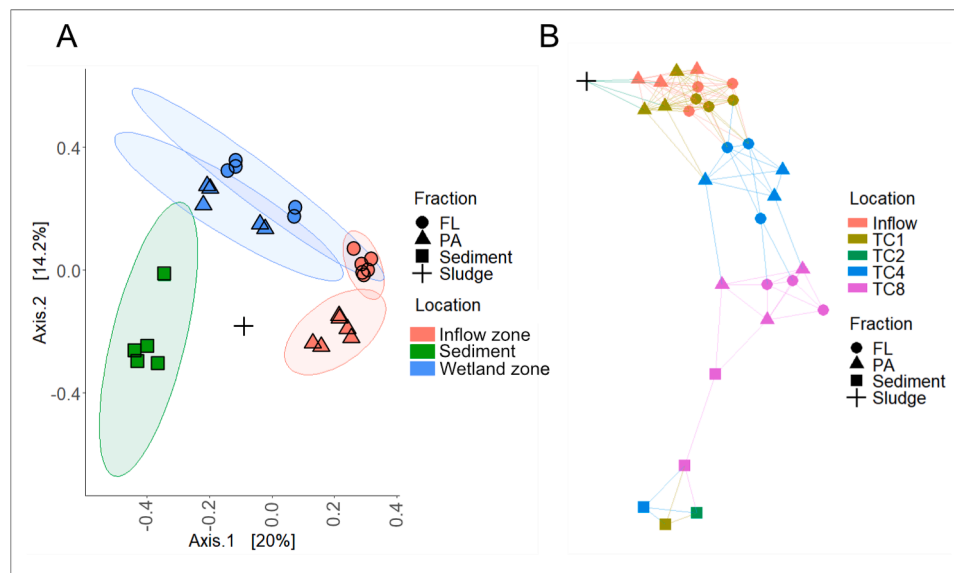


Fig. 4. Principal coordinate analysis (PCoA, A) and network analysis (B) plots illustrate the spatial similarities and 1 longitudinal succession of PA and FL communities in relation to sediments and sludge accumulation. Group clusters reflect CW design, where inflow and TC1 points indicate a CW inflow zone, and TC4 and TC8 denote a wetland-type zone. The confidence ellipse in fig. A encompasses 95 % of potential samples from the multivariate (Gaussian) distribution. The selected PCoA ordination plane accounts for 34 % of dataset variance.

observed across nutrient gradient in estuary ecosystem (Li et al., 2018). Sediment data are clearly distinguished from PA (and FL) data, signifying that the influence of the WWTP PA community on the sediment community composition was low. However, a minor overlap in the 95 % confidence intervals was attributed to the TC8B data point because of the unique sediment community of this cell, as discussed in the previous section.

Network analysis (Fig. 4B) captured the succession of microbial community composition along the CW system and revealed gradually growing dissimilarities between inflow and effluent zones (Semenov et al., 2020). FL and PA showed connections primarily within their respective locations, while connections within each fraction (FL or PA) also extended beyond their immediate treatment cell. This indicates that while a distinct fraction-specific community continuum existed from the inflow to the effluent zone, individual treatment cells imparted their influence on both FL and PA communities. Sludge exhibited the highest similarity to the inflow and TC1 WWTP PA community, rather than the downstream FL and PA communities, suggesting that sludge accumulation did not influence the composition of either sediment or planktonic communities in those locations.

3.4. Prediction of potential functions and metabolic pathways of FL and PA bacteria in CWs

The metabolic profile of both FA and PA communities in the studied CW was predicted using 16S rRNA genes and assembled via the PICRUSt2 pipeline. The primary differentiating factor between the community-level metabolic potential in the studied planktonic community was sample location, and community fractions demonstrated similar metabolic potentials within the same location, as suggested by the data dispersion on the PCoA plot (Fig. 5A). The structure of the community-level metabolic profile, expressed through relative abundance, presented a consistent overview of the metabolic activity of the CW planktonic community in relation to the main metabolic classes (Fig. S5). However, differences were observed when considering individual, specialized metabolic pathways. Constructed wetlands are primarily designed for nutrient removal, and identifying metabolic pathways related to nitrogen, phosphorus, and other key biochemical elements can help understand the system's response to nutrient input

and internal dynamics (Cao et al., 2022). The metabolic profile analysis revealed the presence of a total of 11 non-carbon nutrient assimilation pathways, including denitrification, sulfate reduction, and sulfur oxidation (Fig. 5B).

Nitrification-denitrification, contributes 60–70 % of total nitrogen removal in CWs and is typically the most pronounced nitrogen removal pathway (Lee et al., 2009; Khajah et al., 2023.) At Cromhall CW, the denitrification community primarily consisted of generalist species from the *Arcobacter* and *Flavobacterium* genera, with species from *Rhodobacter* or *Dechloromonas* emerging in specific fractions (>10 %) (Fig. 5C). A similar taxonomic profile of the denitrifying community has been observed in both constructed and natural wetland systems (Xia et al., 2020; Chen et al., 2020). The presence of the sulfolactate degradation pathway, primarily driven by *Flavobacterium* and *Pseudomonas* species, suggests the CW system's potential to degrade odor-causing organo-sulfonate compounds (Wang et al., 2022). Meanwhile, the identification of the methylphosphonate degradation pathway, notably supported by *Polynucleobacter* and *Rubrivivax* in the effluent FL and PA communities, might indicate phosphate-limited conditions that could encourage methane emissions from the system (Yao et al., 2016). However, it should be noted that these functional predictions of the FL and PA community are based on predictive analysis of 16S rRNA gene taxonomic data, and a detailed metabolic profile would require a comprehensive -metagenomic approach (Toole et al., 2021).

3.5. AMR dynamics in FL and PA communities

The CW received an inflow containing an average cumulative load of investigated ARGs of approximately $1.5 \cdot 10^5$ GC/mL (Table S6). *Int11* was the most prevalent AMR gene, accounting for 60 % of the cumulative AMR load, while the remaining AMR genes were present at comparable levels (1.5 – $2.0 \cdot 10^4$ GC/mL). The ARGs content in the CW inflow was consistent with typical ARGs concentrations found previously in secondary municipal WWTP effluent (Pallares-Vega et al., 2019). The PA fraction constituted over 80 % of the bacterial biomass in water samples and contained the majority (60–90 %) of AMR genes (Table S6). Although PA is the dominant effluent biomass fraction, the proportion of FL/PA biomass varies mainly due to the higher variability of PA biomass input, reflecting WWTP performance (Yu et al., 2020).

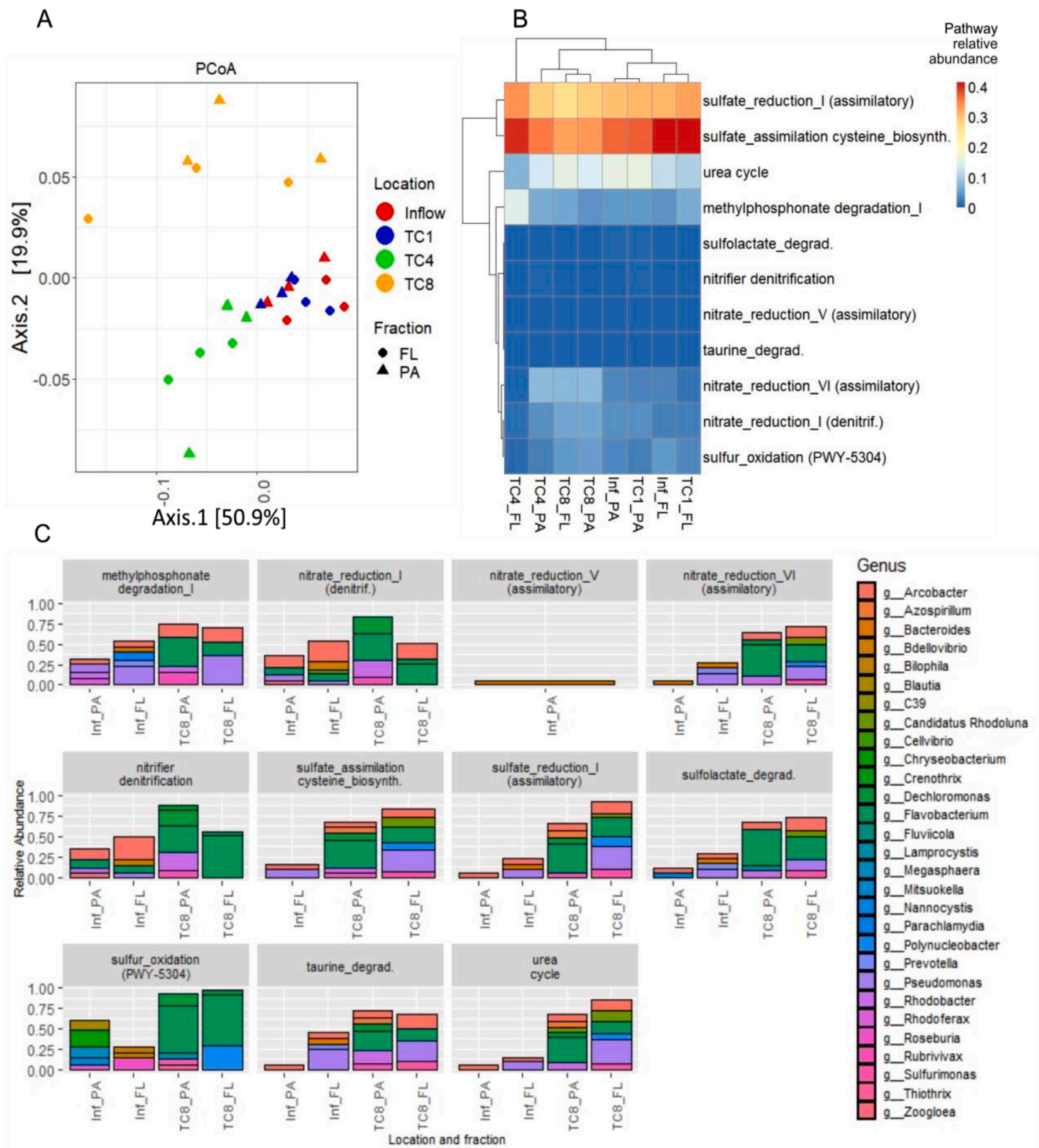


Fig. 5. (A) Principal coordinate analysis (PCoA) using Bray-Curtis dissimilarity metrics shows the predicted metabolic pathway abundance (PICRUSt2, MetaCyc database) in FL and PA community fractions of the studied CW. (B) Displays the relative abundance of community- and location-specific predicted metabolic pathways (PICRUSt2) linked to the assimilation of non-carbon nutrients (PICRUSt2, MetaCyc). (C) Highlights key taxa (selected >5 % of relative abundance for clarity), at the genus level, contributing to metabolic pathways within the noncarbon-nutrients class in the MetaCyc database.

Variability was found in the influx of targeted ARGs into the CW across the five sampling events, with significant differences being observed ($p < 0.05$) in both FL and PA fractions (Table S6). Overall, the distribution of combined load of investigated ARGs shifted from PA carrying 70 ± 6 % in the inflow towards 63 ± 9 % carried by FL fraction in the effluent.

During passage through the CW system, PA and FL fractions

exhibited different losses of ARGs load (Fig. 6). The PA fraction displayed significantly lower ($p < 0.05$) levels of AMR genes in the effluent, with reductions ranging from a 1-log decrease for *sul1* to a 3.5-log decrease in *ermB*. Conversely, the FL fraction maintained constant levels of *sul1* and *int11* genes (0–0.5 log decrease; $p > 0.05$) while showing a significant reduction (approximately 3-log; $p < 0.05$) of *tetW*

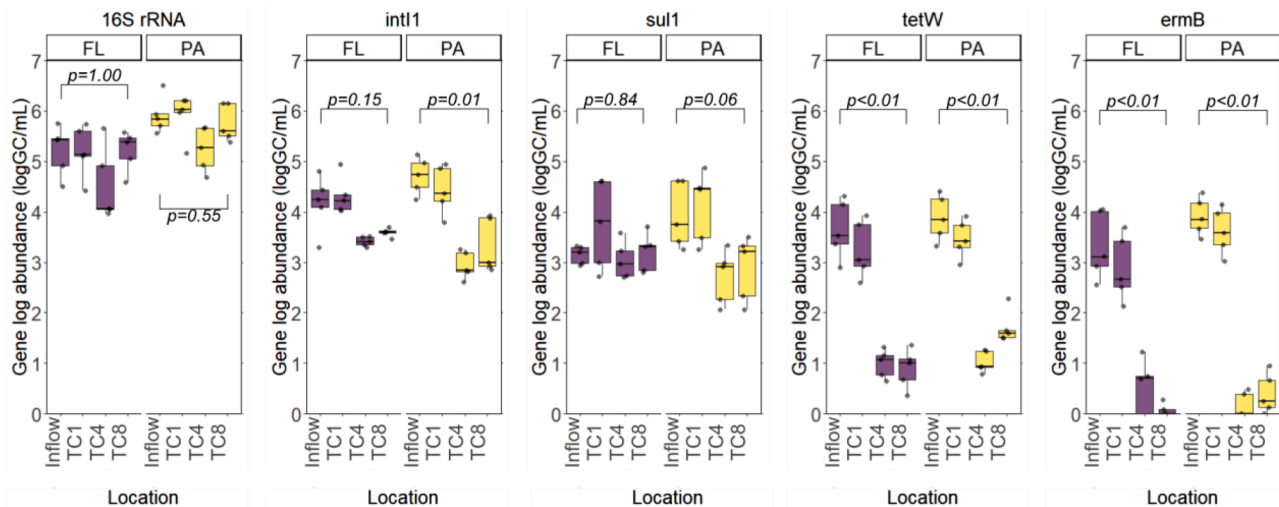


Fig. 6. Bacterial biomass (16S rRNA) and AMR genes (*intI1*, *sul1*, *tetW*, *ermB*) absolute abundance in FL and PA community fractions. Absolute abundance is presented as log gene copies (log GC) per mL of water sample. Values below 1 logGC/mL are considered approximations due to qPCR assay and machine detection limits. P-values signify the significance levels of differences between inflow and effluent (TC8) points. Due to non-normal distribution in *tetW* and *ermB* data, a non-parametric Wilcoxon rank sum test was used.

and *ermB*. Considering the cumulative reduction of PA and FL ARGs load, the results align with several studies on CWs and conventional WWTPs (Hazra et al., 2022).

Nevertheless, the observed variations in AMR gene dynamics between PA and FL fractions indicated that the fate of AMR within the planktonic community was governed by the distinct internal interactions within each respective niche. Similar conclusions arose in studies of other environments, including conventional WWTPs, lakes, and rivers, where ARGs dissemination was differentially mediated by FL and PA communities (Guo et al., 2018; Proia et al., 2018; K. Yu et al., 2020). This highlights the importance of examining community fractions to better understand and potentially prevent AMR dissemination.

Further analysis of fraction-specific ARGs dynamics in relation to the microbial community can be represented through the measure of relative abundance. Relative abundance indicates the proportion of AMR bacteria in the entire community, estimated by the ratio of the number of AMR gene copies to 16S rRNA gene copies. In the inflow wastewater, the relative abundance of AMR genes among the FL bacterial population was either higher ($p < 0.05$) or comparable ($p > 0.05$) with the PA community.

Conditions within the settling basin (TC1) did not significantly alter the relative abundance of the investigated ARGs (Fig. 6) ($p > 0.05$). However, the recurrent presence of algal blooms (Fig. S8), teeming with diverse photosynthetic and non-photosynthetic cyanobacterial communities (including *Oscillatoriales* and MLE1–12), raised concerns. An increasing body of evidence suggests that cyanobacteria, specifically cyanobacterial algal blooms, facilitate the spread of AMR across various aquatic environments (Dias et al., 2019). Although the precise role of cyanobacteria in AMR dissemination remains unclear, cyanobacteria are renowned for acquiring and transferring ARGs, thanks to a diverse pool of mobile genetic elements that enhance their capability for horizontal gene transfer (Volk and Lee, 2023). Moreover, the sludge layer developed in the settling basing and consisting of algal blooms and incoming particulate matter, could potentially promote horizontal gene transfer and serve as an AMR reservoir comparable to role of activated sludge in conventional wastewater treatment plants (Guo et al., 2017).

In the CW moving towards the CW effluent, the decrease in the relative abundance of AMR genes was much more pronounced among the PA community. PA AMR genes reached their lowest relative abundance levels at TC4, and no significant changes were observed in the downstream treatment cells until the final effluent point. This pattern coincided with the previously mentioned suspended solids dynamics

(Table S2). In the FL community, a substantial decrease, by as much as two orders of magnitude, was observed in the relative abundance of *ermB* and *tetW* genes within the TC1-TC4 zone. The relative abundance of AMR genes revealed significant intra-location fluctuation as indicated by the standard error of the measurements (Fig. 7). Gene intra-location fluctuation was particularly visible among the PA resistome at TC1 where the relative standard error approached 90 % for the respective AMR gene dataset. This could be partially explained by the reoccurring presence of sludge and algal blooms in the settling pond which, as discussed previously, could have triggered acquisition of AMR within the PA population. Relative abundance of AMR genes may exhibit seasonal variability due to prescription antibiotic usage patterns and temperature effects on drugs and key AMR hosts (Schages et al., 2020; Sui et al., 2017). Recent findings indicate significant day-to-day and intra-day variability of ARGs in wastewater (Chau et al., 2022; S. Sun et al., 2021). Moreover, small (<2000 PE.) WWTPs such Cromhall CW typically experience very high fluctuation of ARGs (Harnisz et al., 2020). A detailed explanation of the variability observed here would require additional water quality analysis and was outside the scope of the study. Within the initial 20 % of the CW (TC1-TC4), the PA resistome was almost entirely immobilized, while the FL community maintained a 10–20 times higher proportion of *sul1* and *intI1* genes than the PA community. Moreover, FL bacteria harbored *sul1* and *intI1* genes until the final effluent, showing a limited selective pressure on these genes in the FL community.

3.6. Inter-gene relationships and AMR host identification

Shifts in AMR gene abundance throughout the CW were equally evident across the PA fraction, showing strong ($r^2 > 80\%$) and significant ($p < 0.05$) correlations between the internal dynamics of targeted AMR genes (Fig. S11). In contrast, the FL community only exhibited a significant ($p < 0.5$) correlation for the *tetW/ermB* pair (Fig. S11).

This observation might suggest the possibility of a host-specific nature in the resistome of FL bacteria, and a community-specific nature in the resistome of PA bacteria. This further emphasizes the role of particle-associated biofilms as a reservoir and hotspot for AMR acquisition and exchange (Kaur et al., 2022).

Suspended solids consist of various organic and inorganic particles (e.g., microplastics) with differing hydrodynamic and physicochemical properties, as well as affinity towards antimicrobial compounds (i.e. HMs) which could affect the composition and travel distance of mobile

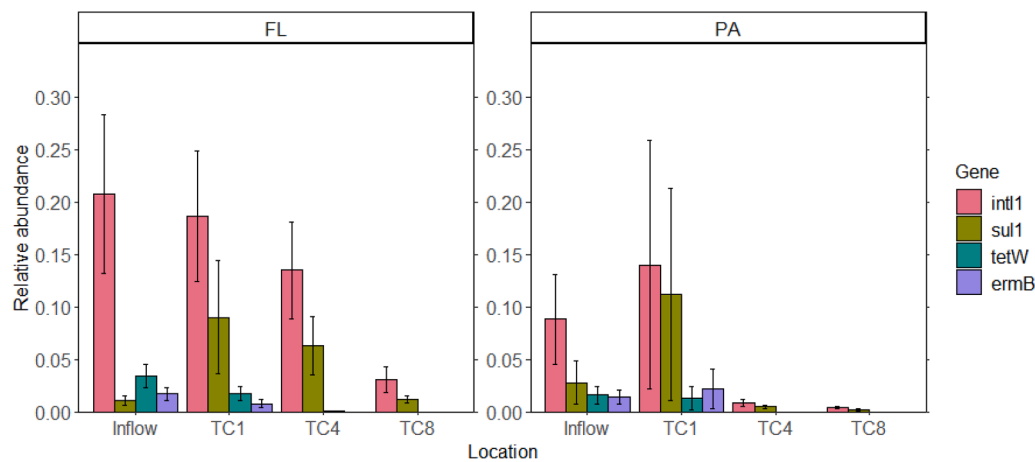


Fig. 7. Relative abundance of AMR genes in FL and PA community fractions in water samples. For the clarity of the graphs values corresponding with *tetW* and *ermB* at the TC4 and TC8 sampling points were below 0.1 %.

genetic elements and AMR bacteria (López-Pérez et al., 2016; Mughini-Gras et al., 2021; Mulling, 2013).

ARGs spread within the FL community was driven by host-specific dynamics. In the FL fraction, *tetW* and *ermB* relative abundance dynamics displayed a strong correlation ($r^2 > 80\%$, $n = 12$) with several ASVs, accounting for a total of 11 % and 1 % of community composition in the inflow and effluent samples, respectively (Table S5). The identified ASVs matched sequences belonging to members of the Bacteroides, Enterobacteriaceae, Aeromonadaceae, and Lachnospiraceae, among others. These taxa represent a broad group of gram-negative, fermentative bacteria common in the human gut and include known pathogenic species. Many studies have identified a high correlation of AMR in WWTPs with members of these families, which, due to their physiology (high plasmid permissiveness), are considered keystone taxa involved in AMR spread (Nguyen et al., 2021; Rice et al., 2020). However, AMR can also be harbored by non-pathogenic species, often allochthonous or well-adapted for survival in environmental conditions of CWs, such as nitrogen-cycling *Nitrosomonas* or *Nitrospira*, complicating targeted AMR approaches (Kumar et al., 2023; Ping et al., 2022).

Sul1 and *intl1* did not exhibit a strong correlation ($r^2 < 80\%$) with any ASVs, displaying greater variability than *tetW* and *ermB*. This is likely because *sul1* and *intl1* can be carried on a broader range of plasmid types and transposons, expanding the list of possible hosts and facilitating the dispersion of these genes within the community (An et al., 2018; Jiang et al., 2019).

None of the strongly correlated ASVs represented unique taxa for either FL fraction. This suggests that potential AMR hosts could alternate between FL and PA lifestyles depending on environmental conditions. This mobility is unfavorable towards targeted AMR design considerations and requires a comprehensive design approach considering both PA and FL bacteria. However, correlation analysis must be carefully interpreted, as observed correlations may not imply causation. Detailed AMR-host dynamics in the system can only be revealed through metagenomic sequencing, functional assays, and genetic manipulation (Behruznia and Gordon, 2022; Calderón-Franco et al., 2022). The observed correlations provide an overview of the metabolic and physiological profile of the bacterial community sharing AMR dynamics in the system. The fact that the effluent FL fraction still contained a relatively high abundance of potentially pathogenic taxa and a notable potential for AMR acquisition and spread (expressed as *intl1* gene) highlights the FL fraction as a key escape route for biohazards from the CW.

3.7. CW design considerations

Effective solids immobilization in CWs shifted the problem of AMR

and pathogen spread toward the FL fraction. FL bacteria may be eliminated primarily through photoinactivation or predation. Photoinactivation capacity can be enhanced by dedicating open and shallow water sections for effective sunlight penetration; however, this requires specific weather conditions and suitable water quality without particulate UV screens (Farrell et al., 2018; Nelson et al., 2018). Predation is enhanced in low-flow zones and plant-based habitats that attract predatory protozoa and bacterivorous bacterial species (Decamp and Warren, 1998; Pedescoll et al., 2016). In the studied CW system, community composition revealed a notable (0.1–0.5 %) presence of *Bdellovibrio* spp., known to prey on various bacteria, including human pathogens (Sokkett and Lambert, 2004).

Correlation analysis indicated that taxa adaptable to both FL and PA lifestyles primarily shaped AMR spread. Most of the FL fractions, which proved most relevant for health hazard consideration, consisted of bacteria that could adopt PA and biofilm lifestyles. Therefore, the design should facilitate the migration of the FL fraction into biofilms. This could be achieved by increasing retention time to facilitate adsorption of FL bacteria onto plant biomass, limiting water depth to enhance vertical migration towards sediments, or creating subsurface flow sections to increase contact time and exchange with substrate biofilms. Future studies should focus on quantifying the specific design effects on ARGs removal in CWs, considering the dynamics of related PA and FL communities. The results of this study were obtained during the summer period with stable hydraulic conditions. Seasonal changes in weather and wastewater quality could alter dynamics and taxonomic composition of PA and FL. Future studies should investigate for example if PA bacteria, including their ARGs and pathogen load are also effectively retained at higher hydraulic loading and in winter conditions. Future research could also extend beyond analyzing the interaction between planktonic and sediment microbial communities to exploring the interplay between planktonic PA and FL fractions and the epiphytic biofilm that is maintained and shaped by diverse plant communities. Seasonal changes in water temperature can influence bacterial motility and biofilm formation abilities (Tang et al., 2017; Alotaibi and Bukhari, 2021), thereby potentially altering PA and FL dynamics. For a more accurate prediction of PA and FL dynamics and the corresponding dissemination routes of pathogens and AMR bacteria in CW, seasonal studies are recommended.

4. Conclusions

- The PA and FL fractions of planktonic communities exhibit distinct taxonomic diversity, which shapes the diverse spatial distribution of associated pathogenic and AMR load in various aquatic

environments. Understanding the composition of the PA and FL microbial community, including the associated pathogen and AMR fractions, can enhance predictions of pathogen and AMR removal in wastewater treatment systems like constructed wetlands.

- The PA community was largely responsible for the ARGs load introduced into the CW, while the FL community served as the primary escape route for ARGs from the CW under study.
- The FL bacterial community in the planktonic phase of the studied constructed wetland carried a wastewater-related footprint throughout the system. This significantly influenced the microbiome and resistome in the planktonic community over a greater distance than the corresponding PA fraction.
- Under stable hydraulic conditions, the sediments did not significantly influence the composition of the PA and FL planktonic communities. Conversely, the planktonic community had a negligible impact on the composition of the sediment microbial community.
- AMR removal in the constructed wetland was both fraction- and gene-specific. However, the PA fraction lost at least 90 % of its carried ARGs, while the FL fraction managed to preserve some of its resistome unchanged during the treatment process.

CRedit authorship contribution statement

Franciszek Bydalek: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Gordon Webster:** Conceptualization, Formal analysis, Investigation, Methodology, Writing – review & editing. **Ruth Barden:** Resources, Supervision, Writing – review & editing. **Andrew J. Weightman:** Conceptualization, Supervision, Writing – review & editing. **Barbara Kasprzyk-Hordern:** Supervision, Writing – review & editing. **Jannis Wenk:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request. Raw genetic sequencing data are available at NCBI repository <https://www.ncbi.nlm.nih.gov/bio-project/980,007> Accession: PRJNA 980,007.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.watres.2024.121408](https://doi.org/10.1016/j.watres.2024.121408).

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