water

MDPI

Article

Contrasting Distribution of Microbial Communities, Functional Genes, and Antibiotic Resistance Genes in Produced Water Treatment Plants with Different Treatment Technologies

Kunpeng Xie, Qianzhi Zeng, Sihui Yu, Hongjing Luo, Yongsheng Zhang, Changwei Ma, Haoyu Hu, Shengnan Shi * and Zheng Gong *

Key Laboratory of Plant Biotechnology of Liaoning Province, School of Life Sciences, Liaoning Normal University, Dalian 116081, China; xkp_1995@sina.com (K.X.); zqz070011@163.com (Q.Z.); ysh322159@163.com (S.Y.); luo54500@163.com (H.L.); zhangys641@163.com (Y.Z.); mcw13470177639@163.com (C.M.); h1366942510@163.com (H.H.)

* Correspondence: shishengnan013@163.com (S.S.); 103596@lnnu.edu.cn (Z.G.)

Abstract: A massive volume of produced water (PW) generated in the process of oil extraction must be treated effectively due to its threat to the ecosystems and human health. Different biological treatment technologies have been used in wastewater treatment plant (WWTP) systems to treat PW. However, their influence on treatment performance has not been investigated. In this study, three PW treatment plants (PWTPs) with different treatment technologies were compared in the following aspects: microbial community structure and assembly, functional genes, and the spread of antibiotic resistance genes (ARGs). The results indicated that different biological treatment technologies led to the variations in the diversity and composition of the microbial community. Phylogenetic bin-based null model analysis (iCAMP) revealed that different treatment technologies deterministically drove the assembly of microbial communities, especially the genera associated with the removal of petroleum hydrocarbons. The results of the metagenomic analysis showed that the genes related to the degradation of alkanes and aromatic hydrocarbons were the most abundant in PWTP3, suggesting it had the highest petroleum degradation potential. In addition, the highest abundance of ARGs in PWTP1 indicated the potential facilitation of ARG dissemination in activated sludge systems. Network analysis indicated that the dissemination of ARGs in the PWTPs might be mediated by transposases.

Keywords: produced water treatment plant; different treatment technologies; microbial community assembly; petroleum degradation genes; antibiotic resistance gene

Citation: Xie, K.; Zeng, Q.; Yu, S.; Luo, H.; Zhang, Y.; Ma, C.; Hu, H.; Shi, S.; Gong, Z. Contrasting Distribution of Microbial Communities, Functional Genes, and Antibiotic Resistance Genes in Produced Water Treatment Plants with Different Treatment Technologies. *Water* **2024**, *16*, 195. https://doi.org/10.3390/w16020195

Academic Editor: Alejandro Gonzalez-Martinez

Received: 29 November 2023
Revised: 29 December 2023
Accepted: 2 January 2024
Published: 5 January 2024

1. Introduction

Produced water (PW), the largest volume byproduct of the petroleum industry, is generated in the process of oil extraction [1,2]. The composition of PW is complex, and it generally contains organic and inorganic compounds such as petroleum hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), nitrate, sulfur compounds, fungicides, heavy metals, and some other chemicals needed for fracturing [3,4]. Due to the toxicity of many components of oil-production water, the discharge of PW can cause serious harm to ecosystems and human health [5]. Therefore, the massive amounts of PW must be treated effectively.

At present, the technologies applied in wastewater treatment plant (WWTP) systems are mainly divided into three categories: physical treatment, chemical treatment, and biological treatment [6,7]. The WWTPs consisting of these processes are also applied to treat PW [8–10]. Mature physical treatments include gravity separation, air floatation, adsorption, and filtration [11–13]. However, the operation and maintenance are relatively

complex and costly, and can easily cause secondary pollution. Catalytic oxidation, advanced oxidation, and flocculation are frequently used chemical treatments [14–16]. The main disadvantages of chemical treatment are the high cost of chemicals, the need for regular maintenance and calibration of the chemical pump, and the difficulty of the removal of reaction byproducts generated through the process. Biological processes have become good supplementary and alternative methods, owing to their low cost and better performance in treating a wide range of dissolved compounds without secondary pollution [17]. Previous studies have investigated and estimated various biological reactors, including membrane bioreactors, biological aerated filters, moving bed biofilm reactors, etc. [18,19]. To understand whether the adopted biological treatment processes led to different treatment performances, different WWTPs treating various types of wastewater were compared. A previous study indicated that the better performance of the anaerobic/anoxic/oxic (AAO)-membrane bioreactor (MBR)-integrated process and sequencing batch reactor (SBR) was superior to the AAO and oxidation ditch (OD) processes for treating pharmaceuticals [20]. Cao et al. [21] demonstrated that the anoxic/oxic (AO) and AAO systems of cyclic activated sludge systems (CASSs) have distinct nitrogen removal efficiencies. However, comparative studies of PW treatment plants (PWTPs) using different biological treatment technologies are still scarce. With the purpose of guiding the design and operation of WWTPs for better PW treatment efficiency, filling this knowledge gap is necessary. In addition, WWTPs that employ biological treatment technologies rely primarily on major microbial activity and interactions within highly diverse communities. The structure, diversity, and shifts of the microbial community will directly affect the WWTP's performance [22]. Determining the functional genera and exploring their roles will facilitate the optimization of wastewater treatment plants [21]. Still, information related to this field in PWTPs is limited. A previous study indicated that environmental factors are the main drivers affecting microbial community diversity in oil-contaminated soils [23]. In a full-scale heavy oil-produced water treatment plant, the composition of the microbial community also changed remarkably with changes in environmental conditions, especially the artificially regulated dissolved oxygen (DO) [24]. However, to date, detailed comparative research of the impact of the environment on the variations in the distribution of microbiota in WWTPs treating PW using different treatment technologies remains scarce.

The mechanism of community assembly is an essential question in microbial community ecology, and investigating the controlling factors of community assembly contributes to the understanding of species composition and association patterns [25,26]. According to previous theory, the species abundance, composition, and distribution patterns are largely controlled by deterministic processes [27,28]. By contrast, stochastic processes largely control species dynamics, such as birth, speciation, and immigration [27,29]. Homogeneous selection (HoS) and heterogeneous selection (HeS) are deterministic processes, and dispersal limitation (DL), homogenizing dispersal (HD), and drift (DR) are stochastic processes. Selection under persistent environmental conditions (persistent selective pressures) is referred to as HoS, which leads to low compositional turnover [30]. By comparison, HeS represents a condition in which environmental condition changes (changed selection pressure) result in high composition turnover. DL indicates that the composition turnover is attributed to low dispersal rates, whereas HD indicates that low composition turnover is attributed to high dispersal rates. Community assembly is governed by DR, when neither dispersal nor selection processes are likely to dominate [31,32]. Based on the concept proposed by Vellend, a comprehensive analytical framework was proposed and used to quantitatively infer the process of community assembly [33–35]. Phylogenetic bin-based null model analysis (iCAMP) is a newly developed model proved to be efficient and steady, and has been widely used for investigating the ecological assembly processes in bioreactors, considering the contributions of different processes to the microbial community assembly with different origins, such as variations in wastewater composition, priority effects, disparate microbial development stages, and heterogeneous microbial inoculum [35–38]. At present, the assembly of microbial communities in WWTPs treating PW using differ-

ent treatment technologies remains largely unknown and the breakthrough scheme, i.e., iCAMP, is critical for elucidating the ecological mechanisms.

The release and residues of antibiotics into the environment exert selective pressure on microorganisms in the ecosystem, leading to the development of antibiotic resistance mechanisms in bacteria, as well as the enrichment of antibiotic resistance genes (ARGs) [39]. The widespread occurrence of ARGs is a major public health issue and an emerging global challenge [40,41]. WWTPs are among the most important sinks for antibiotic resistance [31]. Discharging of treated municipal sewage has increased the number of ARGs in the receiving surface waters [42,43]. Thus, it is essential to investigate the dissemination of ARGs in WWTP systems. It is well known that the PW is usually subjected to biocidal treatment before water injection for secondary oil recovery, with the purpose of preventing the contamination caused by microorganisms in the well. Glutaraldehyde, sodium hypochlorite, chlorine dioxide, dibromonitrilopropionamide, etc., were detected in the PW [44,45]. Previous studies suggested that biocides can induce the emergence of ARGs [46]. Therefore, it is likely that the ARGs can occur in PWTPs due to the existence of biocides in PW, and it is necessary to investigate the risk of transmission of ARGs [47]. However, corresponding research is still rarely reported.

The Xingjiang Oilfield is among the major oilfields in China. In this study, three PWTPs in the Xingjiang Oilfield were chosen for the investigation of (1) water quality indexes, (2) microbial community structure analysis and microbial community assembly, (3) functional gene analysis, and (4) the spread of ARGs in different PWTPs. Hopefully, this investigation will assist the development of effective strategies for PW treatment processes in the future.

2. Materials and Methods

2.1. Treatment Plants and Sample Collection

The locations and detailed information of each plant are shown in Table 1. Different PWTPs with different bioprocesses, activated sludge processes, biological aerated filters, and biological contact oxidation processes were applied to the three plants and named as PWTP1, PWTP2, and PWTP3. Samples were taken from the aerobic biochemical pools of PWTP1 and PWTP3, and the filter samples of PWTP2, in June 2023. Nine samples for each PWTP were collected and stored at −80 °C until further testing.

Table 1. Characteristics of the three PWTPs.

		PWTP1	PWTP2	PWTP3
Location		Karamay, Xinjiang	Karamay, Xinjiang	Karamay, Xinjiang
Name		Heavy oil sewage standard discharge station	Heavy oil saline sewage standard discharge station	Fracturing flowback fluid standard discharge station
Bioprocess		Activated sludge process	Biological aerated filter	Biological contact oxidation process
COD (mg/L)	Influent	300–350	350–400	400–500
	Effluent	180–200	150–200	100–150
NH_4-N (mg/L)	Influent	7–8	13–14	15–16
	Effluent	1–2	6–7	5–6
Petroleum (mg/L)	Influent	7–8	5–6	9–10
	Effluent	1–2	1–2	2–3
Flow rate (m^3/h)		80	60	70
T (°C)		30–32	25–27	28–30
pH		7.0–7.5	7.0–7.5	7.5–8.0
DO (mg/L)		2–4	2–3	3–4
Volatile Phenol (mg/L)		0.074	0.135	0.035

2.2. Analytical Methods

The physical and chemical indexes of wastewater detected during the experiment included pH, temperature, dissolved oxygen (DO), chemical oxygen demand (COD), ammonia nitrogen (NH_4-N), and petroleum. The pH was determined by a pH meter (HI98191, Hanna®, Italy). The dissolved oxygen meter (HI9146, Hanna®, Italy) was used for the detection of temperature and DO. COD was detected using dichromate titration. Nesser's reagent method was used for the measurement of NH_4-N (visible spectrophotometer), and infrared spectrometry was used for the measurement of petroleum (infrared oil meter).

2.3. DNA Extraction and PCR Amplification

The E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) was used to extract microbial DNA, referring to the manufacturer's instructions. Primers 515F and 907R (BIOZERON Co., Ltd., Shanghai, China) were used to amplify the V4–V5 region of the 16S ribosomal RNA gene of bacteria. The AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) was used for the extraction of amplicons, following the instructions of the manufacturer. According to the Illumina genomic DNA library preparation procedure, the pooled DNA products were used to construct the Illumina Pair-End library. The amplicon libraries were then sequenced (2 × 250) on the Illumina MiSeq platform (BIOZERON Co., Ltd., Shanghai, China), referring to standard methods.

2.4. Metagenomic Sequencing

Metagenomic shotgun sequencing libraries were constructed and sequenced (Biozeron Biological Technology Co., Ltd., Shanghai, China). Using pair-end 150 bp (PE150) mode, all samples were sequenced in the NovaSeq 6000 instrument (Illumina Inc., California, USA). Trimmomatic v0.36 (http://www.usadellab.org/cms/index.php?page=trimmomatic) was applied for the quality trimming of raw sequence reads to remove low-quality reads and adaptor contaminants. The reads removing low-quality data and host-genome contaminations were called clean reads and used for the further analysis. The standardization of gene abundance referred to Liu et al. [48], and the unit was transcripts per million (TPM).

2.5. Statistical Analysis and Data Visualization

Statistical analyses were performed using SPSS (v.26, IBM SPSS Inc., Chicago, USA). The online tool (http://circos.ca/, (accessed on 13 November 2023)) was used for depicting Circos plots. For the analysis of co-occurrence networks among ARGs, mobile genetic elements (MGEs), and microbial taxa, the correlation data was calculated in the R environment using the "psych" package 2.3.12 [49]. Gephi 0.10.1 (https://gephi.org/) was used for visualizing networks. Spearman's correlation was identified as statistically robust with the correlation of $R > 0.8$ and $p < 0.01$.

3. Results and Discussion

3.1. The Water Quality Indexes of Different PWTPs

The water quality indexes of three PWTPs with different treatment technologies were monitored (Table 1). The petroleum in the influent water ranged from 7 to 8, 5 to 6, and 9 to 10 mg/L. The average concentration of COD ranged from 300 to 350, 350 to 400, and 400 to 500 mg/L, and the average concentration of NH_4-N ranged from 7 to 8, 13 to 14, and 15 to 16 mg/L in PWTP1, PWTP2, and PWTP3. In the effluent water, the petroleum concentrations were 1–2, 1–2, and 2–3 mg/L in PWTP1, PWTP2, and PWTP3; COD concentrations were 180–200, 150–200, and 100–150 mg/L in PWTP1, PWTP2, and PWTP3; and NH_4-N concentrations ranged from 1 to 2, 6 to 7, and 5 to 6 mg/L in PWTP1, PWTP2, and PWTP3. The results of statistical analyses showed that all the water quality indexes exhibited significant differences among three different PWTPs ($p < 0.05$), which indicated that the treatment efficiency of PWTPs with different treatment technologies was remarkably different. Additionally, these indexes could also have an impact on the structure of microbial community, which will be discussed later in Section 3.2.4.

3.2. Analysis of Microbial Community Structure in Different PWTPs

3.2.1. Overview of Microbial Diversity

The microbial community diversity and structure of the different PWTPs were investigated using Illumina high-throughput sequencing. After the low-quality sequences and chimeras were removed, 32,918–41,974 effective sequences with an average length of 408.88–421.88 bp were obtained. The sequence number of each sample was normalized and 631–1071 OTUs were generated with a threshold of 0.97.

For microbial communities in different PWTPs, Chao1 and ACE were the highest in PWTP3 (1118.38 ± 87.35 and 1152.92 ± 82.70), followed by PWTP2 (980.92 ± 120.73 and 1021.71 ± 134.09) and PWTP1 (883.22 ± 54.52 and 900.95 ± 62.86), suggesting that the richness of PWTP3 was higher than PWTP2 and PWTP1 (Figure S1). The Shannon index followed a trend similar to that of Chao1 and ACE except for PWTP2, which indicated that the diversity of the microbial community was PWTP3 > PWTP1 > PWTP2. A previous study compared the A/O contact oxidation process with bioaugmentation and the A/O conventional activated sludge process without bioaugmentation treatment of petrochemical wastewater, and the diversity of the bacterial community in the contact oxidation system was better than that of the activated sludge system, which is consistent with the results of this study [50]. These results implied that the richness and diversity showed differences among PWTPs with different treatment technologies.

3.2.2. Microbial Community Analysis at the Phylum Level

In order to investigate the distribution of the microbial community in WWTPs treating PW with different treatment technologies, the samples were analyzed using Illumina high-throughput sequencing. The taxa with relative abundance > 1% (at least in one group) were taken into consideration.

The main phyla of each plant are shown in Figure S2. The phylum Proteobacteria was the most abundant, with a relative abundance of 85.36%, 61.56%, and 74.23% in PWTP1, PWTP2, and PWTP3, respectively. These were consistent with the results of previous studies, which indicated that this phylum could effectively degrade refractory petroleum hydrocarbons and was predominant in the microbial community in petroleum hydrocarbon wastewater treatment systems [51,52]. The study of Ma et al. [51] showed that the relative abundance of Proteobacteria accounted for 67.6% and 69.6% in micro-electrolysis with a biological reactor and conventional activated sludge technology treating petroleum hydrocarbon wastewater. Kim et al. [53] also indicated the predominance of Proteobacteria (62.8 ± 10.7% in relative abundance) in a full-scale WWTP fed with petroleum refining wastewater. However, the second most abundant phylum in the microbial community of each plant was different. In PWTP1, Dadabacteria had the second highest relative abundance of 6.49%, whereas it was not the main phylum in PWTP2 (0.02%) or PWTP3 (0.004%). Bacteroidota was the second most dominant phylum in PWTP2, and its relative abundance in PWTP1, PWTP2, and PWTP3 was 4.29%, 12.25%, and 6.52%, respectively. The relative abundance of Desulfobacterota was 6.53% in PWTP3, whereas it was 1.47% and 0.22% in PWTP2 and PWTP1, respectively. It was reported that Dadabacteria was associated with the persistence and possible potential for degradation of aromatic compounds, and Bacteroidota and Desulfobacterota were commonly found in petroleum wastewater [54–56]. The other two main phyla in PWTP3 were Firmicutes (5.28%) and Campylobacterota (4.43%). Firmicutes was also dominant in PWTP2 (4.33%), while it had low relative abundance in PWTP1 (0.12%). Campylobacterota appeared in PWTP1 and PWTP2 with low relative abundance (0.09% and 0.24%, respectively). Firmicutes was able to degrade refractory organic matter, and Campylobacterota was found in oil pollution sediments [51,57]. Deinococcota, Acetothermia, Thermotogota, Chloroflexi, and Armatimonadota were only predominant in PWTP2. Among them, Deinococcota, Acetothermia, Thermotogota, and Chloroflexi were related to oil contamination, oil degradation, and oil reservoirs [58–61]. Recently, it has been found that the relative content of Armatimonadota might be related to the concentration of phenol [62]. Spearman correlation analysis showed

that the relative abundance of Armatimonadota was significantly positively correlated with the concentration of volatile phenol (r_s = 1.00, $p < 0.01$), which is consistent with a previous study.

3.2.3. Microbial Community Analysis at the Genus Level

Figure 1 showed the relative abundance of microbial communities in different PWTPs at the genus level. In general, significant differences were observed between groups. In the PWTP1, *Hyphomicrobium*, *Novosphingobium*, *Hydrogenophaga*, *Pedomicrobium*, and *Methylocystis* accounted for the highest relative abundances. As the most abundant genera, *Hyphomicrobium* is capable of aromatic hydrocarbon degradation [63]. *Novosphingobium* is able to utilize multiple polycyclic aromatic hydrocarbons (PAHs) as the sole carbon sources [64]. A previous study indicated that *Hydrogenophaga* showed positive correlation with the removal of C21–C34 petroleum hydrocarbon fractions [65]. The *Pedomicrobium* genus could have the ability of hydrocarbonoclastic [66]. *Methylocystis* was used for the biodegradation of PAHs in the study of Magdy et al. [67].

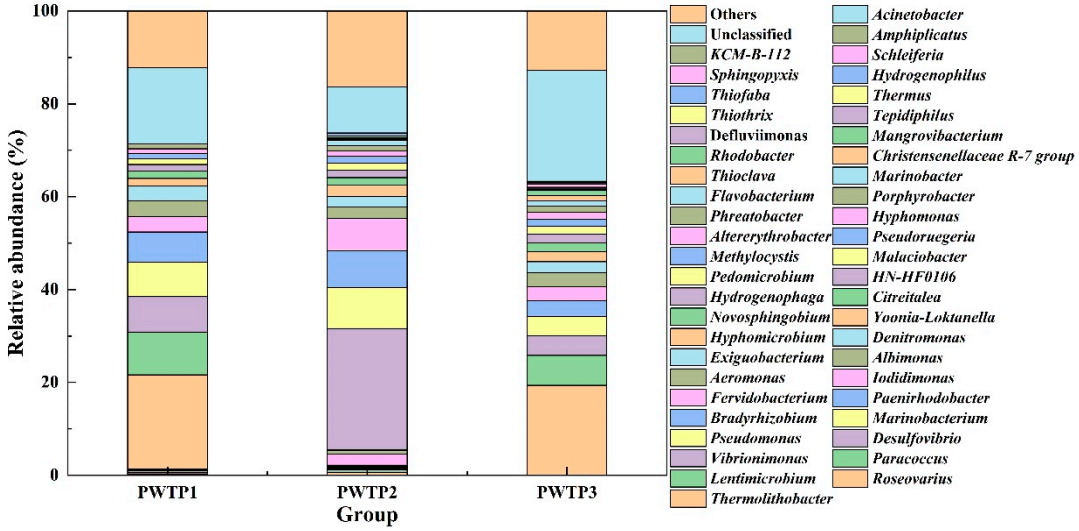


Figure 1. Bacterial community composition at the genus level in different PWTPs.

Tepidiphilus, *Thermus*, *Hydrogenophilus*, *Schleiferia*, and *Amphiplicatus* were the predominant genera in PWTP2. *Tepidiphilus* was the dominant bacterial genera which might contribute to the degradation of petroleum compounds [68]. *Thermus* comprises extreme thermophilic microorganisms with the ability to degrade PAH compounds and PAH/alkane mixtures [69]. *Hydrogenophilus* belongs to the class Betaproteobacteria, which is representative in oil reservoir samples and the members of this class were proven to be the carrier of hydrocarbon degradation functional genes [70]. *Schleiferia* is a heat-resistant bacterium (30–60 °C) that has not been well investigated at oil contamination sites [71], whereas *Amphiplicatus* is capable of reducing nitrates and biodegrading organic matter in wastewater.

In PWTP3, the top five genera in relative abundance were *Roseovarius* (19.34%), *Paracoccus* (6.44%), *Desulfovibrio* (4.25%), *Marinobacterium* (4.15%), and *Iodidimonas* (2.97%). Previous studies indicated that *Roseovarius* was involved in the degradation of crude oil and PAH degradation in seawater [61,72,73]. The genus *Paracoccus* was previously found in activated sludge from a petrochemical plant and was proven to be capable of simultaneous nitrification and denitrification [54,74]. The study of Qian et al. [75] confirmed the sulfate-reduction coupling PAH degradation of *Desulfovibrio* and *Petrimonas*. Dworkin et al. [76] demonstrated that *Marinobacterium* could grow under various conditions, particularly in the presence of petroleum hydrocarbons and in oil reservoirs at both mesophilic and high temperatures and high concentrations of salts. In addition, *Iodidimonas* has been found in PW/hydrocarbon-contaminated environments, which might have the potential for the

degradation of hydrocarbon degradation [77]. The results indicated that different treatment technologies could lead to different microbial community compositions, and the mechanism for the microbial community assembly is discussed in Section 3.2.5.

3.2.4. Correlation Analysis of Environmental Factors and Microbial Community Structure

The possible correlations between the microbial community structure and environmental factors were analyzed using canonical correspondence analysis (CCA) (Figure 2). There were nine environmental factors (influent COD, effluent COD, influent NH_4-N, effluent NH_4-N, influent petroleum, effluent petroleum, flow rate, temperature, and pH) that showed significance on the basis of the permutation test analysis ($p < 0.05$). In the CCA plot, the length of the arrows represents the degree to which each variable is related to the community structure. As shown in Figure 2, the most significant environmental factor was the temperature. This result was consistent with the results of previous studies, which reported that temperature might have the strongest influence on the composition of the bacterial community in bioreactors [78].

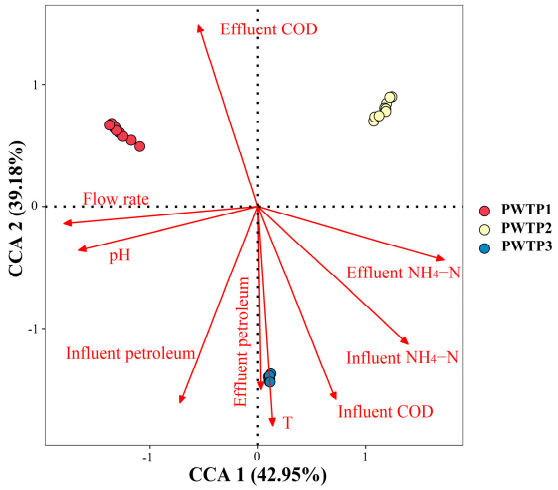


Figure 2. CCA (canonical correspondence analysis) of bacterial communities in different PWTPs.

3.2.5. Microbial Community Assembly in in Different PWTPs

The assembly mechanism of microbial community is one of the solid theoretical foundations for the regulation of microbial community in WWTP systems [79]. Because both deterministic and stochastic processes are considered to be important in the local community assembly, determining the relative importance of different ecological processes and the mechanisms of assembly is of great importance [26].

Previous studies acknowledged that homogeneous selection (HoS) and heterogeneous selection (HeS) are deterministic processes, whereas dispersal limitation (DL), homogenizing dispersal (HD), and drift (DR) are stochastic processes [35]. According to the iCAMP results, the relative importance of the deterministic process was 71.49%, 68.18%, and 74.62% for PWTP1, PWTP2, and PWTP3, respectively (Figure 3a). The deterministic ratios showed large effect sizes (Cohen's d = 2.51, 1.69, and 0.83 for PWTP1, PWTP2, and PWTP3, respectively), indicating that microbial community assembly was the most deterministic in PWTP3, followed by PWTP2 and PWTP1. Furthermore, the relative importance of the HoS, HeS, DL, HD, and DR was determined. As shown in Figure 3a, HoS, DR, and HD were the three most dominant processes in the assembly of the microbial community in all the groups, with an average importance of 68.17–74.59%, 14.53–20.69%, and 4.97–11.24%, respectively. In different PWTPs, the relative importance of different processes was altered with large or medium effect sizes (|Cohen's d| = 0.79–9.94), implying the relative importance of the deterministic process changed with the different treatment technologies applied. This could be due to the constant imposed selective pressure by different treatment

technologies. As different deterministic forces, they decreased stochasticity to varying degrees, leading to the changes in deterministicity.

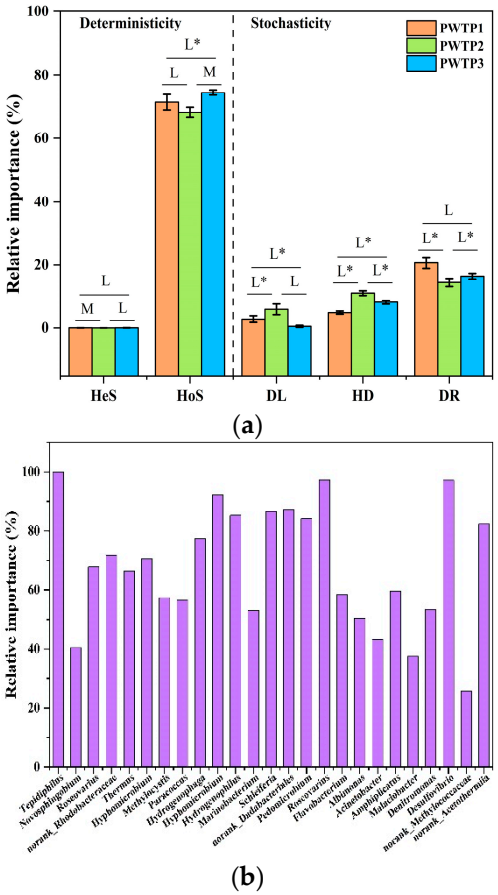


Figure 3. Relative importance of different ecological processes based on iCAMP (**a**). Relative importance of the genus with greatest relative abundance in each bin (**b**). (One-side significance based on bootstrapping test was expressed as * $p < 0.05$. L, M, S, and N represent large ($|d| > 0.8$), medium ($0.5 < |d| \leq 0.8$), small ($0.2 < |d| \leq 0.5$), and negligible ($|d| \leq 0.2$) effect sizes, based on Cohen's d).

In addition, the observed OTUs fell into 67 phylogenetic groups (termed "bins"), and the corresponding assembly mechanisms were analyzed. The deterministic process, which mainly consisted of HoS, dominated 21 bins (31.34% of the bin numbers), 24 bins (35.82% of the bin numbers), and 27 bins (40.30% of the bin numbers) for PWTP1, PWTP2, and PWTP3, respectively. The taxonomy of the bins was also demonstrated, and the ecological processes of the bins containing the predominant genera were further investigated (Figure 3b). Investigations on these genera can help to better understand the effects of different treatment technologies on microbial diversity, structure, functional gene composition, and activities.

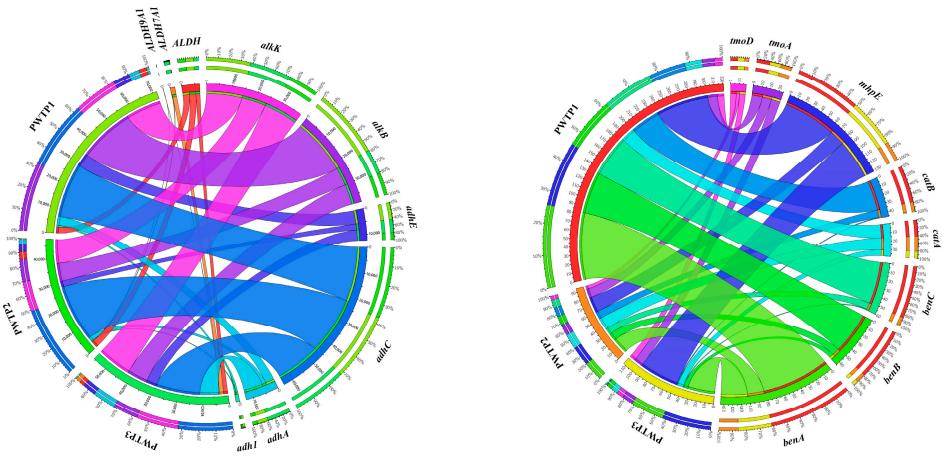
The main bin was categorized as *Tepidiphilus* (bin 32, 8.60% of all bins), the dominant genus in PWTP2 with the ability to degrade petroleum compounds, and it was dominated by HD (67.29%), HoS (100.00%), and HoS (64.19%) in PWTP1, PWTP2, and PWTP3 [68]. In contrast, the predominant genus in PWTP1, *Novosphingobium* (bin 14, 7.60% of all bins), was mainly regulated by stochastic processes in PWTP1 (HD: 100.00%) and PWTP2 (HD: 100.00%), while it was regulated by deterministic processes in PWTP3 (100.00%). Bin 25 (6.58%), which was mainly ascribed to *Roseovarius*, the most abundant genus in PWTP3, was controlled by HoS in all three plants (88.05% in PWTP1, 100.00% in PWTP2, and 100.00% in PWTP3). Bin 23 (assigned to *norank_f_Rhodobacteraceae*) was totally HD-dominant in PWTP1, whereas it was totally HoS-dominant in PWTP2 and PWTP3. Belonging to the Alphaproteobacteria class, this family was found in soils extracted from oil wells and may be

associated with the metabolism of the anaerobically activated aromatic hydrocarbons [80]. These results suggested that different treatment technologies might result in different genera being driven deterministically, particularly those associated with the removal of petroleum hydrocarbons. It was speculated that different treatment technologies might provide persistent environmental conditions, which had persistent selective pressures on specific genera.

3.3. Analysis of Petroleum Hydrocarbon Degradation-Related Genes in Different PWTPs

To further compare the potential of petroleum hydrocarbon degradation in different PWTPs, the related genes of samples from PWTP1, PWTP2, and PWTP3 were annotated, and the KEGG entry number and the description of genes are listed in Table S1.

A previous study reported that alkane hydroxylase, alcohol dehydrogenase, aldehyde dehydrogenase, and acetyl-CoA synthetase are involved in the alkane degradation pathway [81]. Therefore, the corresponding genes were investigated (Figure 4a). *alkB* is the gene encoding alkane 1-monooxygenase, which oxidizes the methyl end of an alkane to a hydroxyl group. The abundance of *alkB* was the highest in PWTP3 (227.27 TPM), followed by PWTP1 (82.56 TPM) and PWTP2 (73.97 TPM). The primary alcohol generated is then catalyzed by alcohol dehydrogenase to form the corresponding aldehydes. The total abundance of the related genes (*adhA*, *adhC*, and *adh1*) was similar to the results of *alkB*, increasing by 4.38% and 2.27% in PWTP3 and PWTP1, respectively, compared with that in PWTP2. The aldehyde dehydrogenase genes (*ALDH*, *ALDH7A1*, *ALDH9A1*, and *adhE*) participate in the process of catalytic oxidation of aldehydes to fatty acids, and the sum of their abundances was also greater in PWTP3 and PWTP1, which were 1.56 and 1.50 times of that in PWTP2. The gene *alkK* is associated with acyl-CoA synthetase, which is involved in the conversion of fatty acids into acyl-CoA. The abundance of this gene was remarkably higher in PWTP3 (46.25%) and PWTP1 (39.64%) than that in PWTP2. The results could be attributed to the robust microbial community in PWTP3. According to a previous report, attached biofilms with extracellular polymeric substances (EPS) can protect microorganisms from environmental and chemical stresses [82]. Therefore, the genera with alkane-degrading genes could have better stress tolerance in PWTP3, which was applied with biological contact oxidation. In addition, the oily substances could stimulate the growth of oil-degrading microbes [83]. According to Table 1, the petroleum concentration in the influent of PWTP2 was the lowest, which indicated the lowest relative abundance of alkane-degrading genera. This might explain the lowest abundance of alkane-degrading genes in PWTP2.



(**a**) (**b**)

Figure 4. The Circos plots of petroleum hydrocarbon degradation-related genes in different PWTPs. The abundance of genes involved in the degradation of alkane (**a**) and aromatic hydrocarbons (**b**).

Genes associated with aromatic hydrocarbon degradation were also investigated. *tmoA* and *tmoD* genes are the genes encoding the enzymes (toluene monooxygenases) involved in the degradation of toluene to benzyl alcohol. The abundances of these two genes in PWTP3 were 15.23% and 74.41% higher than those in PWTP2, whereas they were 13.32% and 5.66% higher than those in PWTP1, respectively. The enzyme benzoate 1,2-dioxygenase, which contributes to the transformation of benzoate to catechol, is encoded by *benA*, *benB*, and *benC*. The abundance of these genes was all highest in PWTP1, and the sum abundance was PWTP3 (184.29 TPM) > PWTP1 (39.05 TPM) > PWTP2 (36.23 TPM). The genes *catA* and *catB* encode catechol 1,2-dioxygenase and muconate cycloisomerase, respectively, which are the key enzymes involved in the catalytic oxidation of catechol and 4-methyl catechol. In the aromatic hydrocarbon degradation pathway, catechol is the intermediate product of toluene degradation, and catechol 1,2-dioxygenase is the key enzyme for cleaving the benzene ring during toluene degradation. As shown in Figure 4b, the abundance of *catA* and *catB* was the highest in PWTP3, compared with PWTP2 and PWTP1. In addition, *mhpE* is an enzyme-coding gene that catalyzes the oxidation of 4-hydroxy 2-oxovalerate to acetaldehyde and pyruvic acid, which is the intermediate link of the synthesis of acetyl-CoA. This gene showed a similar changing trend to *catA* and *catB* genes. These results showed that PWTP3 might degrade petroleum hydrocarbon more effectively than PWTP1 and PWTP2, indicating that different treatment technologies could lead to different treatment efficiencies. The relationships between the petroleum hydrocarbon degradation-related genes and the environmental factors/dominant genera were assessed on the basis of the Mantel test to investigate the driving factors. As shown in Figure S3a, both alkane and aromatic hydrocarbon-degrading genes had significant positive correlations with influent COD ($r_p = 0.35$, $p < 0.01$; $r_p = 0.44$, $p < 0.01$), effluent COD ($r_p = 0.21$, $p < 0.01$; $r_p = 0.28$, $p < 0.01$), influent NH_4-N ($r_p = 0.82$, $p < 0.01$; $r_p = 0.94$, $p < 0.01$), effluent NH_4-N ($r_p = 0.93$, $p < 0.01$; $r_p = 0.97$, $p < 0.01$), influent petroleum ($r_p = 0.36$, $p < 0.01$; $r_p = 0.15$, $p < 0.05$), flow rate ($r_p = 0.97$, $p < 0.01$; $r_p = 0.84$, $p < 0.01$), T ($r_p = 0.75$, $p < 0.01$; $r_p = 0.56$, $p < 0.01$), and DO ($r_p = 0.45$, $p < 0.01$; $r_p = 0.24$, $p < 0.01$). Figure S3b showed that the top five genera in PWTP1 (*Hyphomicrobium*, *Novosphingobium*, *Hydrogenophaga*, *Pedomicrobium*, and *Methylocystis*), PWTP2 (*Tepidiphilus*, *Thermus*, *Hydrogenophilus*, *Schleiferia*, and *Amphiplicatus*), and PWTP3 (*Roseovarius*, *Paracoccus*, *Desulfovibrio*, *Marinobacterium*, and *Iodidimonas*) were all significantly positively correlated with both alkane and aromatic hydrocarbon-degrading genes ($p < 0.05$). These results indicated that the difference between alkane and aromatic hydrocarbon-degrading genes was caused by multiple factors, including abiotic driving factors and biotic driving factors.

3.4. Abundance and Diversity of ARGs in Different PWTPs

The abundance of ARGs in the samples was calculated to analyze the impact of different biological processes on the spread of ARGs. Eighteen ARG types in total were identified (Figure 5a). In general, the total abundances of the detected ARGs were significantly higher in PWTP1 and PWTP2 than in PWTP3 (ANOVA, $p < 0.05$). Among all the ARG types, Bacitracin-resistant genes predominated in PWTP1, with an abundance of 68.18 ± 2.81 TPM. Multidrug-resistant genes were the most abundant in PWTP2 and PWTP3, the abundances of which was 209.69 ± 32.06 and 64.99 ± 1.64 TPM. Consistent with previous studies, genes that can resist Bacitracin and Multidrug were found to be relatively abundant in the WWTPs [84–86]. As for ARG subtypes, a total of 147 were identified in all samples, with 87 subtypes in PWTP1, 114 subtypes in PWTP2, and 87 subtypes in PWTP3. The diversity of ARG subtypes in PWTP2 samples was significantly higher than that in PWTP1 and PWTP3 samples (ANOVA, $p < 0.01$), indicating that the subtypes of ARGs varied among PWTPs with different treatment technologies (Figure 5b).

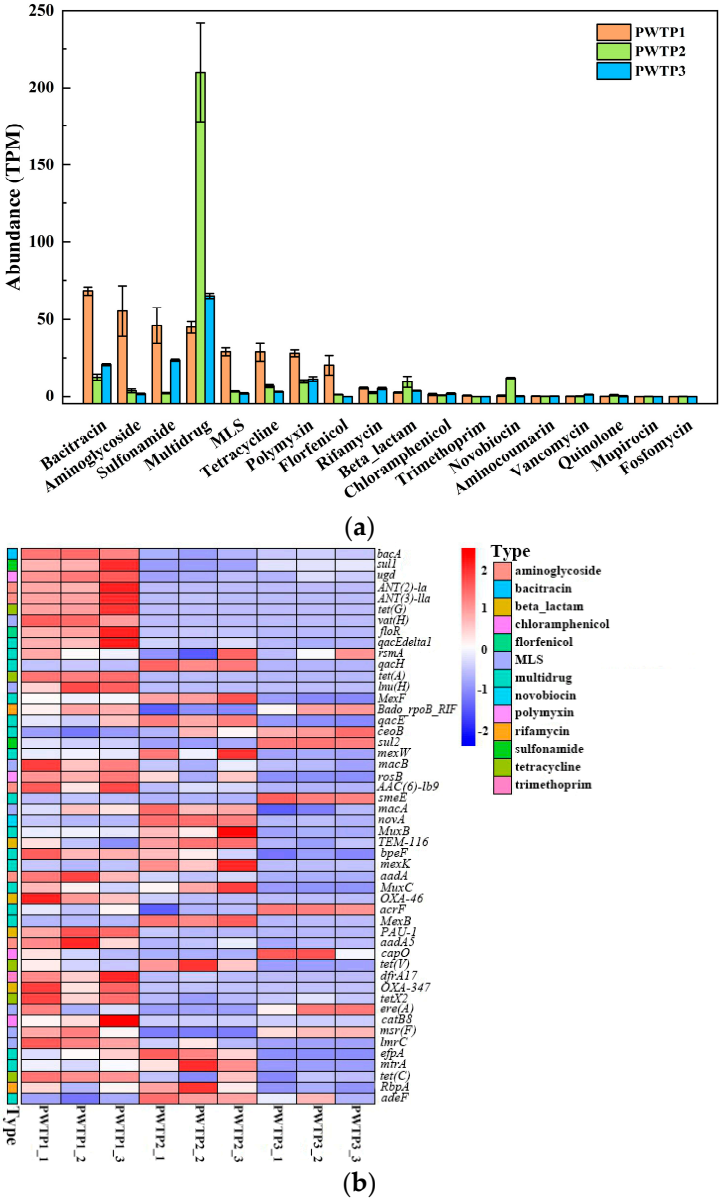


Figure 5. The abundance of ARG types (**a**) and heatmap of ARG subtypes (z−score standardization) (**b**) in different PWTPs.

The fracturing flowback fluid treated in PWTP3 was supplemented with 0.05% formaldehyde, 0.05% quaternary ammonium surfactant, and 0.05% alkyl polyamines before injection into the well, whereas the steam injection-produced liquid treated in PWTP1 and PWTP2 was not supplemented with bactericide after hardness removal in the steam-injection boiler. Thus, ARG dissemination was expected to be the greatest in PWTP3. However, in this study, the ARG sum abundance was PWTP1 > PWTP2 > PWTP3. This unexpected result might be associated with the different wastewater treatment processes applied in three different PWTPs (PWTP1: activated sludge process; PWTP2: biological aerated filter; PWTP3: biological contact oxidation process). Firstly, Section 3.2 indicated that the different wastewater treatment processes led to the variation in the microbial community. It could be speculated that this result might be related to the shift of ARG hosts with the different wastewater treatment processes applied, which caused the corresponding changes in ARG abundance. Additionally, a previous study has reported that in the integrated fixed film activated sludge system of one municipal WWTP, the ARGs detected in activated sludge were more abundant than those in attached biofilms [82]. ARGs have been shown to vary

with the distance from the substrate in the biofilm, which showed higher abundance near the interfaces of the fluid and biofilm than the parts near the substratum [87]. As a kind of suspended biofilm, the fluid–biofilm interface of activated sludge is large, which may be conducive to the development of ARGs [82]. Studies have shown that more ARG donors and receptors were distributed in the top layer of the attached biofilm, where the bacteria have better access to oxygen and nutrients, which could affect drug resistance plasmids transfer [88–91]. This might be the reason for the most abundant ARGs being present in PWTP1, in which the activated sludge process is applied.

3.5. Co-Occurrence between ARGs and MGEs

Horizontal gene transfer (HGT)-mediated process contributes to antibiotic resistance dissemination. Therefore, MGEs, which play an important role in the HGT of ARGs, were investigated. Five types of MGEs were detected in the samples: insertion_sequence, integrase, ist, plasmid, and transposase. Among them, MGEs associated with transposase were the most abundant in all three PWTPs (Figure S4). PWTP2 samples contained up to 34 MGE subtypes, whereas PWTP1 and PWTP3 contained 27 and 28 MGE subtypes, respectively. Based on these results, it could be inferred that the different treatment technologies might lead to differences in the abundance of ARGs and MGEs, although the diversity of ARGs and MGEs varies slightly among different samples.

Network analysis could provide a deeper understanding of the symbiotic patterns between ARGs and MGEs. This study used network inference based on strong ($R^2 > 0.8$) and significant ($p < 0.01$) correlations to explore the co-occurrence patterns of ARGs and MGEs (Figure 6). The *sul1* gene played an important role in conferring resistance to sulfonamide and was the main subtype of ARGs in the sample. The *sul1* gene was positively correlated with *intI1*. Therefore, this might indicate that the horizontal transfer of sulfonamide resistance genes in this experiment was mainly mediated by *intI1*. The most significant MGE subtype in the sample was *tnpA-3*, which connected 51 ARG subtypes. The *tnpA-3* gene belongs to the *tnp* family and is an enzyme capable of performing transposable functions [92]. Studies have shown that there is a strong correlation between multiple ARGs encoding transposable enzymes, indicating the co-selection of horizontal gene transfer and ARGs [93]. Transposable enzymes are involved in the migration of DNA within and possibly between genomes, suggesting that these genes could serve as agents for analyzing the potential of HGT [94]. Therefore, the results indicated that the dissemination of ARGs in the PWTPs might be transposase-mediated.

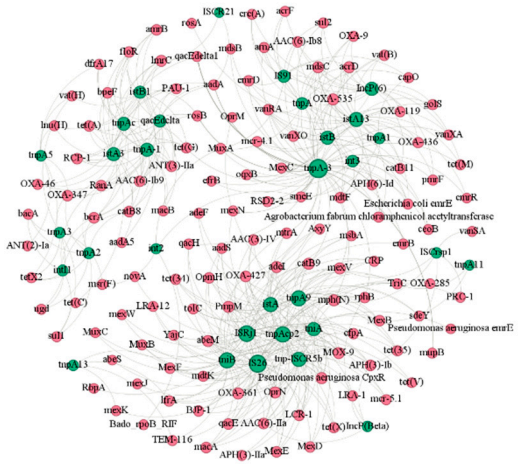


Figure 6. Network analysis of the co-occurrence between ARG subtypes and MGE subtypes.

4. Conclusions

This study compared three PWTPs with different treatment technologies. Different biological treatment technologies caused the changes in the diversity and composition of

microbial communities. The iCAMP results indicated that the assembly of the microbial community by different treatment technologies was deterministic, especially for petroleum hydrocarbon-degrading genera. The abundance of alkane and aromatic hydrocarbon-degrading genes was the highest in PWTP3, suggesting the effectiveness of PWTP3 for petroleum removal. In addition, the sum of ARGs was the most abundant in PWTP1, which implied that the activated sludge system might facilitate the spread of ARGs compared to the attached biofilm systems. According to the co-occurrence network of ARGs and MGEs, the HGT-mediated ARGs transfer in PWTPs was in connection with transposase. This study firstly compared the water quality indexes of PWTPs with different treatment technologies, and focused on the effects of different biological processes on microbial community structure and assembly, functional gene abundance, and ARG dissemination. This work extents our understanding about the effect of PWTPs with different treatment technologies and sheds light on its potential application in treating PW water.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w16020195/s1, Table S1: KEGG entry number and the description of genes; Figure S1: Alpha diversity indices including Chao1, ACE, and Shannon of microbial community in different PWTPs; Figure S2: Bacterial community composition at the phylum level in different PWTPs; Figure S3: The correlation between petroleum hydrocarbon degradation related genes and the environmental factors (a) or dominant genera (b). The color of the edges corresponds to the r value obtained from the Mantel test, and the width of the edges denotes the statistical significance. The pairwise correlation is indicated by the color gradient that denotes Pearson's correlation coefficient; Figure S4: The abundance of MGEs subtypes in different PWTPs.

Author Contributions: Conceptualization, S.S. and Z.G.; methodology, S.Y. and Q.Z.; software, Q.Z.; validation, K.X., S.S. and Z.G.; formal analysis, H.L.; investigation, Y.Z.; data curation, C.M. and H.H.; writing—original draft preparation, K.X.; writing—review and editing, K.X.; supervision, Z.G.; funding acquisition, Z.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the horizontal scientific research project (microbial-enhanced treatment of petroleum hydrocarbon pollutants: technical and engineering demonstration), grant number H2022011.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

Acknowledgments: The authors gratefully acknowledge the horizontal scientific research project (microbial-enhanced treatment of petroleum hydrocarbon pollutants: technical and engineering demonstration, H2022011).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Olajire, A.A. Recent advances on the treatment technology of oil and gas produced water for sustainable energy industry-mechanistic aspects and process chemistry perspectives. *Chem. Eng. J. Adv.* **2020**, *4*, 100049. [CrossRef]
2. Samuel, O.; Othman, M.H.D.; Kamaludin, R.; Sinsamphanh, O.; Abdullah, H.; Puteh, M.H.; Kurniawan, T.A.; Li, T.; Ismail, A.F.; Rahman, M.A.; et al. Oilfield-produced water treatment using conventional and membrane-based technologies for beneficial reuse: A critical review. *J. Environ. Manag.* **2022**, *308*, 114556. [CrossRef] [PubMed]
3. Chittick, E.A.; Srebotnjak, T. An analysis of chemicals and other constituents found in produced water from hydraulically fractured wells in California and the challenges for wastewater management. *J. Environ. Manag.* **2017**, *204*, 502–509. [CrossRef] [PubMed]
4. Al-Ghouti, M.A.; Al-Kaabi, M.A.; Ashfaq, M.Y.; Da'na, D.A. Produced water characteristics, treatment and reuse: A review. *J. Water Process.* **2019**, *28*, 222–239. [CrossRef]
5. Husain, A.; Al-Harthi, M.A. Chemical treatment of oilfield wastewater and the effect of temperature on treatment efficiency: A review. *J. Pet. Sci. Eng.* **2023**, *220*, 111089. [CrossRef]
6. Yang, Y.; Jie, B.; Zhai, Y.; Zeng, Y.; Kang, J.; Cheng, G.; Zhang, X. Performance and mechanism of efficient activation of peroxymonosulfate by Co-Mn-ZIF derived layered double hydroxide for the degradation of enrofloxacin. *J. Mol. Liq.* **2024**, *394*, 123723. [CrossRef]

7. Wang, Y.; Lin, N.; Xu, J.; Jiang, H.; Chen, R.; Zhang, X.; Liu, N. Construction of microwave/PMS combined dual responsive perovskite-MXene system for antibiotic degradation: Synergistic effects of thermal and non-thermal. *Appl. Surf. Sci.* **2023**, *639*, 158263. [CrossRef]
8. Ghafoori, S.; Omar, M.; Koutahzadeh, N.; Zendehboudi, S.; Malhas, R.N.; Mohamed, M.; Al-Zubaidi, S.; Redha, K.; Baraki, F.; Mehrvar, M. New advancements, challenges, and future needs on treatment of oilfield produced water: A state-of-the-art review. *Sep. Purif. Technol.* **2022**, *289*, 120652. [CrossRef]
9. Patni, H.; Ragunathan, B. Recycling and re-usage of oilfield produced water—A review. *Mater. Today Proc.* **2023**, *77*, 307–313. [CrossRef]
10. Akmirza, I.; Pascual, C.; Carvajal, A.; Pérez, R.; Muñoz, R.; Lebrero, R. Anoxic biodegradation of BTEX in a biotrickling filter. *Sci. Total Environ.* **2017**, *587–588*, 457–465. [CrossRef]
11. Forero, J.E.; Ortíz, O.P.; Nariño, F.A.; Díaz, J.; Peña, H. Design and development of a high efficiency tank for crude oil dehydration (I). *CTF-Cienc. Tecnol. Futuro* **2008**, *3*, 185–199. [CrossRef]
12. Wang, C.; Wang, Z.; Wei, X.; Li, X. A numerical study and flotation experiments of bicyclone column flotation for treating of produced water from ASP flooding. *J. Water Process Eng.* **2019**, *32*, 100972. [CrossRef]
13. Kogiso, M.; Aoyagi, M. Produced water treatment using self-assembled organic nanotubes as adsorbents. *J. Jpn. Pet. Inst.* **2018**, *61*, 44–49. [CrossRef]
14. Hassan, A.A.; Al-zobai, K.M.M. Chemical oxidation for oil separation from oilfield produced water under UV irradiation using Titanium dioxide as a nano-photocatalyst by batch and continuous techniques. *Int. J. Chem. Eng.* **2019**, *2019*, 9810728. [CrossRef]
15. Mokhbi, Y.; Korichi, M.; Akchiche, Z. Combined photocatalytic and Fenton oxidation for oily wastewater treatment. *Appl. Water Sci.* **2019**, *9*, 35. [CrossRef]
16. Duan, M.; He, Z.; Wang, X.; Jing, B.; Xu, Z.; Xiong, Y.; Fang, S. A novel interface-active cationic flocculant for the oil-water separation of oily wastewater produced from polymer flooding. *J. Mol. Liq.* **2019**, *286*, 110868. [CrossRef]
17. Lusinier, N.; Seyssiecq, I.; Sambusiti, C.; Jacob, M.; Lesage, N.; Roche, N. Biological treatments of oilfield produced water: A comprehensive review. *SPE J.* **2019**, *24*, 2135–2147. [CrossRef]
18. Guo, C.; Chen, Y.; Chen, J.; Wang, X.; Zhang, G.; Wang, J.; Cui, W.; Zhang, Z. Combined hydrolysis acidification and bio-contact oxidation system with air-lift tubes and activated carbon bioreactor for oilfield wastewater treatment. *Bioresour. Technol.* **2014**, *169*, 630–636. [CrossRef]
19. Sharghi, E.A.; Bonakdarpour, B. The study of organic removal efficiency and halophilic bacterial mixed liquor characteristics in a membrane bioreactor treating hypersaline produced water at varying organic loading rates. *Bioresour. Technol.* **2013**, *149*, 486–495. [CrossRef]
20. Liu, H.; Lam, J.C.W.; Li, W.; Yu, H.; Lam, P.K.S. Spatial distribution and removal performance of pharmaceuticals in municipal wastewater treatment plants in China. *Sci. Total Environ.* **2017**, *586*, 1162–1169. [CrossRef]
21. Cao, J.; Zhang, T.; Wu, Y.; Sun, Y.; Zhang, Y.; Huang, B.; Fu, B.; Yang, E.; Zhang, Q.; Luo, J. Correlations of nitrogen removal and core functional genera in full-scale wastewater treatment plants: Influences of different treatment processes and influent characteristics. *Bioresour. Technol.* **2020**, *297*, 122455. [CrossRef] [PubMed]
22. Wagner, M.; Loy, A. Bacterial community composition and function in sewage treatment systems. *Curr. Opin. Biotechnol.* **2002**, *13*, 218–227. [CrossRef] [PubMed]
23. Jiao, S.; Liu, Z.S.; Lin, Y.B.; Yang, J.; Chen, W.M.; Wei, G.H. Bacterial communities in oil contaminated soils: Biogeography and co-occurrence patterns. *Soil Biol. Biochem.* **2016**, *98*, 64–73. [CrossRef]
24. Wang, X.; Jiang, L.; Gai, Z.; Tao, F.; Tang, H.; Xu, P. The plasticity of indigenous microbial community in a full-scale heavy oil-produced water treatment plant. *J. Hazard. Mater.* **2018**, *358*, 155–164. [CrossRef] [PubMed]
25. Xu, L.; Zhou, J.; Zheng, P.; Wan, X.; Zhu, L.; Huang, J.; Jiang, J.; Chen, Y.; Song, Z. Seasonal variation significantly influenced the stochasticity of community assembly of amphibian symbiotic bacteria. *Environ. Microbiol.* **2022**, *24*, 5734–5748. [CrossRef] [PubMed]
26. Zhou, J.Z.; Ning, D.L. Stochastic Community Assembly: Does It Matter in Microbial Ecology? *Microbiol. Mol. Biol. Rev.* **2017**, *81*, 10–1128. [CrossRef]
27. Chave, J. Neutral theory and community ecology. *Ecol. Lett.* **2004**, *7*, 241–253. [CrossRef]
28. Chesson, P. Mechanisms of maintenance of species diversity. *Annu. Rev. Ecol. Syst.* **2000**, *31*, 343–366. [CrossRef]
29. Hubbell, S.P. *The Unified Neutral Theory of Biodiversity and Biogeography*; Princeton University Press: Princeton, NJ, USA, 2001.
30. Dini-Andreote, F.; Stegen, J.C.; van Elsas, J.D.; Salles, J.F. Disentangling mechanisms that mediate the balance between stochastic and deterministic processes in microbial succession. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, E1326–E1332. [CrossRef]
31. Stegen, J.C.; Lin, X.; Fredrickson, J.K.; Chen, X.; Kennedy, D.W.; Murray, C.J.; Rockhold, M.L. Quantifying community assembly processes and identifying features that impose them. *ISME J.* **2013**, *7*, 2069. [CrossRef]
32. Stegen, J.C.; Lin, X.; Fredrickson, J.K.; Konopka, A.E. Estimating and mapping ecological processes influencing microbial community assembly. *Front. Microbiol.* **2015**, *6*, 370. [CrossRef] [PubMed]
33. Vellend, M. Conceptual synthesis in community ecology. *Q. Rev. Biol.* **2010**, *85*, 183–206. [CrossRef] [PubMed]
34. Vellend, M.; Srivastava, D.S.; Anderson, K.M.; Brown, C.D.; Jankowski, J.E.; Kleynhans, E.J.; Kraft, N.J.B.; Letaw, A.D.; Macdonald, A.A.M.; Maclean, J.E.; et al. Assessing the relative importance of neutral stochasticity in ecological communities. *Oikos* **2014**, *123*, 1420–1430. [CrossRef]

35. Ning, D.; Yuan, M.; Wu, L.; Zhang, Y.; Guo, X.; Zhou, X.; Yang, Y.; Arkin, A.P.; Firestone, M.K. A quantitative framework reveals ecological drivers of grassland microbial community assembly in response to warming. *Nat. Commun.* **2020**, *11*, 4717. [CrossRef] [PubMed]
36. Yuan, S.; Xu, R.; Wang, D.; Lin, Q.; Zhou, S.; Lin, J.; Xia, L.; Fu, Y.; Gan, Z.; Meng, F. Ecological linkages between a biofilm ecosystem and reactor performance: The specificity of biofilm development phases. *Environ. Sci. Technol.* **2021**, *55*, 11948–11960. [CrossRef] [PubMed]
37. Zhang, Z.; Deng, Y.; Feng, K.; Cai, W.; Li, S.; Yin, H.; Xu, M.; Ning, D.; Qu, Y. Deterministic assembly and diversity gradient altered the biofilm community performances of bioreactors. *Environ. Sci. Technol.* **2019**, *53*, 1315–1324. [CrossRef]
38. Zhou, J.; Liu, W.; Deng, Y.; Jiang, Y.H.; Xue, K.; He, Z.; Van Nostrand, J.D.; Wu, L.; Yang, Y.; Wang, A. Stochastic assembly leads to alternative communities with distinct functions in a bioreactor microbial community. *Mbio* **2013**, *4*, 10–1128. [CrossRef] [PubMed]
39. Zielinski, W.; Korzeniewska, E.; Harnisz, M.; Drzymala, J.; Felis, E.; Bajkacz, S. Wastewater treatment plants as a reservoir of integrase and antibiotic resistance genes—An epidemiological threat to workers and environment. *Environ. Int.* **2021**, *156*, 106641. [CrossRef]
40. Pruden, A. Balancing water sustainability and public health goals in the face of growing concerns about antibiotic resistance. *Environ. Sci. Technol.* **2014**, *48*, 5–14. [CrossRef]
41. Berendonk, T.U.; Manaia, C.M.; Merlin, C.; Fatta-Kassinos, D.; Cytryn, E.; Walsh, F.; Bürgmann, H.; Sørum, H.; Norström, M.; Pons, M.N.; et al. Tackling antibiotic resistance: The environmental framework. *Nat. Rev. Microbiol.* **2015**, *13*, 310–317. [CrossRef]
42. Jäger, T.; Hembach, N.; Elpers, C.; Wieland, A.; Alexander, J.; Hiller, C.; Krauter, G.; Schwartz, T. Reduction of antibiotic resistant bacteria during conventional and advanced wastewater treatment, and the disseminated loads released to the environment. *Front. Microbiol.* **2018**, *9*, 2599. [CrossRef] [PubMed]
43. Osińska, A.; Korzeniewska, E.; Harnisz, M.; Felis, E.; Bajkacz, S.; Jachimowicz, P.; Niestępski, S.; Konopka, I. Small-scale wastewater treatment plants as a source of the dissemination of antibiotic resistance genes in the aquatic environment. *J. Hazard. Mater.* **2020**, *381*, 121221. [CrossRef] [PubMed]
44. Orem, W.; Tatu, C.; Varonka, M.; Lerch, H.; Bates, A.; Engle, M.; Crosby, L.; McIntosh, J. Organic substances in produced and formation water from unconventional natural gas extraction in coal and shale. *Int. J. Coal Geol.* **2014**, *126*, 20–31. [CrossRef]
45. Acharya, S.M.; Chakraborty, R.; Tringe, S.G. Emerging trends in biological treatment of wastewater from unconventional oil and gas extraction. *Front. Microbiol.* **2020**, *11*, 569019. [CrossRef] [PubMed]
46. Wu, J.; Xiang, J.; Zeng, Q.; Liu, Q.; Li, Y.; Sun, Y.; Xie, K.; Shi, S.; Gong, Z. Mitigation of the negative impacts of 84 disinfectant exposure for phenol treatment by an electro-enhanced SBR: Performance, oxidative stress, iron homeostasis and antibiotic resistance genes. *J. Water Process.* **2023**, *55*, 104161. [CrossRef]
47. Zhou, S.; Peng, S.; Li, Z.; Zhang, D.; Zhu, Y.; Li, X.; Hong, M.; Li, W.; Lu, P. Characterization of microbial communities and functions in shale gas wastewaters and sludge: Implications for pretreatment. *J. Hazard. Mater.* **2022**, *424*, 127649. [CrossRef]
48. Liu, Q.; Li, Y.; Sun, Y.; Xie, K.; Zeng, Q.; Hao, Y.; Yang, Q.; Pu, Y.; Shi, S.; Gong, Z. Deterioration of sludge characteristics and promotion of antibiotic resistance genes spread with the co-existing of polyvinylchloride microplastics and tetracycline in the sequencing batch reactor. *Sci. Total Environ.* **2024**, *906*, 167544. [CrossRef]
49. Revelle, W.; Revelle, M.W. Package 'psych'. *Compr. R Arch. Netw.* **2015**, *337*. Available online: https://personality-project.org/r/psych/ (accessed on 1 January 2024).
50. Ma, F.; Guo, J.B.; Zhao, L.J.; Chang, C.C.; Cui, D. Application of bioaugmentation to improve the activated sludge system into the contact oxidation system treating petrochemical wastewater. *Bioresour. Technol.* **2009**, *100*, 597–602. [CrossRef]
51. Ma, W.; Han, Y.; Xu, C.; Han, H.; Ma, W.; Zhu, H.; Li, K.; Wang, D. Enhanced degradation of phenolic compounds in coal gasification wastewater by a novel integration of micro-electrolysis with biological reactor (MEBR) under the micro-oxygen condition. *Bioresour. Technol.* **2018**, *251*, 303–310. [CrossRef]
52. Huang, C.; Shi, Y.; Sheng, Z.; El-Din, M.G.; Liu, Y. Characterization of microbial communities during start-up of integrated fixed-film activated sludge (IFAS) systems for the treatment of oil sands process-affected water (OSPW). *Biochem. Eng. J.* **2017**, *122*, 123–132. [CrossRef]
53. Kim, E.; Yulisa, A.; Kim, S.; Hwang, S. Monitoring microbial community structure and variations in a full-scale petroleum refinery wastewater treatment plant. *Bioresour. Technol.* **2020**, *306*, 123178. [CrossRef] [PubMed]
54. Wang, Q.; Kong, J.; Liang, J.; El-Din, M.G.; Zhao, P.; Xie, W.; Chen, C. Nitrogen removal intensification of aerobic granular sludge through bioaugmentation with "heterotrophic nitrification-aerobic denitrification" consortium during petroleum wastewater treatment. *Bioresour. Technol.* **2022**, *361*, 127719. [CrossRef] [PubMed]
55. Chu, L.; Wang, J. Treatment of oil-field produced wastewater by electron beam technology: Demulsification, disinfection and oil removal. *J. Clean. Prod.* **2022**, *378*, 134532. [CrossRef]
56. Graham, E.D.; Tully, B.J. Marine *Dadabacteria* exhibit genome streamlining and phototrophy-driven niche partitioning. *ISME J.* **2021**, *15*, 1248–1256. [CrossRef] [PubMed]
57. Li, H.; Sun, H.; Wang, X.; Li, F.; Cao, L.; Li, Y.; Dong, R.; Sun, Y.; Sun, P.; Bao, M. Temporal and spatial variation of petroleum hydrocarbons and microbial communities during static release of oil pollution sediments. *Front. Mar. Sci.* **2022**, *9*, 1025612. [CrossRef]

58. Gao, Y.; Yuan, L.; Du, J.; Wang, H.; Yang, X.; Duan, L.; Zheng, L.; Bahar, M.M.; Zhao, Q.; Zhang, W.; et al. Bacterial community profile of the crude oil-contaminated saline soil in the Yellow River Delta Natural Reserve, China. *Chemosphere* **2022**, *289*, 133207. [CrossRef] [PubMed]
59. Hu, P.; Tom, L.; Singh, A.; Thomas, B.C.; Baker, B.J.; Piceno, Y.M.; Andersen, G.L.; Banfield, J.F. Genome-resolved metagenomic analysis reveals roles for Candidate phyla and other microbial community members in biogeochemical transformations in oil reservoirs. *mBio* **2016**, *7*, 10–1128. [CrossRef]
60. Nesbø, C.L.; Farrell, A.A.; Zhaxybayeva, O.; L'Haridon, S. *Thermotoga, Bergey's Manual of Systematics of Archaea and Bacteria*; Wiley: Hoboken, NJ, USA, 2019; pp. 1–13. [CrossRef]
61. Shahi, A.; Aydin, S.; Ince, B.; Ince, O. Reconstruction of bacterial community structure and variation for enhanced petroleum hydrocarbons degradation through biostimulation of oil contaminated soil. *Chem. Eng. J.* **2016**, *306*, 60–66. [CrossRef]
62. He, J.; Luo, T.; Shi, Z.; Angelidaki, I.; Zhang, S.; Luo, G. Microbial shifts in anaerobic digestion towards phenol inhibition with and without hydrochar as revealed by metagenomic binning. *J. Hazard. Mater.* **2022**, *440*, 129718. [CrossRef]
63. Yoshikawa, M.; Zhang, M.; Toyota, K. Integrated anaerobic-aerobic biodegradation of multiple contaminants including chlorinated ethylenes, benzene, toluene, and dichloromethane. *Water Air Soil Pollut.* **2017**, *228*, 25. [CrossRef] [PubMed]
64. Segura, A.; Hernández-Sánchez, V.; Marqués, S.; Molina, L. Insights in the regulation of the degradation of PAHs in *Novosphingobium* sp. HR1a and utilization of this regulatory system as a tool for the detection of PAHs. *Sci. Total Environ.* **2017**, *590*, 381–393. [CrossRef] [PubMed]
65. Hou, J.Y.; Liu, W.X.; Wang, B.B.; Wang, Q.L.; Luo, Y.M.; Franks, A.E. PGPR enhanced phytoremediation of petroleum contaminated soil and rhizosphere microbial community response. *Chemosphere* **2015**, *138*, 592–598. [CrossRef] [PubMed]
66. de la Cueva, S.C.; Rodríguez, C.H.; Cruz, N.O.S.; Contreras, J.A.R.; Miranda, J.L. Changes in bacterial populations during bioremediation of soil contaminated with petroleum hydrocarbons. *Water Air Soil Pollut.* **2016**, *227*, 91. [CrossRef]
67. Magdy, M.M.; Gaber, Y.; Sebak, M.; Azmy, A.F.; AbdelGhani, S. Different metabolic pathways involved in anthracene biodegradation by *Brevibacillus*, *Pseudomonas* and *Methylocystis* Species. *Beni-Suef Univ. J. Basic Appl. Sci.* **2022**, *11*, 4. [CrossRef]
68. Liang, J.; Chen, C.; Yoza, B.A.; Liang, Y.; Li, J.; Ke, M. Hydrolysis and acidification of activated sludge from a petroleum refinery. *Pet. Sci.* **2019**, *16*, 428–438. [CrossRef]
69. Feitkenhauer, H.; Müller, R. Degradation of polycyclic aromatic hydrocarbons and long chain alkanes at 6070 °C by *Thermus* and *Bacillus* spp. *Biodegradation* **2003**, *14*, 367–372. [CrossRef]
70. Verde, L.C.L.; Silva, T.R.E.; Dellagnezze, B.M.; Neto, E.V.D.S.; de Oliveira, V.M. Diversity of Hydrocarbon-Related Catabolic Genes in Oil Samples from Potiguar Basin (Rn, Brazil). *Biotechnology* **2013**, *2013*, 1–7.
71. Albuquerque, L.; Rainey, F.A.; Nobre, M.F.; da Costa, M.S. *Schleiferia thermophila* gen. nov., sp. nov., a slightly thermophilic bacterium of the phylum '*Bacteroidetes*' and the proposal of *Schleiferiaceae* fam. Nov. *Int. J. Syst. Evol. Microbiol.* **2011**, *61*, 2450–2455. [CrossRef]
72. McKew, B.A.; Coulon, F.; Osborn, A.M.; Timmis, K.N.; McGenity, T.J. Determining the identity and roles of oil-metabolizing marine bacteria from the Thames estuary, UK. *Environ. Microbiol.* **2007**, *9*, 165–176. [CrossRef]
73. Wang, B.; Lai, Q.; Cui, Z.; Tan, T.; Shao, Z. A pyrene-degrading consortium from deep-sea sediment of the West Pacific and its key member *Cycloclasticus* sp. P1. *Environ. Microbiol.* **2008**, *10*, 1948–1963. [CrossRef] [PubMed]
74. Guo, J.; Wu, X.; Liu, S.; Zhang, Y.; Cai, Z. Screening of three ammonia-oxidizing bacteria and construction of compounding agent CCZU C6 in high-efficiency ammonia-oxidizing. *J. Water Process Eng.* **2021**, *40*, 101862. [CrossRef]
75. Qian, Y.; Xu, M.; Deng, T.; Hu, W.; He, Z.; Yang, X.; Wang, B.; Song, D.; Chen, L.; Huang, Y.; et al. Synergistic interactions of *Desulfovibrio* and *Petrimonas* for sulfate-reduction coupling polycyclic aromatic hydrocarbon degradation. *J. Hazard. Mater.* **2021**, *407*, 124385. [CrossRef] [PubMed]
76. Dworkin, M.; Falkow, S.; Rosenberg, E.; Schleifer, K.H.; Stackebrandt, E. *The Prokaryotes: A Handbook on the Biology of Bacteria*; Archaea. Bacteria: Firmicutes, Actinomycetes; Springer: New York, NY, USA, 2006; Volume 1. [CrossRef]
77. Van Houghton, B.D.; Acharya, S.M.; Rosenblum, J.S.; Chakraborty, R.; Tringe, S.G.; Cath, T.Y. Membrane bioreactor pretreatment of high-salinity O&G produced water. *ACS ES&T Water* **2022**, *2*, 484–494. [CrossRef]
78. Valentín-Vargas, A.; Toro-Labrador, G.; Massol-Deyá, A.A. Bacterial community dynamics in full-scale activated sludge bioreactors: Operational and ecological factors driving community assembly and performance. *PLoS ONE* **2012**, *7*, e42524. [CrossRef] [PubMed]
79. Sun, C.; Zhang, B.; Ning, D.; Zhang, Y.; Dai, T.; Wu, L.; Li, T.; Liu, W.; Zhou, J.; Wen, X. Seasonal dynamics of the microbial community in two full-scale wastewater treatment plants: Diversity, composition, phylogenetic group based assembly and co-occurrence pattern. *Water Res.* **2021**, *200*, 117295. [CrossRef] [PubMed]
80. Sierra-Garcia, I.N.; Belgini, D.R.B.; Torres-Ballesteros, A.; Paez-Espino, D.; Capilla, R.; Neto, E.V.S.; Gray, N.; de Oliveira, V.M. In depth metagenomic analysis in contrasting oil wells reveals syntrophic bacterial and archaeal associations for oil biodegradation in petroleum reservoirs. *Sci. Total Environ.* **2020**, *715*, 136646. [CrossRef]
81. Das, N.; Chandran, P. Microbial degradation of petroleum hydrocarbon contaminants: An overview. *Biotechnol. Res. Int.* **2011**, *2011*, 941810. [CrossRef]
82. Li, B.B.; Zhi, L.L.; Peng, Z.Y.; Ma, X.X.; Li, J. Contrasting distribution of antibiotic resistance genes and microbial communities in suspended activated sludge versus attached biofilms in an integrated fixed film activated sludge (IFAS) system. *Sci. Total Environ.* **2020**, *742*, 140481. [CrossRef]

83. Geissen, V.; Gomez-Rivera, P.; Lwanga, E.H.; Mendoza, R.B.; Narcías, A.T.; Mar-cías, E.B. Using earthworms to test the efficiency of remediation of oil-polluted soil in tropical Mexico. *Ecotox. Environ. Saf.* **2008**, *71*, 638–642. [CrossRef]
84. Du, J.; Ren, H.Q.; Geng, J.J.; Zhang, Y.; Xu, K.; Ding, L.L. Occurrence and abundance of tetracycline, sulfonamide resistance genes, and class 1 integron in five wastewater treatment plants. *Environ. Sci. Pollut. Res.* **2014**, *21*, 7276–7284. [CrossRef]
85. Sun, H.; Zhang, H.; Wu, D.; Ding, J.; Niu, Y.; Jiang, T.; Yang, X.; Liu, Y. Deciphering the antibiotic resistome and microbial community in municipal wastewater treatment plants at different elevations in eastern and western China. *Water Res.* **2023**, *229*, 119461. [CrossRef]
86. Xu, M.; Gao, P.; Chen, H.Q.; Shen, X.X.; Xu, R.Z.; Cao, J.S. Metagenomic insight into the prevalence and driving forces of antibiotic resistance genes in the whole process of three full-scale wastewater treatment plants. *J. Environ. Manag.* **2023**, *344*, 118369. [CrossRef]
87. Petrovich, M.; Chu, B.; Wright, D.; Griffin, J.; Elfeki, M.; Murphy, B.T.; Poretsky, R.; Wells, G. Antibiotic resistance genes show enhanced mobilization through suspended growth and biofilm-based wastewater treatment processes. *FEMS Microbiol. Ecol.* **2018**, *94*, fiy041. [CrossRef]
88. Li, B.; Qiu, Y.; Zhang, J.; Huang, X.; Shi, H.C.; Yin, H.B. Real-time study of rapid spread of antibiotic resistance plasmid in biofilm using microfluidics. *Environ. Sci. Technol.* **2018**, *52*, 11132–11141. [CrossRef]
89. Qiu, Y.; Zhang, J.; Li, B.; Wen, X.H.; Liang, P.; Huang, X. A novel microfluidic system enables visualization and analysis of antibiotic resistance gene transfer to activated sludge bacteria in biofilm. *Sci. Total Environ.* **2018**, *642*, 582–590. [CrossRef]
90. Król, J.E.; Nguyen, H.D.; Rogers, L.M.; Beyenal, H.; Krone, S.M.; Top, E.M. Increased transfer of a multidrug resistance plasmid in *Escherichia coli* biofilms at the air-liquid interface. *Appl. Environ. Microbiol.* **2011**, *77*, 5079–5088. [CrossRef] [PubMed]
91. Stalder, T.; Top, E. Plasmid transfer in biofilms: A perspective on limitations and opportunities. *NPJ Biofilms Microbiomes* **2016**, *2*, 16022. [CrossRef]
92. Wu, Y.; Qi, D.; Yao, H.; Ren, J.; Hu, J.; Lyu, Y.; Yang, S.; Sun, W. Antibiotic resistome and its driving factors in an urban river in northern China. *Sci. Total Environ.* **2022**, *838*, 156536. [CrossRef] [PubMed]
93. Zeng, J.Y.; Li, Y.Y.; Jin, G.P.; Su, J.Q.; Yao, H.Y. Short-term benzalkonium chloride (C_{12}) exposure induced the occurrence of wide-spectrum antibiotic resistance in agricultural soils. *Environ. Sci. Technol.* **2022**, *56*, 15054–15063. [CrossRef] [PubMed]
94. Ochman, H.; Lawrence, J.G.; Groisman, E.A. Lateral gene transfer and the nature of bacterial innovation. *Nature* **2000**, *405*, 299–304. [CrossRef] [PubMed]