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Recovery trajectories and community resilience of biofilms in receiving rivers after wastewater treatment plant upgrade

Hui Lin [a,b,1], Qiaojuan Wang [a,b,1], Jie Zhou [a,b], Donglin Wang [a,b], Yujie Men [c], Yaohui Bai [a,*], Jiuhui Qu [a]

[a] Key Laboratory of Drinking Water Science and Technology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, 100085, China
[b] University of Chinese Academy of Sciences, Beijing, 100049, China
[c] Department of Chemical and Environmental Engineering, University of California, Riverside, CA, 92521, United States

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ABSTRACT

Wastewater treatment plant (WWTP) upgrades can reduce both nutrient and micropollutant emissions into receiving rivers, thus modifying the composition and function of biological communities. However, how microbial communities vary and whether they can be restored to levels found in less-polluted rivers remains uncertain. Aquatic biofilms are sensitive to environmental change and respond rapidly to bottom-up pressure. Thus, we used 12 flumes configured in three experimental treatments to mimic the dynamic processes of biofilm microbial communities occurring in a wastewater-receiving river following WWTP upgrade, with rivers containing two levels of nutrients and micropollutants used as references. We compared the biofilm microbial biomass, carbon source utilization, and community composition among the three "blocks". Results showed that the metabolic patterns of the carbon sources and composition of the biofilm bacterial communities in the flumes mimicking a receiving river with WWTP upgrade recovered over time to those mimicking a less-disturbed river. The restoration of potential carboxylic acid-consuming denitrifying bacteria (i.e., *Zoogloea*, *Comamonas*, *Dechloromonas*, and *Acinetobacter*) likely played a significant role in this process. Combining quantitative analysis of the denitrification genes *nirS* and *nosZ*, we confirmed that the denitrification function of the river biofilms recovered after WWTP upgrade, consistent with our previous field investigation.

1. Introduction

Compared with direct untreated wastewater discharge, wastewater treatment plants (WWTPs) have improved the water quality of many receiving river ecosystems (Carey and Migliaccio, 2009; Marti et al., 2004). However, WWTP discharge remains a major source of nutrients and micropollutants (MPs) in receiving rivers (Halliday et al., 2015; Kostich et al., 2014; Luo et al., 2014). MPs reach the aquatic environment in low yet steady concentrations because of their persistence and continuous release (Gros et al., 2012; Halliday et al., 2015; Luo et al., 2014). Many MPs, e.g., pharmaceuticals and personal care products (PPCPs), are pharmacologically active in animals, and thus may have long-term effects on non-target aquatic organisms and communities. Notably, these communities may respond by shifting toward more tolerant species, with an associated impairment in many functions, such as algal growth, community structure, respiration, and biofilm production (Lawrence et al., 2005; Munn et al., 2002; Tlili et al., 2020).

To avoid these potential negative ecological effects, various countries have introduced measures to reduce the concentrations of nutrients and MPs. For instance, Switzerland and Germany have upgraded many of their WWTPs, resulting in over 80% removal of MPs by additional ozonation and powdered activated carbon (PAC) treatment (Boehler et al., 2012; Hollender et al., 2009; Margot et al., 2013). China, especially Beijing, has also introduced several treatment technologies in existing WWTPs (e.g., ozonation, PAC adsorption, and membrane filtration), thereby greatly reducing pollutant discharge into receiving aquatic ecosystems (Wang et al., 2020). In addition, other measures have been adopted to intensify water remediation, including control of pollution sources (e.g., water conservation forests and ecological slopes) and mitigation of diffuse pollution (e.g., ecological floating beds and constructed wetlands) (Sun et al., 2017; Wu et al., 2015; Zhao et al., 2010).

* Corresponding author.
E-mail address: yhbai@rcees.ac.cn (Y. Bai).
[1] These authors contributed equally to this work.

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River/stream biofilms are the first biological compartment to be exposed to sewage effluent. Epilithic biofilms are composed of heterotrophic (bacteria, protozoa, fungi, and meiofauna) and autotrophic organisms (algae and cyanobacteria) embedded in a matrix of hydrated extracellular polymeric substances (Sabater et al., 2016). These river microbial communities not only dominate the metabolism of many aquatic systems, but are also major components for the uptake, storage, and cycling of carbon, nitrogen, and phosphorous, and thus are crucial elements in water purification (Battin et al., 2016; Cadenasso et al., 2003). In addition, changes in environmental factors in aquatic ecosystems can lead to changes in biofilm characteristics. As an important component of biofilms, bacteria respond rapidly as a consortium of microorganisms following anthropogenic disturbance, and therefore can be used as an "early warning system" for the detection of human impact on aquatic systems (Sabater et al., 2007a,b).

With the development of the microbial loop concept in the early 1980s (Azam et al., 1983), substantial research efforts have evaluated both bottom-up (resources) and top-down (predation) processes in regulating bacterial populations in biofilms. Bottom-up effects are common in streams, where river biofilms integrate external changes and respond rapidly to bottom-up pressure (Hung and Li, 2013; Sabater et al., 2007a,b). For instance, Tlili et al. (2020, 2017) found that as pollution levels increase in wastewater-impacted streams, the variability of bacterial populations in biofilms decreases while tolerance to MP mixtures in biofilms increases. Shifts in biofilm bacterial community composition and function are an inevitable chronic effect of pollution. However, knowledge on the dynamic processes and recovery trajectories of river biofilms following water remediation remains scarce. The recovery of river biofilms also indicates the restoration of water ecology. Given this background, we performed a laboratory experiment using flumes to track the dynamics and resilience of natural microbial biofilm communities when pollution pressure decreases, in which potentially confounding environmental factors are strongly simplified. Accordingly, different variables were selected to assess microbial responses following a decrease in wastewater exposure, including biomass (adenosine triphosphate (ATP) content), metabolic characteristics (Biolog carbon utilization), and community composition (16S rRNA gene amplicon sequencing). We tested the hypothesis that a reduction in MP and nutrient discharge due to WWTP upgrade would shift the composition and function of the biofilm microbial community in the receiving river to resemble more closely that of a less-disturbed river.

2. Material and methods

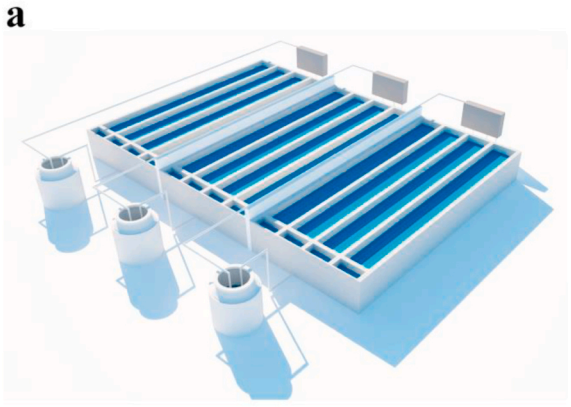
2.1. Construction of flumes

Experiments were performed in 12 flumes (length, 60 cm; width, 8 cm; depth, 8 cm) configured in three experimental "blocks" (Fig. 1a). We collected biofilm sediment and cobblestones from an unpolluted site upstream of the Chaobai River in Beijing, China (117.1610°E, 40.6915 °N) (Liao et al., 2018) and then added and distributed these biofilm-colonized substrata to the flumes to create an even plane bed (~5 cm height). Each experimental "block" received a constant flow of influent (200 mL/min) from the water tank (50 L), with water depth (~5.5 cm) maintained under continuous recirculation for 24 h. Influents for all flumes were renewed each day. Before running the flume experiment, we domesticated the biofilms using unpolluted water collected upstream of the Chaobai River as influent for 15 d. Water properties are provided in Table S1. After this, the flumes received their respective treatments from November 13, 2019 to January 18, 2020.

2.2. Flume experiments

The flume influent was prepared by adding certain concentrations of nutrients (CH_3COONa, $K_2HPO_4{\cdot}3H_2O$, NH_4Cl, and KNO_3) and MPs to the unpolluted upstream water. The MPs were composed of three sulfonamides (sulfamethoxazole, pyrimethamine, and sulfadiazine), one beta blocker (atenolol), one acetanilide (paracetamol), caffeine, atrazine, ibuprofen, naproxen, and carbamazepine (Fig. 1b). The MP mixture was first dissolved in 50% (vol/vol) methanol-water and then spiked in tap water as the influent. These pharmaceuticals were selected as they cover a variety of medicine classes, are frequently prescribed for human use and are commonly detected in surface waters (Kolpin et al., 2002).

Most rivers in Beijing are wastewater-dominated, but there are also



b

Compounds			Higher	Lower
Nutrients (mg/L)		$C_2H_3O_2Na$	20.5	20.5
		$K_2HPO_4{\cdot}3H_2O$	1.8387	1.8387
		NH_4Cl	3.8214	1.9107
		KNO_3	180.357	50.5
MPs (ng/L)	Sulfonamides	Sulfamethoxazole	300	30
		Sulfadiazine	200	20
		Trimethoprim	200	20
	Beta blockers	Atenolol	500	50
	Acetanilides	Paracetamol	200	20
	Others	Caffeine	1000	100
		Atrazine	500	50
		Ibuprofen	500	50
		Naproxen	500	50
		Carbamazepine	300	30

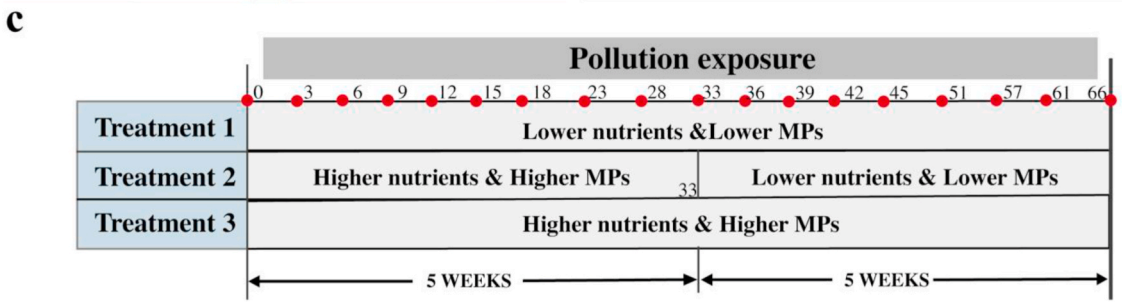


Fig. 1. Design of flume experiment.

some less disturbed rivers (non-effluent but human-impacted outfalls) in suburban areas. Here, we assumed that the levels of nutrients and MPs discharged into the receiving river after WWTP upgrade were similar to those in less-disturbed rivers. Based on the concentrations of nutrients and MPs downstream of the Tonghui River before and after upgrade of the Gaobeidian Wastewater Treatment Plant (116.5°E, 40.0°N) (Su et al., 2020; Wang et al., 2020), we used three types of influent treatment containing different concentrations of nutrients and MPs to explore how a WWTP upgrade affects microbial ecology in the receiving river ecosystem. The three treatments included (i) L_{NM}: influent with lower nutrients and MPs, representing a less-disturbed river; (ii) H_{NM}: influent with higher nutrients and MPs, representing a receiving river of conventional WWTPs; and (iii) H_{NM}-L_{NM}: influent with the change from higher nutrients and MPs to lower nutrients and MPs, representing a receiving river of an upgraded WWTP (Fig. 1c).

Each treatment contained four replicates for a total of 12 flumes. The 12 flumes and three treatments were run for 66 days to investigate variation in the microbial community under different influent treatment.

2.3. Biofilm sampling

Biofilm samples were collected every 2 or 4 d during flume operation (Fig. 1c.), with 20 g of similarly shaped and sized sand samples collected in clean sealed bags each time. Each collected biofilm sample was used for measurement of biomass and metabolic characteristics within 1 h of collection, and biofilm samples collected from days 3, 9, 18, 33, 36, 42, 51, and 66 were used for DNA extraction.

2.4. Biofilm analysis

2.4.1. Biomass measurements

Active biomass was measured using the adenosine triphosphate (ATP) method (Holm-Hansen and Booth, 1966). During operation, 3 g of biofilm sand and 20 mL of 10% trichloroacetic acid (TCA) were mixed in a 50-mL sterile polypropylene centrifuge tube for 3 min using an ultrasonic cell crusher (Biosafer 900–92, Safer Co., Ltd., China). After 10 min, 100 μL of 0.22-μm filtered supernatant from each sample was added to a 96-well microplate containing 100 μL of luciferin/luciferase reagent (Promega ENLITEN®ATP kit), which was measured with a GloMax® 96 Microplate Luminometer (Promega Corporation, Switzerland). The samples were then incubated for 2 s at room temperature and luminescence was measured over a 10 s integration time with five successive concentration levels ranging from 1×10^{-11} to 1×10^{-7} M ATP.

2.4.2. 2 carbon metabolism of biofilm microbial community

The metabolic patterns of the biofilm microorganisms and their functional diversities were determined using a Biolog EcoPlate™ system (Biolog Inc., CA, USA). In total, 1.9 g of each sand sample was deposited in a sterile Falcon tube. Detachment of biofilm from the sand was performed using a previous protocol (Weber and Legge, 2010b). Briefly, sand samples were immersed in 9 mL of NaCl (buffered saline) and shaken on a Vortex Genie2 (Scientific Industries Inc, Bohemia, NY, USA) for 30 min at room temperature and 3 000 rpm. After standing for 1 h, 15 μL of the supernatant was inoculated into the Biolog EcoPlates™. After inoculation, the lidded EcoPlates were incubated at 36 °C in the dark under humid conditions. Absorbance at 590 nm and 750 nm was measured every 24 h for 7 d using a SPARK 10-M multifunctional microplate reader (Tecan Group Ltd., Männedorf, Switzerland).

Data processing and analysis followed previous procedures (Weber et al., 2007; Weber and Legge, 2010a). Microbial activity for each sample that expressed average well color development (AWCD) was determined as follows: $AWCD = \sum(C_{590\text{-}750} - R_{590\text{-}750})_i/31$ (Wu et al., 2013), where C represents the difference in optical density values at 590 nm and 750 nm from the 31 response wells, and R is the optical density (OD) value of the blank well. All negative OD values were set to zero (Garland, 1996). We then plotted the sigmoidal color development curve for all read times, with the maximum metabolic rate at the largest absolute value of the curve slope (Schmitt et al., 2004; Stefanowicz et al., 2008; Tlili et al., 2011).

In addition, to explore the differences in the different types of carbon source utilization among the three treatments, the 31 carbon sources were classified into six specific groups (Table S2): i) carbohydrates, ii) esters, iii) amino acids, iv) alcohols, v) carboxylic acids, and vi) amides (Weber and Legge, 2009). We used the OD values ($C_{590\text{-}750} - R_{590\text{-}750}$) obtained at 120 h as the maximum carbon source utilization level as they represented the optimal range of OD readings, as per previous studies (Frac et al., 2012; Gryta et al., 2014; Zsuzsanna Magdolna et al., 2013).

2.4.3. 16S rRNA gene amplicon sequencing

Approximately 3 g of biofilm sand was used for DNA extraction using a PowerBiofilm DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA, USA) based on the manufacturer's recommendations. A total of 107 DNA samples were then sent to the Beijing Genomics Institute (BGI, China) for 16S rRNA sequencing using the V4-specific primer pair 515F/806R, leading to 75 044 to 75 234 reads per sample with an amplicon size of 250–300 bp. The 16S rRNA sequences were analyzed using the QIIME2 pipeline (v2019.4) (Bolyen et al., 2019) to infer amplicon sequence variants (ASVs). Based on the ASVs obtained, we then calculated the alpha diversities, including Shannon, Simpson, Chao1, and Pielou evenness. Classification annotation of ASVs was performed based on the naïve Bayesian classifier method and the SILVA database (v138) (Pruesse et al., 2007). All clean reads were deposited in the NCBI Sequence Read Archive database under accession number PRJNA418866.

2.4.4. Quantitative real-time polymerase chain reaction (qRT-PCR)

The abundances of bacterial 16S rRNA genes and two denitrifying functional genes, i.e., nitrite reductase (*nirS*) and nitrous oxide reductase (*nosZ*), were quantified using a LightCycler 96 Real-Time PCR system (Roche Diagnostics, Switzerland). The *nirS* gene is a key gene that catalyzes the first step in the reduction of soluble nitrite to gaseous product and *nosZ* is the only gene known to catalyze the final step in denitrification (Harter et al., 2016; Philippot et al., 2007).

Primers and thermal parameters are summarized in Table S4. The reaction mixtures used for RT-PCR were comprised of 10 μL of SuperReal Color PreMix (SYBR Green, Tiangen Biotech, Beijing, China), 2 μL of template DNA (sample DNA or plasmid DNA for standard curves), primer pairs, and RNase-free ddH_2O. Standard curves were generated from serial dilutions of plasmid DNA carrying the specific target gene inserts, with R^2 values of 0.99, 0.99, and 0.96 obtained for *nirK*, *nosZ*, and 16S, respectively. The concentrations of *nirS* and *nosZ* were normalized to bacterial 16S rRNA concentrations to calculate the abundance of denitrifying genes.

2.4.5. Statistical analysis

Analysis of similarities (ANOSIM) was applied to identify whether the microbial community composition and carbon metabolism differed significantly among treatments. Based on Bray-Curtis dissimilarity, the community compositions of all samples were analyzed by nonmetric multidimensional scaling (NMDS), and carbon metabolism was analyzed by principal coordinate analysis (PCoA) using the vegan package in R (v4.0.2) (Dixon, 2003). Networks were constructed using extended local similarity analysis (eLSA) (Xia et al., 2011) to evaluate the interaction patterns of the biofilm microbial community and were then visualized with the interactive platform Gephi (v0.9.2) (Bastian et al., 2009).

The dissimilarities of carbon source utilization and community composition among the three treatments over the operation period were evaluated using principal response curves (PRCs). These response curves represent temporal trajectories for different treatments (Van den Brink

and Ter Braak, 1999). Each treatment is presented as a single response curve in a plot where the horizontal axis represents time and vertical axis represents PRC score. It should be noted that the reference level of the treatment (receiving river of conventional WWTPs) had PRC values of zero to exclude the influence of decreasing temperature. Differences in carbon source utilization among the three treatments at the end of the experiment were tested using a Monte Carlo permutation test and redundancy analysis (RDA) with the vegan package in R (v4.0.2). To test for significance of differences, 999 permutations were performed and a significance level of 0.05 was chosen.

3. Results

3.1. Active biomass of biofilms

The biofilms in the 12 flumes were biologically active throughout the experiment (Fig. 2) and showed ATP values in the same range as those obtained from biofilms in drinking water (Lehtola et al., 2002; Prest et al., 2016). The ATP trends across time revealed that there were no significant differences in the active biofilm biomass among the L_{NM} (less-disturbed river), H_{NM} (receiving river of conventional WWTPs), and H_{NM}-L_{NM} (receiving river of upgraded WWTPs) treatments (ANOVA, $p = 0.932$). It should be noted that on day 33, the temperature dropped sharply from 15 °C to 9 °C due to heavy snow, resulting in a significant decrease in active biomass in the three treatments (from $1.02 \times 10^{-10} \pm 5.09 \times 10^{-11}$ M/g biofilm to $1.35 \times 10^{-11} \pm 8.48 \times 10^{-12}$ M/g biofilm). After this, active biomass remained at a lower level in all flumes due to the lower ambient temperature in winter.

3.2. Recovery of biofilm metabolic patterns

The Biolog EcoPlate assay was adopted to examine the capability of biofilms to assimilate the 31 carbon sources, revealing their metabolic activity levels. We calculated the maximum metabolic rate of the carbon sources based on the AWCD sigmoidal color change curve for each sample (Fig. 3a). Comparing the three treatments, the metabolic rates in the H_{NM} flumes were significantly higher than those in the L_{NM} flumes (*t*-test, $p < 0.05$), suggesting that the input of nutrients and MPs accelerated the metabolic activity of the aquatic microorganisms. Moreover, when the pollution level decreased on day 33, the maximum metabolic rate of H_{NM}-L_{NM} gradually decreased to a level similar to that of L_{NM}. To establish the recovery trajectories, we used PRCs to identify the dissimilarities in the utilization of carbon sources among treatments. We set the values of H_{NM} to zero as the reference to exclude the influence of decreasing temperature. An increase in the canonical coefficient indicated that more substrates were used by the biofilm microbial community than in the reference. PRC analysis demonstrated that the differences between H_{NM}-L_{NM} and the reference (H_{NM}) gradually

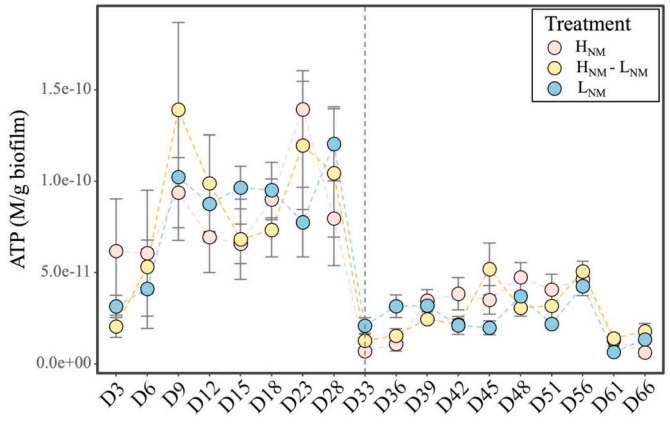


Fig. 2. Comparison of microbial biomass among biofilms in three treatments.

became larger after the decrease in pollution exposure on day 33 and the substrate utilization level of the bacterial communities in the H_{NM}-L_{NM} flumes tended to converge towards L_{NM} (Fig. 3b). At the end of the experiment (day 66), no significant differences were observed in the utilization of carbon sources between L_{NM} and H_{NM}-L_{NM} (Monte Carlo permutation: $p = 0.86$), and bacterial communities utilized more carbon sources under L_{NM} conditions than under H_{NM} conditions. Moreover, based on the PRCs of the six carbon source categories, the utilization of carboxylic acids and esters showed a similar trend to total carbon sources (Fig. 3c and d), whereas the utilization of amino acids, carbohydrates, alcohols, and amides showed no significant differences among the three treatments during operation (Fig. S1). Thus, we inferred that the shifts in total carbon source utilization by the biofilms in the H_{NM}-L_{NM} flumes were primarily due to the change in carboxylic acid and ester utilization.

3.3. Recovery of biofilm bacterial composition

The alpha diversity indices (i.e., Shannon, Simpson, Pielou evenness, and Chao1) showed no significant differences among the three treatments (Fig. S2). However, the composition of the bacterial community in the H_{NM}-L_{NM} flumes clearly changed over time. The NMDS results showed that the dissimilarity in biofilm bacterial composition between H_{NM}-L_{NM} and L_{NM} decreased after the pollution level declined at day 33 (Fig. S3). For shifts over time, PRC showed that the bacterial composition in the H_{NM}-L_{NM} flumes tended to recover towards L_{NM} as the pollution level decreased, with no significant differences found by the end of the experiment (Monte Carlo permutation: $p = 0.54$) (Fig. 4a).

By analyzing the taxonomic classification of the 16S rRNA gene sequences, we detected a total of 49 bacterial phyla in all biofilm samples, but with no obvious changes found at the phylum level among the three treatments (Fig. S4). The most abundant phylum for all biofilms was Proteobacteria, accounting for 56.8% of the total bacterial community, followed by Bacteroidetes (11.4%), Actinobacteria (5.8%), and Planctomycetes (4.3%) (Fig. S4). We subsequently screened 40 dominant genera (with significant differences (Wilcoxon-Test, $p < 0.05$) and >2-fold changes in relative abundance before and after pollution level decrease) and explored carbon source utilization for each of the dominant genera (Table S3). As a result, we identified four bacterial genera (relative abundance >0.1% and showing a recovery trend), i.e., *Zoogloea*, *Comamonas*, *Dechloromonas*, and *Acinetobacter*, with significantly increased abundance in the H_{NM}-L_{NM} flumes after the pollution level decreased (Fig. 4b and S5) (Wilcoxon-Test, $p < 0.05$, $n = 3$). We further analyzed the recovery trajectories of these four genera. Results demonstrated that their relative abundances in the H_{NM}-L_{NM} flumes were restored to the levels detected in the L_{NM} flumes (Fig. S5). These genera are potential denitrifying bacteria that use carboxylic acids as their main carbon source (Gao et al., 2018; Huang et al., 2015; Osaka et al., 2006), suggesting that the increase in abundance of these four denitrifying bacteria may lead to changes in the utilization of carboxylic acid carbon sources among the three treatments.

We next constructed co-occurrence networks for the three treatments using the 109 dominant ASVs (>0.1% abundance) filtered by local similarity and significance ($|LS| \geq 0.8$, $p < 0.05$) (Figs. S6a–d). Comparison of edges among the three treatments revealed that the biofilm microbe interactions in the H_{NM} flumes were more extensive than those in the L_{NM} flumes (Table 1). It should be noted that the H_{NM}-L_{NM} network after day 33 displayed similar topological parameters as L_{NM}, e.g., average degree, clustering coefficient, and graph density. These results suggest that upgraded WWTPs restore the microbial interactions in receiving rivers to that of less-disturbed rivers.

3.4. Recovery of biofilm denitrification populations

To explore the variations in denitrification populations in the H_{NM}-L_{NM} flumes, we quantified the abundances of denitrifying functional

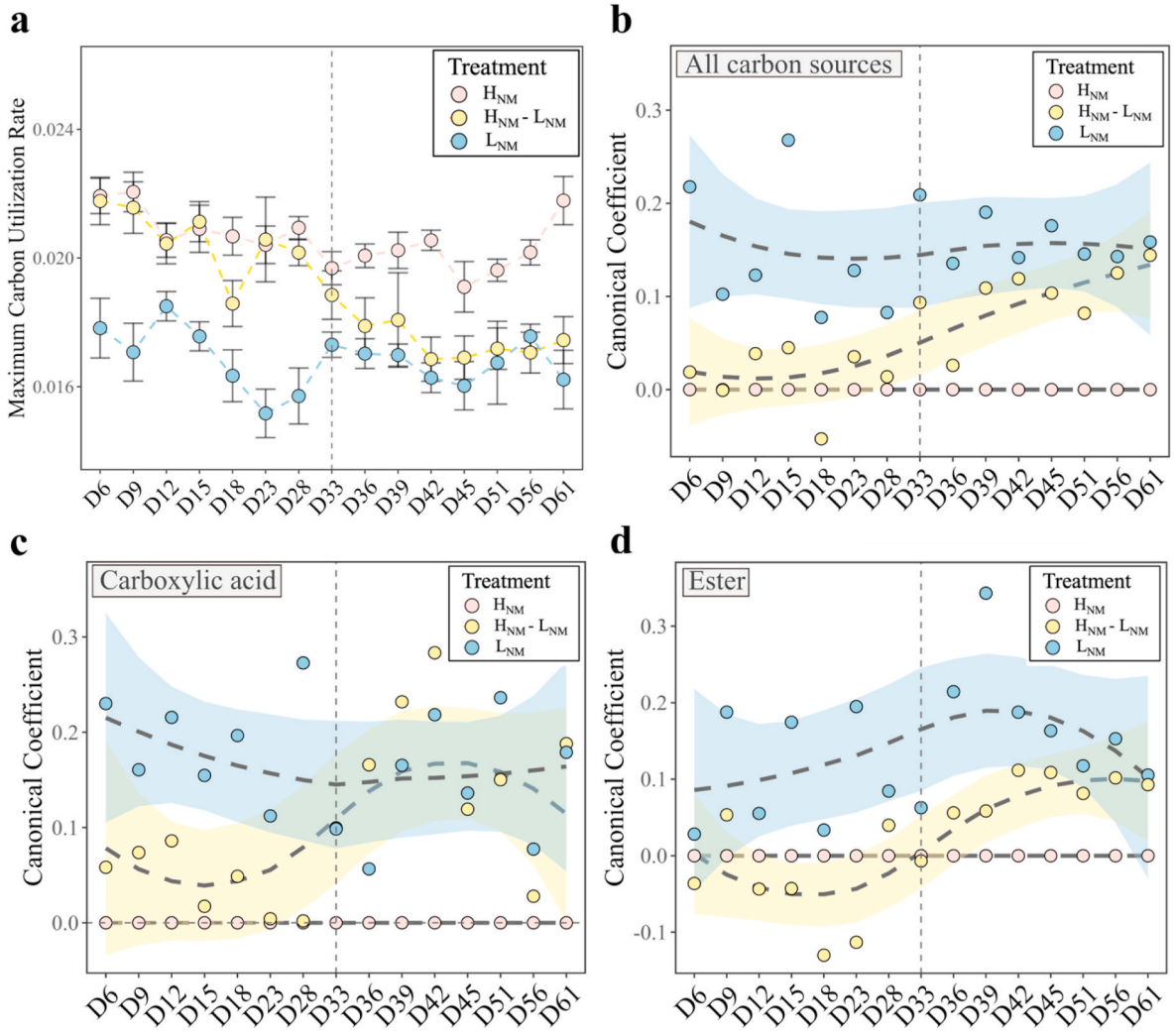


Fig. 3. Comparison of utilization of 31 carbon sources among biofilms in three treatments, including maximum utilization rates of sources among three treatments.

genes coding nitrite reductase (*nirS*) and nitrous oxide reductase (*nosZ*). As expected, the absolute copy numbers of the *nirS* and *nosZ* genes in H_{NM}-L_{NM} increased significantly after day 33 (Figs. S7a and S7b). Furthermore, we found that the abundances of *nirS* and *nosZ* normalized to bacterial 16S rRNA gene copy numbers showed similar increasing trends (Fig. 5a and b). Notably, both the copy numbers and relative abundances of biofilm *nirS* and *nosZ* genes in the H_{NM}-L_{NM} flumes showed convergence towards those in the L_{HM} flumes. These results corroborate the findings obtained from the community composition analysis, which demonstrated that the biofilm denitrification populations in the H_{NM}-L_{NM} flumes were restored to the levels found in the L_{NM} flumes.

4. Discussion

Ozonation plus ultrafiltration membrane technology have been adopted by many WWTPs in Beijing, resulting in the effective removal of excess nutrients (i.e., nitrogen) and MPs in the receiving rivers (Wang et al., 2020). In general, the removal of nutrients in wastewater inhibits the proliferation of microbes (Dunck et al., 2015; Wagenhoff et al., 2011), whereas the removal of MPs can promote the proliferation and metabolic function of microbes (Aristi et al., 2016; Pasquini et al., 2013). As a result, both the composition and function of the microbial community in river biofilms are altered but the variation tendency can be difficult to confirm. Such information could provide a more complete understanding of the potential impacts of these stressors to the ecosystem as a whole. To explore this, we used a series of flumes (easy-to-handle and repeatable) to mimic biofilm development in receiving rivers with WWTP upgrade.

Our results showed that there were no significant differences in microbial biomass among the three treatments (Fig. 2). This may be because the negative effects of decreased nutrient levels on bacterial biomass were offset by the removal of the inhibitory effects of MPs. However, after the exposure level was decreased, metabolic pattern shifts in the biofilm bacterial community were observed in the H_{NM}-L_{NM} flumes, especially an increase in carboxylic acid- and ester-consuming functions (Fig. 3c and d). These results indicate that the biofilm microbes related to consumption of these two carbon sources were more sensitive to MPs. In addition, the four identified bacterial genera (i.e., *Zoogloea*, *Comamonas*, *Dechloromonas*, and *Acinetobacter*) that showed significant changes (from 16S rRNA sequencing, Fig. 4b) in the H_{NM}-L_{NM} flumes were all potential denitrifying bacteria that can utilize carboxylic acids as a carbon source (Goel et al., 2005; Huang et al., 2015; Osaka et al., 2006). These results suggest that the carboxylic acid-consuming denitrifying bacteria were more sensitive to the concentrations of nutrients and micropollutants in the restoration process. However, we did not find that the identified ester-consuming bacteria established a recovery trend (Fig. 4b), probably due to the role of functional redundancy of biofilm communities (Dopheide et al., 2015). Functional redundancy is a keystone of ecological insurance allowing the recovery of microbial

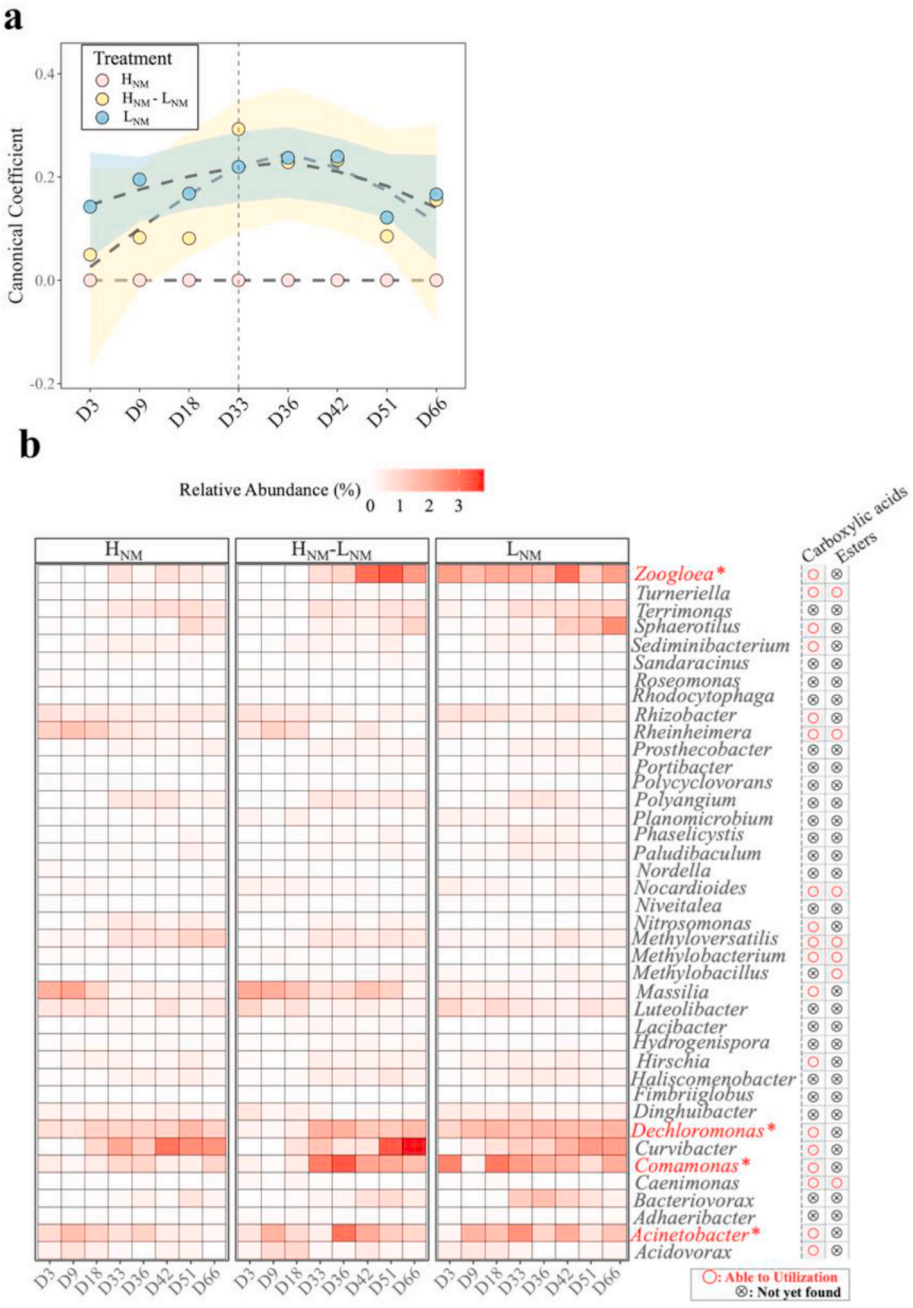


Fig. 4. Comparison of bacterial composition among biofilms in three treatments.

Table 1
Topological features of microbial network. H_{NM}: Higher nutrients and micropollutants (MPs) in feed water; L_{NM}: Lower nutrients and MPs in feed water; H_{NM}-L_{NM} < D33: Before pollution level decreased (level of H_{NM}, < day33) in feed water; H_{NM}-L_{NM} > D33: After pollution level decreased (level of L_{NM}, > day 33) in feed water.

Treatment	Node	Edge	Average Degree	Clustering Coefficient	Graph Density	Modularity	Positive Cor	Negative Cor
H_{NM}	104	340	6.239	0.33	0.063	0.435	60.59%	39.41%
H_{NM}-L_{NM} < D33	104	360	7.44	0.539	0.078	0.641	59.35%	40.65%
H_{NM}-L_{NM} > D33	105	501	9.543	0.963	0.092	0.853	48.60%	51.40%
L_{NM}	98	447	9.122	0.827	0.094	0.455	62.64%	37.36%

functions following stress. Several studies have explored the potential of biofilm bacterial communities to recover following pollution exposure (Arini et al., 2012; Pandey and Bergey, 2018; Pesce et al., 2016). Generally, community function recovers, rather than the community composition. For instance, Boivin et al. (2006) showed that functional changes in bacterial communities following Cu exposure can be restored within 28 d, but community compositions cannot. Here, we highlighted that carboxylic acid-consuming denitrifying bacteria showed a trend of recovery not only in function but also in composition.

In our previous Tonghui River study, after WWTP upgrade, the

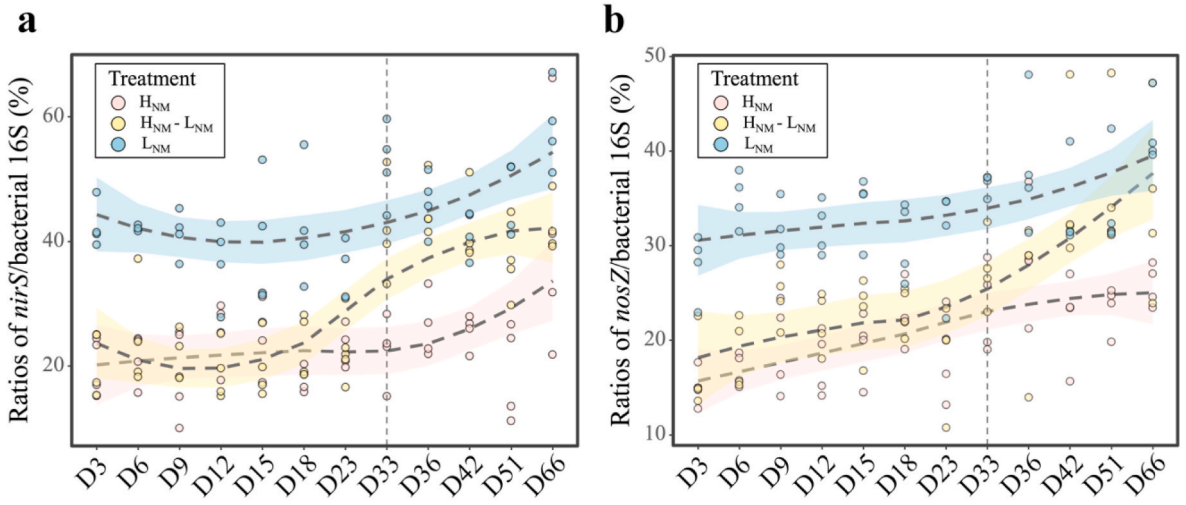


Fig. 5. Ratios of *nirS*/bacterial 16S and *nosZ*/bacterial 16S in biofilms among three treatments.

receiving river ecosystem provided sufficient bioavailable NH_4^+-N for plankton growth (Bronk et al., 2007) by increasing the abundance of denitrifying and dissimilatory nitrate/nitrite reduction to ammonium (DNRA) bacteria and decreasing nitrification (Sanders et al., 2013; Wang et al., 2020). This was also partly evidenced in the current study, i.e., (i) abundance of denitrifying bacteria, including *Zoogloea*, *Comamonas*, *Dechloromonas*, and *Acinetobacter*, significantly increased after the pollution level decreased in the H_{NM}-L_{NM} flumes (Fig. 4b); and, (ii) based on RT-PCR, the abundances of the *nirS* and *nosZ* genes in the H_{NM}-L_{NM} flumes also increased after the pollution level decreased, similar to the levels detected in the L_{NM} flumes (Fig. 5a and b). Furthermore, we compared the 16S rRNA sequences from this study with those obtained from biofilms in the Tonghui River after WWTP upgrade (no biofilm samples were obtained from the river before the upgrade) (Mao et al., 2021). Results demonstrated that the above four denitrifying genera were also present in the biofilms of the Tonghui River after WWTP upgrade (Fig. 6a). We then compared the relative abundances of the four genera in the surface water of the Tonghui River before and after WWTP upgrade and found that, except for *Zoogloea*, the relative abundances of the other three denitrifying genera increased after WWTP upgrade (Fig. 6b). *Zoogloea* species are known to facilitate cell adhesion and biofilm formation (Gao et al., 2018), thereby effectively decreasing the impact of low temperature on other microorganisms in biofilms (Wang et al., 2012). Hence, we speculated that most *Zoogloea* species were deposited in the river biofilm, resulting in there being no significant differences in *Zoogloea* abundance in the surface water before and after WWTP upgrade. Overall, our observations on the variations in denitrification in the flumes were consistent with our previous findings. We deduced that the increase in denitrifying bacteria was likely due to the mitigation of MP inhibition. Several other studies have also observed that a certain concentration of MPs can change the denitrification level in river biofilms (Li et al., 2019; Robson et al., 2020). However, the mechanism underlying the effects of MPs on the activity of denitrifying species is not clear and should be further investigated.

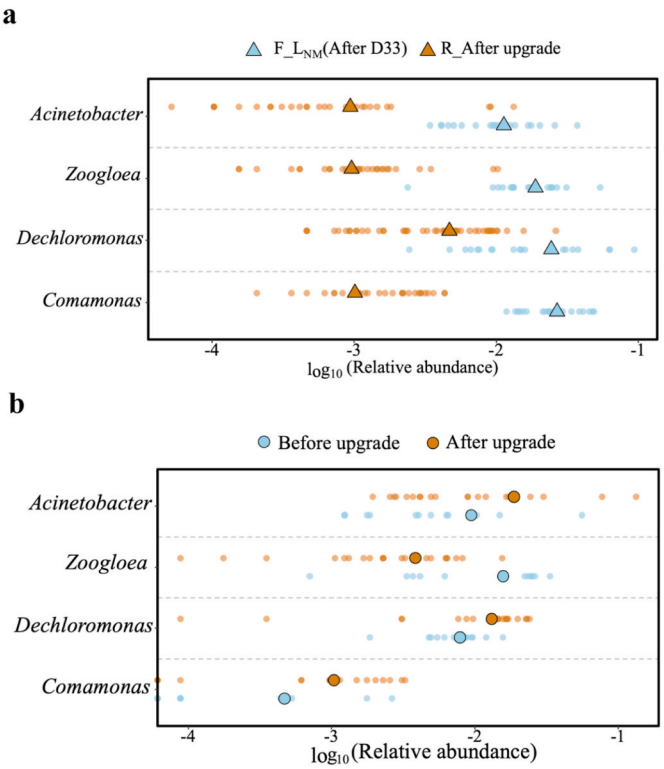


Fig. 6. Comparison of relative abundances of four potential denitrifying genera (i.e., *Zoogloea*, *Comamonas*, *Dechloromonas*, and *Acinetobacter*).

5. Conclusions

To summarize, we conducted a comprehensive evaluation of the restoration process of the biofilm microbial community in flumes following decreased pollution levels (simulating upgraded WWTP receiving river). Our results clearly demonstrated that the composition and function of the biofilm in the WWTP-upgraded receiving river gradually recovered to that observed in the less-disturbed river across time, which is critical to ensure diversity, function, and resilience of an ecosystem. Our findings also highlighted the restoration of potential carboxylic acid-consuming denitrifying bacteria in the river biofilms. These findings advance our understanding of the ecological outcomes of improvement in water quality and should benefit the management of river ecosystems.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2021.111349.

Credit author statement

Hui Lin: Data curation, Writing – original draft. Qiaojuan Wang: Methodology, Investigation. Jie Zhou: Investigation. Donglin Wang: Investigation. Yujie Men: Validation. Yaohui Bai: Investigation, Writing – review & editing. Jiuhui Qu: Funding acquisition.

References

Arini, A., Feurtet-Mazel, A., Maury-Brachet, R., Coste, M., Delmas, F., 2012. Field translocation of diatom biofilms impacted by Cd and Zn to assess decontamination and community restructuring capacities. Ecol. Indicat. 18, 520–531.

Aristi, I., Casellas, M., Elosegi, A., Insa, S., Petrovic, M., Sabater, S., Acuña, V., 2016. Nutrients versus emerging contaminants–Or a dynamic match between subsidy and stress effects on stream biofilms. Environ. Pollut. 212, 208–215.

Azam, F., Fenchel, T., Field, J.G., Gray, J.S., Meyerreil, L.A., Thingstad, F., 1983. The ecological role water-column microbes in the sea. Mar. Ecol. Prog. Ser. 10 (3), 257–263.

Bastian, M., Heymann, S., Jacomy, M., 2009. Gephi: an Open Source Software for Exploring and Manipulating Networks.

Battin, T.J., Besemer, K., Bengtsson, M.M., Romani, A.M., Packmann, A.I., 2016. The ecology and biogeochemistry of stream biofilms. Nat. Rev. Microbiol. 14 (4), 251–263.

Boehler, M., Zwickenpflug, B., Hollender, J., Ternes, T., Joss, A., Siegrist, H., 2012. Removal of micropollutants in municipal wastewater treatment plants by powder-activated carbon. Water Sci. Technol. 66 (10), 2115–2121.

Boivin, M.-E.Y., Massieux, B., Breure, A.M., Greve, G.D., Rutgers, M., Admiraal, W., 2006. Functional recovery of biofilm bacterial communities after copper exposure. Environ. Pollut. 140 (2), 239–246.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J.E., Bittinger, K., Brejnrod, A., Brislawn, C.J., Brown, C.T., Callahan, B.J., Caraballo-Rodriguez, A.M., Chase, J., Cope, E.K., Da Silva, R., Diener, C., Dorrestein, P.C., Douglas, G.M., Durall, D.M., Duvallet, C., Edwardson, C.F., Ernst, M., Estaki, M., Fouquier, J., Gauglitz, J.M., Gibbons, S.M., Gibson, D.L., Gonzalez, A., Gorlick, K., Guo, J., Hillmann, B., Holmes, S., Holste, H., Huttenhower, C., Huttley, G.A., Janssen, S., Jarmusch, A.K., Jiang, L., Kaehler, B.D., Bin Kang, K., Keefe, C.R., Keim, P., Kelley, S.T., Knights, D., Koester, I., Kosciolek, T., Kreps, J., Langille, M.G. I., Lee, J., Ley, R., Liu, Y.-X., Loftfield, E., Lozupone, C., Maher, M., Marotz, C., Martin, B.D., McDonald, D., McIver, L.J., Melnik, A.V., Metcalf, J.L., Morgan, S.C., Morton, J.T., Naimey, A.T., Navas-Molina, J.A., Nothias, L.F., Orchanian, S.B., Pearson, T., Peoples, S.L., Petras, D., Preuss, M.L., Pruesse, E., Rasmussen, L.B., Rivers, A., Robeson, M.S., , II, Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A., Sinha, R., Song, S.J., Spear, J.R., Swafford, A.D., Thompson, L.R., Torres, P.J., Trinh, P., Tripathi, A., Turnbaugh, P.J., Ul-Hasan, S., van der Hooft, J.J.J., Vargas, F., Vazquez-Baeza, Y., Vogtmann, E., von Hippel, M., Walters, W., Walters, W., Wan, Y., Wang, M., Warren, J., Weber, K.C., Williamson, C.H.D., Willis, A.D., Xu, Z.Z., Zaneveld, J.R., Zhang, Y., Zhu, Q., Knight, R., Caporaso, J.G., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2 (vol 37, pg 852, 2019). Nat. Biotechnol. 37 (9), 1091-1091.

Bronk, D.A., See, J.H., Bradley, P., Killberg, L., 2007. DON as a source of bioavailable nitrogen for phytoplankton. Biogeosciences 4 (3), 283–296.

Cadenasso, M.L., Pickett, S.T.A., Weathers, K.C., Bell, S.S., Benning, T.L., Carreiro, M.M., Dawson, T.E., 2003. An interdisciplinary and synthetic approach to ecological boundaries. Bioscience 53 (8), 717–722.

Carey, R.O., Migliaccio, K.W., 2009. Contribution of wastewater treatment plant effluents to nutrient dynamics in aquatic systems: a review. Environ. Manag. 44 (2), 205–217.

Dixon, P., 2003. VEGAN, a package of R functions for community ecology. J. Veg. Sci. 14 (6), 927–930.

Dopheide, A., Lear, G., He, Z., Zhou, J., Lewis, G.D., 2015. Functional gene composition, diversity and redundancy in microbial stream biofilm communities. PloS One 10 (4).

Dunck, B., Lima-Fernandes, E., Cassio, F., Cunha, A., Rodrigues, L., Pascoal, C., 2015. Responses of primary production, leaf litter decomposition and associated. communities to stream eutrophication. Environ. Pollut. 202, 32–40.

Frac, M., Oszust, K., Lipiec, J., 2012. Community level physiological profiles (CLPP), characterization and microbial activity of soil amended with dairy sewage sludge. Sensors 12 (3), 3253–3268.

Gao, N., Xia, M., Dai, J., Yu, D., An, W., Li, S., Liu, S., He, P., Zhang, L., Wu, Z., Bi, X., Chen, S., Haft, D.H., Qiu, D., 2018. Both widespread PEP-CTERM proteins and exopolysaccharides are required for floc formation of Zoogloea resiniphila and other activated sludge bacteria. Environ. Microbiol. 20 (5), 1677–1692.

Garland, J.L., 1996. Analytical approaches to the characterization of samples of microbial communities using patterns of potential C source utilization. Soil Biol. Biochem. 28 (2), 213–221.

Goel, R.K., Sanhueza, P., Noguera, D.R., 2005. Evidence of Dechloromonas Sp. participating in enhanced biological phosphorus removal (EBPR) in a bench-scale aerated-anoxic reactor. Proceedings of the Water Environment Federation (12), 3864–3871, 2005.

Gros, M., Rodriguez-Mozaz, S., Barcelo, D., 2012. Fast and comprehensive multi-residue analysis of a broad range of human and veterinary pharmaceuticals and some of their metabolites in surface and treated waters by ultra-high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem mass spectrometry. J. Chromatogr. A 1248, 104–121.

Gryta, A., Frac, M., Oszust, K., 2014. The application of the biolog ecoplate approach in ecotoxicological evaluation of dairy sewage sludge. Appl. Biochem. Biotechnol. 174 (4), 1434–1443.

Halliday, S.J., Skeffington, R.A., Wade, A.J., Bowes, M.J., Gozzard, E., Newman, J.R., Loewenthal, M., Palmer-Felgate, E.J., Jarvie, H.P., 2015. High-frequency water quality monitoring in an urban catchment: hydrochemical dynamics, primary production and implications for the Water Framework Directive. Hydrol. Process. 29 (15), 3388–3407.

Harter, J., Weigold, P., El-Hadidi, M., Huson, D.H., Kappler, A., Behrens, S., 2016. Soil biochar amendment shapes the composition of N2O-reducing microbial communities. Sci. Total Environ. 562, 379–390.

Hollender, J., Zimmermann, S.G., Koepke, S., Krauss, M., McArdell, C.S., Ort, C., Singer, H., von Gunten, U., Siegrist, H., 2009. Elimination of organic micropollutants in a municipal wastewater treatment plant upgraded with a full-scale post-ozonation followed by sand filtration. Environ. Sci. Technol. 43 (20), 7862–7869.

Holm-Hansen, O., Booth, C.R., 1966. The measurement of adenosine triphosphate in the ocean and its ecological significance. Limnol. Oceanogr. 11 (4), 510–519.

Huang, T.-L., Zhou, S.-L., Zhang, H.-H., Bai, S.-Y., He, X.-X., Yang, X., 2015. Nitrogen removal characteristics of a newly isolated indigenous aerobic denitrifier from oligotrophic drinking water reservoir, Zoogloea sp N299. Int. J. Mol. Sci. 16 (5), 10038–10060.

Hung, R., Li, M., 2013. Effects of Human Activities on the Ecological Processes of River Biofilms in a Highly Urbanized River.

Kostich, M.S., Batt, A.L., Lazorchak, J.M., 2014. Concentrations of prioritized pharmaceuticals in effluents from 50 large wastewater treatment plants in the US and implications for risk estimation. Environ. Pollut. 184, 354–359.

Lawrence, J.R., Swerhone, G.D.W., Wassenaar, L.I., Neu, T.R., 2005. Effects of selected pharmaceuticals on riverine biofilm communities. Can. J. Microbiol. 51 (8), 655–669.

Lehtola, M.J., Miettinen, I.T., Martikainen, P.J., 2002. Biofilm formation in drinking water affected by low concentrations of phosphorus. Can. J. Microbiol. 48 (6), 494–499.

Li, Y., Sun, Y., Zhang, H., Wang, L., Zhang, W., Niu, L., Wang, P., Wang, C., 2019. The responses of bacterial community and N2O emission to nitrogen input in lake sediment: estrogen as a co-pollutant. Environ. Res. 179, 108769.

Liao, K., Bai, Y., Huo, Y., Jian, Z., Hu, W., Zhao, C., Qu, J., 2018. Integrating microbial biomass, composition and function to discern the level of anthropogenic activity in a river ecosystem. Environ. Int. 116, 147–155.

Luo, Y., Guo, W., Ngo, H.H., Long Duc, N., Hai, F.I., Zhang, J., Liang, S., Wang, X.C., 2014. A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. Sci. Total Environ. 473, 619–641.

Mao, G., Liang, J., Wang, Q., Zhao, C., Bai, Y., Liu, R., Liu, H., Qu, J., 2021. Epilithic biofilm as a reservoir for functional virulence factors in wastewater-dominant rivers after WWTP upgrade. J. Environ. Sci. (China) 101, 27–35.

Margot, J., Kienle, C., Magnet, A., Weil, M., Rossi, L., de Alencastro, L.F., Abegglen, C., Thonney, D., Chevre, N., Schaerer, M., Barry, D.A., 2013. Treatment of micropollutants in municipal wastewater: ozone or powdered activated carbon? Sci. Total Environ. 461, 480–498.

Marti, E., Aumatell, J., Gode, L., Poch, M., Sabater, F., 2004. Nutrient retention efficiency in streams receiving inputs from wastewater treatment plants. J. Environ. Qual. 33 (1), 285–293.

Munn, M.D., Black, R.W., Gruber, S.J., 2002. Response of benthic algae to environmental gradients in an agriculturally dominated landscape. J. North Am. Benthol. Soc. 21 (2), 221–237.

Osaka, T., Yoshie, S., Tsuneda, S., Hirata, A., Iwami, N., Inamori, Y., 2006. Identification of acetate- or methanol-assimilating bacteria under nitrate-reducing conditions by stable-isotope probing. Microb. Ecol. 52 (2), 253–266.

Pandey, L.K., Bergey, E.A., 2018. Metal toxicity and recovery response of riverine periphytic algae. Sci. Total Environ. 642, 1020–1031.

Pasquini, L., Merlin, C., Hassenboehler, L., Munoz, J.-F., Pons, M.-N., Goerner, T., 2013. Impact of certain household micropollutants on bacterial behavior. Toxicity tests/study of extracellular polymeric substances in sludge. Sci. Total Environ. 463, 355–365.

Pesce, S., Margoum, C., Foulquier, A., 2016. Pollution-induced community tolerance for in situ assessment of recovery in river microbial communities following the ban of the herbicide diuron. Agric. Ecosyst. Environ. 221, 79–86.

Philippot, L., Hallin, S., Schloter, M., 2007. In: Sparks, D.L. (Ed.), Advances in Agronomy, vol. 96, pp. 249–305.
Prest, E.I., Weissbrodt, D.G., Hammes, F., van Loosdrecht, M.C.M., Vrouwenvelder, J.S., 2016. Long-term bacterial dynamics in a full-scale drinking water distribution system. PloS One 11 (10).
Pruesse, E., Quast, C., Knittel, K., Fuchs, B.M., Ludwig, W., Peplies, J., Glöckner, F.O., 2007. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nucleic Acids Res. 35 (21), 7188–7196.
Robson, S.V., Rosi, E.J., Richmond, E.K., Grace, M.R., 2020. Environmental concentrations of pharmaceuticals alter metabolism, denitrification, and diatom assemblages in artificial streams. Freshw. Sci. 39 (2), 256–267.
Sabater, S., Artigas, J., Corcoll, N., Proia, L., Timoner, X., Tornes, E., 2016. Ecophysiology of River Algae.
Sabater, S., Guasch, H., Ricart, M., Romani, A., Vidal, G., Kluender, C., Schmitt-Jansen, M., 2007a. Monitoring the effect of chemicals on biological communities. The biofilm as an interface. Anal. Bioanal. Chem. 387 (4), 1425–1434.
Sabater, S., Guasch, H., Ricart, M., Romaní, A., Vidal, G., Klünder, C., Schmitt-Jansen, M., 2007b. Monitoring the effect of chemicals on biological communities. The biofilm as an interface. Anal. Bioanal. Chem. 387 (4), 1425–1434.
Sanders, J.G., Beinart, R.A., Stewart, F.J., Delong, E.F., Girguis, P.R., 2013. Metatranscriptomics reveal differences in in situ energy and nitrogen metabolism among hydrothermal vent snail symbionts. ISME J. 7 (8), 1556–1567.
Schmitt, H., Van Beelen, P., Tolls, J., Van Leeuwen, C.L., 2004. Pollution-induced community tolerance of soil microbial communities caused by the antibiotic sulfachloropyridazine. Environ. Sci. Technol. 38 (4), 1148–1153.
Stefanowicz, A.M., Niklinska, M., Laskowski, R., 2008. Metals affect soil bacterial and fungal functional diversity differently materials and methods. Environ. Toxicol. Chem. 27 (3), 591–598.
Su, D., Ben, W., Strobel, B.W., Qiang, Z., 2020. Occurrence, source estimation and risk assessment of pharmaceuticals in the Chaobai River characterized by adjacent land use. Sci. Total Environ. 712.
Sun, S., Sheng, Y., Zhao, G., Li, Z., Yang, J., 2017. Feasibility assessment: application of ecological floating beds for polluted tidal river remediation. Environ. Monit. Assess. 189 (12).
Tlili, A., Corcoll, N., Arrhenius, A., Backhaus, T., Hollender, J., Creusot, N., Wagner, B., Behra, R., 2020. Tolerance patterns in stream biofilms link complex chemical pollution to ecological impacts. Environ. Sci. Technol. 54 (17), 10735–10743.
Tlili, A., Hollender, J., Kienle, C., Behra, R., 2017. Micropollutant-induced tolerance of in situ periphyton: establishing causality in wastewater-impacted streams. Water Res. 111, 185–194.
Tlili, A., Montuelle, B., Berard, A., Bouchez, A., 2011. Impact of chronic and acute pesticide exposures on periphyton communities. Sci. Total Environ. 409 (11), 2102–2113.
Van den Brink, P.J., Ter Braak, C.J.F., 1999. Principal response curves: analysis of time-dependent multivariate responses of biological community to stress. Environ. Toxicol. Chem. 18 (2), 138–148.
Wagenhoff, A., Townsend, C.R., Phillips, N., Matthaei, C.D., 2011. Subsidy-stress and multiple-stressor effects along gradients of deposited fine sediment and dissolved nutrients in a regional set of streams and rivers. Freshw. Biol. 56 (9), 1916–1936.
Wang, Q., Liang, J., Zhao, C., Bai, Y., Liu, R., Liu, H., Qu, J., 2020. Wastewater treatment plant upgrade induces the receiving river retaining bioavailable nitrogen sources. Environ. Pollut. 263.
Wang, S., Shi, W., Yu, S., Yi, X., Yang, X., 2012. Formation of aerobic granules by Mg2+ and Al3+ augmentation in sequencing batch airlift reactor at low temperature. Bioproc. Biosyst. Eng. 35 (7), 1049–1055.
Weber, K.P., Grove, J.A., Gehder, M., Anderson, W.A., Legge, R.L., 2007. Data transformations in the analysis of community-level substrate utilization data from microplates. J. Microbiol. Methods 69 (3), 461–469.
Weber, K.P., Legge, R.L., 2009. One-dimensional metric for tracking bacterial community divergence using sole carbon source utilization patterns. J. Microbiol. Methods 79 (1), 55–61.
Weber, K.P., Legge, R.L., 2010a. In: Cummings, S.P. (Ed.), Bioremediation: Methods and Protocols, pp. 263–281.
Weber, K.P., Legge, R.L., 2010b. Method for the detachment of culturable bacteria from wetland gravel. J. Microbiol. Methods 80 (3), 242–250.
Wu, H., Zhang, J., Ngo, H.H., Guo, W., Hu, Z., Liang, S., Fan, J., Liu, H., 2015. A review on the sustainability of constructed wetlands for wastewater treatment: Design and operation. Bioresour. Technol. 175, 594–601.
Wu, X.-Q., Yuan, W.-M., Tian, X.-J., Fan, B., Fang, X., Ye, J.-R., Ding, X.-L., 2013. Specific and functional diversity of endophytic bacteria from pine wood nematode bursaphelenchus Xylophilus with Different Virulence. Int. J. Biol. Sci. 9 (1), 34–44.
Xia, L.C., Steele, J.A., Cram, J.A., Cardon, Z.G., Simmons, S.L., Vallino, J.J., Fuhrman, J. A., Sun, F., 2011. Extended local similarity analysis (eLSA) of microbial community and other time series data with replicates. BMC Syst. Biol. 5.
Zhao, G., Cui, X., Zhang, Q., Zhu, Y., 2010. Technology and application for ecology rehabilitation on riparian vegetation restoration. Res. Soil Water Conserv. 17 (1), 252–258.
Zsuzsanna Magdolna, N., Katalin, G., Monika, M., Eva, F., 2013. Comparative evaluation of microbial and chemical methods for assessing 4-chlorophenol biodegradation in soil. Period. Polytech. - Chem. Eng. 57 (1–2), 25–35.