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Original Research

Plastic leachates drive conjugative transfer of antibiotic resistance genes

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ABSTRACT

Plastic pollution pervades aquatic ecosystems worldwide, releasing leachates that interact intimately with microbial communities. Antibiotic resistance genes (ARGs) disseminate rapidly through horizontal gene transfer via plasmid conjugation, posing a severe and accelerating threat to public health and environmental stability. While microplastic particles are known to promote ARG exchange within biofilms, the influence of soluble chemical leachates derived from degrading plastics has remained unclear. Here we show that photodegraded leachate from polyvinyl chloride (PVC)—a widely used material in water infrastructure—substantially enhances conjugative transfer of ARGs in both laboratory model systems and natural aquatic microbiomes. Exposure increased transconjugant abundance up to 26.4-fold and conjugation efficiency up to 44.6-fold, with non-monotonic responses modulated by leachate concentration and microbial community diversity. Characterization of the leachate revealed high proportions of biolabile dissolved organic matter alongside additives; mechanistic assays demonstrated that these effects arise through elevated intracellular reactive oxygen species (21% increase), activation of the SOS response and DNA-repair pathways, increased extracellular protein production facilitating cell–cell contact, and compensatory adjustments in the electron transport chain that maintain ATP homeostasis. These results demonstrate that plastic leachates act as potent but previously overlooked facilitators of ARG dissemination beyond the physical effects of microplastics. Our findings reveal a critical synergy between plastic pollution and the global antimicrobial-resistance crisis, underscoring the urgent need for targeted regulations on plastic additives and degradation products in aquatic systems.

1. Introduction

Antibiotics are widely used in various fields, including clinical treatment and animal husbandry [1,2]. However, their extensive use has accelerated the proliferation of antibiotic resistance genes (ARGs) in both environmental and clinical strains, posing a significant threat to human health [3–6]. ARGs are widely distributed across soil, lakes, oceans, and human-associated environments, such as drinking water and coastal waters [7,8]. Among the mechanisms driving ARG dissemination, horizontal gene transfer (HGT), particularly via plasmid conjugation [9], plays a pivotal role by facilitating gene exchange across broad bacterial taxa, often

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transcending species boundaries [10,11].

Plastic pollution, especially microplastics, has become ubiquitous worldwide and has reached even remote ecosystems such as the Arctic [12]. Microplastics serve as substrates for microbial colonization ("plastisphere") [13–17], altering microbial community composition and promoting the proliferation of resistant microbes, including potential pathogens [18–21]. Plastic particles have been shown to significantly influence plastisphere formation and the associated expansion of the microbial resistome, for example, by increasing bacterial conjugation efficiency by 1.4–1.7-fold [13,14,22]. However, the effects of plastic leachate have received comparably less attention [23]. Leachates are highly mobile in water [24] and release substantial amounts of dissolved organic carbon, potentially enhancing microbial growth and ARG dissemination [25].

Plastic additives, including plasticizers, stabilizers, and flame retardants [26], often leach into the environment and exert toxic effects on aquatic organisms. For instance, Bejgarn et al. demonstrated that plastic leachate under sunlight exposure can cause acute toxicity in *Daphnia magna* [27,28]. Leachates containing compounds such as di-2-ethylhexyl phthalate (DEHP) and dimethyl phthalate (DMP) can modulate ARG transfer and bacterial mobility in a concentration-dependent manner [29,30]. Additionally, dissolved organic matter (DOM) released from plastics during mechanical, photochemical, and biological degradation may serve as an additional carbon source, thereby stimulating bacterial growth [31–33]. However, studies examining the ecological effects of plastic leachate on microbiome metabolic activity, community stability, and ARG dissemination remain limited. Although some studies have investigated the effects of specific leachate components (e.g., DEHP and DMP) on ARG spread [29,30], such single-compound approaches cannot reflect the realistic mixture effects of whole plastic leachate. This leads to considerable uncertainty in environmental risk assessment and modeling, as the combined impacts of complex plastic leachate on conjugative ARG transfer in aquatic ecosystems remain largely underexplored.

Given the widespread use of polyvinyl chloride (PVC), whose global production increased from over 20 million tons annually in 2004 to 38 million tons in 2015 [34,35], particularly in pipeline systems within wastewater and sewage infrastructure [36,37], and its relatively high chemical instability and leaching potential, PVC was selected as the target material in this study. Compared with other plastics such as polyethylene (PE), polypropylene (PP), and polyethylene terephthalate (PET), PVC contains chlorine-based stabilizers and plasticizers (e.g., phthalates) that can degrade into chlorinated organic compounds, heavy metal ions, and endocrine-disrupting chemicals, thereby posing more complex environmental risks [28]. To simulate environmental aging, photodegradation was employed because it accelerates DOM release, including plastic additives, and better represents environmental conditions than thermal or biodegradation processes [25,33].

This study investigated the effects of plastic leachate on the conjugative transfer of ARGs in aquatic environments, analyzed the molecular composition of leachate-derived DOM using Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS), and assessed its effects on conjugative plasmid transfer in both natural and anthropogenically impacted waters. To elucidate underlying mechanisms, intracellular reactive oxygen species (ROS), extracellular polymeric substances (EPS), and adenosine triphosphate (ATP) were measured in donor and recipient *Escherichia coli* (*E. coli*). Overall, this study highlights the ecological consequences of plastic leachate and emphasizes the need for strategies to mitigate associated public health risks [38,39].

2. Materials and methods

2.1. Preparation of plastic leachate

PVC chips with an average size of 0.075 mm were produced by shredding water pipes with a shredder (FW100, Tianjin Taisite Instrument Co., Ltd., Tianjin, China) and sieving through a stainless-steel mesh (150 mesh). The shredding chamber was made of stainless steel, and the blades were composed of alloy steel. Prior to leachate preparation, the shredder chamber, blades, stainless-steel mesh, PVC chips, and all glassware were thoroughly rinsed with deionized (DI) water to minimize potential contamination during sample processing.

Plastic leachate was prepared following a previously reported protocol [25], based on the general procedure described by Romera-Castillo et al., with modifications for the present study. Specifically, a single plastic type was used; exposure was conducted under natural solar radiation for 17 days instead of artificial light for 6–30 days; filtration was performed using a 0.22-μm polytetrafluoroethylene (PTFE) filter membrane (Tianjin Taisite Instrument Co., Ltd., Tianjin, China) rather than 0.8-μm glass microfiber filter; and dilution ratios and the experimental design were adjusted accordingly. Briefly, PVC chips were soaked in 500 mL of water at a mass ratio of 1:25 (PVC:water) in a glass flask. The mixture was then exposed to solar radiation for 17 days to simulate natural leaching.

This PVC-to-water ratio and particle size were selected to generate a high-concentration, scenario-based leachate representing intensified exposure conditions (e.g., near-shore or accumulation zones with elevated plastic density) rather than average environmental concentrations. Samples collected on day 0 (prior to solar exposure) and day 17 (after photodegradation) were used for comparative analyses to assess compositional changes during leaching. Daily solar and ultraviolet (UV) irradiance data were obtained from the prediction of worldwide energy resources (NASA/POWER) database (Supplementary Table S1).

After irradiation, the mixture was filtered through a 0.22-μm PTFE membrane. The filtrate was defined as the leachate and stored at 4 °C for subsequent use. The same batch of leachate was used consistently across all subsequent physicochemical analyses and exposure experiments. During total organic carbon (TOC) and FT-ICR MS analyses, DI water was used for instrument calibration and baseline correction. Prior to experiments, the leachate was diluted with DI water at ratios of 1:1, 1:5, 1:10, 1:20, and 1:40 to approximate environmentally relevant exposure scenarios. These treatments were designated as undiluted, L5, L10, L20, and L40, respectively.

2.2. Analysis of plastic leachate composition

TOC contents in leachate samples at different dilutions were measured using a TOC-L CSH/CSN (Shimadzu Corp., Kyoto, Japan) (Supplementary Table S2). The molecular composition of DOM in the leachate was characterized using FT-ICR MS (see Supplementary Text S1 for details).

2.3. Determination of bacterial conjugation efficiency of aquatic microbiomes

2.3.1. Water sample collection

Surface water samples were collected in November 2023 from two sites: the Dasha River (22.59° N, 114.00° E), representing less disturbed water, and the receiving water of the Shenzhen Futian Wastewater Treatment Plant (22.52° N, 113.96° E), representing highly disturbed water (hereafter referred to as receiving water)

(Supplementary Fig. S1). The Dasha River is a natural water body with minimal anthropogenic influence, whereas the receiving water is strongly affected by terrestrial inputs.

To assess the impact of anthropogenic activity on microbial communities and gene transfer, these two contrasting environments were selected. The comparison between less-disturbed water (LDW) and highly disturbed water (HDW) provides insight into how environmental conditions influence microbial community structure and the potential for horizontal gene transfer. At each site, 2 L of surface water was collected and transported to the laboratory under cooled conditions. Upon arrival, samples were pre-filtered through a 0.45-μm polyethersulfone membrane using vacuum filtration to remove debris. Microbial biomass retained on the filters was resuspended in phosphate-buffered saline (PBS) and used as donor communities in subsequent filter mating experiments (Supplementary Text S2).

2.3.2. Filter mating experiment under PVC leachate exposure

Filter-mating experiments were conducted with modifications based on previous studies [40]. Environmental bacterial communities collected on filter membranes (Section 2.3.1) were used as putative plasmid-donor communities, representing potential sources of mobile resistance plasmids rather than predefined donor strains. For each environmental water sample, three independent biological replicates were prepared.

The recipient strain, *E. coli* strain sp. 758 (*E. coli* gfp758), was labeled with green fluorescent protein (GFP) and exhibited resistance to kanamycin (KAN) and tetracycline (TET). Its antibiotic resistance profile was validated using control cultures, confirming resistance to KAN and TET and sensitivity to ampicillin (AMP) (Supplementary Fig. S2). The strain has been fully sequenced using long-read technology and assembled into a complete genome (BioProject PRJNA968142), providing a reference for genetic and plasmid analyses. Thus, colonies appearing on selective LB agar containing 50 μg mL^{-1} KAN, 20 μg mL^{-1} TET, and 100 mg L^{-1} AMP were identified as true transconjugants that acquired AMP resistance via donor plasmids.

After cultivation and quantification of donor and recipient cells (Supplementary Text S3), the two populations were mixed at a 10:1 ratio. PVC leachate was added at a range of dilution gradients to the experimental groups, while control groups without leachate were included to determine baseline conjugation efficiency. The mixtures were immediately dispensed onto 25-mm sterilized membranes (GN-6, Pall Corp., Port Washington, New York, USA) and incubated overnight at 37 °C following established environmental plasmid conjugation protocols [9,41] to maximize conjugation efficiency and detection sensitivity.

Following incubation, mating complexes were recovered by vortexing membranes in 10 mL PBS. From each replicate and each treatment (including controls), 100 μL of the suspension was plated onto selective Luria–Bertani (LB) agar containing 50 μg mL^{-1} KAN, 20 μg mL^{-1} TET, and 100 mg L^{-1} AMP to isolate transconjugants. GFP fluorescence was used as a qualitative genetic marker to confirm the presence of recipient-derived transconjugants. The total experimental duration was approximately 24 h, including conjugation (~16 h) and plate incubation (~8 h). Fluorescence detection was conducted using brief UV/blue light exposure to minimize photobleaching. AMP was selected as the plasmid capture marker because it is a representative β-lactam antibiotic widely used to screen plasmid-mediated resistance and is relevant to clinically important carbapenem-associated resistance pathways [42–44].

Each filter-mating experiment was conducted in three independent biological replicates. For each replicate, the resulting mating mixture was serially diluted and plated onto multiple selective LB agar (technical replicates). To ensure reliable and consistent quantification of conjugation efficiency, we used only LB agar containing 20–30 well-isolated, non-overlapping green-fluorescent *E. coli* gfp758 colonies for enumeration. LB agar with fewer colonies are prone to stochastic variation, whereas LB agar with higher colony densities compromise accurate counting and subsequent single-colony isolation due to colony overlap and interference from background-resistant environmental bacteria. Only colonies exhibiting clearly detectable green fluorescence were counted, thereby minimizing potential misidentification arising from weak or ambiguous signals. Conjugation efficiency was calculated as follows:

$$\eta = \frac{C_{\text{trans}}}{C_{\text{donor}}} \quad (1)$$

where η denotes conjugation efficiency; C_{trans} is the measured transconjugant concentration, which refers to the colony forming units (CFUs) of colonies resistant to AMP, KAN, and TET and exhibiting green fluorescence under excitation; C_{donor} is the donor concentration, which refers to the CFUs of potential donor cells present in the environmental sample used in the filter mating experiment.

2.3.3. Plasmid and genomic DNA extraction and sequencing

For each mating experiment, we collected plasmids derived from the conjugation process (Section 2.3.2) by selecting at least 300 green fluorescent colonies from selective LB agar and transferring them into 5 mL of PBS. Subsequently, 100 μL of this suspension was inoculated into 10 mL of selective liquid LB medium containing the same antibiotic combination, and the mixture was incubated overnight. Conjugative plasmids and metagenomic DNA from environmental water samples were then extracted and sequenced using a PE150 strategy on the Illumina Novaseq platform (see Supplementary Text S4 for details).

In the filter-mating experiments, biological replicates from the two environmental water samples were not pooled; instead, they were sequenced separately, resulting in six metagenomic datasets. Similarly, plasmid DNA extracted from selected colonies was sequenced independently, yielding 12 plasmidome datasets (Supplementary Table S3).

2.3.4. Analysis of microbial community

Metagenomic sequencing reads from the two environmental water samples were deduplicated and quality-filtered using Trimmomatic [45]. The processed reads were taxonomically annotated using the MetaPhlAn pipeline to generate species abundance profiles [46]. Alpha diversity was assessed using the Shannon and Simpson diversity indices, and principal component analysis (PCA) of microbial composition was performed using the vegan, dplyr, tidyr, and ggplot2 packages in R [47,48].

2.3.5. Annotation of plasmids

Raw plasmidome data were first quality-filtered using FASTP [49]. Plasmid reads were aligned to the PLSDB database [50] using BLASTN [51], with a similarity threshold of 90% for plasmid identification. We further mapped the aligned reads to the plasmid database using Minimap2 [52], generating SAM files, which were converted to BAM files using Samtools [53]. We used these files to calculate mapping coverage and fragment length. A plasmid was considered present if more than 70% of its reference length was covered by sequencing reads.

Plasmid abundance was normalized by read count and plasmid length and expressed as reads per kilobase per million mapped reads (RPKM, *r*), calculated as follows:

$$r = \frac{N}{M} \times \frac{10^9}{L} \quad (2)$$

where N denotes mapped reads to plasmid, referring to the number of sequencing reads aligned to a specific plasmid; M is the total mapped reads, which refers to the total number of reads mapped to all reference sequences; and L denotes plasmid length (bp), referring to the length of the plasmid in base pairs. Differences in plasmid abundance during the conjugation process were determined based on RPKM values.

We annotated detected plasmids using the PlasmidFinder platform to determine incompatibility groups [54]. Predicted genes were identified using Prodigal [55] and annotated against the Argannot, VFDB, and CARD databases [56–58]. Sequence alignment was performed using BLASTN, with a threshold of >80% similarity and >70% coverage for the identification of ARGs and virulence factors (VFs) [59].

2.4. Plasmid RP4-Coupled transfer system exposed to PVC leachate for conjugative mechanism investigation

2.4.1. Construction of the RP4 transfer system

Two *E. coli* strains were used: *E. coli* HB101, which carries the RP4 plasmid encoding resistance to AMP, KAN, and TET (Amp^R, Kan^R, and Tet^R), and *E. coli* NK5449, which carries rifampicin resistance (Rif^R) on its chromosome and does not harbor a plasmid. The conjugation system was established in LB liquid medium. The donor strain HB101 with the RP4 plasmid (Amp^R, Kan^R, and Tet^R) and the recipient strain NK5449 (Rif^R) were used as the donor and recipient, respectively.

We prepared three independent biological replicates, each initiated and processed independently from donor–recipient mixing through transconjugant recovery. Donor and recipient strains were cultured in antibiotic-supplemented LB medium, and cell concentrations were determined by plating on selective media (Supplementary Text S5). Optical density (OD600) was measured using a UVmini-1285 spectrophotometer (Shimadzu Corp., Kyoto, Japan), and both cultures were adjusted to an OD600 of 1. The donor and recipient cells were then mixed at a 1:1 ratio.

To evaluate the impact of plastic leachate, we added leachate at different concentrations to the conjugation transfer system, while control groups without leachate were included to establish a baseline conjugation efficiency. After incubation, the mixtures were plated on selective media, and transconjugant colonies were counted. For each biological replicate, colony enumeration was performed using a single selective plate. Individual colonies were subsequently transferred to a liquid medium for the collection of transconjugants (Supplementary Text S6). Additionally, donor–recipient mixtures were collected for further physiological and biochemical analyses [60].

2.4.2. Qualification of EPS content and intracellular ROS levels

EPS were extracted using a thermal method as described by Liao et al. [61]. Briefly, bacterial cells from each biological replicate were resuspended in 2 mL of 0.05% sterile NaCl solution and heated at 60 °C for 30 min. The samples were centrifuged at 4500 rpm for 5 min, and the supernatant was collected as total EPS. The EPS fractions were filtered through a 0.22-μm PTFE membrane. Polysaccharide content was quantified using the phenol-sulfuric acid method (with glucose as the standard), and protein content was measured using a Bradford protein assay kit (Nanjing Yifeixue Bioengineering Institute, Nanjing, Jiangsu, China) [61,62].

Intracellular ROS levels were measured by staining *E. coli* cells with 10 μmol L^{-1} 2′,7′-dichlorofluorescein diacetate (DCFH-DA) (Invitrogen Corp., Carlsbad, California, USA; λ_{ex} = 480 nm, λ_{em} = 525 nm) at 37 °C for 20 min. Fluorescence intensity was measured using a multifunctional microplate reader (Varioskan LUX, Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA). All measurements were performed with at least three independent biological replicates, including untreated controls.

2.4.3. Determination of antioxidative enzyme activity and ATP content

Antioxidative enzymes were extracted from *E. coli* cells following Gravina et al., with minor modifications [63–65]. Cells were collected and washed by centrifugation (15,000 rpm, 30 min, 4 °C) with PBS (1–2 washes), then disrupted using an ultrasonic cell pulverizer (SCIENTZ-IID, Ningbo Scientz Biotechnology Co., Ltd., Ningbo, Zhejiang, China) at 4 °C and 20 KHz for 15 min to obtain cell homogenates. The homogenates were centrifuged (15,000 rpm, 30 min, 4 °C), and the supernatants were used for enzyme activity assays. Total superoxide dismutase activity was measured using a nitro-blue tetrazolium (NBT)-based assay kit (Beyotime Biotechnology Co., Ltd., Shanghai, China) [66,67].

ATP content was determined using an Enhanced ATP Assay Kit (Beyotime Biotechnology Co., Ltd., Shanghai, China) [68,69]. Briefly, cells were lysed by adding 200 μL of lysis buffer to each well of a 96-well plate, followed by vortexing. The lysates were centrifuged at 12,000 rpm for 5 min at 4 °C, and the supernatants were used for colorimetric analysis.

2.4.4. RNA extraction, sequencing, and bioinformatic analysis

Samples were derived from conjugated cells exposed to PVC leachate, with three biological replicates per condition (Section 2.4.1). Each replicate was treated as an independent transcriptome sample for analysis. RNA was extracted, reverse-transcribed into cDNA, and sequenced. Sequencing data were processed for quality control, aligned to plasmid genomes, and quantified for gene expression. Differential expression analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed to identify significantly affected genes and associated biological pathways (Supplementary Text S7).

2.5. Statistical analysis

We used MetaPhlAn to obtain relative abundances of microbial taxa at the phylum and genus levels. Community composition was visualized using stacked bar charts (phylum level) and heatmaps (genus level). Besides, we conducted PCA on genus-level data to assess differences in microbial communities, followed by k-means clustering. Finally, we used one-way analysis of variance (ANOVA) and Tukey's honest significant difference test to evaluate statistical significance (Supplementary Text S8).

3. Results and discussion

3.1. Exploration of plastic leachate composition

This study aims to investigate the effects of plastic leachate on the horizontal transfer of ARGs in aquatic microbiomes. To this end, it is necessary to characterize both the quantity and molecular composition of DOM in the leachate. DOC concentrations in the leachate were 1.2 and 33.0 mg C L^{-1} before and after sunlight exposure, respectively (Supplementary Table S2). FT-ICR MS analysis further revealed an increase in the number of molecular formulas from 112 to 1,458, along with an increase in the average O/C ratio from 0.2 to 0.3 following sunlight exposure. Corresponding FT-ICR-MS parameters and data are provided in

Supplementary Table S4. These results indicate the formation of a substantial amount of oxidized, soluble products following photodegradation.

According to the molecular lability boundary (MLB) concept proposed by D'Andrilli et al., natural organic matter constituents above the MLB (H/C $\geq$ 1.5) correspond to more labile and less stable molecules, whereas those below the MLB (H/C < 1.5) are more recalcitrant [70]. Sheridan et al. further demonstrated that water samples containing higher proportions of labile molecules exhibit greater microbial biomass and diversity [33]. Based on these frameworks, molecules with H/C $\geq$ 1.5 were defined here as biolabile and readily available for microbial utilization. In this study, biolabile molecules accounted for 76.8% and 64.0% of the total molecules in the leachate before and after sunlight exposure, respectively (Fig. 1). Although the relative proportion of labile molecules decreased after 17 days of photodegradation, their absolute number increased substantially. This finding indicates that the leachate contains a greater abundance of labile compounds available for microbial utilization than natural freshwater standards, which typically contain only 19–30% labile molecules [71]. Therefore, plastic leachate contains a high proportion of biolabile compounds that can stimulate bacterial growth [72,73].

Previous research indicates that plastic leachate can contribute to DOC in aquatic environments, although the magnitude varies between freshwater and marine systems. Marine studies have reported that DOC released from plastic debris can substantially exceed natural background levels, with an annual contribution of up to 23,600 tons; in heavily polluted areas, leached DOC may account for up to 10% of DOC in the surface microlayer, a biologically active interface where organic matter and pollutants accumulate [25]. In freshwater systems, Sheridan et al. estimated an average leachate concentration of approximately 0.100–0.113 mg C L^{-1} in lakes based on typical plastic abundance and leaching rates, providing a reference for natural conditions [33]. In the present study, the plastic leachate prepared in freshwater contained 1.202 mg C L^{-1} DOC before and 32.96 mg C L^{-1} after light exposure, both of which substantially exceeded environmental reference values. Even after 40-fold dilution, the DOC concentration remained 0.99 mg C L^{-1}, still above the reference value but within the same order of magnitude. Given that DOC concentrations are likely elevated near shorelines or plastic accumulation zones, the exposure conditions used here represent a high-exposure scenario rather than average freshwater conditions. Accordingly, these results provide insight into microbial and plasmid responses under elevated exposure, rather than direct *in situ* measurements. Notably, the biolabile fraction of DOC derived from plastic leachate may enhance microbial growth and activity, potentially reshaping community structure and influencing carbon and nutrient cycling in plastic-affected ecosystems.

We analyzed the molecular composition of PVC leachate to predict putative plastic-derived DOM compounds (Supplementary Table S4). To evaluate environmental (ENV) and human health (HH) hazards, each compound was assigned a numerical hazard score based on harmonized classification and labeling under the European Union's Classification, Labeling and Packaging (CLP) classifications [74], following the method of Lithner et al. [75]. In this framework, hazard categories (e.g., carcinogenicity, acute toxicity, aquatic toxicity) are converted into numerical scores (10–10,000) and summed separately for ENV and HH risks, yielding total hazard scores ranging from 0 to 11,000, with higher values indicating greater potential hazard.

We identified 15 molecular components corresponding to common plastic additives (Table 1), including plasticizers, stabilizers, softeners, surfactants, and their degradation products. Several compounds (e.g., $C_{17}H_{28}O_2$, $C_{19}H_{32}O_3$, and $C_{20}H_{30}O_4$) were classified as endocrine-disrupting chemicals (EDCs). As reported by Tetu et al., high concentrations of plastic leachate may inhibit bacterial growth due to the presence of such toxic compounds [26,76]. In addition, certain additives in PVC leachate, particularly phthalates, have been shown to promote the spread of ARGs, thereby exacerbating environmental risks [30]. The analysis also identified compounds such as $C_{16}H_{22}O_4$, a plasticizer associated with both ENV and HH risks and classified as an EDC by the United Nations Environment Programme [77]. Although these toxic compounds account for only 0.088% of total detected molecules, their persistence and potential for bioaccumulation may influence

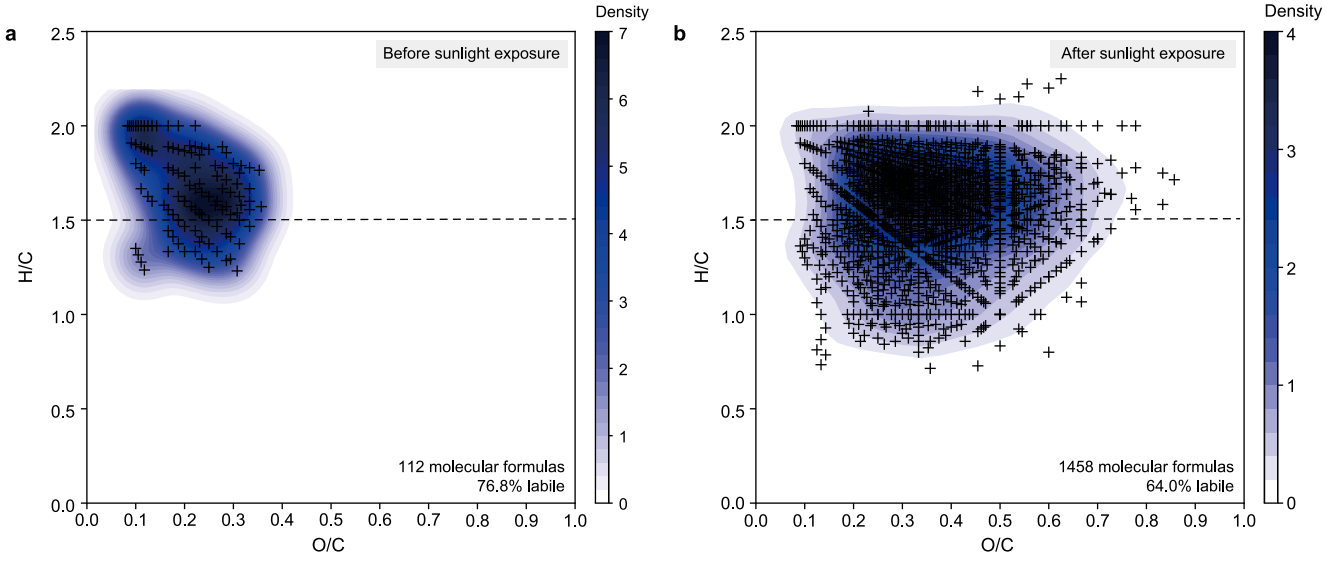

Fig. 1. Molecular composition and biolabile index of polyvinyl chloride (PVC) plastic leachate. Molecular formulas retrieved from Fourier transform ion cyclotron resonance mass spectrometry analysis of PVC leachate before (day 0; **a**) and after (day 17; **b**) sunlight exposure. "+" represents individual molecular formulae, and density represents the number of identical formulae along the H:C and O:C axes. Molecules with an H:C ratio ≥1.5 were categorized as biolabile based on the molecular lability boundary concept and evidence that water with higher proportions of labile molecules exhibits greater microbial biomass and diversity.

Table 1
Molecular formulas and toxicity analysis of assumed compounds unique for plastic leachate dissolved organic matter.

Molecular formula	Putative name	Chemical group	Main application	CAS No.	Sum hazard score[b]		EDC[c]	Abundance (%)[d]
					ENV	HH		
$C_{10}H_8O_6$	Naphthalene	Others	Monomer or intermediate	91-20-3	1100^{++}	110^{+}	-	0.008%
$C_{12}H_{14}O_4$	Diethyl phthalate	Phthalate	Plasticizer	84-66-2	NA	NA	UNEP	0.098%
$C_{14}H_{12}O_3$	2-hydroxy-4-methoxybenzophenone	Benzophenone	Stabilizer	131-57-7	NA	NA	UNEP	0.025%
$C_{14}H_{14}O_4$	Di-allyl phthalate	Phthalate	Plasticizer	131-17-9	1100^{++}	10^{+}	-	0.023%
$C_{14}H_{18}O_6$	Dimethoxyethyl phthalate	Phthalate	Plasticizer	117-82-8	NA	10000^{+++}	-	0.079%
$C_{16}H_{22}O_4$	Dibutyl phthalate (DBP)	Phthalate	Plasticizer	84-74-2	100^{+}	10000^{+++}	HH/REACH/ UNEP	0.088%
	Di-isobutyl phthalate (DiBP)			84-69-5	NA	10000^{+++}	REACH/UNEP	
$C_{17}H_{24}O_3$	7.9-di-tert-butyl-1-oxaspiro(4.5)deca-6.9-diene-2.8-dione	Others	Breakdown product	82304-66-3	NA	NA	-	0.021%
$C_{17}H_{28}O_2$	4-nonylphenol, branched, ethoxylated	NP/OP/NPS[a]	Surfactant or its degradation product	127087-87-0	NA	NA	ENV/REACH/ UNEP	0.009%
$C_{18}H_{18}O_5$	Oxydiethylene dibenzoate	Phthalate	Plasticizer/softener	120-55-8	NA	NA	-	0.014%
$C_{18}H_{24}O_6$	Butoxycarbonylmethyl butyl phthalate	Phthalate	Plasticizer	85-70-1	NA	NA	-	0.305%
$C_{18}H_{26}O_4$	Dipentyl phthalate	Methyl-phenoxide	Plasticizer	121-00-6	NA	NA	-	0.156%
$C_{19}H_{32}O_3$	Nonylphenol, ethoxylated	NP/OP/NPS[a]	Surfactant or its degradation product	9016-45-9	NA	NA	ENV/REACH/ UNEP	0.061%
$C_{20}H_{30}O_4$	Dihexyl phthalate	Phthalate	Plasticizer	84-75-3	NA	10000^{+++}	UNEP	0.017%
$C_{23}H_{40}O_5$	2- [2- [2- [2- (4-nonylphenoxy)Ethoxy]ethoxy]ethoxy]ethanol	NP/OP/NPS[a]	Surfactant or its degradation product	7311-27-5	NA	NA	ENV/REACH/ UNEP	0.032%
$C_{28}H_{50}O_8$	Nonoxynol-1	NP/OP/NPS[a]	Surfactant or its degradation product	26027-38-3	NA	NA	ENV/REACH/ UNEP	0.063%

Note.
[a] NP/OP/NPS: nonyl-phenol, octyl-phenol, and nonyl-phenol-related.
[b] Unified classification of environmental hazards (ENV) and human health (HH) hazards. Total hazard scores were derived from European Union's Classification, Labeling and Packaging (CLP) and European Chemical Agency (ECHA) using Lithner et al.'s gradation method [75]. Scores for ENV and HH ranged from 0 to 11,000: +, (0, 110]; ++, (110, 1100]; +++, (1,100, 11,000], with higher scores indicating greater hazards [74]. NA, not available.
[c] Classification as an endocrine-disrupting chemical (EDC) for ENV or HH effects within the Registration, Evaluation, Authorization, and Restriction of Chemicals (REACH) legislation [78] or recognition in the 2018 report by the United Nations Environment Programme (UNEP) as EDC [77]. -, not an EDC.
[d] The table was sorted in descending order based on the relative abundance of the corresponding chemicals.

microbial behavior and induce long-term biochemical changes in aquatic systems, warranting further investigation.

Overall, plastic leachate contains both biolabile DOM and mildly toxic additives and exhibits high chemical instability. This combination may simultaneously stimulate microbial growth and influence conjugative behavior. Accordingly, subsequent analyses focus on the effects of plastic leachate on microbial conjugative transfer, particularly its role in ARG dissemination.

3.2. Plastic leaching solution promotes plasmid conjugative behavior in aquatic microbiomes

The widespread use of plastics and their additives has led to a substantial increase in environmental plastic waste [35,79]. Since the mid-20th century, global plastic production has risen sharply, leading to the accumulation of plastic debris in aquatic ecosystems [35]. Compared with solid plastic particles, plastic leachates release a diverse array of additives and degradation products with higher bioactivity and toxicity [26,38]. These chemical constituents can significantly alter microbial community structure and function by influencing bacterial growth and metabolism [33]. Such changes may disrupt cellular physiology or increase selective pressure, thereby affecting conjugative transfer processes [80]. Previous studies have demonstrated that plastic biofilms facilitate the HGT of ARGs, highlighting the potential for plastic leachate to promote the dissemination of resistance in exposed microbial communities [20,81].

In this study, filter mating experiments were conducted using samples from LDW and HDW to assess the impact of plastic leachate on conjugative transfer across contrasting environmental conditions. Compared with the control group, low concentrations of PVC leachate (L40 and L10) significantly increased both the number of transconjugants and conjugation efficiency in samples from both LDW and HDW (Fig. 2a and b). The variability observed in the L10 treatment likely reflects the multi-step nature of the filter mating assay, which involves independent quantification of donor abundance, recovery of transconjugants, and subsequent calculation of conjugation efficiency.

At higher concentrations (L5), PVC leachate reduced conjugation efficiency in LDW but continued to enhance it in HDW. This non-linear response suggests that conjugation does not follow a simple concentration-dependent pattern. Alpha diversity analysis (Fig. 2c) showed that both the Shannon index ($P = 2.67 \times 10^{-5}$) and Simpson index ($P = 9.42 \times 10^{-6}$) were significantly higher in HDW than in LDW. Metagenomic profiling further indicated that LDW communities were dominated by Proteobacteria, whereas HDW communities were enriched in Actinobacteria and Bacteroidetes (Fig. 2c). Genus-level PCA and heatmap analyses confirmed these differences: *Polynucleobacter* and *Limnohabitans* dominated LDW, whereas *Flavobacterium* and *Cloacibacterium* were more prevalent in HDW (Fig. 2d; Supplementary Fig. S3).

To explain the observed non-linear responses and differences between LDW and HDW, we further considered potential mechanistic and ecological drivers. Chemical components in plastic leachate may promote conjugation by inducing bacterial stress responses; however, at higher concentrations, these compounds can exert toxic effects that inhibit microbial activity [20,26]. As shown in Section 3.1, PVC leachate contains both a high proportion of labile molecules and a smaller fraction of potentially toxic additives. The coexistence of these components likely underlies the observed non-monotonic response in conjugative transfer.

This pattern can also be interpreted within the framework of ecological resilience and critical slowing down near tipping points. As microbial systems approach stress-induced thresholds, reduced

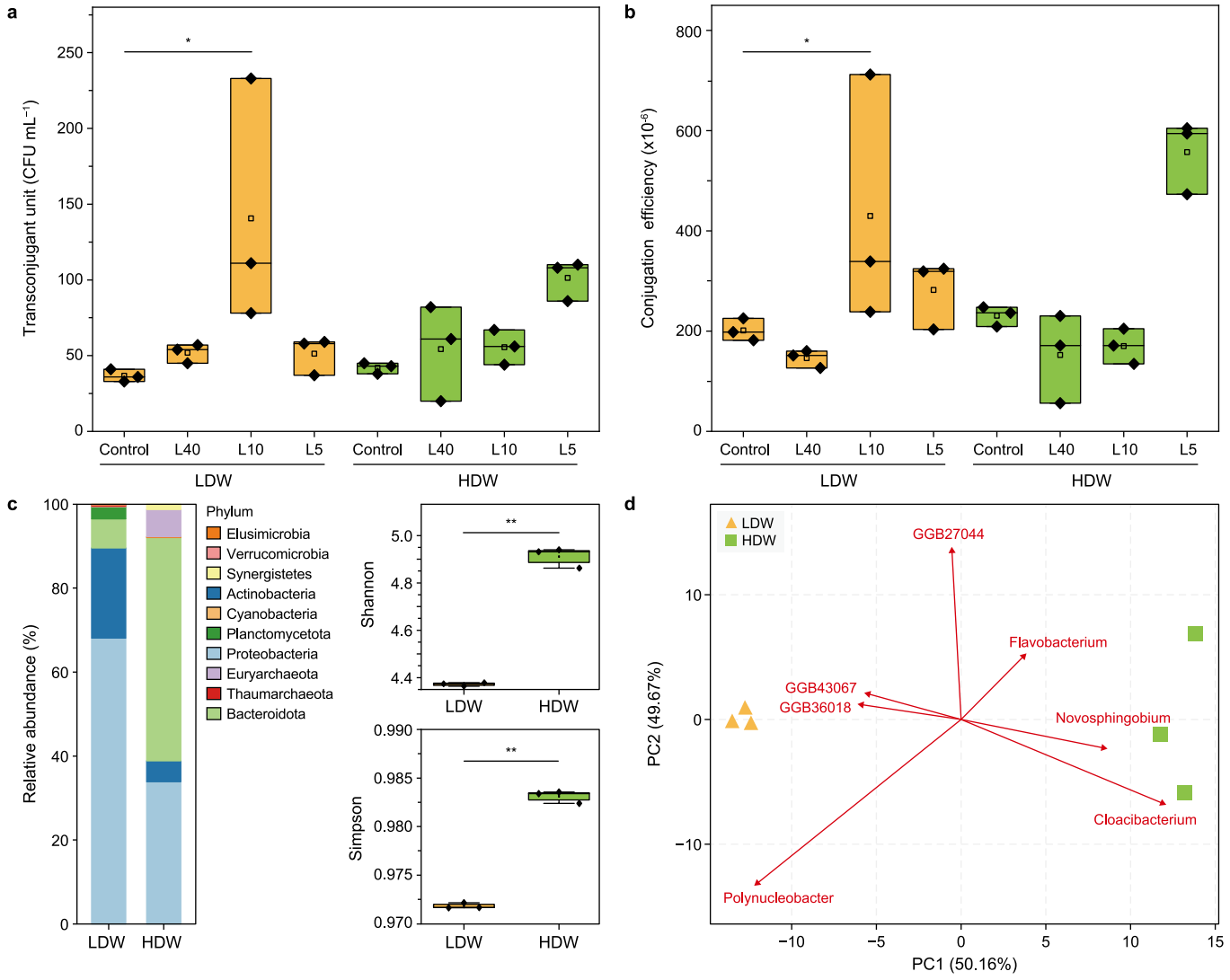

Fig. 2. Microbial community composition and the impact of polyvinyl chloride (PVC) leachate on conjugation efficiency in the microbiome of less-disturbed water (LDW) and highly disturbed water (HDW). Prior to the experiment, the leachate was diluted with deionized (DI) water at ratios of 1:5, 1:10, and 1:40 to obtain concentrations designated L5, L10, and L40, respectively. In the control group, DI water was added instead of PVC leachate. **a**, Transconjugant units, indicating the number of transconjugant cells capable of forming colonies per milliliter of bacterial suspension. CFU, colony-forming unit. **b**, Plasmid conjugation efficiency in LDW and HDW. **c**, Microbial community composition at the phylum level and diversity indices (Shannon and Simpson) of the LDW and HDW samples. **d**, Principal component analysis (PCA) based on genus-level microbial composition of the LDW and HDW samples. Statistical significance was determined using Tukey's honestly significant difference test. * indicates $0.01 < P \leq 0.05$, ** indicates $P \leq 0.01$. In panels **a**–**c**, diamonds indicate individual biological replicates, squares denote the mean value, horizontal lines within columns represent the median, and error bars represent standard deviations.

resilience increases sensitivity to perturbations, resulting in amplified and non-linear biological responses rather than simple dose–response relationships. Under moderate leachate exposure, sublethal stress may transiently enhance conjugation as an adaptive response, whereas higher stress levels may suppress microbial growth and plasmid transfer [82]. Excessive concentrations of organic compounds in leachate can inhibit overall microbial activity, and different water bodies may exhibit varying levels of tolerance due to differences in community composition and resilience between LDW and HDW systems.

Microbial diversity is closely linked to the stability of physiological and biochemical processes [83,84]. In this context, the higher diversity observed in HDW may confer greater functional resilience under chemical stress, allowing conjugative transfer to persist or even increase at higher leachate concentrations. In contrast, the lower-diversity LDW communities may approach critical thresholds more rapidly, resulting in stronger inhibitory and non-linear responses. These findings highlight the importance of microbial community composition, diversity, and resilience in shaping conjugative transfer dynamics under environmental chemical stress.

To elucidate the impact of PVC leachate exposure on the composition of the conjugative plasmidome in aquatic environments, we sequenced pooled samples comprising more than 300 randomly selected transconjugant colonies from each treatment (Supplementary Table S5), obtained from the filter mating experiments. The total number and diversity of conjugative plasmids were higher in LDW than in HDW, reflecting differences in community structure and baseline plasmid abundance. Specifically, 41 conjugative plasmid genomes were detected in LDW, compared to only 12 in HDW. Moreover, plasmids identified in HDW were more frequently associated with VFs and ARGs (Fig. 3a).

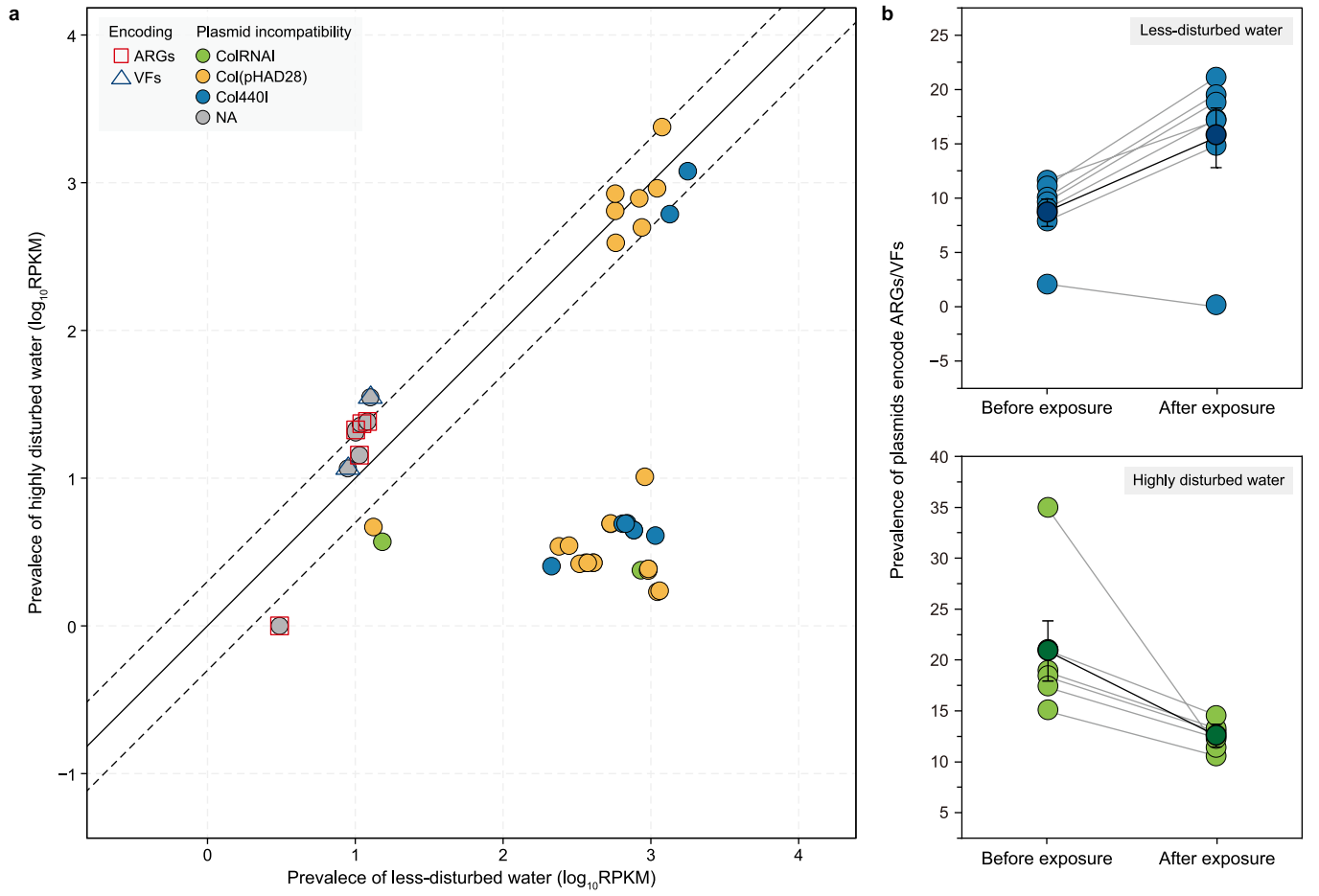

Fig. 3. Impact of plastic leachate on plasmidome in less-disturbed water (LDW) and highly disturbed water (HDW). a, Comparison of plasmid prevalence between LDW and HDW under original conditions (without plastic leachate treatment). Plasmid prevalence within a plasmidome is shown as log-transformed reads per kilobase per million mapped reads (RPKM) values, where mapped reads are defined as the number of reads mapped to a plasmid sequence multiplied by 10^9, and the RPKM value is calculated by further dividing by the total mapped reads and the plasmid length in base pairs. Plasmids with different incompatibility types are illustrated by different colors, "NA" represents unclassified plasmid incompatibility types. While plasmids that encode antibiotic resistance genes (ARGs) and virulence factors (VFs) were highlighted by red rectangular and blue triangular frames, respectively. The dashed line along the diagonal indicates a 2 × variation in plasmid abundance between LDW and HDW. **b**, Changes in prevalence of plasmids carrying ARGs or VFs based on RPKM in LDW and HDW before and after exposure to undiluted plastic leachate. Light circles represent RPKM values of individual plasmids carrying ARGs or VFs, dark circles indicate the mean RPKM values, and error bars represent standard deviations.

Following exposure to plastic leachate (Fig. 3b; Supplementary Fig. S4), plasmid abundance, measured by RPKM, differed between the two systems. In LDW, RPKM values for plasmids carrying ARGs and VFs increased, whereas in HDW, these values decreased. The increase in LDW likely reflects higher microbial diversity and functional resilience, as more diverse communities are better able to maintain genetic diversity and support plasmid propagation under chemical stress [85]. In contrast, the decrease in HDW is consistent with highly anthropogenically impacted environments, where resistance genes are already prevalent; additional chemical stress may not further promote plasmid proliferation and may instead induce competitive suppression among plasmids [86].

The LDW microbial community exhibited substantial propagation of resistance genes under leachate exposure, highlighting the role of community diversity in modulating responses to chemical stressors. Specifically, the more diverse LDW community appears capable of adapting and expanding plasmid prevalence, whereas the less diverse HDW community is more susceptible to inhibitory effects. These findings indicate that plastic leachate imposes selective pressure on gene expression and dissemination [19,80], consistent with previous studies showing that it can enrich ARGs [20]. Collectively, these results further underscore the ecological risks of plastic leachate by demonstrating its capacity to drive the spread of resistance genes in aquatic environments.

In summary, PVC leachate influences microbial conjugative transfer through interactions among community structure, environmental conditions, and chemical composition. High concentrations inhibit conjugation in the less diverse LDW community, whereas the more diverse effluent-receiving water (HDW) exhibits greater adaptability to chemical stress, maintaining or enhancing conjugation. These findings emphasize the importance of ecological diversity in conferring resilience to pollution stress [83,84]. Furthermore, exposure to leachate alters the prevalence of plasmids associated with resistance and virulence in natural waters, suggesting that plastic leachate may exacerbate antibiotic resistance by facilitating gene transfer.

3.3. Conjugative transfer of the RP4 plasmid in the presence of plastic leachate

To elucidate the molecular mechanisms underlying enhanced conjugation, a model system based on RP4 plasmid transfer in liquid LB medium was employed [87]. This system represents a standard experimental framework for studying conjugation mechanisms. In contrast to the filter mating experiment described in Section 3.2, where conjugation occurred on solid filters, the liquid system enables assessment of physiological and biochemical changes during conjugation. In this experiment, *E. coli* HB101 (donor), carrying the RP4 plasmid conferring resistance to KAN, TET, and AMP, and *E. coli* NK5449 (recipient), resistant to rifampicin, were used to evaluate changes in conjugation efficiency under different leachate concentrations.

Based on the findings in Section 3.1, plastic leachate may promote both microbial growth and conjugation transfer. To distinguish these effects, we established a bulk group, in which cellulose served as a carbon source to support bacterial growth without directly affecting conjugation efficiency. The control group represented baseline growth in the absence of leachate. This design enabled assessment of the effects of plastic leachate on conjugation independent of growth stimulation.

When exposed to a low concentration of leachate (L40), no significant difference in transconjugant abundance was observed relative to the control. However, as leachate concentration increased, the number of transconjugants increased significantly. In the undiluted leachate treatment, the number of transconjugants was 26.4-fold higher than in the control group (Fig. 4a). Correspondingly, conjugation efficiency in the undiluted treatment reached 2.98×10^5, representing a 44.6-fold increase relative to the control group. In the low-dose groups (L20 and L40), conjugation efficiencies were 2.88×10^6 and 8.78×10^7, corresponding to 4.3- and 1.3-fold increases, respectively (Fig. 4b). Previous studies have reported that polystyrene microplastics and phthalate esters (PAEs, a typical plastic additive) can increase conjugation efficiency by tenfold and 4.8-fold, respectively [30,88]. The present results further demonstrate that plastic leachate can significantly enhance conjugation-mediated ARG dissemination.

Notably, although donor CFU decreased by 41% in the undiluted leachate group relative to the control group (Fig. 4a), exposure to plastic leachate did not significantly reduce donor biomass compared to the bulk group. Conjugation efficiency is defined as the ratio of transconjugants to donor cells. Although previous studies have reported that heavy metals, non-antibiotic drugs, and other pollutants enhance conjugation efficiency [80,89,90], a significant portion of this increase can be attributed to a reduction in the number of donor cells, typically due to the toxic effects of these chemicals. For example, exposure to cerium oxide nanoparticles resulted in reduced donor and recipient cell counts by 29% and 33%, respectively, while increasing conjugation efficiency by 120% [87]. Similar trends have been reported for plastic additives such as DMP at low concentrations [30]. However, studies involving microplastic particles have shown enhanced conjugation without growth inhibition [88].

Consistent with these findings, the present results suggest that DOM in plastic leachate does not substantially inhibit microbial growth unless present at very high concentrations. Indeed, some studies indicate that leachate-derived DOM may stimulate microbial growth [33]. Because the number of transconjugants is determined by both conjugation efficiency and population density, higher microbial biomass can amplify overall conjugation activity within a community [91]. Therefore, unlike many aquatic pollutants that suppress bacterial growth, plastic leachate may promote microbial proliferation and conjugative transfer, resulting in a greater absolute number of transconjugants and enhanced horizontal transfer of resistance genes, with significant ecological implications.

3.4. Effects of plastic leachate on oxidative stress, intercellular contact, and energetic drivers of conjugative transfer

To investigate the mechanisms underlying enhanced conjugation, we collected samples from the liquid conjugation system for physiological and biochemical analyses. In parallel, we extracted RNA from conjugating cells exposed to plastic leachate, and transcriptome analyses were performed (Supplementary Table S3). Differential gene expression analysis (Supplementary Table S6), along with Gene Ontology (GO) and KEGG enrichment analyses (Supplementary Fig. S5–S6), was conducted to assess the effects of

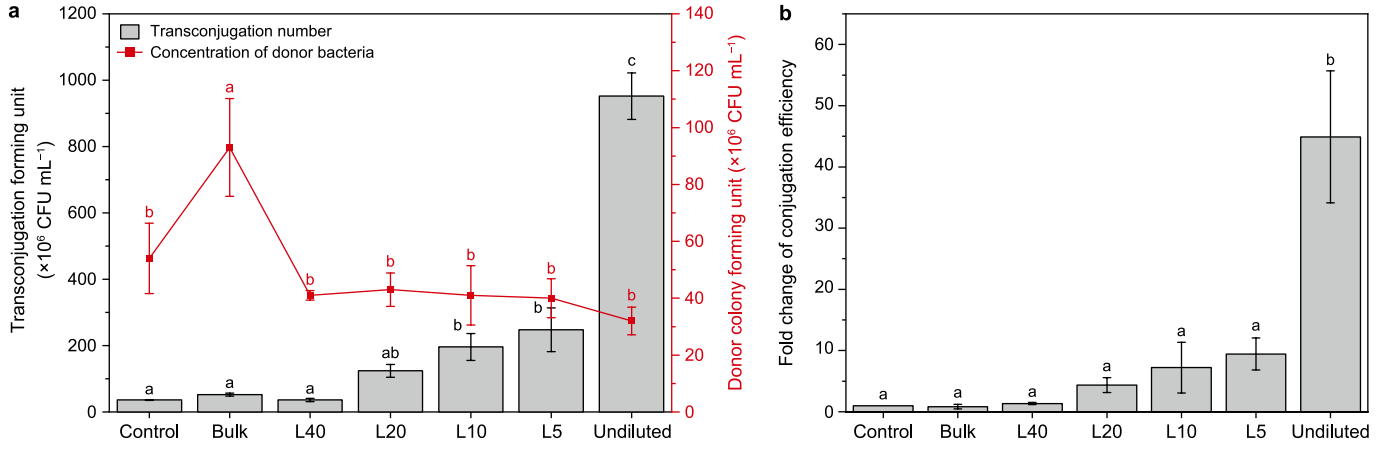

Fig. 4. Conjugation transfer of the RP4 plasmid, a model conjugative plasmid used in this study, in the presence of plastic leaching solutions. a, Transconjugant forming unit and Donor colony forming unit after RP4 plasmid conjugation under polyvinyl chloride (PVC) leachate exposure. Transconjugants were selected on Luria–Bertani agar containing the appropriate antibiotics to ensure plasmid acquisition. **b**, Fold change of conjugation efficiency of *E. coli* under different concentrations of PVC leachate. In panels **a** and **b**, PVC leachate was diluted with deionized water at ratios of 1:1, 1:5, 1:10, 1:20, and 1:40, designated as undiluted, L5, L10, L20, and L40, respectively. The control group was a negative control, in which no PVC leachate or other additives were added, and bacteria were cultured only in medium supplemented with deionized water. The bulk group is a test group in which PVC leachate was replaced with cellulose, which could serve as a recalcitrant carbon source to promote growth without affecting conjugation efficiency. Statistical significance was assessed using Tukey's honestly significant difference test; different letters indicate statistically significant differences among treatments ($P < 0.05$).

plastic leachate on gene expression and metabolic pathways, with the aim of elucidating mechanisms driving enhanced conjugation in aquatic microbial communities.

ROS play a critical role in HGT by acting as signaling molecules that alter membrane permeability, induce SOS responses, and promote ARG transfer. Previous studies have shown that environmental stressors, including plastic leachate, can elevate intracellular ROS levels in bacteria.

In this study, exposure of *E. coli* to PVC leachate resulted in a significant 21% increase in intracellular ROS levels, accompanied by upregulation of superoxide dismutase (SOD) activity (*sodA, sodB,* and *sodC*; Fig. 5a and b,f), a key enzyme responsible for ROS detoxification [92,93]. Elevated ROS levels can cause DNA damage, thereby triggering the SOS response—a well-established mechanism for DNA repair and recombination in bacteria [94]. Consistent with this, SOS response genes (*lexA* and *yedK*) and DNA repair genes (*recN* and *uvrB*) were upregulated in *E. coli* exposed to PVC leachate. Mechanistically, ROS-induced DNA damage promotes autocleavage of the *LexA* repressor, relieving repression of SOS-regulated genes, including plasmid transfer operons [95]. Activation of these operons enhances transcription of conjugation-related genes, such as those encoding pili and transfer proteins, thereby facilitating assembly of the conjugation machinery and promoting plasmid transfer to recipient cells [96]. This provides a molecular explanation for the observed increase in horizontal transfer of ARGs under oxidative stress. Concurrently, upregulation of DNA repair genes such as *recN* and *uvrB* helps maintain genomic and plasmid stability under these conditions (Fig. 5f). These findings are consistent with previous studies demonstrating that environmental stressors, including heavy metals, can induce the SOS response and enhance plasmid conjugation [97,98].

Conjugative transfer is initiated by direct cell-to-cell contact, in which EPS play a critical role in mediating adhesion and intercellular interactions. Under PVC leachate exposure, however, polysaccharide content in *E. coli* did not increase (Fig. 5c). In contrast, extracellular protein content increased significantly by 2.92-fold relative to the control (Fig. 5d), suggesting that plastic leachate primarily modulates cell–cell interactions through changes in

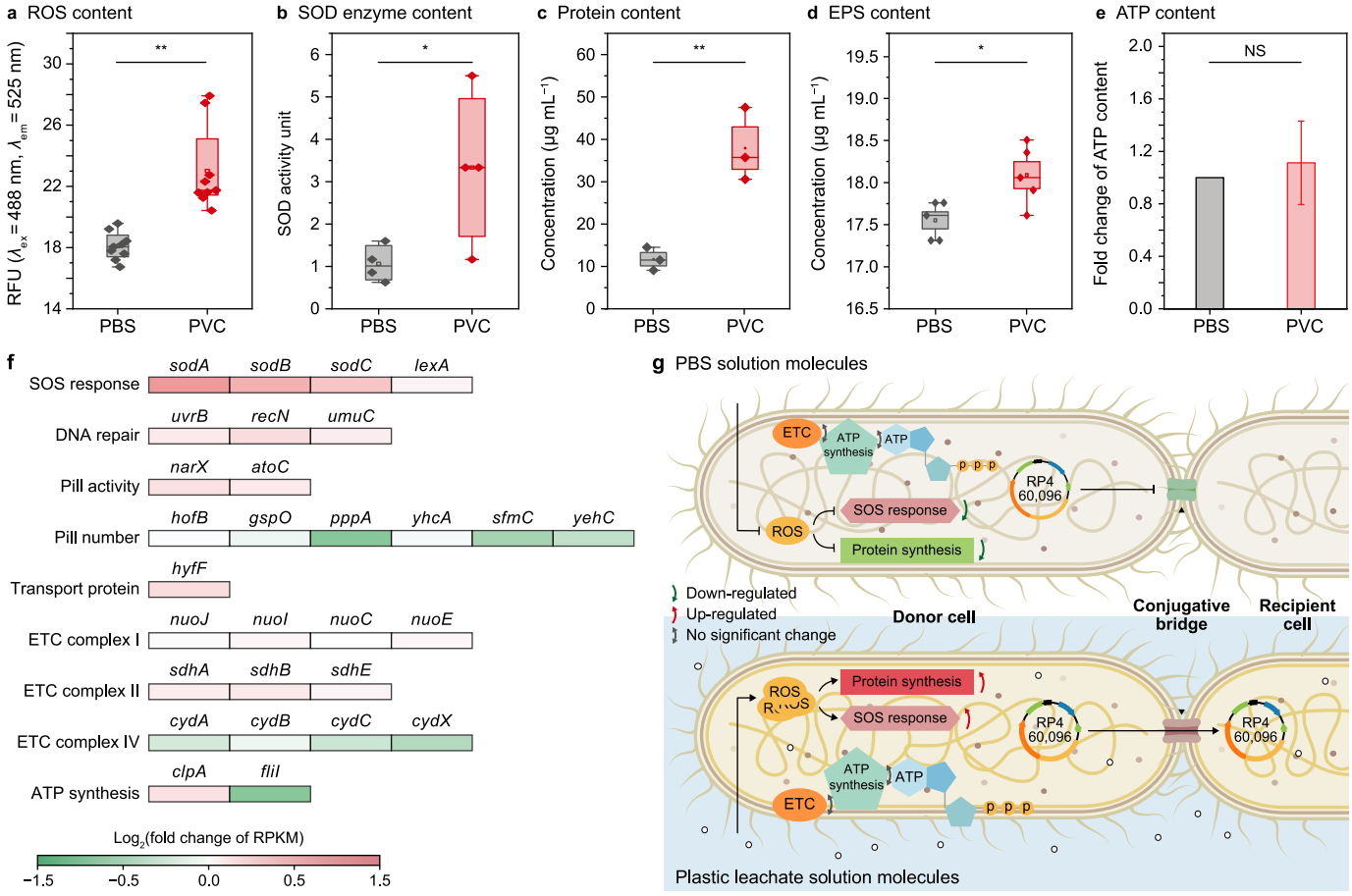

Fig. 5. Effects of polyvinyl chloride (PVC) leachate on oxidative stress, intercellular contact, and energy-driven conjugation transfer in *Escherichia coli*. RFU: relative fluorescence unit; PBS: phosphate-buffered saline; ETC: electron transport chain. **a**, Intracellular reactive oxygen species (ROS) levels. **b**, Superoxide dismutase (SOD) activity indicating the oxidative stress response. **c**, Total cellular protein content. **d**, extracellular polymeric substances (EPS) content, including extracellular polysaccharides and proteins. **e**, Intracellular adenosine triphosphate (ATP) content, reflecting cellular energy status, was quantified following exposure to plastic leachate. In panels **a**–**e**, the horizontal line within each column represents the median, diamonds indicate individual biological replicates, squares denote the mean value, and error bars represent standard deviations. **f**, Heatmap shows differential gene expression associated with oxidative stress response, intercellular contact, and energy supply for conjugative transfer in *E. coli* exposed to PVC leachate, with gene expression presented as $\log_2$-transformed fold changes of RPKM values relative to the control group without plastic leachate exposure; red and green indicate up-regulated and down-regulated genes, respectively. **g**, A proposed mechanistic model summarizes the upregulation of RP4 plasmid conjugative transfer under exposure to plastic leachate. The left bacterium represents the donor cell, the middle structure indicates the conjugative bridge, and the right bacterium represents the recipient cell. The number below RP4 indicates the length of the RP4 plasmid. The model highlights the combined roles of oxidative stress induction, enhanced cell contact, and increased intracellular energy availability. Statistical significance was determined using Tukey's honestly significant difference test; * indicates $0.01 < P \leq 0.05$, ** indicates $P \leq 0.01$, and NS indicates no significant difference.

extracellular protein composition rather than polysaccharides [99]. Transcriptomic analysis further revealed that genes involved in pilus synthesis and assembly were downregulated, whereas genes associated with pilus function and activity were upregulated (Fig. 5f). These results suggest that, despite reduced pilus production, functional modifications in pilus activity may occur, potentially enhancing the efficiency of conjugative transfer [100].

The energy required for conjugative transfer is primarily supplied by ATP, which is generated through the electron transport chain (ETC) during aerobic respiration. Notably, ATP levels in *E. coli* did not change significantly following exposure to plastic leachate (Fig. 5e). However, transcriptome analysis revealed significant upregulation of genes associated with ETC complexes I and II (e.g., *nuoC, nuoF, nuoI, nuoJ, nuoE, sdhA, sdhB*, and *sdhE*), which facilitate electron and proton transfer during ATP synthesis. In contrast, genes associated with ETC complex IV (*cydA*, *cydB*, *cydC*, and *cydX*) were downregulated (Fig. 5f). These compensatory changes likely maintain stable ATP levels and ensure a consistent cellular energy supply [101,102].

Although ATP levels remained unchanged, ATP-dependent processes were actively involved in the cellular response to PVC leachate, including ROS detoxification, EPS production, and SOD activation [103]. Moreover, upregulation of genes involved in ATP metabolism (e.g., *clpA*) and downregulation of genes such as *fliI* support the notion that PVC leachate induces metabolic adjustments to maintain energy homeostasis during plasmid transfer [96,104]. These metabolic responses, including the upregulation of genes involved in maintaining ATP synthesis homeostasis and oxidative stress responses, are consistent with the enrichment of biological processes related to the SOS response and DNA repair identified by GO and KEGG analyses (Supplementary Fig. S5–S6).

Overall, exposure to plastic leachate induces oxidative stress in bacteria, activates the SOS response, and enhances conjugative transfer through the coordinated upregulation of ROS-scavenging and DNA-repair pathways. At the same time, compensatory regulation of the electron transport chain maintains ATP homeostasis, supporting the energy-intensive processes required for plasmid transfer. This integrated physiological response promotes bacterial adaptation under environmental stress and facilitates horizontal gene transfer and ARG dissemination (Fig. 5g).

4. Conclusion

This study demonstrates the potential ecological risks posed by plastic leachate in promoting the horizontal transfer of ARGs in aquatic environments. Plastic leachate contains a complex mixture of DOM that is more chemically unstable than that found in natural waters, which can stimulate microbial growth while simultaneously exerting selective pressure through persistent, potentially toxic compounds. The results show that plastic leachate enhances plasmid-mediated conjugation efficiency, particularly at low concentrations, whereas higher concentrations may inhibit microbial communities with lower diversity.

Mechanistic analyses further indicate that plastic leachate enhances conjugation efficiency via pathways that exceed those previously reported for microplastic particles and individual plastic additives. These findings suggest that plastic leachate can accelerate the dissemination of ARGs, posing a significant threat to aquatic ecosystems and public health.

PVC is chemically unstable due to its chlorinated backbone, relatively low crystallinity, and the presence of leachable additives such as phthalate plasticizers. These components can degrade and release toxic compounds, further promoting plasmid-mediated gene transfer. Accordingly, stricter regulatory measures are warranted, including replacing PVC with more stable materials (e.g., PP, PE, PET), mandating toxicity assessments of plastic leachates, and establishing maximum allowable limits for leachate-derived pollutants. Long-term environmental impact assessments are also needed to inform effective strategies for pollution prevention and mitigation.

Despite these findings, several limitations should be acknowledged. First, FT-ICR MS provides detailed molecular characterization but does not yield absolute quantitative concentrations. Second, although filter mating and liquid conjugation experiments demonstrate that plastic leachate can modulate conjugative transfer under controlled laboratory conditions, the long-term ecological impacts and *in situ* dynamics of ARG dissemination in natural aquatic systems remain unclear and require further validation through field studies and metagenomic analyses. It should also be noted that the conjugation efficiencies reported here reflect relative changes in transfer potential rather than absolute *in situ* transfer rates. Finally, because this study focuses on freshwater systems, the findings may not be directly transferable to marine environments, where microbial communities and environmental conditions differ. Moreover, the leachate concentrations used represent scenario-based exposure levels designed to probe mechanistic responses; actual environmental impacts will depend on factors such as local plastic abundance, hydrodynamic conditions, and community composition.

CRediT authorship contribution statement

Yongsheng Chen: Writing – original draft, Visualization, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Kaiqiang Yu:** Methodology, Investigation, Conceptualization. **Yuhong Sun:** Methodology, Investigation. **Yuxi Yan:** Methodology, Investigation. **Gege Yin:** Methodology, Investigation. **Junjian Wang:** Writing – review & editing, Supervision. **Xia Li:** Writing – review & editing, Supervision. **Song Tang:** Writing – review & editing, Supervision. **Paul Pronyk:** Writing – review & editing, Supervision. **Yu Xia:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Ethical statement

Sampling complied with local regulations; no permits were required for non-endangered aquatic systems.

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.ese.2026.100705.

Data availability

All raw sequencing reads are available at the National Center for Biotechnology Information (NCBI) under BioProjects PRJNA1233896 and PRJNA1233468.

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