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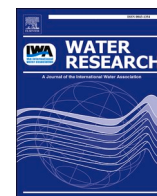
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Metagenomic insights into microbial drivers of organic micropollutant removal in wastewater-impacted riverbank filtration

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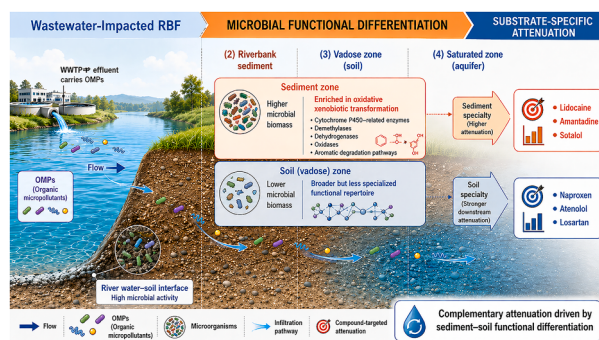
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HIGHLIGHTS

- Substrate heterogeneity drives OMP removal in WWTP effluent-fed RBF.
- Sediment and soil show complementary, compound-specific removal.
- Metagenomics links OMP removal to microbial functional differentiation.
- Sediment enriched in oxidative xenobiotic degradation pathways.
- Maintaining oxic, active zones enhances RBF treatment performance.

GRAPHICAL ABSTRACT



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ABSTRACT

Organic micropollutants (OMPs) in wastewater treatment plant (WWTP) effluent pose persistent risks to aquatic ecosystems and drinking water sources. Riverbank filtration (RBF) is a nature-based treatment process, yet the compartment-specific roles of riverbed sediment and downstream soil in OMP attenuation remain poorly resolved under wastewater-impacted conditions. Here, we combined targeted chemical analysis, OMP property compilation, shotgun metagenomics, EnviPath-based biotransformation annotation, and exploratory network analysis to investigate OMP attenuation in a laboratory-scale RBF system treating real WWTP effluent for 10 months. Nineteen OMPs were monitored along a sequential sediment–soil filtration pathway. Sediment preferentially attenuated hydrophilic or charged compounds, including lidocaine, amantadine, and sotalol, whereas soil contributed more strongly to the attenuation of naproxen, atenolol, and losartan. Metagenomic profiling

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revealed distinct microbial communities and functional gene repertoires between sediment and soil after long-term operation. Sediment harbored higher relative abundances of genes associated with oxidative xenobiotic transformation, including cytochrome P450-related enzymes, demethylases, dehydrogenases, oxidases, and aromatic compound degradation pathways. An exploratory Spearman network further identified associations among microbial genera, EnviPath-annotated candidate biotransformation genes, and OMP removal rates, including 17 KO–OMP links supported by both correlation and pathway annotation. These findings indicate that sediment and soil develop complementary microbial functional potentials that may support compound-specific OMP attenuation. This study provides a mechanistic basis for optimizing sediment–soil configurations in wastewater-impacted RBF systems and for improving nature-based barriers against diverse OMP mixtures.

1. Introduction

Organic micropollutants (OMPs), including pharmaceuticals, personal care products, pesticides, and industrial chemicals, are increasingly detected in aquatic environments worldwide and have raised significant environmental and public health concerns (Su et al., 2020; Barbosa et al., 2016; Albergamo et al., 2019; Hermes et al., 2018; de Oliveira et al., 2020). Although typically present at trace concentrations (ng/L to µg/L), many OMPs exhibit persistence, bioaccumulation potential, and chronic toxicity, and may contribute to the development of antibiotic resistance (Mathias et al., 2018; Pesqueira et al., 2020; Etchepare and van der Hoek, 2015; Pereira et al., 2015; Lin et al., 2016; Danner et al., 2019; Tarafdar et al., 2022; Delbes et al., 2022). Their continuous release into water bodies, particularly via wastewater treatment plant (WWTP) effluents, has made them a critical challenge for water quality management.

Conventional wastewater treatment processes are not specifically designed to remove OMPs and often achieve only partial removal (Tiwari et al., 2017; Sharma et al., 2019). As a result, WWTP effluents remain a major source of OMPs in surface waters, where they may pose long-term ecological and human health risks (Benson et al., 2017; Grenni et al., 2018; Kim et al., 2017). To address these limitations, nature-based treatment systems such as riverbank filtration (RBF) have gained increasing attention as sustainable and cost-effective approaches for improving water quality (Maeng et al., 2010; Zhao et al., 2022; Gavrilescu, 2005; Hiscock and Grischek, 2002; Storck et al., 2012; Hamann et al., 2016; Ahmed and Marhaba, 2017). RBF involves the infiltration of surface water through riverbed sediments and adjacent aquifer materials toward extraction wells, during which a combination of physical, chemical, and biological processes contributes to contaminant attenuation (Maeng et al., 2010; Zhao et al., 2022; Gavrilescu, 2005; Hiscock and Grischek, 2002; Storck et al., 2012). During RBF and related managed aquifer recharge (MAR) systems, OMP attenuation may involve filtration, dilution, sorption/desorption, redox reactions, and microbial biotransformation, with the relative importance of these processes depending on contaminant properties, hydraulic conditions, source-water quality, and microbial activity (Li et al., 2020; Regnery et al., 2017; Hübner et al., 2022).

Previous studies have demonstrated that RBF can effectively reduce turbidity, pathogens, and certain organic micropollutants through sorption, biodegradation, and redox-driven transformations (Zhao et al., 2022; Hamann et al., 2016; Ahmed and Marhaba, 2017). However, most existing research has focused on relatively clean surface water, while the application of RBF to WWTP effluent—characterized by elevated organic matter, complex contaminant mixtures, and distinct microbial communities—remains insufficiently understood (Albergamo et al., 2019; Gillefalk et al., 2018; Maeng and Lee, 2019; Yu et al., 2020).

In particular, the respective roles of different filtration media, especially riverbed sediment and soil, have not been systematically distinguished. Most previous RBF or MAR studies have treated the subsurface filtration zone as a relatively homogeneous reactive medium or relied on mixed groundwater samples from monitoring wells, thereby obscuring the distinct contributions of riverbed sediment and downstream soil or aquifer materials (Regnery et al., 2017; Barba et al., 2019;

Modrzyński et al., 2021; Erdélyi et al., 2025). These media differ substantially in physicochemical properties, redox conditions, and microbial habitats, which are likely to influence OMP removal pathways and efficiencies. A mechanistic understanding of how microbial communities associated with sediment and soil contribute to OMP attenuation under wastewater-impacted conditions is therefore essential for optimizing RBF performance.

Metagenomics provides a powerful, culture-independent approach to characterize microbial communities and their functional potential in environmental systems (Tringe and Rubin, 2005; Devarapalli and Kumavath, 2015; Zhu et al., 2020). By enabling the identification of taxonomic composition and metabolic capabilities, metagenomic analysis offers unique opportunities to link microbial functions with contaminant transformation processes (Rahimi et al., 2018; Nascimento et al., 2020; Wang et al., 2026; Sharma et al., 2022). Despite its potential, the application of metagenomics to elucidate substrate-specific microbial mechanisms of OMP removal in RBF systems treating WWTP effluent remains limited.

In this study, we investigate the removal of OMPs in a laboratory-scale RBF system fed with WWTP effluent, with a particular focus on the contrasting roles of sediment and soil. We hypothesized that sediment and soil would harbor taxonomically and functionally distinct microbial communities, resulting in complementary, compound-specific OMP attenuation patterns under long-term WWTP effluent exposure. By integrating targeted chemical analysis with metagenomic profiling, we aim to (i) quantify substrate-specific removal patterns of OMPs, (ii) characterize shifts in microbial community composition and functional gene profiles, and (iii) elucidate the microbial functional drivers underlying OMP attenuation. In addition, EnviPath-based candidate biotransformation gene annotation and exploratory Spearman rank correlation network analysis were used to evaluate potential associations among microbial genera, functional genes, and individual OMP attenuation rates. This work provides mechanistic insights to support the design and optimization of RBF systems for the treatment of wastewater-impacted water sources. We hypothesized that sediment and soil would harbour taxonomically and functionally distinct microbial communities, leading to complementary, compound-specific OMP removal patterns under long-term wastewater effluent exposure.

2. Materials and methods

2.1. Simulated riverbank filtration set-up

Laboratory-scale column experiments were conducted at the Water Lab of Civil Engineering and Geosciences, Delft University of Technology (The Netherlands). The experimental system consisted of two identical sets of three PVC columns (length: 1 m; inner diameter: 60 mm) to simulate riverbank filtration (RBF) conditions (Fig. 1a). Specifically, the system consisted of two independent duplicate column sets. Each set included one upstream sediment column followed by three soil columns in series, hereafter referred to as sediment, soil-1, soil-2, and soil-3. Columns were packed with soil collected from the riverbank of the Lek River (Krimpen aan de Lek, The Netherlands; 51°53'44.2"N, 4°38'29.9"E). To better represent natural RBF conditions, an additional

10 cm column filled with riverbed sediment from the same location was installed upstream of the soil columns, reflecting the biologically active interface zone.

Packing was performed incrementally in 4–5 cm layers with gentle compaction to minimize preferential flow. Columns were equipped with sampling ports and valves at both inlet and outlet for water and solid sampling. Flow was maintained in an upward direction to prevent air entrapment. The system was operated in the dark at room temperature for 10 months. Secondary effluent from the WWTP Harnaschpolder (Delft, The Netherlands) was continuously supplied at a hydraulic loading rate of 3 L d^{-1} , corresponding to a filtration velocity of approximately 1 m d^{-1} . Detailed physicochemical properties of the sediment and soil media, such as organic carbon content, cation exchange capacity, mineral composition, and particle-size distribution, were not measured in the present study. Therefore, substrate-specific OMP attenuation was interpreted in relation to the sequential filtration configuration, OMP physicochemical properties, redox profiles, and microbial functional potential rather than measured sediment or soil physicochemical characteristics.

2.2. Sampling and sample preservation

Influent (WWTP effluent) and effluent (post-filtration water) samples were collected monthly to quantify OMP concentrations and removal

efficiencies. Intermediate water samples after sediment filtration were also used to evaluate compartment-specific attenuation and to describe the dissolved oxygen and nitrogen-species profiles along the sediment–soil flow path. Water samples were filtered through $0.2 \mu\text{m}$ polycarbonate filters (Merck Millipore, Germany) immediately after collection.

Sediment and soil samples were collected from the sediment column and the second soil column before operation (initial condition) and after 10 months of continuous filtration for metagenomic analysis. All samples were collected in duplicate. In total, eight solid samples were analyzed by shotgun metagenomics: sediment before operation, sediment after operation, soil before operation, and soil after operation, each with duplicate samples. Water samples were transported on ice and stored at -20°C prior to analysis. Solid samples were transferred to sterile cryotubes and stored at -80°C until DNA extraction. Because the experimental system was operated sequentially, soil attenuation represents additional downstream removal after upstream sediment filtration rather than removal under identical influent concentrations as the sediment compartment.

2.3. Organic micropollutants (OMPs) analysis

OMPs were extracted from water samples using solid-phase extraction (SPE) with Oasis Prime HLB cartridges (Waters, USA) and analyzed

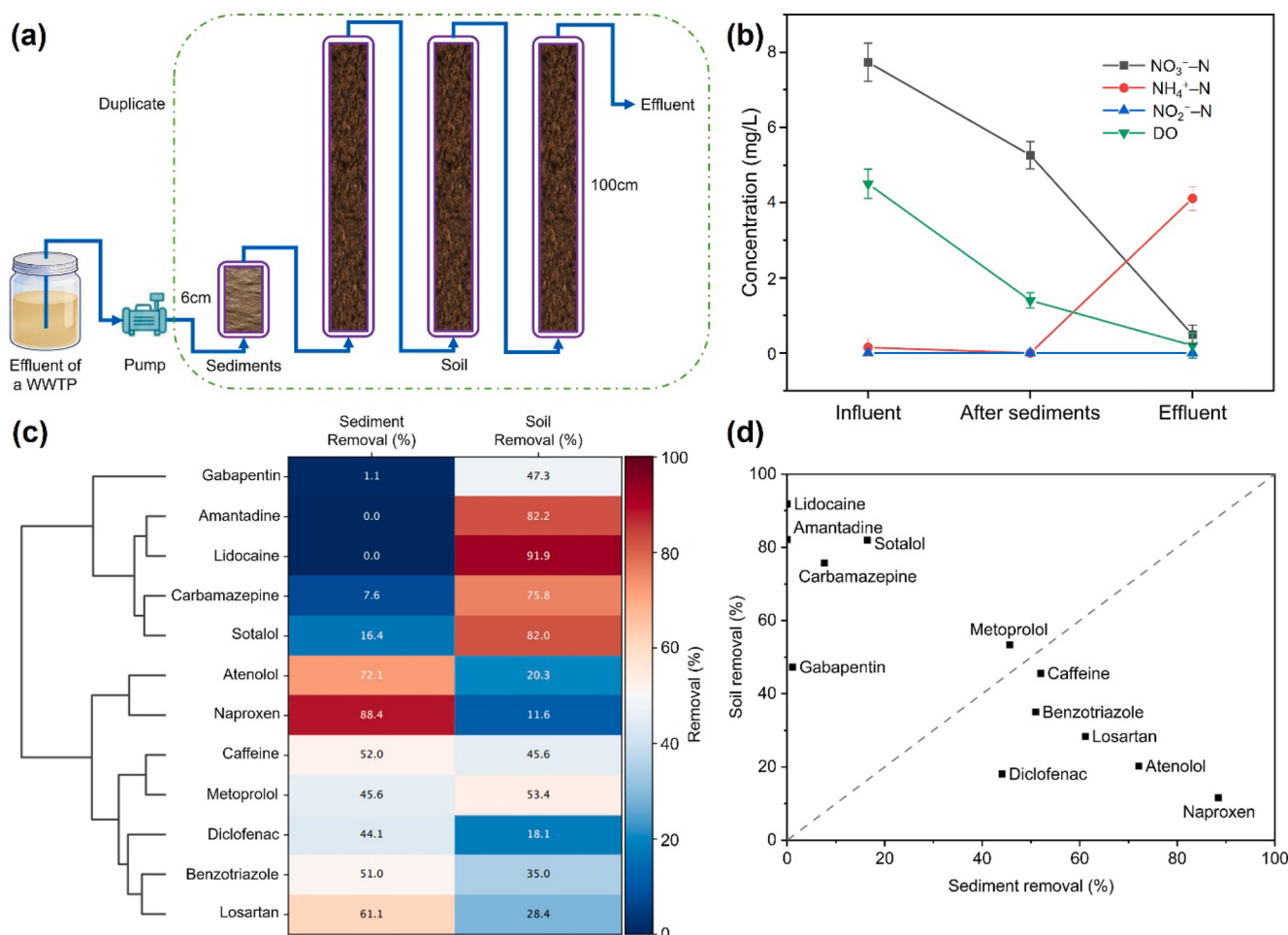


Fig. 1. Experimental design and removal of OMPs by sediments and soil. (a) Experimental setup of the laboratory-scale riverbank filtration (RBF) system fed with wastewater treatment plant (WWTP) effluent, consisting of a sediment layer followed by soil columns under upward flow conditions. (b) Spatial profiles of nitrogen species (NO_3^- -N, NH_4^+ -N, NO_2^- -N) and dissolved oxygen (DO) along the RBF flow path. (c) Substrate-specific removal efficiencies of selected organic micropollutants (OMPs) in sediment and soil media. (d) Substrate-specific differences in organic micropollutant (OMP) removal between sediment and soil. The dashed line represents equal removal efficiency (1:1). Deviations from the line indicate preferential removal in either sediment or soil.

by high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC–MS/MS, API 6500 QTRAP). Analytical procedures followed established methods (Česen et al., 2018), with detailed information provided in Text S1 and Table S1. Nineteen OMPs were monitored throughout the experiment. Removal efficiencies were calculated based on measured aqueous concentrations across the corresponding filtration compartments.

To support interpretation of compound-specific attenuation patterns, key physicochemical properties of the investigated OMPs were compiled from publicly available chemical databases and literature values. These properties included dominant charge state under the experimental pH range, and hydrophilicity/hydrophobicity classification. The compiled OMP properties are summarized in Table S3. These data were used to provide qualitative context for interpreting preferential attenuation of different OMPs in sediment and soil, but were not used to calculate quantitative sorption coefficients.

2.4. DNA extraction and sequencing

DNA was extracted from the filters using the DNeasy Powersoil Kit (Qiagen, Germany) following the protocol, bead beating was set to 10 min, followed by 4 min centrifugation at 4000 g. The incubation time at 4 °C after addition of inhibitor removal solution (IRS) was extended to 10 min. DNA was eluted in 60 µL elution buffer. Subsequently, DNA concentration was measured on a Qubit2.0 fluorometer using a Qubit® dsDNA HS Assay Kit (Invitrogen, OR, United States) following manufacturer instructions. One µL DNA solution was added to 199 µL working solution for measurement. Genomic DNA as well as all further DNA products were stored at –80 °C until processing.

Sequencing libraries were generated using NEB Next® Ultra™ DNA Library Prep Kit for Illumina® (New England Biolabs, MA, USA) following manufacturer's recommendations and index codes were added. The library quality was assessed on the Qubit4.0 Fluorometer (Life Technologies, Grand Island, NY) and Qsep 400 High-Throughput Nucleic Acid Protein Analysis system (Houze Biological Technology Co, Hangzhou, China) system. At last, the library was sequenced on an Illumina NovaSeq 6000 platform and 150 bp paired-end reads were generated.

2.5. Metagenomics analysis

The quality of the sequenced raw reads was assessed by FastQC (version 0.11.7) with default parameters (Wingett and Andrews, 2018) and visualized with MultiQC (version 1.0) (Ewels et al., 2016). Low-quality paired-end reads were trimmed and filtered by Trimmomatic version 0.39 on the paired-end mode (Bolger et al., 2014). Taxonomic classification of raw reads was performed to profile the microbiome from each sample using the standard Kraken2 database (Wood et al., 2019). Clean reads were assembled into contigs using MetaSPAdes (version 3.13.0) with a k-mer spectrum of 21, 33, 71, 101, and 121 (Nurk et al., 2017). Open reading frames (ORFs) were predicted using Prodigal (Hyatt et al., 2010), followed by clustering with MMseqs2 under stringent parameters (sequence identity ≥ 0.9 and coverage ≥ 0.9) (Steinegger and Söding, 2017). Functional genes were annotated using eggNOG-mapper with recommended parameters (diamond, e-value = $1e^{-5}$) (Cantalapiedra et al., 2021). And relative abundance (transcripts per million, TPM) of each sample was quantified using CoverM with stringent parameters to ensure accuracy: –min-read-percent-identity 90, –min-read-aligned-percent 50, –min-covered-fraction 0.5 (Aroney et al., 2024). Functional profiles were summarized at the KEGG orthology group level. Pathway-level TPM values were transformed into Z-scores on a per-pathway basis using $Z = (x - \mu_{row}) / \sigma_{row}$, where x is the TPM value of a given pathway in a given sample, μ_{row} is the mean TPM of that pathway across all samples, and σ_{row} is the corresponding standard deviation.

2.6. Biotransformation rules extraction and analysis

The EAWAG (Sivakumar et al., 2017) and EnviPath (Wicker et al., 2016) databases were utilized to predict the biotransformation pathways of organic micropollutants. The associated EC numbers at the sub-subclass level and corresponding genes for each biotransformation rule were extracted and summarized. For each OMP, candidate degradation genes were retrieved from metagenomic data using Python. Specifically, third-level EC numbers were compiled from EnviPath, and genes with matching IDs were extracted from the metagenomic dataset into new Excel sheets via search and copy functions. A list of relevant biotransformation rules (bt rules), functional groups, and reaction types is provided in Table S2, along with the corresponding EC numbers for all 12 organic micropollutants. Additionally, heatmaps were generated using the ggplot2 package in R for visualization (Wickham, 2011). For reconstructed transformation pathways, a pathway enzyme was considered detected when the corresponding KEGG orthology or enzyme annotation was present with TPM > 0 in at least one sample. Missing enzymes were interpreted cautiously because apparent absence may reflect incomplete assembly, low gene abundance, ORF prediction limitations, or incomplete reference database coverage rather than true absence of the corresponding metabolic capability.

2.7. Statistical analysis of OMP attenuation, functional genes, and microbial consortia

Statistical analyses were performed to evaluate relationships among OMP attenuation, microbial functional potential, and microbial community composition. Differential abundance analyses were conducted to compare genes, pathways, and genera between sediment and soil samples after long-term filtration. Features enriched in sediment or soil were identified based on log₂ fold change and adjusted p-value thresholds, as specified in the figure captions. To formally evaluate associations between microbial features and OMP attenuation, Spearman rank correlations were calculated between microbial genera or KEGG orthology groups (KOs) and the removal rate of each OMP across all eight metagenomic samples. For gene-level analysis, only KOs predicted as candidate biotransformation genes by EnviPath were included. Multiple testing correction was performed using the Benjamini–Hochberg method. Edges were retained at $|\rho| \geq 0.70$ and BH-adjusted $p < 0.25$. For each MP, the top five KOs and top three genera ranked by $|\rho|$ were selected for visualization. A bipartite correlation network was constructed using the igraph and ggraph packages in R. In the network, KO nodes and genus nodes were independently connected to OMP nodes. KO–OMP edges supported by both Spearman correlation and EnviPath biotransformation pathway annotation were highlighted as dual-evidence-supported associations. Because the sample size was limited ($n = 8$), a conventional BH-adjusted threshold of $p < 0.05$ was overly conservative for this exploratory analysis. Therefore, BH-adjusted $p < 0.25$ was applied as a relaxed discovery threshold together with a stringent correlation-effect threshold of $|\rho| \geq 0.70$. The resulting network was interpreted as exploratory and hypothesis-generating rather than as definitive evidence of causality or active biodegradation. All statistical analyses and visualizations were performed in R unless otherwise specified.

3. Results

3.1. OMPs removal in sediments and soil

The experimental results revealed notable differences in OMPs removal efficiency between sediments and soil columns, as presented in Fig. 1. It shows the compartment-specific attenuation along the sequential sediment–soil filtration pathway, together with the measured dissolved oxygen (DO) profile.

In sediments columns, lidocaine, amantadine, and sotalol exhibited

the highest removal rates, of approx. 85%, 70%, and 60%, respectively. These compounds, known for their hydrophilic and charged characteristics, may be influenced by a combination of physicochemical retention and microbial transformation potential. Their high attenuation in sediment should therefore not be attributed solely to biodegradation. The measured DO profile showed that DO decrease from the influent to after the sediment column but remained detectable after sediment filtration, indicating that the sediment compartment represented an oxidative to low-oxygen reactive interface rather than a fully anaerobic zone. In contrast, compounds such as naproxen, diclofenac, and losartan displayed minimal removal (<20%) in sediments, indicating that sediment attenuation was strongly compound specific rather than uniformly effective for all OMPs.

Conversely, soil columns demonstrated a distinct removal pattern. Naproxen, atenolol, and losartan were efficiently removed, with removal rates exceeding 80%, while compounds such as caffeine, benzotriazole, and metoprolol showed moderate removal (50–70%). However, lidocaine and amantadine, which were highly removed in sediments, showed significantly lower removal in soil. Because the columns were operated in series, soil removal represents additional downstream attenuation after upstream sediment filtration rather than an independent removal test under identical influent concentrations.

Overall, the results highlight a substrate-specificity in the attenuation potential for individual OMPs. Sediment and soil provided complementary attenuation functions along the RBF flow path. The OMP property information summarized in the Supplementary Information, including charge state and hydrophilicity/hydrophobicity classification, provides additional context for interpreting these compound-specific attenuation patterns.

3.2. PCoA of genes, pathways, and genera

To assess microbial community responses to long-term exposure to wastewater-derived organic micropollutants (OMPs), principal coordinates analysis (PCoA) was performed based on metagenomic profiles at three levels: functional genes, metabolic pathways, and taxonomic composition at the genus level (Fig. 2). Sediment and soil samples were compared before (Be) and after (Af) 10 months of continuous filtration of wastewater treatment plant (WWTP) effluent.

At the gene level (Fig. 2a), a clear separation between pre- and post-treatment samples was observed along PCoA1, which explained 86.37% of the total variation. Both sediment and soil samples exhibited pronounced shifts after long-term operation. In particular, Sedi-Af samples were distinctly separated from Sedi-Be samples, indicating substantial restructuring of functional gene profiles. A similar divergence was observed in soil, where Soil-Af samples were clearly separated from Soil-Be samples, reflecting strong shifts in microbial functional potential. At

the pathway level (Fig. 2b), comparable separation patterns were observed, with PCoA1 explaining 81.93% of the variation. Post-treatment samples (Sedi-Af and Soil-Af) were clearly distinguished from their initial states. Given the duplicate sampling design, minor differences in within-group dispersion were interpreted cautiously rather than used as the main basis for biological inference.

At the genus level (Fig. 2c), microbial community composition also exhibited clear divergence after long-term filtration. Separation along PCoA1 (85.55%) distinguished pre- and post-treatment samples in both sediment and soil. Overall, the most robust pattern supported by the PCoA results is the clear separation between pre- and post-operation microbial profiles and the differentiation between sediment and soil compartments after long-term filtration.

3.3. Enrichment of genes, pathways, and genera

Differential abundance analysis revealed clear separation between sediment and soil communities at the gene, pathway, and genus levels after 10 months of wastewater treatment plant (WWTP) effluent exposure (Fig. 3). In all panels, positive log₂ fold change values indicate enrichment in sediment, whereas negative values indicate enrichment in soil.

At the gene level (Fig. 3a), numerous genes were significantly enriched in sediment, including K21209 (acyltransferase), K21159 (epoxide hydrolase), and K03391 (pentachlorophenol monooxygenase), while others such as K20305 (trafficking protein particle complex subunit 8), K18533 (adenylate/nucleoside-diphosphate kinase), and K10527 (enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase) were more abundant in soil. This substrate-specific gene distribution coincided with the compound-specific attenuation patterns observed in Fig. 1, where lidocaine, amantadine, and sotalol were preferentially removed in sediment, whereas naproxen, atenolol, and losartan showed higher removal in soil.

At the pathway level (Fig. 3b), several metabolic pathways exhibited clear substrate-specific enrichment. Pathways such as ko03450 (Non-homologous end-joining) and ko01059 (Biosynthesis of enediyne antibiotics) were enriched in sediment, while pathways including ko04110 (Cell cycle), ko04136 (Autophagy - other), and ko04141 (Protein processing in endoplasmic reticulum) were more abundant in soil. These pathway-level differences suggest different microbial functional potentials between sediment and soil, with sediment-associated pathways aligning with the higher removal of lidocaine and amantadine, and soil-associated pathways aligning with the enhanced removal of naproxen and atenolol.

At the genus level (Fig. 3c), clear taxonomic differentiation was observed. Genera such as *Conexibacter*, *Intrasporangium*, *Arthrobacter*, and *Geodermatophilus* were enriched in sediment, whereas *Blastococcus*,

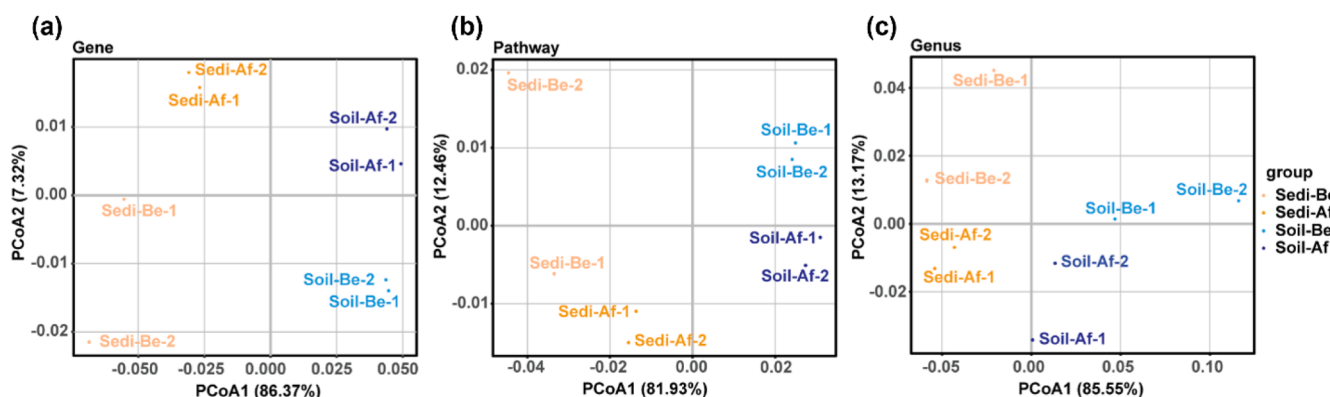


Fig. 2. Principal coordinates analysis (PCoA) of microbial communities based on metagenomic data at three levels. (a) functional genes, (b) metabolic pathways, and (c) genus-level taxonomic composition. Samples represent sediment (Sedi) and soil (Soil) collected before (Be) and after (Af) 10 months of riverbank filtration with wastewater treatment plant (WWTP) effluent. The percentage of variation explained by each principal coordinate axis is indicated.

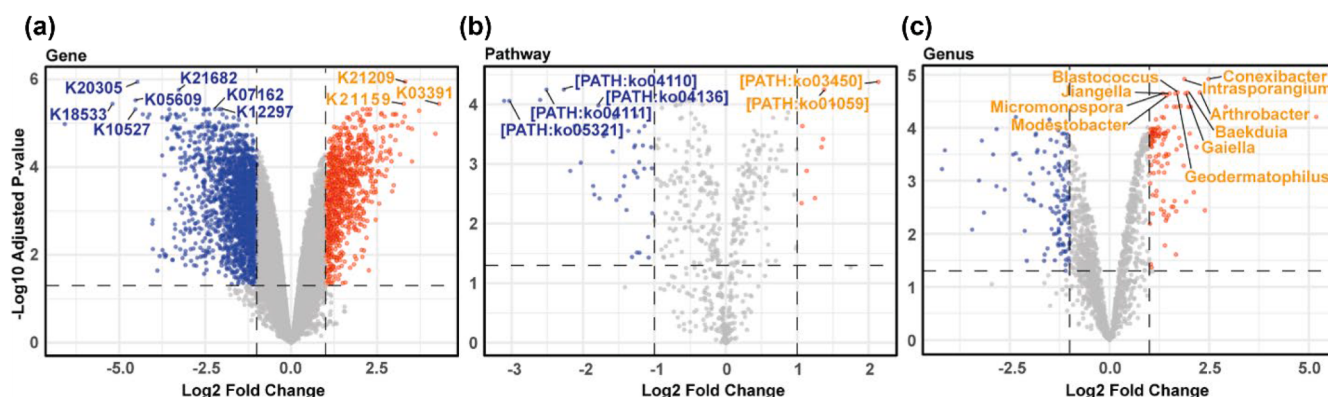


Fig. 3. Volcano plots showing differential abundance between sediment and soil after 10 months of wastewater treatment plant (WWTP) effluent filtration: at (a) gene, (b) pathway, and (c) genus levels. The x-axis represents $\log_2$ fold change (sediment vs soil), and the y-axis shows $-\log_{10}$ adjusted p-value ($|\log_2FC| > 1$, adjusted $p < 0.05$). Features with positive values are enriched in sediment, whereas those with negative values are enriched in soil. Colored points indicate significantly enriched features.

Jangella, *Micromonospora*, and *Modestobacter* were more abundant in soil. These differentially enriched taxa coincided with distinct OMP attenuation patterns, suggesting potential functional associations between microbial community structure and compound-specific removal. However, differential abundance analysis alone does not establish direct causal relationships between specific taxa, genes, pathways, and individual OMP attenuation.

3.4. Key xenobiotics metabolic pathways and enzymes of OMPs degradation

The heatmap illustrates the relative abundance (Z-score) were calculated on a per-pathway (row-wise) basis of key xenobiotic degradation pathways in sediment and soil samples before (Be) and after (Af) 10 months of wastewater treatment plant (WWTP) effluent exposure (Fig. 4). Z-scores were calculated on a per-pathway basis by mean-

centering and scaling TPM values across all samples, allowing relative enrichment or depletion of each pathway to be compared among sample groups. Across all samples, sediment exhibited consistently higher abundances of most xenobiotic degradation pathways compared to soil. This pattern was evident both before and after operation, with sediment samples showing predominantly higher Z-scores, whereas soil samples remained comparatively lower. Pathways involved in the degradation of caprolactam, bisphenol, dioxin, naphthalene, styrene, and various chlorinated and aromatic compounds were particularly enriched in sediment.

After 10 months of operation, both sediment (Sedi-Af) and soil (Soil-Af) samples showed shifts in pathway profiles; however, the substrate-dependent distinction remained pronounced. Sediment samples after operation clustered closely and maintained high relative abundances of xenobiotic degradation pathways. In contrast, soil samples exhibited more moderate changes and remained lower in overall pathway

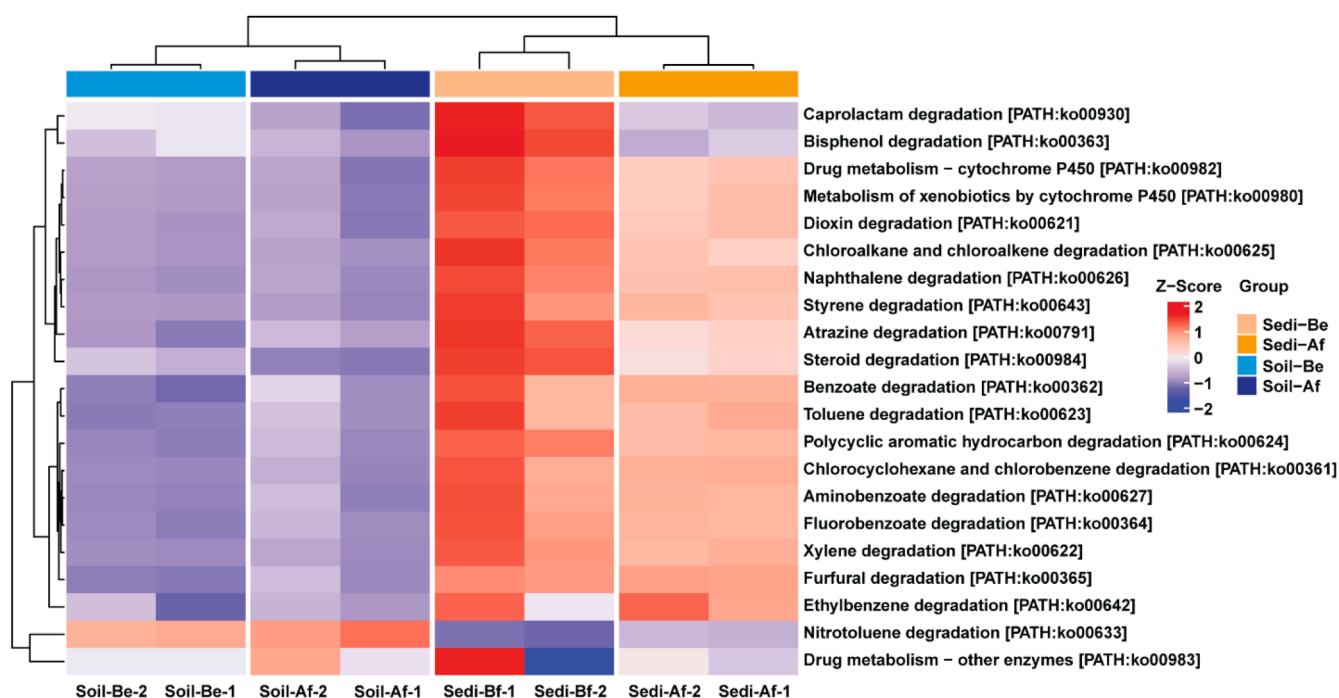


Fig. 4. Heatmap showing the relative abundance (Z-score) of xenobiotic degradation pathways in sediment and soil samples before (Be) and after (Af) 10 months of filtration of wastewater treatment plant (WWTP) effluent. Rows represent metabolic pathways and columns represent samples. Warmer colors indicate higher relative abundance. Clustering reflects similarities in pathway profiles across samples. Z-scores were calculated row-wise for each pathway.

abundance. These pathway-level differences are consistent with the compound-specific removal patterns observed in Fig. 1b, where sediment showed higher removal efficiencies for compounds such as lidocaine and amantadine. The enrichment of diverse xenobiotic degradation pathways in sediment suggests a greater functional potential for the transformation of a broad range of OMPs compared to soil.

3.5. Functional gene and pathway associations with OMP degradation

The distribution of key functional genes and reconstructed metabolic pathways associated with the degradation of representative organic micropollutants (OMPs) further highlighted the functional differentiation between sediment and soil (Fig. 5). These genes are considered

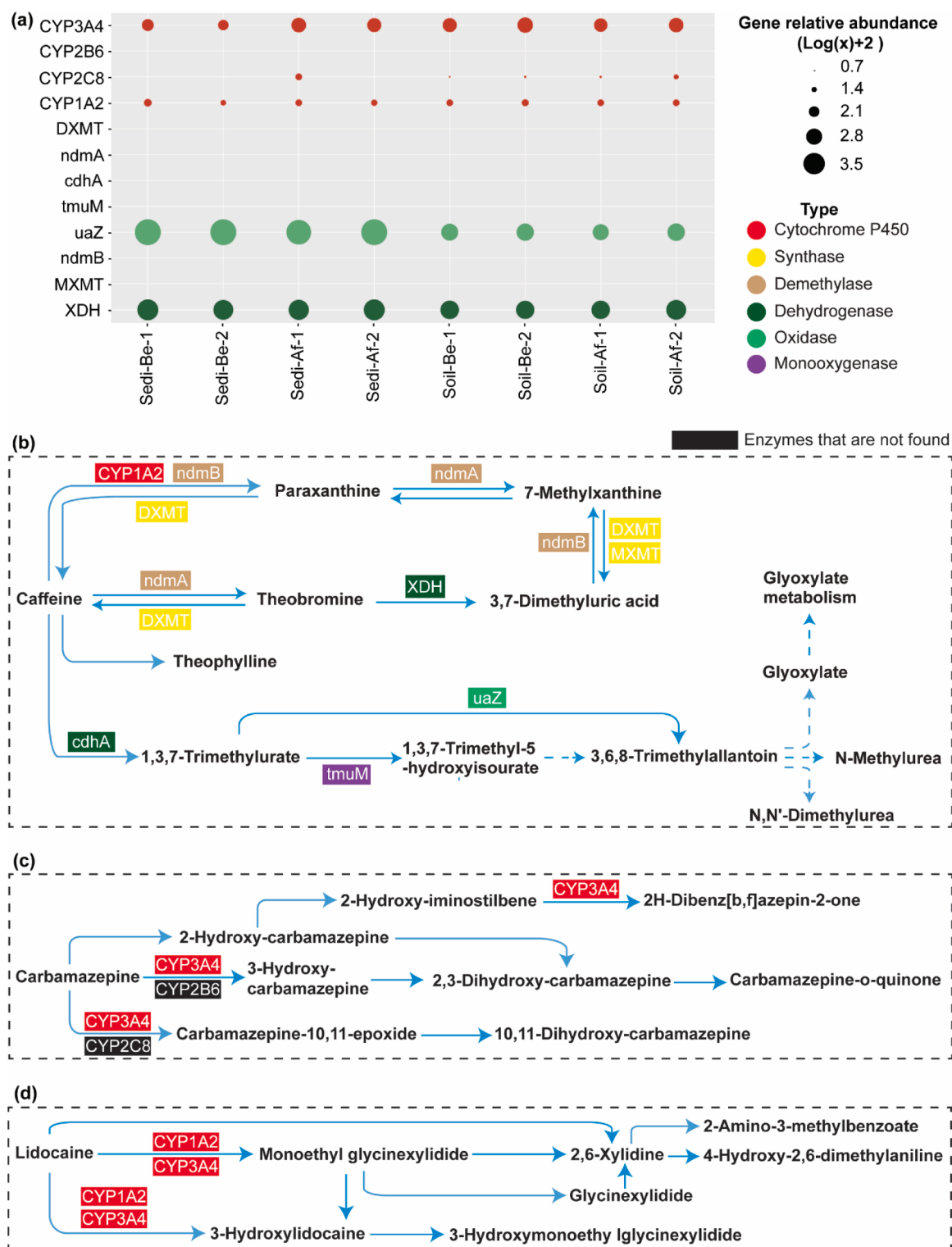


Fig. 5. Functional gene abundance and reconstructed metabolic pathways associated with the degradation of representative organic micropollutants (OMPs) in sediment and soil. (a) Relative abundance of key genes involved in oxidative and demethylation processes. Dot size represents gene abundance, and colors indicate enzyme classes. (b–d) Reconstructed metabolic pathways for caffeine, carbamazepine, and lidocaine based on annotated genes. Colored labels indicate detected enzymes, while black boxes represent missing enzymes.

candidate transformation-related genes and are discussed as functional potential rather than direct evidence of degradation activity. At the gene level (Fig. 5a), enzymes involved in oxidative transformation and demethylation were consistently more abundant in sediment. Cytochrome P450-related genes (e.g., CYP3A4, CYP2B6, CYP2C8, and CYP1A2) showed higher relative abundance in sediment across both pre- and post-operation samples. Similarly, genes encoding demethylases (ndmA, ndmB, DXMT), dehydrogenases (XDH, cdhA), and oxidases (uaZ) were enriched in sediment compared to soil. In contrast, several of these genes were present at lower abundance or absent in soil samples.

Pathway reconstruction revealed that these genes collectively covered key transformation steps for caffeine, carbamazepine, and lidocaine (Fig. 5b–d). For caffeine (Fig. 5b), multiple demethylation and oxidation-related genes were detected in sediment, whereas fewer steps were represented in soil. For carbamazepine (Fig. 5c), cytochrome P450-associated reactions were predominantly supported by genes enriched in sediment. A similar pattern was observed for lidocaine (Fig. 5d), where P450-related genes were more abundant in sediment than in soil. These gene- and pathway-level differences are consistent with the compound-specific removal patterns observed in Fig. 1b, where lidocaine exhibited higher removal in sediment compared to soil.

Overall, sediment showed a broader and more abundant set of genes associated with oxidative and demethylation processes, indicating a higher functional potential for the transformation of selected OMPs

relative to soil. It should be noted that a pathway enzyme was considered detected when the corresponding KEGG orthology or enzyme annotation was present with TPM > 0 in at least one sample. Apparent absence of pathway enzymes may reflect incomplete assembly, low abundance, ORF prediction limitations, or incomplete database annotation rather than true absence of metabolic capability.

3.6. Exploratory gene–OMP removal association network

To further evaluate the relationships between microbial functional potential genes and OMP attenuation, we constructed an exploratory bipartite Spearman rank correlation network linking microbial genera, EnviPath-annotated candidate biotransformation genes, and individual OMP removal rates across all eight metagenomic samples (Fig. 6). KO and genus nodes were connected independently to OMP nodes. KO–OMP edges supported by both Spearman correlation and EnviPath biotransformation annotation were highlighted as EnviPath-supported associations.

The resulting network contained 28 genera, 43 KOs, and 12 OMPs. A total of 54 KO–OMP edges and 34 genus–OMP edges were retained under the criteria $|\rho| \geq 0.70$ and BH-adjusted $p < 0.25$. Among the retained KO–OMP associations, 17 edges were supported by both Spearman correlation and EnviPath biotransformation annotation. These dual-evidence-supported links connected candidate

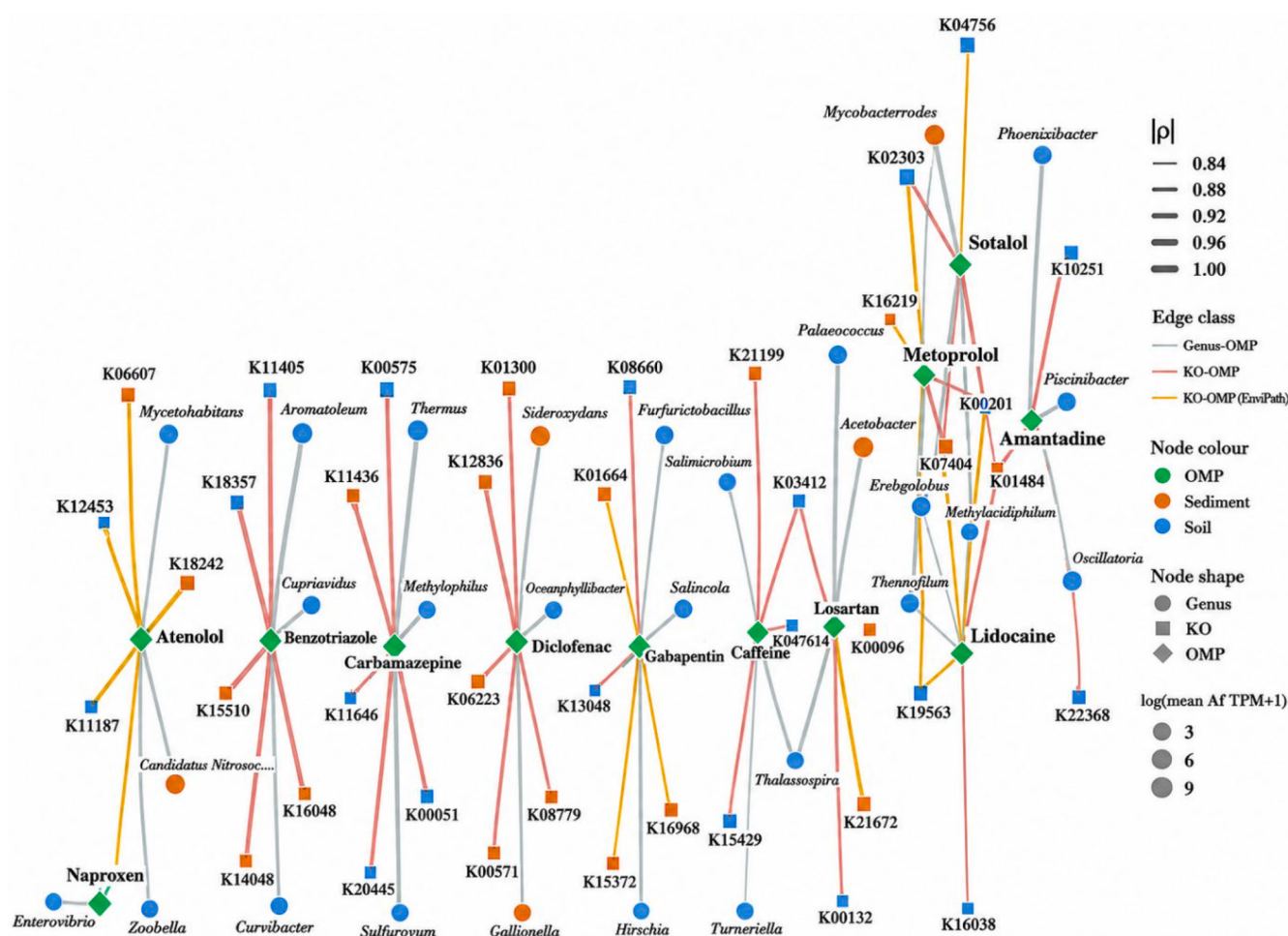


Fig. 6. Exploratory gene/genera–OMP removal association network based on Spearman rank correlation and EnviPath-supported candidate biotransformation genes. Nodes represent microbial genera, KEGG orthology groups (KOs), and OMPs. Edges indicate Spearman rank correlations between microbial features and OMP removal rates across all eight samples. Edges indicate genus–OMP associations, red edges indicate KO–OMP associations, and gold edges indicate KO–OMP associations supported by both Spearman correlation and EnviPath biotransformation annotation. Node color indicates enrichment group, and node shape indicates node type. The network is interpreted as exploratory and hypothesis-generating rather than as definitive evidence of causality.

biotransformation genes with the attenuation of several OMPs, including atenolol, gabapentin, lidocaine, losartan, metoprolol, and sotalol.

The network further showed that individual OMPs were associated with distinct combinations of microbial genera and functional genes. EnviPath-supported KO–OMP edges provide database-based support for potential biotransformation links, whereas non-EnviPath-supported edges may represent statistically supported associations beyond currently annotated biotransformation pathways.

Nevertheless, the network should be interpreted as exploratory and hypothesis-generating. The sample size was limited, and BH-adjusted $p < 0.25$ was used as a relaxed discovery threshold together with a stringent correlation-effect threshold of $|\rho| \geq 0.70$. Moreover, gene-centric metagenomics identifies functional potential rather than gene expression, enzyme activity, or transformation-product formation. Therefore, the network provides statistical and database-supported evidence for potential microbial associations with OMP attenuation, though not establish direct causality or confirm active biodegradation.

4. Discussion

This study shows that OMP attenuation in wastewater-impacted riverbank filtration (RBF) is compartment dependent. Sediment and soil exhibited distinct and complementary attenuation patterns, accompanied by differences in microbial community composition, functional gene profiles, and gene-OMP association networks. These results indicate that RBF should not be treated as a homogeneous reactive zone. Instead, riverbed sediment and downstream soil function as sequential compartments with different physicochemical and microbial characteristics. This interpretation is consistent with broader RBF and managed aquifer recharge (MAR) studies showing that trace organic contaminant attenuation depends on retention time, redox conditions, source-water quality, primary substrate availability, hydraulic flow paths, microbial community structure, and compound-specific properties (Li et al., 2020; Regnery et al., 2017; Hübner et al., 2022; Erdélyi et al., 2025; Kondor et al., 2024). Previous MAR and reactive-barrier studies also showed that sediment or engineered media can shape microbial communities and improve the removal of some OMPs, although treatment performance remains compound specific (Barba et al., 2019; Modrzyński et al., 2021). Therefore, OMP attenuation in the present system should be interpreted as the combined outcome of compound properties, sequential sediment-soil filtration, physicochemical retention, redox development, and microbial transformation potential.

4.1. Compound- and compartment-specific controls on OMP attenuation

The removal of OMPs differed markedly between sediment and soil. Sediment preferentially attenuated lidocaine, amantadine, and sotalol, whereas soil showed higher attenuation of naproxen, atenolol, and losartan. These patterns likely reflect the combined influence of OMP charge state, hydrophilicity, sequential exposure conditions, sorption potential, redox environment, and microbial functional differentiation.

To support compound-specific interpretation, key OMP properties, including charge state under the experimental pH range and hydrophilicity/hydrophobicity classification, were summarized in the Supplementary Information. These properties are important because contaminant structure and speciation influence partitioning, bioavailability, and susceptibility to enzymatic transformation during MAR (Hübner et al., 2022; Erdélyi et al., 2025; Kondor et al., 2024). For example, cationic and hydrophilic compounds such as lidocaine and amantadine may interact with sediment surfaces through electrostatic attraction, cation exchange, or biofilm-associated retention. Their high attenuation in sediment should therefore not be attributed solely to biodegradation.

The measured dissolved oxygen profile provides important redox context. DO decreased from the influent to the outlet of the sediment

column, but remained detectable after sediment filtration, indicating that the sediment compartment represented an oxidative to low-oxygen reactive interface rather than a fully anaerobic zone. DO further declined toward the final effluent, accompanied by changes in nitrogen species. This gradient indicates active biogeochemical processing along the sediment-soil pathway and supports the interpretation that sediment and soil provided different redox-related microbial habitats.

Redox conditions are widely recognized as key controls on microbial activity and trace organic contaminant attenuation in MAR and related subsurface systems (Regnery et al., 2017; Hübner et al., 2022; Stiegler et al., 2023; Adler et al., 2025; Hellauer et al., 2018; Munz et al., 2019). Oxygen-available conditions can favor oxidative transformation and biofilm activity for many micropollutants, whereas more reducing conditions may promote transformation of certain persistent compounds (Hübner et al., 2022; Stiegler et al., 2023; Adler et al., 2025). In the present system, measurable DO in sediment, preferential attenuation of selected OMPs, and enrichment of oxidative transformation-related genes together support the interpretation that sediment functioned as a reactive interface with oxidative microbial functional potential.

The temporal increase in attenuation provides additional mechanistic support. In a fixed sediment-soil filtration system, purely abiotic sorption would generally be expected to be strongest initially and then approach saturation or steady state. Therefore, progressive attenuation during long-term operation is more consistent with microbial acclimation, biofilm development, and enrichment of transformation-related functions than with instantaneous abiotic sorption alone. This agrees with MAR studies showing that primary substrate composition, biodegradable dissolved organic carbon, humic content, and microbial adaptation can influence TOC attenuation and xenobiotic-transformation potential (Li et al., 2020; Li et al., 2014; Alidina et al., 2014).

Nevertheless, the relative contributions of sorption and biotransformation cannot be quantitatively deconvoluted here. Detailed sediment and soil properties, including organic carbon content, cation exchange capacity, mineral composition, and particle-size distribution, were not measured. Sorption isotherms, sterile controls, transformation-product analysis, isotope fractionation, and time-resolved activity measurements were also not included. Thus, sediment-soil differences should be interpreted as the cumulative outcome of sequential filtration, OMP properties, redox development, sorption-related retention, and microbial functional potential. Because sediment was upstream of soil, soil attenuation represents additional downstream removal after partial sediment attenuation, rather than an independent test under identical influent concentrations.

4.2. Microbial functional differentiation and gene-OMP associations

Metagenomic profiles revealed clear differences between sediment and soil after long-term exposure to WWTP effluent. PCoA based on genes, pathways, and genus-level taxonomy showed separation between pre- and post-operation samples, indicating that continuous filtration substantially altered microbial community composition and functional potential. These shifts were compartment specific, supporting the hypothesis that sediment and soil harbor distinct microbial assemblages and functional repertoires.

Sediment harbored higher relative abundances of genes and pathways associated with oxidative xenobiotic transformation, including cytochrome P450-related enzymes, demethylases, dehydrogenases, oxidases, and aromatic compound degradation pathways. These functions are consistent with potential hydroxylation, demethylation, oxidation, and ring-modification reactions involved in the initial transformation of structurally diverse trace organic contaminants (Hübner et al., 2022). Previous metagenomic and MAR studies similarly showed that microbial functional potential varies with infiltration depth, carbon limitation, humic content, and recharge pathways (Li et al., 2020; Barba et al., 2019; Li et al., 2014). These studies support the broader concept that

subsurface microbial communities can develop spatially structured functional repertoires relevant to contaminant attenuation.

Soil exhibited a different functional and taxonomic profile. Although soil showed lower relative enrichment of several oxidative xenobiotic pathways compared with sediment, it contributed substantially to the attenuation of naproxen, atenolol, and losartan. Soil should therefore not be viewed as less important, but as functionally distinct. Soil-associated communities may provide complementary transformation potential, redox microenvironments, or sorption-related retention conditions that favor attenuation of compounds different from those preferentially attenuated in sediment.

To move beyond differential abundance alone, we constructed an exploratory bipartite Spearman rank correlation network linking microbial genera, EnviPath-annotated candidate biotransformation genes, and individual OMP removal rates. The network contained 28 genera, 43 KOs, and 12 OMPs, with 54 KO—OMP edges and 34 genus—OMP edges retained under $|\rho| \geq 0.70$ and BH-adjusted $p < 0.25$. Among them, 17 KO—OMP associations were supported by both Spearman correlation and EnviPath biotransformation annotation. These dual-evidence-supported links provide statistical and database-based support for potential microbial functional associations with OMP attenuation.

However, the network should be interpreted cautiously. The sample size was limited, and BH-adjusted $p < 0.25$ was used as an exploratory discovery threshold together with a stringent effect-size filter. Moreover, gene-centric metagenomics identifies functional potential rather than expression, enzyme activity, transformation-product formation, or in situ degradation rates. Therefore, the network is hypothesis-generating rather than definitive evidence of causality. Future metatranscriptomics, metaproteomics, transformation-product analysis, isotope tracing, and targeted microcosm experiments are needed to validate the inferred functional roles (Stiegler et al., 2023; Prieto-Espinoza et al., 2025).

4.3. Environmental implications for wastewater-impacted RBF systems

The results of this study provide important insights for the design and optimization of RBF systems treating wastewater-derived effluents. While previous studies have emphasized the importance of redox zonation and sequential oxic–anoxic conditions for enhancing OMP removal (Hellauer et al., 2018; Munz et al., 2019; Zhai et al., 2026; Zeeshan et al., 2026), our findings demonstrate that substrate configuration and influent composition must be considered equally critical design parameters. In particular, sediment layer should not be regarded merely as a passive physical filter. Instead, it may act as a reactive interface where measurable oxygen availability, biofilm development, sorption-related retention, and oxidative microbial functional potential jointly contribute to OMP attenuation. Preserving this sediment layer may therefore be important for achieving complementary attenuation of chemically diverse OMP mixtures. Moreover, RBF performance depends on the sequence and interaction of multiple reactive compartments. Sediment and soil did not provide identical attenuation functions; rather, they contributed differently to overall OMP removal. This supports a compartment-based view of RBF, in which reactive sediment, downstream soil, redox gradients, hydraulic residence time, OMP properties, and microbial functional differentiation jointly determine treatment performance (Regnery et al., 2017; Hübner et al., 2022; Modrzyński et al., 2021; Erdélyi et al., 2025; Kondor et al., 2024).

From an engineering perspective, these findings suggest that simply replicating redox gradients may not be sufficient to achieve optimal OMP removal in wastewater-impacted systems. Instead, RBF design should aim to preserve or engineer substrate heterogeneity, enabling the coexistence of distinct microbial functional niches. This includes maintaining biologically active sediment zones, optimizing hydraulic retention time, and managing organic carbon availability to sustain co-metabolic activity (Zhao et al., 2022; Hamann et al., 2016; Hellauer

et al., 2018). Such strategies could significantly enhance the robustness and efficiency of nature-based treatment systems, particularly in scenarios where conventional RBF is extended to treat WWTP effluents rather than relatively less complex river water matrices.

Despite providing mechanistic insights into OMP attenuation during riverbank filtration, this study has several limitations that should be addressed in future work. First, although the removal of parent OMPs was systematically monitored, the formation, identity, and fate of transformation products were not characterized. This represents an important knowledge gap because some transformation products may exhibit persistence or toxicity comparable to, or even greater than, their parent compounds. Second, our metagenomic profiling focused primarily on the prokaryotic community. Other biological components, including eukaryotic microorganisms such as fungi and protozoa, as well as viruses, may also contribute to OMP degradation or regulate microbial community structure through processes such as predation, symbiosis, and horizontal gene transfer. Their roles therefore warrant further investigation. Third, although the column system was operated for 10 months, the continuous loading of organic-rich WWTP effluent could promote biofilm accumulation and bio-clogging during long-term field operation over years to decades. Such processes may modify pore structure, hydraulic conductivity, and retention time, thereby influencing the long-term stability of OMP removal. Finally, this study was conducted using laboratory-scale columns under controlled conditions. Field-scale validation is needed to account for the combined effects of temperature fluctuations, sediment heterogeneity, variable flow paths, and dynamic riverbank hydrology, and to support the practical implementation of RBF as a nature-based barrier for wastewater-impacted source waters.

In summary, this study demonstrates that OMP attenuation in wastewater-impacted RBF systems is governed by a coupled interplay between substrate heterogeneity, redox dynamics, influent composition, and microbial functional potential. By explicitly distinguishing between sediment and soil compartments and linking their physicochemical and microbial characteristics to compound-specific removal patterns, this work advances beyond existing redox-centric frameworks. In contrast to previous studies based on river water infiltration through homogeneous media, our results reveal that substrate-driven microbial functional differentiation plays a central role in controlling OMP biotransformation under complex wastewater conditions. These findings provide new mechanistic insight into OMP fate in subsurface environments and offer a foundation for the rational design of next-generation nature-based treatment systems targeting micropollutant removal.

Code availability

All codes used in this study are publicly available.

CRediT authorship contribution statement

Yujia Zhai: Conceptualization, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Xun Wang:** Conceptualization, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Xuhan Deng:** Formal analysis, Visualization, Writing – review & editing. **Xiaoming Li:** Writing – original draft, Writing – review & editing. **Bin Hu:** Writing – original draft, Writing – review & editing. **Walter van der Meer:** Supervision, Writing – review & editing. **Mark C.M. van Loosdrecht:** Supervision, Writing – review & editing. **Gang Liu:** Conceptualization, Formal analysis, Funding acquisition, Supervision, Visualization, Writing – original draft, Writing – review & editing. **Martin Pabst:** Conceptualization, 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.

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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.2026.126421.

Data availability

Data will be made available on request.

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