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# Journal of Cleaner Production

## Safe reuse of wastewater: effect of disinfection methods on microbial community

--Manuscript Draft--

<b>Manuscript Number:</b>	JCLEPRO-D-23-08618R3
<b>Article Type:</b>	Original article
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<b>Corresponding Author:</b>	Robert Duran University of Pau and Pays de l'Adour Pau, FRANCE
<b>First Author:</b>	Boxi Wang
<b>Order of Authors:</b>	Boxi Wang Robert Duran Thierry Pigot Cristiana Cravo-Laureau
<b>Abstract:</b>	<p>Performic acid (PFA), a new generation of disinfectant, has shown outstanding performance for wastewater treatment. However, the effect of PFA on wastewater microbial community changes as well as the differences in comparison to the commonly disinfectants used are poorly documented. We provide an overview of the PFA effect and that of chlorine and UV, two commonly used disinfectants, on microbial community diversity and structure. The study was performed using wastewaters from three municipal wastewater treatment plants (WWTPs) processed by activated sludge, lagoon and reed beds, and biofilter. We focused on disinfection of pathogen microorganisms, identifying sensitive and resistant bacteria to disinfectants that can serve as biomarkers for evaluating the disinfectant performances. As well, we investigated the modifications of microbial functions after treatment by functional predictive analysis. The more efficient <i>E. coli</i> inactivation was obtained with initial concentration of 4 mg/L of PFA with 15 min disinfectant contact time that achieved 95.71-99.99% inactivation efficiency. Although the origin of wastewater was the main factor determining the microbial community diversity and composition, the microbial communities were specific to the disinfectant used. Linear discriminant analysis Effect Size (LEfSe) identified 18 and 10 biomarkers, including pathogenic microorganisms affiliated to <i>Chromobacterium</i>, <i>Flavobacterium</i>, <i>Aeromonas</i>, <i>Enterobacteriaceae</i>, <i>Escherichia_Shigella</i> and <i>Fusobacteriaceae</i>, which were sensitive to PFA, while being resistant to chlorine and/or UV. Consistently, the predicted bacterial functional patterns diverged according to the wastewater origin and the disinfectant used. Pathogen microorganisms were more sensitive to PFA than the other treatments, demonstrating its potential for implementing a safe reuse of wastewater.</p>
<b>Suggested Reviewers:</b>	Maren Ortiz-Zarragoitia maren.ortiz@ehu.eus  Jean Baptiste Ramond jbramond@bio.puc.cl  Tibor Benedek benedek.tibor@fh.szie.hu  Jianli Li ljl2489@163.com



July the 26<sup>th</sup>, 2023

Dear Editor,

Please find as attached file, a revised version of the manuscript JCLEPRO-D-23-08618R3. As you will see, we have made revisions to the manuscript in line with the reviewers' comments and criticisms.

The modifications have been highlighted in blue. The responses to reviewer's comments are in the rebuttal letter.

We hope that this new version will be now acceptable for publication.

If there are any points we haven't addressed, please contact us and we will be happy to clarify them.

On behalf of authors,

Sincerely,

Pr. Robert Duran



**Prof Robert Duran**  
**IPREM**

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## **Rebuttal letter**

### **Reviewer #4:**

The article has been strongly improved. Still, it is not easy to be read and, in my opinion, some informations (i.e.the characterization of the effluents rom the three WWTPs) should be included in the text and the English should be revised. However, it contains interesting data and now I thing it could be published after minor revisions.

[Response]: Thank you for the comments that are useful to further improve the MS.

The information of the characterization of WWTP, which was presented in supplementary material Table S1, it is now described in the text as follows:

"Indeed, the WWTPs' effluents showed different physical-chemical characteristics (Table S1). The conductivity was lower in WWTP1 effluent (718  $\mu\text{S}/\text{cm}$ ) than in WWTP2 (1000  $\mu\text{S}/\text{cm}$ ) and WWTP3 (1254  $\mu\text{S}/\text{cm}$ ) effluents (Table S1). Consistently, WWTP1 effluent exhibited the lower contents of COD (36 mg/L), TSS (2 mg/L), TOC (18 mg/L), and TN (3.5 mg/L), while WWTP2 effluent showed the higher COD (80 mg/L) and NT (60.6 mg/L) contents (Table S1)."

Also the information of statistics comparing the WWTP and treatments presented in supplementary material tables has been included in the text.

The modifications of text for English revision are also indicated.

## **Safe reuse of wastewater: effect of disinfection methods on microbial community**

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## **Highlights**

- PFA was **an** efficient disinfectant comparing to chlorine and UV
- Microbial communities from treated water were specific to the disinfectant used
- Biomarkers sensitive to PFA and resistant to chlorine and/or UV were identified
- Bacterial predicted functions diverge according to WWTP and disinfectants

# 1 Safe reuse of wastewater: effect of disinfection methods on microbial community

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19 **Abstract**

20 Performic acid (PFA), a new generation of disinfectant, has shown outstanding  
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44  
45 **Keywords:** Wastewater reuse; Performic acid; Disinfection efficiency; Disinfectant  
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## 47 1. Introduction

48 Water scarcity is becoming a global concern in recent decades. The  
49 overexploitation of water is one of the major threats to the EU freshwater  
50 environment, especially for agricultural irrigation (EC, 2018). More than 40,000  
51 million m<sup>3</sup> wastewater is treated each year in the EU, only less than 3% being reused  
52 (EC, 2022). Thus, there is place to improve water reuse, which represents a potential  
53 on easing water supply pressure, also contributing to a sustainable agriculture.

54 Domestic wastewater owns microorganism associated to human diseases  
55 (Diamond et al., 2022; McCall et al., 2020). It is thus critical to establish efficient and  
56 economical disinfection frameworks in wastewater treatment in order to supply safe  
57 water. Tertiary treatment aims to remove stubborn pollutants (organics, turbidity,  
58 nitrogen, phosphorus, metals, and pathogens), including also an additional  
59 disinfection step (Pei et al., 2019). The tertiary treatment provides additional  
60 assurance of effluent quality, protecting thus wildlife in the discharged water system.  
61 It also ensures the safety of wastewater reuse for various purposes, such as irrigation  
62 and industrial applications (Gerba and Pepper, 2019).

63 Several disinfection approaches are commonly implemented in tertiary treatment  
64 for the inactivation of pathogen microorganisms, such as chlorination, ultraviolet (UV)  
65 irradiation, ozonation and filtration (USEPA, 2004). Chlorine and its various forms  
66 are powerful oxidants for the inactivation of pathogens (Furst et al., 2018), but it  
67 forms toxic by-products that might impact the receiving environment threatening the  
68 human health (Bull et al., 1995; Nieuwenhuijsen et al., 2000; Victoria, 2002). The UV  
69 irradiation has been considered as an alternative to chlorine for wastewater treatment  
70 due to its short contact time, and it has the advantage that toxic by-products are not  
71 produced (Zyara et al., 2016). However, UV irradiation has the disadvantage to be  
72 expensive because of high-energy demand, high costs of the equipment and its  
73 management. The investment cost is estimated to be 10 times more expensive than  
74 that of chlorine and PFA treatments (Ragazzo et al., 2013). Also, the practical  
75 application of UV irradiation carries many limitations because it is dependent on

1 76 water quality parameters, such as turbidity, colour and suspended solid (Oguma et al.,  
2 77 2001; Zimmer and Slawson, 2002).

3  
4 78 Performic acid (PFA), a high oxidative peracid, has been considered as an ideal  
5  
6 79 alternative for industrially wastewater disinfection due to its outstanding  
7  
8 80 characteristics (Gehr et al., 2009; Ragazzo et al., 2013). PFA is a wide-spectrum  
9  
10 81 disinfectant with deodorizing ability and low corrosive effect, exhibiting toxicity to  
11  
12 82 viruses, bacteria and bacterial spores, mycobacteria as well as microscopic fungi, but  
13  
14 83 not to higher forms of life (Gehr et al., 2009; Luukkonen and Pehkonen, 2017). The  
15  
16 84 PFA treatment cost has been estimated at 0.0114 €/m<sup>3</sup> (Luukkonen and Pehkonen,  
17  
18 85 2017), while that of chlorination at 0.004 €/m<sup>3</sup> (Zagklis and Bampos, 2022). Although  
19  
20 86 the operation cost of PFA treatment is estimated to be 2.5 times more expensive than  
21  
22 87 that of chlorine (Luukkonen et al., 2015). It has the advantage to produce by-products  
23  
24 88 (hydrogen peroxide and formic acid) that are nontoxic to aquatic fauna at the  
25  
26 89 disinfection doses (Gehr et al., 2009; Ragazzo et al., 2013). Thus, its reasonable cost  
27  
28 90 and splendid performance at ambient temperature demonstrate that PFA has potential  
29  
30 91 applicability for large wastewater treatment plants (WWTPs) (Luukkonen et al.,  
31  
32 92 2015).

33  
34  
35 93 Several studies have reported on the effect of the commonly used disinfectants on  
36  
37 94 microorganisms. Most studies have considered the decrease of bacterial cell counts  
38  
39 95 and species richness, the inactivation of viruses, and the removal of microorganisms  
40  
41 96 carrying antibiotic resistance genes (ARGs) (Kantor et al., 2019; Kulkarni et al., 2018;  
42  
43 97 Ng et al., 2019; Stamps et al., 2018; Zaouri et al., 2020). Nevertheless, the effect of  
44  
45 98 disinfectant treatments on the microbial community structure and functions is scarce,  
46  
47 99 especially for the PFA treatment (Kantor et al., 2019; Numberger et al., 2019; Stamps  
48  
49 100 et al., 2018). The release of potential pathogens and the modification of microbial  
50  
51 101 community in the receiving environment have been reported during reclaimed water  
52  
53 102 discharging process (Numberger et al., 2019). Thus, it is important to consider the  
54  
55 103 effect of wastewater treatments on the microbial community. Precisely, the objective  
56  
57 104 of our study was to examine the effect of PFA treatment on the microbial diversity,  
58  
59 105 microbial structure and function. The disinfection efficiency of PFA treatment against

106 pathogens was also evaluated and compared to that of other conventional treatments.  
107 We hypothesize that the microbial communities will be modified differently  
108 according to the treatment because they involve different action mechanisms.

109 In order to examine the possibility to safely reuse treated wastewater for irrigation,  
110 industrial applications, groundwater recharge and other agricultural activities, this  
111 study aimed to compare PFA, UV and chlorine treatments regarding their effect on 1)  
112 the microbial community diversity and structure, and 2) the disinfection efficiency for  
113 the inactivation of pathogens. For this purpose different WWTPs were treated in order  
114 to identify sensitive and resistant bacteria to each disinfectant, representing potential  
115 microbial biomarkers according to treatment. The bacterial functions were predicted  
116 and correlated with the wastewaters resulting from the treatments.

## 117 **2. Materials and methods**

### 118 **2.1 Sampling sites and physical-chemical characterization of wastewaters**

119 Three domestic WWTPs with different treatment processes, activated sludge,  
120 lagoon and reed bed and biofilter were selected. Brief information of the sampling  
121 WWTPs is listed in the [Table S1](#).

122 The main physical-chemical parameters were determined on the wastewater  
123 samples. Conductivity was measured by 3510 WTW multiparameter-probe. Chemical  
124 oxygen demand (COD) was determined using colorimetric method by Hach reactants  
125 and Hach DR3900 spectrophotometer. Total suspended solid (TSS) was determined  
126 after filtration through 0.45  $\mu\text{m}$  on 47 mm glass fiber filter and drying at 105  $^{\circ}\text{C}$  until  
127 constant mass. Total organic carbon (TOC) and total nitrogen (TN) were determined  
128 by a Shimadzu TOC VCN analyzer with a TN unit (chemiluminescence measurement  
129 of  $\text{NO}_x$ ) according to (Behera et al., 2017). TOC was obtained by the difference of  
130 total carbon and inorganic carbon analyses.

## 131 2.2 Sample collection and disinfection

132 The effluent wastewater samples were collected from each WWTP with sterile  
133 Scott bottles. All the samples were collected in the morning and transported to the  
134 laboratory at 4 °C. The disinfections were generated by three disinfectants (PFA,  
135 chlorine and UV) just right after the water samples arrived to the laboratory. Each  
136 disinfection treatment was performed in true biological triplicates. Thus, a total of 45  
137 samples (3 WWTP x triplicate x 5 treatments (PFA\_L, PFA\_H, Chlorine, UV and  
138 untreated wastewater as control)) were analysed. All samples were maintained at 4 °C  
139 prior further processing.

140 The optimal disinfection concentration of all the disinfectants used was  
141 determined in a pre-experiment using *E. coli* and total coliforms as indicators. The  
142 initial doses range were determined based on official wastewater treatment manual  
143 and previous studies, also adjusted according to the wastewater treatment plants  
144 conditions (Anastasi et al., 2013; EPA, 2011; Gehr et al., 2009; Ragazzo et al., 2013;  
145 USEPA, 2003). More specifically, previous reports indicated that UV was effective at  
146 10 mJ/cm<sup>2</sup> although a higher dose (30-40 mJ/cm<sup>2</sup>) would be required for complete  
147 disinfection (Andreadakis et al., 1999), while hypochlorite was found effective at 2  
148 mg/L (Collivignarelli et al., 2017) and PFA in 1-4 mg/L range (Chhetri et al., 2014).

### 149 2.2.1 PFA preparation and disinfection

150 For preparing PFA, 10 mL of formic acid was mixed with 1 mL sulfuric acid  
151 (98%, Aldrich) as a catalyst on ice and wait about 10 min to cool. 14.2 mL of  
152 hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>, 50% wt, Aldrich) was then added slowly into the mixture.  
153 Then, the mixed solution was maintained for 60 min to get ready to use. The whole  
154 process was performed in ice bath and mixed with a magnetic stirrer. 100 mg of the  
155 mixture was then mixed with 0.1M H<sub>2</sub>SO<sub>4</sub> solution and titrated to determine the  
156 concentrations of H<sub>2</sub>O<sub>2</sub> and PFA. The H<sub>2</sub>O<sub>2</sub> titration was conducted with potassium  
157 permanganate solution (KMnO<sub>4</sub>, 0.02 mol/L, Aldrich). An excess of potassium iodide  
158 was then added and oxidized by PFA. Iodine was then titrated with sodium thiosulfate

159 (Na<sub>2</sub>SO<sub>3</sub>, 0.02 mol/L, Aldrich). The PFA solution was kept at -20 °C and titrated each  
160 time right before use.

161 Because PFA is rapidly decomposed (Karpova et al., 2013), two doses of PFA, a  
162 lower initial concentration at 1 mg/L with 15 min disinfectant contact time (PFA\_L)  
163 corresponding to a dose know to inactive most microorganisms (Karpova et al., 2013)  
164 and a higher initial concentration at 4 mg/L with 15 min disinfectant contact time  
165 (PFA\_H) corresponding to the optimal dose for disinfection (Karpova et al., 2013;  
166 Chhetri et al., 2014), were used in the disinfection. Na<sub>2</sub>SO<sub>3</sub> solution (0.1 mol/L) was  
167 used for quenching the reaction.

### 168 **2.2.2 Chlorination**

169 A solution of 100 mg/L of total chlorine was prepared from pure calcium  
170 hypochlorite (Aldrich) and stored at 4 °C. The prepared solution was titrated by  
171 iodometric titration in acetic acid medium before use. The chlorination was performed  
172 with the initial concentration at 2 mg/L for 30 min (Chl). Na<sub>2</sub>SO<sub>3</sub> solution was used  
173 for quenching the reaction.

### 174 **2.2.3 UV disinfection**

175 The laboratory scale UV disinfection was performed using low-pressure mercury  
176 vapour lamp to perform the disinfection at the dose of 10 mJ/cm<sup>2</sup>. The irradiance rate  
177 was measured by UV radiometer (Avantes Avaspec 2048L equipped with a 600 µm  
178 core optical fiber and a corrector cosinus (4.9 mm diameter)). The solutions were  
179 irradiated by a home-made device consisting of four Phillips TL UV 8W 30 cm  
180 fluorescent lamps. The device was placed on top of the solutions and the height was  
181 adjusted until obtaining the desired irradiance. The lamp was preheated for more than  
182 15 min before disinfection. 250 mL of wastewater was treated each time in the  
183 specific glass beaker. The exposure time was calculated by the desired dose,  
184 irradiance rate and transmittance value of the wastewater sample.

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## 185 2.3 Microbial analysis

186 The *Escherichia coli* (*E. coli*) counts were determined by a standard  
187 beta-glucuronidase positive method (APHA, 2005).

188 The water samples were filtered by sterile disposable filtration units (MicroFunnel,  
189 Pall Corporation, USA) with 0.2 µm Supor membrane to concentrate the microbial  
190 biomass. The iQ-Check Free DNA Removal Solution (FDRS, Bio-Rad Laboratories,  
191 USA), which degrades free DNA by enzymatic treatment, was used to ensure that the  
192 samples are devoid of free DNA prior to DNA extraction. For the free DNA  
193 elimination, we followed standard protocol. In short, the membranes were folded into  
194 cones and transferred into Eppendorf tubes containing 40 µl FDRS and 460 µl  
195 distilled Milli-Q water, followed by 30 min incubation at 37 °C. To inactivate the  
196 FDRS and for DNA extraction, 1 mL lysis solution (R1) from Aquadien™ Bacterial  
197 DNA Extraction and Purification Kit (Bio-Rad Laboratories, USA) were then added  
198 and incubated for 15 min at 95 °C with 1,300 rpm in a shaking heating block  
199 (Eppendorf ThermoMixer C, Eppendorf, Germany). We followed the standard  
200 protocol for the Aquadien™ Bacterial DNA Extraction and Purification Kit for the  
201 following steps.

202 The V3-V4 region of 16S rRNA gene was amplified using the primer pair 341F  
203 (5' - CCTAYGGGRBGCASCAG - 3') and 806R (5' -  
204 GGACTACNNGGGTATCTAAT - 3') (Gao et al., 2020) connecting with barcodes.  
205 PCR amplification, library preparation and sequencing were conducted by Novogene  
206 Europe (Cambridge, UK) using their proprietary methods. To ensure an accurate  
207 recognition of the samples, the index pairs were specific for each sample. Quality  
208 control was performed at each step of the procedure. The PCR products were purified  
209 using AMPureXP magnetic beads, pooled in equimolar and the libraries generated  
210 with NEBNext® Ultra™ DNA Library Prep Kit for Illumina and quantified via  
211 Qubit and Q-PCR. Paired-end 250 bp sequencing was then performed on Illumina  
212 NovaSeq 6000 sequencer (Illumina, San Diego, CA, USA).

## 213 2.4 Bioinformatics analysis and visualization

214 Paired-end reads were assigned to samples based on the sample-specific barcode  
215 and truncated by cutting off the barcode and primer sequences, and were merged  
216 using FLASH (V1.2.7) (Magoc and Salzberg, 2011). Qiime2 (v 2019.4) was used for  
217 the data processing and analysis. The sequence quality control and amplicon  
218 sequencing variants (ASVs) assignment were performed using DADA2 plugin.  
219 Taxonomy assignment was completed using the q2-feature-classifier plugin against  
220 SILVA database (v132) with a clustering threshold of 97% similarity. The ASVs  
221 assigned to Eukaryota, Chloroplast, Mitochondria and unassigned ASVs were  
222 removed and the filtered dataset was then rarefied at the depth of 71673 using  
223 *alpha-rarefaction.py* script. The rarefied data were then used for the following  
224 analyses without threshold. The complete dataset was deposited in the Sequence Read  
225 Archive (SRA) of the National Center for Biotechnology Information (NCBI) and is  
226 available under the Bioproject ID PRJNA907723.

227 For the alpha-diversity, the Shannon, Simpson, observed features, Chao1, ace  
228 indices were analyzed using *alpha\_diversity.py* in Qiime2. Statistical analysis  
229 (ANOVA, Tukey HSD) and visualization were performed in GraphPad Prism.  
230 Beta-diversity was calculated in Qiime2 and R based on weighted Unifrac and  
231 Bray-Curtis dissimilarity. Permutational multivariate analysis of variance  
232 (PERMANOVA) was performed with 999 times permutations using the  
233 *beta\_significance.py* script in Qiime2 and the “adonis” function in the vegan package  
234 in R. Principal coordinate analyses (PCoA) were performed and visualized by  
235 EMPERor (Vazquez-Baeza et al., 2013) and the Ape and ggplot2 packages in R. The  
236 significances of physical-chemical parameters associated with microbial community  
237 were identified by “envfit” function in the “vegan” package in R.

238 Linear discriminant analysis Effect Size (LEfSe, v 1.0) (Segata et al., 2011) was  
239 performed to identify the biomarkers between the groups, which emphasizes both  
240 statistical significance and biological relevance. LEfSe analysis was performed using  
241 the ASV table, at the ASV level spanning all the taxonomic levels. The

1 242 non-parametric factorial Kruskal-Wallis sum-rank test was performed first to detect  
2 243 features with significant differential abundance between the classes (groups). The  
3  
4 244 pairwise Wilcoxon test was then applied to check if the subclasses agree with the  
5  
6 245 class level trend, which was performed only among subclasses in different classes  
7  
8 246 with the same name. The resulting subset of vectors was then used to build a Linear  
9  
10 247 Discriminant Analysis model. The alpha-value for both Kruskal-Wallis and Wilcoxon  
11  
12 248 tests were at 0.05 and the logarithmic LDA threshold score for discriminative features  
13  
14 249 was 2.  
15

16 250 The PICRUST2 was used for the prediction of microbial community functions  
17  
18 251 following the following 4 steps: (1) sequence placement, (2) hidden-state prediction  
19  
20 252 of genomes, (3) metagenome prediction, (4) pathway-level predictions. The  
21  
22 253 pathway-level predictions were performed using a customized mapping file based on  
23  
24 254 the KEGG database (KEGG Brite orthologs to pathways, updated on 22 Nov. 2020,  
25  
26 255 [Table S2](#)). The descriptions were then added to the Enzyme Classification (EC)  
27  
28 256 numbers, KEGG orthologs (K) with default mapping table, and a customized mapping  
29  
30 257 table including descriptions of higher hierarchical levels was used for the KEGG  
31  
32 258 pathways (ko, [Table S3](#)). STAMP v 2.1.3 was used for visualizing PICRUST2 output  
33  
34 259 data. ANOVA and Kruskal-Wallis H-test were used for the multiple groups' statistical  
35  
36 260 analysis. Tukey-Kramer and Welch's at 0.95 were performed as Post-hoc tests.  
37  
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## 40 261 **3. Results and discussion**

### 41 262 **3.1 Disinfection treatment efficiency**

42  
43 263 The disinfection efficiency of PFA, UV and chlorine was determined for the three  
44  
45 264 different wastewater treatment plants by determining the reduction of the *E. coli*  
46  
47 265 content, which represents a key parameter for characterizing reclaimed water quality  
48  
49 266 in wastewater reuse (EC, 2018; EPA, 2004). The initial *E. coli* cell concentration  
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51 267 (before treatment BT, [Fig. 1](#)) was  $2.8 \times 10^4$ ,  $2.5 \times 10^4$  and  $5.8 \times 10^3$  CFU/100 mL for  
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53 268 WWTP1, 2, and 3, respectively. In WWTP1 and WWTP2, the *E. coli* cell  
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55 269 concentration significantly decreased in all the treated wastewaters ( $p < 0.001$ , [Fig. 1](#)).  
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1 270 Indeed, the WWTPs' effluents showed different physical-chemical characteristics  
2 271 (Table S1). The conductivity was lower in WWTP1 effluent (718  $\mu\text{S}/\text{cm}$ ) than in  
3 272 WWTP2 (1000  $\mu\text{S}/\text{cm}$ ) and WWTP3 (1254  $\mu\text{S}/\text{cm}$ ) effluents (Table S1). Consistently,  
4 273 WWTP1 effluent exhibited the lower contents of COD (36 mg/L), TSS (2 mg/L),  
5 274 TOC (18 mg/L), and TN (3.5 mg/L), while WWTP2 effluent showed the higher COD  
6 275 (80 mg/L) and NT (60.6 mg/L) contents (Table S1).

7 276 Noteworthy, the PFA treatment was more efficient with WWTP1, which exhibited  
8 277 the lowest TSS content (Table S1, Fig. 1). This result is consistent with previous  
9 278 studies showing that the disinfection efficiency of UV and peracetic acid increased  
10 279 with low TSS (Dominguez Henao et al., 2018; Walters et al., 2014).

11 280 The Chl and both low and high doses of PFA (PFA\_L and PFA\_H) treatments  
12 281 showed similar *E. coli* content reduction for WWTP1 and WWTP2 ( $p > 0.05$ ),  
13 282 although the *E. coli* concentration (277 CFU/100 mL) was higher for the PFA\_L  
14 283 treatment of WWTP2. All these three treatments were significantly more efficient in  
15 284 decreasing the *E. coli* concentration than the UV treatment ( $p < 0.001$ ). The Chl and  
16 285 PFA\_H treatments had the best efficiency, reducing the *E. coli* concentration below  
17 286 40 CFU/100 mL. A reduction of *E. coli* counts by 3-4  $\log_{10}$  magnitude was observed  
18 287 with the PFA\_H treatment for WWTP1 and WWTP2, while for WWTP3 the  
19 288 reduction of *E. coli* counts was 1  $\log_{10}$  magnitude (Fig. 1). The treatment of WWTP3  
20 289 showed different trends, except for the PFA\_H treatment that was also the most  
21 290 efficient for reducing the *E. coli* content. These observations suggested that the  
22 291 disinfection efficiency, evaluated by the decrease of *E. coli* concentration, was  
23 292 dependent on the water physical-chemical parameters. The treatment with the high  
24 293 dose of PFA (PFA\_H) provided better performance than chlorine and UV,  
25 294 irrespective of the wastewater origin, observation that was in agreement with a  
26 295 previous report (Ragazzo et al., 2020).

27 296 The UV and PFA treatments were less efficient in decreasing the *E. coli*  
28 297 concentration for the WWTP3 wastewater than for WWTP1 and WWTP2, even if the  
29 298 TSS of WWTP2 and WWTP3 were at a similar level (16 and 18 mg/L, respectively).  
30 299 It is likely that the high TSS content of WWTP3 might relate high *E. coli*

1 300 concentration since WWTP3 exhibited also the highest conductivity (1254  $\mu\text{S}/\text{cm}$ ,  
2 301 Table S1), which has been shown to be related to the *E. coli* concentration (Foschi et  
3 al., 2021). The inactivation efficiency of *E. coli* has been shown to be dependent on  
4 302 the WWTP treatment processes (Biswal et al., 2014). Although UV disinfection  
5 303 significantly decreased *E. coli* concentration, it was not efficient as Chl and PFA in  
6 304 WWTP1 and WWTP2. Among the disinfection treatments, only the PFA\_H was  
7 305 effective for reducing the *E. coli* concentration. Such observation was in agreement  
8 306 with previous studies showing that PFA was more efficient in reducing *E. coli*  
9 307 concentration than the disinfectants commonly used for municipal wastewater  
10 308 treatment (Maffettone et al., 2020; Wang et al., 2023).  
11 309

## 12 310 **3.2 Effect of disinfection treatment on microbial community**

13 311 A total of 5,724,372 raw reads was obtained and assigned to 17,151 amplicon  
14 312 sequence variants (ASVs). The sequences were then rarefied at the minimum 71,673  
15 313 reads per sample. The final dataset, including 3,153,612 reads dispatched within  
16 314 14,838 ASVs, was used for the microbial community analysis.

### 17 315 **3.2.1 Impact of disinfection treatments on microbial community diversity**

18 316 The diversity indices (Shannon, Simpson, observed richness, Chao1 and ace) were  
19 317 significantly different (two-way ANOVA,  $p < 0.001$ ) according to the WWTP origin  
20 318 and the treatment, WWTP origin explaining most of the variation (62.8 %,  $p < 0.001$ ;  
21 319 Table S4). The microbial community of the wastewater from WWTP2 exhibited  
22 320 significantly higher diversity indices than those from WWTP1 and WWTP3 (two-way  
23 321 ANOVA,  $p < 0.001$ ; Fig. S1). The microbial community of wastewater from WWTP3  
24 322 had significantly higher Shannon index than that from WWTP1 (two-way ANOVA,  $p$   
25 323  $< 0.001$ ; Fig. S1).

26 324 Noteworthy, the treatments for a given WWTP decreased the microbial diversity  
27 325 (Shannon index and the observed richness, two-way ANOVA,  $p < 0.05$ ; Fig. S2),  
28 326 except for WWTP2 (Fig. S2). Interestingly, the WWTP2, lagoon and reed beds  
29 327 process, showed the higher COD, TOC and TN contents (Table S1). It has been

1 328 shown that the efficiency of a treatment in decreasing the microbial diversity is  
2 329 dependent on the wastewater physical-chemical characteristics such as COD, TOC  
3 330 and TN (Tian and Wang, 2020; Wu et al., 2022), the treatment processes (Sauter et al.,  
4 331 2023), and even on the different type of reed beds systems (Kowal et al., 2021). Thus,  
5 332 it is likely that the high content of organic matter (COD, TOC and TN) might explain  
6 333 the fact that the microbial diversity was not decreased by the treatments in WWTP2.  
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12 334 The WWTP3 showed higher diversity (Shannon and observed richness) indices  
13 335 before treatment (BT) than the two other WWTPs (two-way ANOVA,  $p < 0.05$ ; Fig.  
14 336 S2), which may explain the lower *E. coli* inactivation observed with WWTP3  
15 337 wastewater (Fig 1) despite that it showed the lowest *E. coli* concentration. Such  
16 338 observation was consistent with a previous report showing that the *E. coli* inactivation  
17 339 is dependent on the microbial diversity (Qian et al., 2022).  
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25 340 For the WWTP1, the Shannon index was significantly lower (two-way ANOVA,  
26 341  $p < 0.001$ ; Fig. S2) for PFA\_H than for before treatment (BT), and for the three other  
27 342 treatments. Similarly, the observed richness for PFA\_H was significantly lower than  
28 343 for Chl (two-way ANOVA,  $p < 0.05$ ; Fig. S2). The WWTP3 treated by PFA\_H and  
29 344 Chl showed significantly lower Shannon index and observed richness than BT  
30 345 (two-way ANOVA,  $p < 0.05$ ; Fig. S2).  
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### 39 346 **3.2.2 Impact of disinfection on microbial community structure**

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41 347 The principal coordinate analysis (PCoA), based on either the weighted UniFrac  
42 348 or Bray-Curtis dissimilarity matrices (Fig. 2), further confirmed that the microbial  
43 349 communities of the wastewaters were different according to the WWTPs, revealing  
44 350 also the treatments effects. The PCoA clustered the microbial communities according  
45 351 to their origin (i.e. WWTP), separating the different WWTPs along the axis 1, which  
46 352 explained 66.7% of the variation (Fig. 2a). This observation was supported by the  
47 353 permutational multivariate ANOVA (PERMANOVA) indicating that the WWTP  
48 354 origin was the main factor explaining the differences between the microbial  
49 355 communities (pseudo-F = 79.68,  $p = 0.001$ ). Within a WWTP cluster, the microbial  
50 356 communities clustered by treatment (Fig. 2a). Thus, in order to reveal the effect of the  
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1 357 disinfection treatments on the microbial community, PCoA were performed for each  
2 358 WWTP (Fig. 2b, 2c, 2d). For WWTP2 and WWTP3, wastewater samples from the  
3  
4 359 same treatment tightly clustered together (Fig. 2c, 2d). Envfit (Fits an Environmental  
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6 360 Vector or Factor onto an Ordination) analysis also revealed that the composition of  
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8 361 wastewater microbial communities significantly varied by treatment in WWTP2 ( $r^2 =$   
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10 362  $0.94, p < 0.001$ ) and WWTP3 ( $r^2 = 0.89, p < 0.001$ ), but not in WWTP1.

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12  
13 363 Before the treatments, COD, TOC and TN were identified as the main factors  
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15 364 driving the microbial communities of WWTP2 (Envfit; COD:  $r^2 = 0.9712, p = 0.004$ ;  
16  
17 365 TOC:  $r^2 = 0.9688, p = 0.004$ ; NT:  $r^2 = 0.9682, p = 0.009$ ), while the conductivity  
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19 366 (Envfit;  $r^2 = 0.9930, p = 0.001$ ) and TSS (Envfit;  $r^2 = 0.9844, p = 0.001$ ) were the  
20  
21 367 main factors driving the microbial communities from WWTP3. After the disinfection  
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23 368 treatments, the same factors were found to drive the microbial communities from  
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25 369 WWTP2 and WWTP3 irrespective of the treatment: COD (Chl:  $r^2 = 0.9568, p = 0.004$ ;  
26  
27 370 UV:  $r^2 = 0.9955, p = 0.004$ ; PFA\_L:  $r^2 = 0.9664, p = 0.003$ ; PFA\_H:  $r^2 = 0.9251, p =$   
28  
29 371  $0.003$ ), TOC (Chl:  $r^2 = 0.9522, p = 0.004$ ; UV:  $r^2 = 0.9946, p = 0.004$ ; PFA\_L:  $r^2 =$   
30  
31 372  $0.9601, p = 0.003$ ; PFA\_H:  $r^2 = 0.9139, p = 0.004$ ) and NT (Chl:  $r^2 = 0.9503, p =$   
32  
33 373  $0.020$ ; UV:  $r^2 = 0.9927, p = 0.004$ ; PFA\_L:  $r^2 = 0.9547, p = 0.009$ ; PFA\_H:  $r^2 =$   
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35 374  $0.9051, p = 0.026$ ) for WWTP2; conductivity (Chl:  $r^2 = 0.9936, p = 0.001$ ; UV:  $r^2 =$   
36  
37 375  $0.9907, p = 0.008$ ; PFA\_L:  $r^2 = 0.9968, p = 0.001$ ; PFA\_H:  $r^2 = 0.9866, p = 0.002$ )  
38  
39 376 and TSS (Chl:  $r^2 = 0.9798, p = 0.006$ ; UV:  $r^2 = 0.9943, p = 0.009$ ; PFA\_L:  $r^2 = 0.9883,$   
40  
41 377  $p = 0.006$ ; PFA\_H:  $r^2 = 0.9678, p = 0.006$ ) for WWTP3.

42  
43 378 These results suggested that the origin (i.e. WWTP) was the main factor  
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45 379 determining the composition of the microbial community. The different disinfection  
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47 380 treatments resulted in specific microbial communities, which effects were different  
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49 381 according to the wastewater origin (WWTP).

### 50 51 52 53 382 **3.2.3 Microbial composition differences between WWTPs: identification of** 54 55 383 **specific biomarkers**

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58 384 The distinction of the microbial communities according to their origin (i.e.  
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60 385 WWTP) was confirmed by the presence of different dominant phyla (Fig. 3).

1 386 Proteobacteria (60.5%) was the most dominant bacterial phylum in all three WWTPs,  
2 387 consistent with previous studies in both domestic and industrial WWTPs (Sharma et  
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4 388 al., 2021; Tang et al., 2020a). The relative abundance of the Proteobacteria  
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6 389 significantly decreased from WWTP2 (70.7%) to WWTP3 (61.9%), and to WWTP1  
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8 390 (48.9%) (ANOVA,  $p < 0.001$ ).

10 391 The second dominant phylum in the three WWTPs was Bacteroidetes (11.4%),  
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12 392 followed then by Actinobacteria (8.1%), Nitrospirae (4.3%) and Nanoarchaeaeota  
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14 393 (3.7%). The relative abundance of the Actinobacteria significantly decreased from  
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16 394 WWTP1 (15.1%) to WWTP2 (7.1%), and to WWTP3 (2.3%) (ANOVA,  $p < 0.01$ ).  
17  
18 395 Nitrospirae was significantly more abundant in WWTP3 (11.9%) than in WWTP1  
19  
20 396 (0.6%) and WWTP2 (0.6%) (ANOVA,  $p < 0.001$ ). Nitrospirae includes nitrifying  
21  
22 397 taxa, nitrite-oxidizing bacteria (NOB) and comammox bacteria (Koch et al., 2019).  
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24 398 Nitrospira, the largest class, have been found to be the dominant nitrifiers in  
25  
26 399 wastewater treatment plants (Forrez et al., 2011). Nitrospira, an aerobic  
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28 400 chemolithoautotrophic nitrite-oxidizing bacterium, plays important roles in  
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30 401 nitrification and oxidizes nitrite to nitrate (Daims et al., 2015; Daims and Wagner,  
31  
32 402 2018). It has been identified as keystone taxon, an ideal partner for establishing  
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34 403 microbial relationships under various conditions, in aquatic environment (Veloso et  
35  
36 404 al., 2023).

39 405 The linear discriminant effect size (LEfSe) analysis revealed microbial taxa  
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41 406 specifically more abundant according to the origin of the WWTP. Such taxa found  
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43 407 significantly more abundant to a specific condition by LEfSe analysis has been  
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45 408 proposed to serve as biomarkers (Segata et al., 2011). The LEfSe comparing the  
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47 409 microbial communities before treatment (BT) revealed biomarkers for each WWTP  
48  
49 410 (Fig. 4). For WWTP1, Actinobacteria, Elusimicrobia, Epsilonbacteraeota,  
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51 411 Latescibacteria, Omnitrophicaeota and Spirochaetes phyla, and Parcubacteria and  
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53 412 Campylobacteria class were identified as biomarkers (Fig. 4). Actinobacteria, known  
54  
55 413 for carrying antibiotic resistant genes (ARGs) (Tang et al., 2020b), have been reported  
56  
57 414 dominant in activated sludge processed municipal wastewaters (Kang et al., 2020; Xu  
58  
59 415 et al., 2017). Bacteria affiliated to Parcubacteria have been reported to be involved in

1 416 sulphamethoxazole antibiotic degradation (Song et al., 2019). *Campylobacter* species  
2 417 are known as bacterial indicators for faecal contamination by animals (Ogden et al.,  
3 418 2009).

4 419 For WWTP2, the LEfSe analysis revealed Proteobacteria, Cyanobacteria phyla  
5 420 and Oxyphotobacteria class as biomarkers (Fig. 4). Cyanobacteria are considered to  
6 421 be well adapted to high organic and low oxygen contents (Saqrane et al., 2020).  
7 422 WWTP2 process is based on lagoon system and reed beds, providing probably  
8 423 appropriate growth condition for Cyanobacteria. Cyanobacteria are known to release  
9 424 cyanotoxins (Saqrane et al., 2020). Nevertheless, after disinfection treatments, the  
10 425 relative abundance of Cyanobacteria in WWTP2 decreased to the same level of that  
11 426 found in WWTP1 and WWTP3 (Fig. S3).

12 427 For WWTP3, the biofiltration based process, the LEfSe analysis revealed  
13 428 *Armatimonadetes*, *Verrucomicrobia*, *Acidobacteria*, *Nitrospirae* and  
14 429 *Gemmatimonadetes* phyla, and the *Acidobacteria*, *Nitrospira* and *Planctomycetacia*  
15 430 classes as biomarkers (Fig. 4). Such observation was consistent with previous studies  
16 431 showing that these taxa were found dominant in three different types of biofilters  
17 432 (Gulay et al., 2019; Lautenschlager et al., 2014; Li et al., 2020). *Nitrospirae* and  
18 433 *Verrucomicrobia* have been demonstrated to be involved in the transformation of  
19 434 organic nitrogen (Tang et al., 2021).

20 435 After the disinfection treatments, most of the detected biomarkers differed from  
21 436 those of the non-treated WWTPs (Fig. S3). However, the *Actinobacteria*,  
22 437 *Alphaproteobacteria* and *Acidobacteria* classes were still identified as biomarkers for  
23 438 WWTP1, WWTP2 and WWTP3 respectively, irrespective of the disinfection  
24 439 treatment (Fig. 4, Fig. S3). Such result further confirmed that the origin of  
25 440 wastewaters is an important factor shaping the microbial community (habitat filtering).  
26 441 Thus, it is likely that the WWTP specific biomarkers are reflecting not only the  
27 442 differences on wastewater composition, but also the processing methods involved in  
28 443 the wastewater treatment systems (activated sludge, lagoon and reed beds, and  
29 444 biofilter, for WWTP1, WWTP2 and WWTP3 respectively).

### 3.3 Identification of biomarkers sensitive and resistant to the disinfection treatments

The LEfSe analysis comparing the bacterial communities before (BT) and after each treatment revealed biomarkers, taxa significantly more abundant in a specific condition. Such biomarkers reflect thus the disinfection efficiency of each treatment towards microbial taxa (Fig. 5). Two groups of biomarkers were detected: i) treatment-sensitive biomarkers corresponding to taxa significantly found more abundant in BT (i.e. depleted in the wastewater treated by a disinfectant), and ii) treatment-resistant biomarkers corresponding to taxa significantly found more abundant in treated water.

The numbers of treatment-sensitive biomarkers decreased as follow PFA\_H, PFA\_L, UV, and chlorine. For PFA\_H, 39 treatment-sensitive biomarkers were detected including ASVs related to Flavobacteriaceae, Chromobacteriaceae, *Chromobacterium* and *Escherichia\_Shigella* (Fig. 5), members of which have been reported as dominant taxa in sewage and WWTP (Ahmed et al., 2017; Leroy-Freitas et al., 2022; Yousra et al., 2017). For the PFA\_L, 11 treatment-sensitive biomarkers including the previous discussed taxa Chromobacteriaceae, *Chromobacterium* and *Escherichia\_Shigella* were revealed. For chlorine and UV, only 2 and 4 treatment-sensitive biomarkers were detected, respectively. Additionally, 11, 3, 3 and 1 treatment-resistant biomarkers were detected for the UV, chlorine, PFA\_H and PFA\_L, respectively (Fig. 5). Paracaedibacteraceae, identified as treatment-resistant biomarker for both Chl and UV, has been reported to be significantly enriched after hydrogen peroxide treatment (Piel et al., 2021). *Acetoanaerobium* and *Acetobacterium* were identified as treatment-resistant biomarker of Chl and UV, respectively. *Ottowia*, identified as UV resistant biomarker, was isolated from different types of wastewater treated by activated sludge (Spring et al., 2004). Obscuribacterales, identified as treatment-resistant biomarkers for both PFA\_L and PFA\_H, have been found in activated sludge of aerobic reactor, aquifer, soil and animal (McGorum et al., 2015; Salmaso et al., 2018; Soo et al., 2014). The genome analyses of bacteria belonging to

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474 Obscuribacterales revealed their ability to perform both aerobic and anaerobic  
475 respiration, store polyphosphate, utilize nitrate, as well as their potential to use  
476 carbohydrates and/or fatty acids, but their harmful potential has not been reported so  
477 far (Dueholm et al., 2022; Salmaso et al., 2018).

478 Such results, showing more sensitive biomarkers and less resistant biomarkers for  
479 PFA than for the other treatments, demonstrated a more efficient disinfection of  
480 wastewater by the PFA treatment than the treatments involving UV and chlorine  
481 disinfectants.

482 In order to better understand the differences in effectiveness of disinfectants, we  
483 further performed LefSe analysis comparing all pairs of two treatments (Fig. 6).  
484 Twelve taxa were sensitive to PFA\_L and resistant to chlorine, including  
485 microorganisms affiliated to i) Aeromonadales, Aeromonadaceae and *Aeromonas* that  
486 have been reported as typical taxa in WWTP (Gao et al., 2023; VandeWalle et al.,  
487 2012), and ii) Enterobacteriales and Enterobacteriaceae (Fig. 6, Fig. S4a), members of  
488 which represent faecal indicators (Kausar et al., 2019). Also, 10 taxa were sensitive to  
489 PFA\_L and resistant to UV corresponding, in addition to the above-mentioned taxa, to  
490 *Chromobacterium* and Fusobacteriaceae (Fig. 6, Fig. S4b), also known as faecal  
491 indicators (Hutinel et al., 2022). Thus, PFA exhibits superior disinfection efficacy over  
492 chlorine and UV because taxa related to faecal bacteria have shown increased  
493 sensitivity to PFA.

494 Additionally, biomarkers sensitive to the PFA\_H treatment were found to be  
495 resistant to either Chl (18 biomarkers) or UV (26 biomarkers) treatments (Fig. S4e,f).  
496 The 18 PFA\_H treatment-sensitive biomarkers resistant to Chl included taxa affiliated  
497 to faecal indicators such as Chromobacteriaceae, *Chromobacterium*,  
498 Flavobacteriaceae and *Flavobacterium* (Fig. S4e). Similarly, the 26 PFA\_H  
499 treatment-sensitive biomarkers resistant to UV included faecal indicators such as  
500 Aeromonadales and Aeromonadaceae (Fig. S4f). These observations further  
501 confirmed the higher disinfection efficiency of PFA. Moreover, PFA was also found  
502 to be more efficient when increasing the PFA dose since 11 biomarkers sensitive to  
503 PFA\_H were found resistant to PFA\_L (Fig. 6, Fig. S4c).



1 504 Previous studies have noted that the reaction pathways of *E. coli* inactivation by  
2 505 PFA included both direct and indirect free radical reactions, in which the PFA,  $\bullet$  OH  
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4 506 and peroxide radicals accounted for 73 %, 20 % and 6 %, respectively (Ding et al.,  
5  
6 507 2023). Thus, PFA exhibit higher efficiency and effectiveness than the free radicals. It  
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8 508 has been shown that PFA is predominantly responsible of cell inactivation by  
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10 509 penetrating the microbial cell walls and membranes to damage functional proteins. In  
11  
12 510 contrast, the  $\bullet$  OH and peroxide radicals effectively oxidize the cell interior  
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14 511 (Koivunen and Heinonen-Tanski, 2005; Luukkonen and Pehkonen, 2017). PFA has  
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16 512 been shown to exhibit high reactivity with cysteine and methionine residues through  
17  
18 513 an oxygen transfer pathway (Du et al., 2018; Wang et al., 2023). It has been shown  
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20 514 that the number of broken and cross-linked bacterial cells increases after PFA  
21  
22 515 disinfection causing cell damage like wrinkles, chapped and rough surface on *E. coli*,  
23  
24 516 *S. aureus*, and *B. subtilis* (Ding et al., 2023). The intracellular oxidative stress has  
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26 517 been shown to be higher after PFA treatment (Wang et al., 2023). Thus, the PFA  
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28 518 destroys the cell walls and membranes of microorganisms, resulting in the release of  
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30 519 the cellular content.

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33 520 The regrowth and recovery of pathogenic bacteria and antibiotic resistant bacteria  
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35 521 (ARBs) carrying antibiotic resistance genes (ARGs) can potentially lead to  
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37 522 subsequent contamination of the surface and ground water reservoirs (Shekhawat et  
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39 523 al., 2020). Previous studies showed that bacteria were not able to regrow 24 hours  
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41 524 after PFA disinfection of wastewater without irradiation (Ragazzo et al., 2013).  
42  
43 525 Similarly, *E. coli* and *enterococci* cells showed irreversible inactivation (Rocher et al.,  
44  
45 526 2021). Additionally, PFA has been shown to be beneficial in controlling antibiotic  
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47 527 resistance using antibiotic resistant *E. coli* as typical indicator (Campo et al., 2020).  
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### 50 528 **3.4 Functional predictions**

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54 529 In order to estimate the functional capabilities of the microbial communities,  
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56 530 functional profiles were determined by PICRUSt2. The nearest sequenced taxon  
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58 531 indices (NSTI) indicated that PICRUSt2 prediction was accurate (mean NSTI = 0.11  
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60 532  $\pm$  0.05 SD). Although predictive, with the limitation that no direct evidence of a

1 533 function is provided, such approach provides an overview of the microbial processes  
2 534 (Menendez-Serra et al., 2019; Bourhane et al., 2022, 2023). The comparison of the  
3  
4 535 PICRUSt2 predicted functions of bacterial communities showed that the wastewater  
5  
6 536 samples from the same WWTP clustered together at both low and high KEGG  
7  
8 537 hierarchy levels (Fig. 7a, e). Most of the KEGG orthologs at level 2 had  
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10 538 enrichment/depletion variations among the three WWTPs. They were more abundant  
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12 539 in WWTP2 and WWTP3 than in WWTP1 (Fig. 7e).

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14 540 PCA analysis considering each WWTP revealed that the bacterial communities  
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16 541 clustered according to the disinfectant used for the treatment (Fig. 7b, c, d), showing  
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18 542 the functional shifts within the bacterial communities caused by the different  
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20 543 disinfectants. The heatmap of the abundant functional patterns demonstrated that the  
21  
22 544 wastewater owns a specific functional pattern according to the origin (Fig. 7e).  
23  
24 545 Noteworthy, the functional pattern was also modified by the treatment depending on  
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26 546 the disinfectant used. Consistently with the microbial community differences  
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28 547 observed (Fig. 2), the physical-chemical characteristics prevailing at the WWTP are  
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30 548 the main factors shaping the microbial community structure, composition and  
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32 549 functions. Such observation was in agreement with previous studies reporting that  
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34 550 microbial changes are linked with effluent quality and system performance (Cao et al.,  
35  
36 551 2020; ElNaker et al., 2019).

37  
38 552 The relative abundance of genes involved in cell cycle pathway of *Caulobacter*  
39  
40 553 [PATH: ko04112] significantly decreased in PFA\_H and Chl compared with BT  
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42 554 (ANOVA,  $p < 0.001$ ; Fig. S5). It demonstrates the effects of PFA and chlorine on  
43  
44 555 *Caulobacter* life cycle, which was consistent with a previous study showing that cell  
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46 556 damages (e.g. discharge of cellular materials) were observed after chlorination in  
47  
48 557 *Caulobacter* (Kim et al., 1982). Similarly, the relative abundance of genes associated  
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50 558 to cell motility pathway significantly decreased in PFA\_H compared with BT, being  
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52 559 at lower abundance in PFA\_H and PFA\_L than in UV and Chl (ANOVA,  $p < 0.001$ ;  
53  
54 560 Fig. S6). This result demonstrated the effect of PFA on decreasing the motility and  
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56 561 activity of microorganisms, which was consistent with the reduction of the microbial  
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58 562 diversity and the reduction of *E. coli* and other faecal microorganisms counts  
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1 563 observed in PFA treatment (Fig. 1, Fig. 5, Fig. 6). Such observation further confirmed  
2 564 the effect of PFA on microbial community, which likely relies on the disinfection  
3  
4 565 efficiency. It has been shown that the application of 4 mg/L PFA for 16 h has not  
5  
6 566 toxicological effect using the *V. fischeri* assay (Chhetri et al., 2014), suggesting that  
7  
8 567 PFA has a short decomposition time. PFA was also reported causing less disinfection  
9  
10 568 by-products than other commonly used peracids (Luukkonen and Pehkonen, 2017). In  
11  
12 569 this study, the disinfection was performed with 1 and 4 mg/L PFA for 15 min  
13  
14 570 followed by a quenching step to stop the reaction. Although the residual toxicity test  
15  
16 571 was not conducted in this study, PFA achieved high disinfection efficacy while  
17  
18 572 producing very low residual toxicity according to previous reports (Rocher et al.,  
19  
20 573 2021).

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22  
23 574 Regarding the metabolism associated pathways, the relative abundance of genes  
24  
25 575 involved in the biosynthesis of secondary metabolites and metabolism of amino acids  
26  
27 576 was significantly different (ANOVA,  $p < 0.05$ ) according to the disinfection treatment  
28  
29 577 among all three WWTPs (Fig. 7e, S7-9). Previous studies have also reported that  
30  
31 578 enzymes from the same species may have different activities depending on the  
32  
33 579 treatment and conditions (Delforno et al., 2017; Emmanuel et al., 2019; Yang et al.,  
34  
35 580 2020).

36  
37 581 The genes involved in penicillin and cephalosporin biosynthesis pathway [PATH:  
38  
39 582 ko00311] were significantly depleted on UV and Chl in comparison with PFA\_H and  
40  
41 583 PFA\_L treatments (ANOVA,  $p < 0.05$ ; Fig. S7). Similarly, the abundance of genes  
42  
43 584 involved in betalain biosynthesis pathway [PATH: ko00965] was significantly more  
44  
45 585 abundant in PFA\_H than UV (ANOVA,  $p < 0.05$ ; Fig. S8). Betalain has been reported  
46  
47 586 for its antimicrobial activity on a broad spectrum of microbes such as  
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49 587 multidrug-resistant bacteria, single-species and dual-species biofilm-producing  
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51 588 bacteria, dengue virus and fungi (Wijesinghe and Choo, 2022). Betalains primarily  
52  
53 589 occur in Caryophyllales order plants. The enzymes involved in betalain biosynthesis  
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55 590 have been reported in fungi, *Gluconacetobacter diazotrophicus* and Cyanobacteria  
56  
57 591 (Contreras-Llano et al., 2019; Guerrero-Rubio et al., 2020). However, LEfSe did not  
58  
59 592 identified Cyanobacteria as PFA-resistant biomarkers (Fig. S4), suggesting that

1 593 bacteria producing antimicrobial compounds (betalains), likely present in treated  
2 594 wastewater, were more sensitive to UV and chlorine than to PFA. Since UV and  
3  
4 595 chlorination have been reported for their strong elimination efficiency of antibiotic by  
5  
6 596 photolysis (Ding et al., 2020; Serna-Galvis et al., 2017), such a slight deficiency does  
7  
8 597 not indicate that PFA is incompetent. It nevertheless alerts the risk of antibiotics  
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10 598 release from wastewater into water system that potentially alters microbial ecosystems,  
11  
12 599 that in turn represents a sanitary issue for human health (Baquero et al., 2008). The  
13  
14 600 genes involved in glutathione metabolism pathway [PATH: ko00480] were  
15  
16 601 significantly more abundant in PFA\_H (ANOVA,  $p < 0.05$ ; Fig. S9). The glutathione  
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18 602 metabolism has been suggested to play important role in bacterial defence against  
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20 603 chlorine-based disinfection (Du et al., 2015), demonstrating that PFA has likely  
21  
22 604 similar effect on bacteria.  
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24  
25 605 These results suggested that PFA\_H, even efficient on disinfecting most of the  
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27 606 microorganisms (including pathogens), was not effective on inactivating the  
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29 607 microorganisms carrying penicillin and cephalosporin, and betalain biosynthesis  
30  
31 608 pathways. However, further analyses determining direct functions, such as shotgun  
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33 609 metagenomics, are needed in order to describe in depth the effect of disinfection on  
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35 610 the functional capabilities of microbial communities.  
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#### 37 38 611 **4. Conclusions**

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41 612 Although the operational cost of PFA treatment has been estimated to be  
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43 613 approximately 2.5 times more expensive than that of chlorine (Luukkonen et al.,  
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45 614 2015), the PFA treatment owns several assets to be used as treatment for safe reuse of  
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47 615 wastewater as it does not produce by-product. Also it has been shown that its cost can  
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49 616 be drastically reduced for treatment plants with larger capacity (Ragazzo et al., 2013).  
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51 617 But, even if further analyses are still required to comprehensively evaluate the costs, it  
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53 618 is also necessary to evaluate the disinfection efficiency of PFA treatment in  
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55 619 comparison to other commonly used treatments.  
56

57 620 Our study elucidated the effect of PFA, UV and chlorine on microorganism  
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59 621 disinfection, microbial community diversity, structure and functions crossing three  
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1 622 different WWTPs. It provides compelling evidence that different disinfectants  
2 623 resulted in specific microbial communities and functions, which effects differed  
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4 624 according to the physical-chemical characteristic of the wastewater origin (WWTP).  
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6 625 Our findings indicated that PFA was efficient in *E. coli* disinfection of wastewater,  
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8 626 being able to decrease the microbial community diversity. Additionally, specific  
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10 627 biomarkers of each WWTP were revealed, which likely relies on the processing  
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12 628 treatments methods. Also, our study detected sensitive/resistant biomarkers for each  
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14 629 disinfectant, PFA exhibiting higher number of sensitive biomarkers than chlorine and  
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16 630 UV, PHA\_H having the highest number. Our findings underline the fact that PFA is  
17  
18 631 efficient on wastewater disinfection in comparison with the traditional treatments  
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20 632 involving UV and chlorine disinfectants. Because PFA was particularly effective in  
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22 633 removing known faecal microorganisms, it has high potential for safe reuse of  
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24 634 wastewater.  
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27 635

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42  
43 643 analysis, Writing – original draft. **RD:** Conceptualisation, Investigation, Resources,  
44  
45 644 Formal analysis, Writing – original draft, Supervision, Project administration, Funding  
46  
47 645 acquisition. **TG:** Conceptualisation, Writing – original draft, Supervision, Project  
48  
49 646 administration, Funding acquisition. **CCL:** Conceptualisation, Investigation,  
50  
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2  
3 653 Sequence Read Archive (SRA) of the National Center for Biotechnology Information  
4  
5 654 (NCBI) and is available under the Bioproject ID PRJNA907723.  
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## References

- 655  
656  
657 Ahmed, W., Staley, C., Sidhu, J., Sadowsky, M., Toze, S., 2017. Amplicon-based profiling of bacteria in  
658 raw and secondary treated wastewater from treatment plants across Australia. *Applied Microbiology*  
659 *and Biotechnology* 101, 1253-1266. 10.1007/s00253-016-7959-9.
- 660 Anastasi, E.M., Wohlsen, T.D., Stratton, H.M., Katouli, M., 2013. Survival of *Escherichia coli* in two  
661 sewage treatment plants using UV irradiation and chlorination for disinfection. *Water research* 47,  
662 6670-6679. 10.1016/j.watres.2013.09.008.
- 663 Andreadakis, A., Mamais, D., Christoulas, D., Kabylafka, S., 1999. Ultraviolet Disinfection of Secondary  
664 and Tertiary Effluent in the Mediterranean Region. *Water Science and Technology* 40, 253-260.  
665 10.2166/wst.1999.0598.
- 666 APHA, A.W., 2005. *Standard Methods for the Examination of Water and Wastewater*, 21st Edition ed.  
667 American Public Health Association, American Water Works Association and Water Environment  
668 Federation, Washington, DC, USA
- 669 Baquero, F., Martinez, J.L., Canton, R., 2008. Antibiotics and antibiotic resistance in water  
670 environments. *Current opinion in biotechnology* 19, 260-265. 10.1016/j.copbio.2008.05.006.
- 671 Behera, P., Mahapatra, S., Mohapatra, M., Kim, J.Y., Adhya, T.K., Raina, V., Suar, M., Pattnaik, A.K.,  
672 Rastogi, G., 2017. Salinity and macrophyte drive the biogeography of the sedimentary bacterial  
673 communities in a brackish water tropical coastal lagoon. *Science of the Total Environment* 595,  
674 472-485. 10.1016/j.scitotenv.2017.03.271.
- 675 Biswal, B.K., Khairallah, R., Bibi, K., Mazza, A., Gehr, R., Masson, L., Frigon, D., 2014. Impact of UV and  
676 peracetic acid disinfection on the prevalence of virulence and antimicrobial resistance genes in  
677 uropathogenic *Escherichia coli* in wastewater effluents. *Appl Environ Microb* 80, 3656-3666
- 678 Bourhane, Z., Cagnon, C., Castañeda, C., Rodriguez-Ochoa, R., Alvaro-Fuentes, J., Cravo-Laureau, C.,  
679 Duran, R., 2023. Vertical organisation of microbial communities in Salineta hypersaline wetland, Spain.  
680 *Front Microbiol* 14: 869907.
- 681 Bourhane, Z., Lanzen, A., Cagnon, C., Ben Said, O., Mahmoudi, E., Coulon, F., Atai, E., Borja, A.,  
682 Cravo-Laureau, C., Duran, R., 2022. Microbial diversity alteration reveals biomarkers of contamination  
683 in soil-river-lake continuum. *J Haz Mat* 421, 126789.
- 684 Bull, R.J., Birnbaum, L.S., Cantor, K.P., Rose, J.B., Butterworth, B.E., Pegram, R., Tuomisto, J., 1995.  
685 Water chlorination: essential process or cancer hazard? *Fundamental and applied toxicology : official*  
686 *journal of the Society of Toxicology* 28, 155-166. 10.1006/faat.1995.1156.
- 687 Campo, N., De Flora, C., Maffettone, R., Manoli, K., Sarathy, S., Santoro, D., Gonzalez-Olmos, R., Auset,  
688 M., 2020. Inactivation kinetics of antibiotic resistant *Escherichia coli* in secondary wastewater  
689 effluents by peracetic and performic acids. *Water research* 169, 115227.  
690 10.1016/j.watres.2019.115227.
- 691 Cao, J., Zhang, T., Wu, Y., Sun, Y., Zhang, Y., Huang, B., Fu, B., Yang, E., Zhang, Q., Luo, J., 2020.  
692 Correlations of nitrogen removal and core functional genera in full-scale wastewater treatment plants:  
693 Influences of different treatment processes and influent characteristics. *Bioresource technology* 297,  
694 122455. 10.1016/j.biortech.2019.122455.
- 695 Chhetri, R.K., Thornberg, D., Berner, J., Gramstad, R., Ojstedt, U., Sharma, A.K., Andersen, H.R., 2014.  
696 Chemical disinfection of combined sewer overflow waters using performic acid or peracetic acids. *The*  
697 *Science of the total environment* 490, 1065-1072. 10.1016/j.scitotenv.2014.05.079.
- 698 Collivignarelli, M.C., Abbà, A., Alloisio, G., Gozio, E., Benigna, I., 2017. Disinfection in Wastewater

699 Treatment Plants: Evaluation of Effectiveness and Acute Toxicity Effects. Sustainability 9  
1 700 Contreras-Llano, L.E., Guerrero-Rubio, M.A., Lozada-Ramirez, J.D., Garcia-Carmona, F., Gandia-Herrero,  
2 F., 2019. First Betalain-Producing Bacteria Break the Exclusive Presence of the Pigments in the Plant  
3 Kingdom. *Mbio* 10. 10.1128/mBio.00345-19.  
4 702  
5 703 Daims, H., Lebedeva, E.V., Pjevac, P., Han, P., Herbold, C., Albertsen, M., Jehmlich, N., Palatinszky, M.,  
6 Vierheilg, J., Bulaev, A., Kirkegaard, R.H., von Bergen, M., Rattei, T., Bendinger, B., Nielsen, P.H.,  
7 704 Wagner, M., 2015. Complete nitrification by *Nitrospira* bacteria. *Nature* 528, 504-509.  
8 705 10.1038/nature16461.  
9 706  
10 707 Daims, H., Wagner, M., 2018. *Nitrospira*. *Trends Microbiol* 26, 462-463. 10.1016/j.tim.2018.02.001.  
11 708 Delforno, T.P., Lacerda, G.V., Jr., Sierra-Garcia, I.N., Okada, D.Y., Macedo, T.Z., Varesche, M.B., Oliveira,  
12 V.M., 2017. Metagenomic analysis of the microbiome in three different bioreactor configurations  
13 applied to commercial laundry wastewater treatment. *The Science of the total environment* 587-588,  
14 709 389-398. 10.1016/j.scitotenv.2017.02.170.  
15 710  
16 711 Diamond, M.B., Keshaviah, A., Bento, A.I., Conroy-Ben, O., Driver, E.M., Ensor, K.B., Halden, R.U.,  
17 Hopkins, L.P., Kuhn, K.G., Moe, C.L., Rouchka, E.C., Smith, T., Stevenson, B.S., Susswein, Z., Vogel, J.R.,  
18 712 Wolfe, M.K., Stadler, L.B., Scarpino, S.V., 2022. Wastewater surveillance of pathogens can inform public  
19 713 health responses. *Nat Med* 28, 1992-1995. 10.1038/s41591-022-01940-x.  
20 714  
21 715 Ding, N., Li, Z., Jiang, L., Liu, H., Zhang, Y., Sun, Y., 2023. Kinetics and mechanisms of bacteria  
22 716 disinfection by performic acid in wastewater: In comparison with peracetic acid and sodium  
23 717 hypochlorite. *The Science of the total environment* 878, 162606. 10.1016/j.scitotenv.2023.162606.  
24 718  
25 719 Ding, Y., Jiang, W., Liang, B., Han, J., Cheng, H., Haider, M.R., Wang, H., Liu, W., Liu, S., Wang, A., 2020.  
26 720 UV photolysis as an efficient pretreatment method for antibiotics decomposition and their  
27 721 antibacterial activity elimination. *Journal of hazardous materials* 392, 122321.  
28 722 10.1016/j.jhazmat.2020.122321.  
29 723  
30 724 Dominguez Henao, L., Cascio, M., Turolla, A., Antonelli, M., 2018. Effect of suspended solids on  
31 725 peracetic acid decay and bacterial inactivation kinetics: Experimental assessment and definition of  
32 726 predictive models. *The Science of the total environment* 643, 936-945.  
33 727 10.1016/j.scitotenv.2018.06.219.  
34 728  
35 729 Du, P., Liu, W., Cao, H., Zhao, H., Huang, C.H., 2018. Oxidation of amino acids by peracetic acid:  
36 730 Reaction kinetics, pathways and theoretical calculations. *Water research X* 1, 100002.  
37 731 10.1016/j.wroa.2018.09.002.  
38 732  
39 733 Du, Z., Nandakumar, R., Nickerson, K.W., Li, X., 2015. Proteomic adaptations to starvation prepare  
40 734 *Escherichia coli* for disinfection tolerance. *Water research* 69, 110-119. 10.1016/j.watres.2014.11.016.  
41 735  
42 736 Dueholm, M.K.D., Nierychlo, M., Andersen, K.S., Rudkjoberg, V., Knutsson, S., Mi, D.A.S.G.C., Albertsen,  
43 737 M., Nielsen, P.H., 2022. MiDAS 4: A global catalogue of full-length 16S rRNA gene sequences and  
44 738 taxonomy for studies of bacterial communities in wastewater treatment plants. *Nat Commun* 13, 1908.  
45 739 10.1038/s41467-022-29438-7.  
46 740  
47 741 EC, 2018. Proposal for a Regulation of the European Parliament and of the Council on Minimum  
48 742 Requirements for Water Reuse, in: Commission, E. (Ed.), COM, Brussels  
49 743  
50 744 EC, 2022. Water Reuse Fact Sheet-Water is too precious to waste. European Commission  
51 745  
52 746 ElNaker, N.A., Hasan, S.W., Yousef, A.F., 2019. Impact of current density on the function and microbial  
53 747 community structure in electro-bioreactors. *Journal of hazardous materials* 368, 877-884.  
54 748 10.1016/j.jhazmat.2018.09.016.  
55 749  
56 750 Emmanuel, S.A., Sul, W.J., Seong, H.J., Rhee, C., Ekpheghere, K.I., Kim, I.S., Kim, H.G., Koh, S.C., 2019.



1 743 Metagenomic analysis of relationships between the denitrification process and carbon metabolism in  
2 744 a bioaugmented full-scale tannery wastewater treatment plant. *World journal of microbiology &*  
3 745 *biotechnology* 35, 149. 10.1007/s11274-019-2716-8.  
4 746 EPA, I., 2011. *Water Treatment Manual: Disinfection*. Environmental Protection Agency Wexford,  
5 747 Ireland  
6  
7 748 EPA, U., 2004. *Guidelines for water reuse*. Washington, DC 450  
8 749 Forrez, I., Boon, N., Verstraete, W., Carballa, M., 2011. 6.38 - Biodegradation of Micropollutants and  
9 750 Prospects for Water and Wastewater Biotreatment, in: Moo-Young, M. (Ed.), *Comprehensive*  
10 751 *Biotechnology* (Second Edition). Academic Press, Burlington, pp. 485-494.  
11 752 Foschi, J., Turolla, A., Antonelli, M., 2021. Soft sensor predictor of *E. coli* concentration based on  
12 753 conventional monitoring parameters for wastewater disinfection control. *Water research* 191, 116806.  
13 754 10.1016/j.watres.2021.116806.  
14 755 Furst, K.E., Pecson, B.M., Webber, B.D., Mitch, W.A., 2018. Tradeoffs between pathogen inactivation  
15 756 and disinfection byproduct formation during sequential chlorine and chloramine disinfection for  
16 757 wastewater reuse. *Water research* 143, 579-588. 10.1016/j.watres.2018.05.050.  
17 758 Gao, F.Z., He, L.Y., He, L.X., Zou, H.Y., Zhang, M., Wu, D.L., Liu, Y.S., Shi, Y.J., Bai, H., Ying, G.G., 2020.  
18 759 Untreated swine wastes changed antibiotic resistance and microbial community in the soils and  
19 760 impacted abundances of antibiotic resistance genes in the vegetables. *The Science of the total*  
20 761 *environment* 741, 140482. 10.1016/j.scitotenv.2020.140482.  
21 762 Gao, Y., Shi, X., Jin, X., Wang, X.C., Jin, P., 2023. A critical review of wastewater quality variation and  
22 763 in-sewer processes during conveyance in sewer systems. *Water research* 228, 119398.  
23 764 10.1016/j.watres.2022.119398.  
24 765 Gehr, R., Chen, D., Moreau, M., 2009. Performic acid (PFA): tests on an advanced primary effluent  
25 766 show promising disinfection performance. *Water science and technology : a journal of the*  
26 767 *International Association on Water Pollution Research* 59, 89-96. 10.2166/wst.2009.761.  
27 768 Gerba, C.P., Pepper, I.L., 2019. Chapter 22 - Municipal Wastewater Treatment, in: Brusseau, M.L.,  
28 769 Pepper, I.L., Gerba, C.P. (Eds.), *Environmental and Pollution Science* (Third Edition). Academic Press, pp.  
29 770 393-418.  
30 771 Guerrero-Rubio, M.A., Garcia-Carmona, F., Gandia-Herrero, F., 2020. First description of betalains  
31 772 biosynthesis in an aquatic organism: characterization of 4,5-DOPA-extradial-dioxygenase activity in the  
32 773 cyanobacteria *Anabaena cylindrica*. *Microbial biotechnology* 13, 1948-1959.  
33 774 10.1111/1751-7915.13641.  
34 775 Gulay, A., Fowler, S.J., Tatari, K., Thamdrup, B., Albrechtsen, H.J., Al-Soud, W.A., Sorensen, S.J., Smets,  
35 776 B.F., 2019. DNA- and RNA-SIP Reveal *Nitrospira* spp. as Key Drivers of Nitrification in Groundwater-Fed  
36 777 Biofilters. *Mbio* 10, e01870-01819. 10.1128/mBio.01870-19.  
37 778 Hutinel, M., Larsson, D.G.J., Flach, C.F., 2022. Antibiotic resistance genes of emerging concern in  
38 779 municipal and hospital wastewater from a major Swedish city. *The Science of the total environment*  
39 780 812, 151433. 10.1016/j.scitotenv.2021.151433.  
40 781 Kang, X.H., Leng, Y., O, M.M., Zeng, X.Y., Li, S.W., 2020. The seasonal changes of core bacterial  
41 782 community decide sewage purification in sub-plateau municipal sewage treatment plants. *Bioprocess*  
42 783 *and biosystems engineering* 43, 1609-1617. 10.1007/s00449-020-02352-2.  
43 784 Kantor, R.S., Miller, S.E., Nelson, K.L., 2019. The Water Microbiome Through a Pilot Scale Advanced  
44 785 Treatment Facility for Direct Potable Reuse. *Front Microbiol* 10, 993. 10.3389/fmicb.2019.00993.  
45 786 Kauser, I., Ciesielski, M., Poretsky, R.S., 2019. Ultraviolet disinfection impacts the microbial community

787 composition and function of treated wastewater effluent and the receiving urban river. PeerJ 7, e7455.  
788 10.7717/peerj.7455.

789 Kim, C.-K., Park, M.-K., Yum, K., 1982. Effects of chlorine and chlorine compounds on morphology and  
790 function of *Caulobacter* cells. *Applied Microscopy* 12, 23-32

791 Koch, H., van Kessel, M., Lucker, S., 2019. Complete nitrification: insights into the ecophysiology of  
792 comammox *Nitrospira*. *Appl Microbiol Biotechnol* 103, 177-189. 10.1007/s00253-018-9486-3.

793 Koivunen, J., Heinonen-Tanski, H., 2005. Inactivation of enteric microorganisms with chemical  
794 disinfectants, UV irradiation and combined chemical/UV treatments. *Water research* 39, 1519-1526.  
795 10.1016/j.watres.2005.01.021.

796 Kowal, P., Ciesielski, S., Godzieba, M., Fitobor, K., Gajewska, M., Kolecka, K., 2021. Assessment of  
797 diversity and composition of bacterial community in sludge treatment reed bed systems. *The Science*  
798 *of the total environment* 756, 144060. 10.1016/j.scitotenv.2020.144060.

799 Kulkarni, P., Olson, N.D., Paulson, J.N., Pop, M., Maddox, C., Claye, E., Rosenberg Goldstein, R.E.,  
800 Sharma, M., Gibbs, S.G., Mongodin, E.F., Sapkota, A.R., 2018. Conventional wastewater treatment and  
801 reuse site practices modify bacterial community structure but do not eliminate some opportunistic  
802 pathogens in reclaimed water. *The Science of the total environment* 639, 1126-1137.  
803 10.1016/j.scitotenv.2018.05.178.

804 Lautenschlager, K., Hwang, C., Ling, F., Liu, W.T., Boon, N., Koster, O., Egli, T., Hammes, F., 2014.  
805 Abundance and composition of indigenous bacterial communities in a multi-step biofiltration-based  
806 drinking water treatment plant. *Water research* 62, 40-52. 10.1016/j.watres.2014.05.035.

807 Leroy-Freitas, D., Machado, E.C., Torres-Franco, A.F., Dias, M.F., Leal, C.D., Araujo, J.C., 2022. Exploring  
808 the microbiome, antibiotic resistance genes, mobile genetic element, and potential resistant  
809 pathogens in municipal wastewater treatment plants in Brazil. *The Science of the total environment*  
810 842, 156773. 10.1016/j.scitotenv.2022.156773.

811 Li, Y., Wang, Y., Wan, D., Li, B., Zhang, P., Wang, H., 2020. Pilot-scale application of sulfur-limestone  
812 autotrophic denitrification biofilter for municipal tailwater treatment: Performance and microbial  
813 community structure. *Bioresource technology* 300, 122682. 10.1016/j.biortech.2019.122682.

814 Luukkonen, T., Heynink, T., Ramo, J., Lassi, U., 2015. Comparison of organic peracids in wastewater  
815 treatment: Disinfection, oxidation and corrosion. *Water research* 85, 275-285.  
816 10.1016/j.watres.2015.08.037.

817 Luukkonen, T., Pehkonen, S.O., 2017. Peracids in water treatment: A critical review. *Critical Reviews in*  
818 *Environmental Science and Technology* 47, 1-39. 10.1080/10643389.2016.1272343.

819 Maffettone, R., Manoli, K., Santoro, D., Passalacqua, K.D., Wobus, C.E., Sarathy, S., 2020. Performic  
820 Acid Disinfection of Municipal Secondary Effluent Wastewater: Inactivation of Murine Norovirus, Fecal  
821 Coliforms, and Enterococci. *Environ Sci Technol* 54, 12761-12770. 10.1021/acs.est.0c05144.

822 Magoc, T., Salzberg, S.L., 2011. FLASH: fast length adjustment of short reads to improve genome  
823 assemblies. *Bioinformatics* 27, 2957-2963. 10.1093/bioinformatics/btr507.

824 McCall, C., Wu, H., Miyani, B., Xagorarakis, I., 2020. Identification of multiple potential viral diseases in  
825 a large urban center using wastewater surveillance. *Water research* 184, 116160.  
826 10.1016/j.watres.2020.116160.

827 McGorum, B.C., Pirie, R.S., Glendinning, L., McLachlan, G., Metcalf, J.S., Banack, S.A., Cox, P.A., Codd,  
828 G.A., 2015. Grazing livestock are exposed to terrestrial cyanobacteria. *Veterinary research* 46, 16.  
829 10.1186/s13567-015-0143-x.

830 Menendez-Serra, M., Triado-Margarit, X., Castaneda, C., Herrero, J., Casamayor, E.O., 2019. Microbial

1 831 composition, potential functional roles and genetic novelty in gypsum-rich and hypersaline soils of  
2 832 Monegros and Gallocanta (Spain). *The Science of the total environment* 650, 343-353.  
3 833 10.1016/j.scitotenv.2018.09.050.  
4 834 Ng, C., Tan, B., Jiang, X.T., Gu, X., Chen, H., Schmitz, B.W., Haller, L., Charles, F.R., Zhang, T., Gin, K.,  
5 835 2019. Metagenomic and Resistome Analysis of a Full-Scale Municipal Wastewater Treatment Plant in  
6 836 Singapore Containing Membrane Bioreactors. *Front Microbiol* 10, 172. 10.3389/fmicb.2019.00172.  
7 837 Nieuwenhuijsen, M.J., Toledano, M.B., Eaton, N.E., Fawell, J., Elliott, P., 2000. Chlorination disinfection  
8 838 byproducts in water and their association with adverse reproductive outcomes: a review. *Occup*  
9 839 *Environ Med* 57, 73-85. 10.1136/oem.57.2.73.  
10 840 Numberger, D., Ganzert, L., Zoccarato, L., Muhldorfer, K., Sauer, S., Grossart, H.P., Greenwood, A.D.,  
11 841 2019. Characterization of bacterial communities in wastewater with enhanced taxonomic resolution  
12 842 by full-length 16S rRNA sequencing. *Sci Rep* 9, 9673. 10.1038/s41598-019-46015-z.  
13 843 Ogden, I.D., Dallas, J.F., MacRae, M., Rotariu, O., Reay, K.W., Leitch, M., Thomson, A.P., Sheppard, S.K.,  
14 844 Maiden, M., Forbes, K.J., Strachan, N.J., 2009. *Campylobacter* excreted into the environment by  
15 845 animal sources: prevalence, concentration shed, and host association. *Foodborne Pathog Dis* 6,  
16 846 1161-1170. 10.1089/fpd.2009.0327.  
17 847 Oguma, K., Katayama, H., Mitani, H., Morita, S., Hirata, T., Ohgaki, S., 2001. Determination of  
18 848 pyrimidine dimers in *Escherichia coli* and *Cryptosporidium parvum* during UV light inactivation,  
19 849 photoreactivation, and dark repair. *Applied and environmental microbiology* 67, 4630-4637.  
20 850 10.1128/AEM.67.10.4630-4637.2001.  
21 851 Pei, M., Zhang, B., He, Y., Su, J., Gin, K., Lev, O., Shen, G., Hu, S., 2019. State of the art of tertiary  
22 852 treatment technologies for controlling antibiotic resistance in wastewater treatment plants. *Environ*  
23 853 *Int* 131, 105026. 10.1016/j.envint.2019.105026.  
24 854 Piel, T., Sandrini, G., Muyzer, G., Brussaard, C.P.D., Slot, P.C., van Herk, M.J., Huisman, J., Visser, P.M.,  
25 855 2021. Resilience of Microbial Communities after Hydrogen Peroxide Treatment of a Eutrophic Lake to  
26 856 Suppress Harmful Cyanobacterial Blooms. *Microorganisms* 9. 10.3390/microorganisms9071495.  
27 857 Qian, S., Hou, R., Yuan, R., Zhou, B., Chen, Z., Chen, H., 2022. Removal of *Escherichia coli* from  
28 858 domestic sewage using biological sand filters: Reduction effect and microbial community analysis.  
29 859 *Environmental research* 209, 112908. 10.1016/j.envres.2022.112908.  
30 860 Ragazzo, P., Chiuccini, N., Piccolo, V., Ostoich, M., 2013. A new disinfection system for wastewater  
31 861 treatment: performic acid full-scale trial evaluations. *Water science and technology : a journal of the*  
32 862 *International Association on Water Pollution Research* 67, 2476-2487. 10.2166/wst.2013.137.  
33 863 Ragazzo, P., Chiuccini, N., Piccolo, V., Spadolini, M., Carrer, S., Zanon, F., Gehr, R., 2020. Wastewater  
34 864 disinfection: long-term laboratory and full-scale studies on performic acid in comparison with  
35 865 peracetic acid and chlorine. *Water research* 184, 116169. 10.1016/j.watres.2020.116169.  
36 866 Rocher, V., Azimi, S., Mailler, R., Rechdaoui-Guérin, S., Mèche, P., Pichon, S., Goffin, A., Bernier, J., Roy,  
37 867 J., Varrault, G., 2021. Effectiveness of Disinfecting Wastewater Treatment Plant Discharges: Case of  
38 868 chemical disinfection using performic acid. IWA Publishing.  
39 869 Salmaso, N., Albanese, D., Capelli, C., Boscaini, A., Pindo, M., Donati, C., 2018. Diversity and Cyclical  
40 870 Seasonal Transitions in the Bacterial Community in a Large and Deep Perialpine Lake. *Microb Ecol* 76,  
41 871 125-143. 10.1007/s00248-017-1120-x.  
42 872 Saqrane, S., Oudra, B., El Mhammedi, M.A., 2020. Remediation of Freshwaters Contaminated by  
43 873 Cyanobacteria, in: Inamuddin, Ahamed, M.I., Lichtfouse, E., Asiri, A.M. (Eds.), *Methods for*  
44 874 *Bioremediation of Water and Wastewater Pollution*. Springer International Publishing, Cham, pp.

875 161-180.

1 876 Sauter, D., Steuer, A., Wasmund, K., Hausmann, B., Szewzyk, U., Sperlich, A., Gnirss, R., Cooper, M.,  
2 877 Wintgens, T., 2023. Microbial communities and processes in biofilters for post-treatment of ozonated  
3 wastewater treatment plant effluent. *The Science of the total environment* 856, 159265.  
4 878 10.1016/j.scitotenv.2022.159265.  
5 879

6 880 Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S., Huttenhower, C., 2011.  
7 881 Metagenomic biomarker discovery and explanation. *Genome biology* 12, R60.  
8 882 10.1186/gb-2011-12-6-r60.  
9 883

10 883 Serna-Galvis, E.A., Ferraro, F., Silva-Agredo, J., Torres-Palma, R.A., 2017. Degradation of highly  
11 884 consumed fluoroquinolones, penicillins and cephalosporins in distilled water and simulated hospital  
12 wastewater by UV(254) and UV(254)/persulfate processes. *Water research* 122, 128-138.  
13 885 10.1016/j.watres.2017.05.065.  
14 886

15 887 Sharma, P., Tripathi, S., Chandra, R., 2021. Metagenomic analysis for profiling of microbial  
16 888 communities and tolerance in metal-polluted pulp and paper industry wastewater. *Bioresource  
17 technology* 324, 124681. 10.1016/j.biortech.2021.124681.  
18 889

19 890 Shekhawat, S.S., Kulshreshtha, N.M., Gupta, A.B., 2020. Investigation of chlorine tolerance profile of  
20 891 dominant gram negative bacteria recovered from secondary treated wastewater in Jaipur, India.  
21 *Journal of environmental management* 255, 109827. 10.1016/j.jenvman.2019.109827.  
22 892

23 893 Song, M., Luo, C., Jiang, L., Peng, K., Zhang, D., Zhang, R., Li, Y., Zhang, G., 2019. The presence of in situ  
24 894 sulphamethoxazole degraders and their interactions with other microbes in activated sludge as  
25 895 revealed by DNA stable isotope probing and molecular ecological network analysis. *Environ Int* 124,  
26 896 121-129. 10.1016/j.envint.2018.12.039.  
27 897

28 897 Soo, R.M., Skennerton, C.T., Sekiguchi, Y., Imelfort, M., Paech, S.J., Dennis, P.G., Steen, J.A., Parks, D.H.,  
29 898 Tyson, G.W., Hugenholtz, P., 2014. An expanded genomic representation of the phylum cyanobacteria.  
30 *Genome biology and evolution* 6, 1031-1045. 10.1093/gbe/evu073.  
31 899

32 900 Spring, S., Jackel, U., Wagner, M., Kampfer, P., 2004. *Ottowia thiooxydans* gen. nov., sp. nov., a novel  
33 901 facultatively anaerobic, N<sub>2</sub>O-producing bacterium isolated from activated sludge, and transfer of  
34 902 *Aquaspirillum gracile* to *Hylemonella gracilis* gen. nov., comb. nov. *Int J Syst Evol Microbiol* 54, 99-106.  
35 903 10.1099/ijs.0.02727-0.  
36 904

37 904 Stamps, B.W., Leddy, M.B., Plumlee, M.H., Hasan, N.A., Colwell, R.R., Spear, J.R., 2018. Characterization  
38 905 of the Microbiome at the World's Largest Potable Water Reuse Facility. *Front Microbiol* 9, 2435.  
39 906 10.3389/fmicb.2018.02435.  
40 907

41 907 Tang, G., Li, B., Zhang, B., Wang, C., Zeng, G., Zheng, X., Liu, C., 2021. Dynamics of dissolved organic  
42 908 matter and dissolved organic nitrogen during anaerobic/anoxic/oxic treatment processes. *Bioresource  
43 909 technology* 331, 125026. <https://doi.org/10.1016/j.biortech.2021.125026>.  
44 910

45 910 Tang, G., Zheng, X., Li, X., Liu, T., Wang, Y., Ma, Y., Ji, Y., Qiu, X., Wan, Y., Pan, B., 2020a. Variation of  
46 911 effluent organic matter (EfOM) during anaerobic/anoxic/oxic (A(2)O) wastewater treatment processes.  
47 912 *Water research* 178, 115830. 10.1016/j.watres.2020.115830.  
48 913

49 913 Tang, Z., Xi, B., Huang, C., Tan, W., Li, W., Zhao, X., Liu, K., Xia, X., 2020b. Mobile genetic elements in  
50 914 potential host microorganisms are the key hindrance for the removal of antibiotic resistance genes in  
51 915 industrial-scale composting with municipal solid waste. *Bioresource technology* 301, 122723.  
52 916 10.1016/j.biortech.2019.122723.  
53 917

54 917 Tian, L., Wang, L., 2020. A meta-analysis of microbial community structures and associated metabolic  
55 918 potential of municipal wastewater treatment plants in global scope. *Environmental Pollution* 263,

919 114598. 10.1016/j.envpol.2020.114598.  
1 920 USEPA, 2003. Wastewater Technology Fact Sheet-Disinfection for Small Systems. United States  
2 Environmental Protection Agency  
3 921  
4 922 USEPA, 2004. Primer for municipal wastewater treatment systems, in: USEPA (Ed.). United States  
5 Environmental Protection Agency, Washington, D.C., United States, p. 29 p.  
6 923  
7 924 Vällitalo, P., Massei, R., Heiskanen, I., Behnisch, P., Brack, W., Tindall, A.J., Du Pasquier, D., Küster, E.,  
8 925 Mikola, A., Schulze, T., Sillanpää, M., 2017. Effect-based assessment of toxicity removal during  
9 926 wastewater treatment. *Water research* 126, 153-163. 10.1016/j.watres.2017.09.014.  
10 927 VandeWalle, J.L., Goetz, G.W., Huse, S.M., Morrison, H.G., Sogin, M.L., Hoffmann, R.G., Yan, K.,  
11 928 McLellan, S.L., 2012. *Acinetobacter*, *Aeromonas* and *Trichococcus* populations dominate the microbial  
12 929 community within urban sewer infrastructure. *Environmental microbiology* 14, 2538-2552.  
13 930 <https://doi.org/10.1111/j.1462-2920.2012.02757.x>.  
14 931 Vazquez-Baeza, Y., Pirrung, M., Gonzalez, A., Knight, R., 2013. EMPeror: a tool for visualizing  
15 932 high-throughput microbial community data. *GigaScience* 2, 16. 10.1186/2047-217X-2-16.  
16 933 Veloso, S., Amouroux, D., Lancelour, L., Cagnon, C., Monperrus, M., Deborde, J., Laureau, C.C., Duran,  
17 934 R., 2023. Keystone microbial taxa organize micropollutant-related modules shaping the microbial  
18 935 community structure in estuarine sediments. *Journal of hazardous materials* 448, 130858.  
19 936 10.1016/j.jhazmat.2023.130858.  
20 937 Victoria, E., 2002. *Disinfection of Treated Wastewater-Guidelines for Environmental Management*.  
21 938 Environmental Protection Authority, Australia.  
22 939 Walters, E., Graml, M., Behle, C., Müller, E., Horn, H., 2014. Influence of particle association and  
23 940 suspended solids on UV inactivation of fecal indicator bacteria in an urban river. *Water, Air, & Soil*  
24 941 *Pollution* 225, 1-9  
25 942 Wang, J., Chen, W., Wang, T., Reid, E., Krall, C., Kim, J., Zhang, T., Xie, X., Huang, C.H., 2023. Bacteria  
26 943 and Virus Inactivation: Relative Efficacy and Mechanisms of Peroxyacids and Chlor(am)ine. *Environ Sci*  
27 944 *Technol.* 10.1021/acs.est.2c09824.  
28 945 Wijesinghe, V.N., Choo, W.S., 2022. Antimicrobial betalains. *Journal of applied microbiology* 133,  
29 946 3347-3367. 10.1111/jam.15798.  
30 947 Wu, Q., Ma, H., Su, Z., Lu, W., Ma, B., 2022. Impact of marine aquaculture wastewater discharge on  
31 948 microbial diversity in coastal waters. *Regional Studies in Marine Science* 56, 102702.  
32 949 <https://doi.org/10.1016/j.rsma.2022.102702>.  
33 950 Xu, D., Liu, S., Chen, Q., Ni, J., 2017. Microbial community compositions in different functional zones of  
34 951 Carrousel oxidation ditch system for domestic wastewater treatment. *AMB Express* 7, 40.  
35 952 10.1186/s13568-017-0336-y.  
36 953 Yang, Y., Wang, L., Xiang, F., Zhao, L., Qiao, Z., 2020. Activated Sludge Microbial Community and  
37 954 Treatment Performance of Wastewater Treatment Plants in Industrial and Municipal Zones.  
38 955 *International journal of environmental research and public health* 17. 10.3390/ijerph17020436.  
39 956 Yousra, T., Mehri, I., Lajnef, R., Rejab, A.B., Khessairi, A., Cherif, H., Ouzari, H., Hassen, A., 2017.  
40 957 Biofilms in bioremediation and wastewater treatment: characterization of bacterial community  
41 958 structure and diversity during seasons in municipal wastewater treatment process. *Environmental*  
42 959 *science and pollution research international* 24, 3519-3530. 10.1007/s11356-016-8090-2.  
43 960 Zaouri, N., Jumat, M.R., Cheema, T., Hong, P.Y., 2020. Metagenomics-based evaluation of groundwater  
44 961 microbial profiles in response to treated wastewater discharge. *Environmental research* 180, 108835.  
45 962 10.1016/j.envres.2019.108835.

1 963 Zimmer, J.L., Slawson, R.M., 2002. Potential repair of Escherichia coli DNA following exposure to UV  
2 964 radiation from both medium- and low-pressure UV sources used in drinking water treatment. Applied  
3 965 and environmental microbiology 68, 3293-3299. 10.1128/AEM.68.7.3293-3299.2002.  
4 966 Zyara, A.M., Torvinen, E., Veijalainen, A.M., Heinonen-Tanski, H., 2016. The effect of chlorine and  
5 967 combined chlorine/UV treatment on coliphages in drinking water disinfection. J Water Health 14,  
6 968 640-649. 10.2166/wh.2016.144.  
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8 969  
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10 970  
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971 **Figure captions**

972 Fig. 1. Efficiency of the disinfection treatments on the different WWTPs. The  
973 efficiency of the disinfection treatments was estimated by *E. coli* counts. BT, before  
974 treatment; The treatments are UV, chlorine, performic acid at low (PFA\_L) and high  
975 (PFA\_H) concentrations.

976  
977 Fig. 2. Comparison of microbial communities by Principal Coordinates Analysis  
978 (PCoA) based on weighted UniFrac distances including the three WWTPs (a); PCoA  
979 based on Bray-curtis distances for WWTP1 (b), WWTP2 (c), and WWTP3 (d). The  
980 arrows indicate the significant impact of the parameter (*E. coli* counts) on the  
981 microbial community composition (\*  $p < 0.05$ , \*\*  $p < 0.01$ , Permutations = 999).  
982 Circles: WWTP1; diamond: WWTP2; triangles: WWTP3. Red: chlorine; blue:  
983 performic acid at low concentration (PFA\_L); orange: performic acid at high  
984 concentration (PFA\_H); green: before treatment (BT); purple: UV.

985  
986 Fig. 3. Composition of microbial communities at the phyla level in each treatment and  
987 WWTP. BT, before treatment; The treatments are UV, chlorine, performic acid at low  
988 (PFA\_L) and high (PFA\_H) concentrations.

989  
990 Fig. 4. Bacterial biomarkers (bacterial taxa) for the different WWTPs before treatment  
991 (BT). The Cladogram shows the bacterial taxa identified by linear discriminant  
992 analysis effect size (LEfSe). The biomarkers are colored according to their WWTPs'  
993 specificity indicating that they are significantly more abundant in a WWTP than the  
994 others. The wastewater samples without any treatment were used in the analysis. D1:  
995 Phylum; D2: Class.

996  
997 Fig. 5. Linear discriminant analysis effect size (LEfSe) identifying treatment-sensitive  
998 (external) and treatment-resistant (inner) biomarkers according to treatment PFA\_H,  
999 PFA\_L, Chlorine and UV. The comparisons were performed between the wastewater

1 1000 before (BT) and after each treatment from the three WWTPs. The length of the bars  
2 1001 corresponds to the LDA score. The known pathogens are represented in red. D1:  
3  
4 1002 Phylum; D2: Class; D3: Order; D4: Family; D5: Genus; D6: Species.  
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6 1003

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8 1004 Fig. 6. Disinfection efficiency on pathogen microorganisms according to the different  
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10 1005 treatments. The comparisons were performed between each pair of the treatments  
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12 1006 from all three WWTPs by linear discriminant analysis effect size (LEfSe) identifying  
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14 1007 treatment-sensitive biomarkers. The colored dots correspond to microbial genera that  
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16 1008 sensitive to PFA\_H (red), PFA\_L (orange), Chlorine (purple) and UV (blue)  
17  
18 1009 treatment respectively.  
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21 1010  
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23 1011 Fig. 7. Functional comparison of bacterial communities predicted by PICRUSt2. The  
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25 1012 PCAs show the predicted functions, based on KEGG categories (level 3), of the three  
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27 1013 WWTPs (a), and for the different disinfectants treatments in WWTP1 (b), WWTP2  
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29 1014 (c), WWTP3 (d). (e) Heatmap of the standardized abundance of predicted functions  
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31 1015 based on KEGG categories (level 2). BT, before treatment; the treatments are UV,  
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33 1016 chlorine, performic acid at low (PFA\_L) and high (PFA\_H) concentrations.  
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1 **Safe reuse of wastewater: effect of disinfection methods on microbial community**

2

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12

13 **Highlights**

- 14 ● PFA was an efficient disinfectant comparing to chlorine and UV
- 15 ● Microbial communities from treated water were specific to the disinfectant used
- 16 ● Biomarkers sensitive to PFA and resistant to chlorine and/or UV were identified
- 17 ● Bacterial predicted functions diverge according to WWTP and disinfectants

18

19 **Abstract**

20 Performic acid (PFA), a new generation of disinfectant, has shown outstanding  
21 performance for wastewater treatment. However, the effect of PFA on wastewater  
22 microbial community changes as well as the differences in comparison to the  
23 commonly disinfectants used are poorly documented. We provide an overview of the  
24 PFA effect and that of chlorine and UV, two commonly used disinfectants, on  
25 microbial community diversity and structure. The study was performed using  
26 wastewaters from three municipal wastewater treatment plants (WWTPs) processed  
27 by activated sludge, lagoon and reed beds, and biofilter. We focused on disinfection  
28 of pathogen microorganisms, identifying sensitive and resistant bacteria to  
29 disinfectants that can serve as biomarkers for evaluating the disinfectant performances.  
30 As well, we investigated the modifications of microbial functions after treatment by  
31 functional predictive analysis. The more efficient *E. coli* inactivation was obtained  
32 with initial concentration of 4 mg/L of PFA with 15 min disinfectant contact time that  
33 achieved 95.71-99.99% inactivation efficiency. Although the origin of wastewater  
34 was the main factor determining the microbial community diversity and composition,  
35 the microbial communities were specific to the disinfectant used. Linear discriminant  
36 analysis Effect Size (LEfSe) identified 18 and 10 biomarkers, including pathogenic  
37 microorganisms affiliated to *Chromobacterium*, *Flavobacterium*, *Aeromonas*,  
38 Enterobacteriaceae, *Escherichia\_Shigella* and Fusobacteriaceae, which were sensitive  
39 to PFA, while being resistant to chlorine and/or UV. Consistently, the predicted  
40 bacterial functional patterns diverged according to the wastewater origin and the  
41 disinfectant used. Pathogen microorganisms were more sensitive to PFA than the  
42 other treatments, demonstrating its potential for implementing a safe reuse of  
43 wastewater.

44

45 **Keywords:** Wastewater reuse; Performic acid; Disinfection efficiency; Disinfectant  
46 sensitive biomarker; Microbial community; Microbial function

47 **1. Introduction**

48 Water scarcity is becoming a global concern in recent decades. The  
49 overexploitation of water is one of the major threats to the EU freshwater  
50 environment, especially for agricultural irrigation (EC, 2018). More than 40,000  
51 million m<sup>3</sup> wastewater is treated each year in the EU, only less than 3% being reused  
52 (EC, 2022). Thus, there is place to improve water reuse, which represents a potential  
53 on easing water supply pressure, also contributing to a sustainable agriculture.

54 Domestic wastewater owns microorganism associated to human diseases  
55 (Diamond et al., 2022; McCall et al., 2020). It is thus critical to establish efficient and  
56 economical disinfection frameworks in wastewater treatment in order to supply safe  
57 water. Tertiary treatment aims to remove stubborn pollutants (organics, turbidity,  
58 nitrogen, phosphorus, metals, and pathogens), including also an additional  
59 disinfection step (Pei et al., 2019). The tertiary treatment provides additional  
60 assurance of effluent quality, protecting thus wildlife in the discharged water system.  
61 It also ensures the safety of wastewater reuse for various purposes, such as irrigation  
62 and industrial applications (Gerba and Pepper, 2019).

63 Several disinfection approaches are commonly implemented in tertiary treatment  
64 for the inactivation of pathogen microorganisms, such as chlorination, ultraviolet (UV)  
65 irradiation, ozonation and filtration (USEPA, 2004). Chlorine and its various forms  
66 are powerful oxidants for the inactivation of pathogens (Furst et al., 2018), but it  
67 forms toxic by-products that might impact the receiving environment threatening the  
68 human health (Bull et al., 1995; Nieuwenhuijsen et al., 2000; Victoria, 2002). The UV  
69 irradiation has been considered as an alternative to chlorine for wastewater treatment  
70 due to its short contact time, and it has the advantage that toxic by-products are not  
71 produced (Zyara et al., 2016). However, UV irradiation has the disadvantage to be  
72 expensive because of high-energy demand, high costs of the equipment and its  
73 management. The investment cost is estimated to be 10 times more expensive than  
74 that of chlorine and PFA treatments (Ragazzo et al., 2013). Also, the practical  
75 application of UV irradiation carries many limitations because it is dependent on

76 water quality parameters, such as turbidity, colour and suspended solid (Oguma et al.,  
77 2001; Zimmer and Slawson, 2002).

78 Performic acid (PFA), a high oxidative peracid, has been considered as an ideal  
79 alternative for industrially wastewater disinfection due to its outstanding  
80 characteristics (Gehr et al., 2009; Ragazzo et al., 2013). PFA is a wide-spectrum  
81 disinfectant with deodorizing ability and low corrosive effect, exhibiting toxicity to  
82 viruses, bacteria and bacterial spores, mycobacteria as well as microscopic fungi, but  
83 not to higher forms of life (Gehr et al., 2009; Luukkonen and Pehkonen, 2017). The  
84 PFA treatment cost has been estimated at 0.0114 €/m<sup>3</sup> (Luukkonen and Pehkonen,  
85 2017), while that of chlorination at 0.004 €/m<sup>3</sup> (Zagklis and Bampos, 2022). Although  
86 the operation cost of PFA treatment is estimated to be 2.5 times more expensive than  
87 that of chlorine (Luukkonen et al., 2015). It has the advantage to produce by-products  
88 (hydrogen peroxide and formic acid) that are nontoxic to aquatic fauna at the  
89 disinfection doses (Gehr et al., 2009; Ragazzo et al., 2013). Thus, its reasonable cost  
90 and splendid performance at ambient temperature demonstrate that PFA has potential  
91 applicability for large wastewater treatment plants (WWTPs) (Luukkonen et al.,  
92 2015).

93 Several studies have reported on the effect of the commonly used disinfectants on  
94 microorganisms. Most studies have considered the decrease of bacterial cell counts  
95 and species richness, the inactivation of viruses, and the removal of microorganisms  
96 carrying antibiotic resistance genes (ARGs) (Kantor et al., 2019; Kulkarni et al., 2018;  
97 Ng et al., 2019; Stamps et al., 2018; Zaouri et al., 2020). Nevertheless, the effect of  
98 disinfectant treatments on the microbial community structure and functions is scarce,  
99 especially for the PFA treatment (Kantor et al., 2019; Numberger et al., 2019; Stamps  
100 et al., 2018). The release of potential pathogens and the modification of microbial  
101 community in the receiving environment have been reported during reclaimed water  
102 discharging process (Numberger et al., 2019). Thus, it is important to consider the  
103 effect of wastewater treatments on the microbial community. Precisely, the objective  
104 of our study was to examine the effect of PFA treatment on the microbial diversity,  
105 microbial structure and function. The disinfection efficiency of PFA treatment against

106 pathogens was also evaluated and compared to that of other conventional treatments.

107 We hypothesize that the microbial communities will be modified differently  
108 according to the treatment because they involve different action mechanisms.

109 In order to examine the possibility to safety reuse treated wastewater for irrigation,  
110 industrial applications, groundwater recharge and other agricultural activities, this  
111 study aimed to compare PFA, UV and chlorine treatments regarding their effect on 1)  
112 the microbial community diversity and structure, and 2) the disinfection efficiency for  
113 the inactivation of pathogens. For this purpose different WWTPs were treated in order  
114 to identify sensitive and resistant bacteria to each disinfectant, representing potential  
115 microbial biomarkers according to treatment. The bacterial functions were predicted  
116 and correlated with the wastewaters resulting from the treatments.

## 117 2. Materials and methods

### 118 2.1 Sampling sites and physical-chemical characterization of wastewaters

119 Three domestic WWTPs with different treatment processes, activated sludge,  
120 lagoon and reed bed and biofilter were selected. Brief information of the sampling  
121 WWTPs is listed in the Table S1.

122 The main physical-chemical parameters were determined on the wastewater  
123 samples. Conductivity was measured by 3510 WTW multiparameter-probe. Chemical  
124 oxygen demand (COD) was determined using colorimetric method by Hach reactants  
125 and Hach DR3900 spectrophotometer. Total suspended solid (TSS) was determined  
126 after filtration through 0.45  $\mu\text{m}$  on 47 mm glass fiber filter and drying at 105  $^{\circ}\text{C}$  until  
127 constant mass. Total organic carbon (TOC) and total nitrogen (TN) were determined  
128 by a Shimadzu TOC VCN analyzer with a TN unit (chemiluminescence measurement  
129 of  $\text{NO}_x$ ) according to (Behera et al., 2017). TOC was obtained by the difference of  
130 total carbon and inorganic carbon analyses.

## 131 2.2 Sample collection and disinfection

132 The effluent wastewater samples were collected from each WWTP with sterile  
133 Scott bottles. All the samples were collected in the morning and transported to the  
134 laboratory at 4 °C. The disinfections were generated by three disinfectants (PFA,  
135 chlorine and UV) just right after the water samples arrived to the laboratory. Each  
136 disinfection treatment was performed in true biological triplicates. Thus, a total of 45  
137 samples (3 WWTP x triplicate x 5 treatments (PFA\_L, PFA\_H, Chlorine, UV and  
138 untreated wastewater as control)) were analysed. All samples were maintained at 4 °C  
139 prior further processing.

140 The optimal disinfection concentration of all the disinfectants used was  
141 determined in a pre-experiment using *E. coli* and total coliforms as indicators. The  
142 initial doses range were determined based on official wastewater treatment manual  
143 and previous studies, also adjusted according to the wastewater treatment plants  
144 conditions (Anastasi et al., 2013; EPA, 2011; Gehr et al., 2009; Ragazzo et al., 2013;  
145 USEPA, 2003). More specifically, previous reports indicated that UV was effective at  
146 10 mJ/cm<sup>2</sup> although a higher dose (30-40 mJ/cm<sup>2</sup>) would be required for complete  
147 disinfection (Andreadakis et al., 1999), while hypochlorite was found effective at 2  
148 mg/L (Collivignarelli et al., 2017) and PFA in 1-4 mg/L range (Chhetri et al., 2014).

### 149 2.2.1 PFA preparation and disinfection

150 For preparing PFA, 10 mL of formic acid was mixed with 1 mL sulfuric acid  
151 (98%, Aldrich) as a catalyst on ice and wait about 10 min to cool. 14.2 mL of  
152 hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>, 50% wt, Aldrich) was then added slowly into the mixture.  
153 Then, the mixed solution was maintained for 60 min to get ready to use. The whole  
154 process was performed in ice bath and mixed with a magnetic stirrer. 100 mg of the  
155 mixture was then mixed with 0.1M H<sub>2</sub>SO<sub>4</sub> solution and titrated to determine the  
156 concentrations of H<sub>2</sub>O<sub>2</sub> and PFA. The H<sub>2</sub>O<sub>2</sub> titration was conducted with potassium  
157 permanganate solution (KMnO<sub>4</sub>, 0.02 mol/L, Aldrich). An excess of potassium iodide  
158 was then added and oxidized by PFA. Iodine was then titrated with sodium thiosulfate

159 (Na<sub>2</sub>SO<sub>3</sub>, 0.02 mol/L, Aldrich). The PFA solution was kept at -20 °C and titrated each  
160 time right before use.

161 Because PFA is rapidly decomposed (Karpova et al., 2013), two doses of PFA, a  
162 lower initial concentration at 1 mg/L with 15 min disinfectant contact time (PFA\_L)  
163 corresponding to a dose known to inactivate most microorganisms (Karpova et al., 2013)  
164 and a higher initial concentration at 4 mg/L with 15 min disinfectant contact time  
165 (PFA\_H) corresponding to the optimal dose for disinfection (Karpova et al., 2013;  
166 Chhetri et al., 2014), were used in the disinfection. Na<sub>2</sub>SO<sub>3</sub> solution (0.1 mol/L) was  
167 used for quenching the reaction.

### 168 **2.2.2 Chlorination**

169 A solution of 100 mg/L of total chlorine was prepared from pure calcium  
170 hypochlorite (Aldrich) and stored at 4 °C. The prepared solution was titrated by  
171 iodometric titration in acetic acid medium before use. The chlorination was performed  
172 with the initial concentration at 2 mg/L for 30 min (Chl). Na<sub>2</sub>SO<sub>3</sub> solution was used  
173 for quenching the reaction.

### 174 **2.2.3 UV disinfection**

175 The laboratory scale UV disinfection was performed using low-pressure mercury  
176 vapour lamp to perform the disinfection at the dose of 10 mJ/cm<sup>2</sup>. The irradiance rate  
177 was measured by UV radiometer (Avantes Avaspec 2048L equipped with a 600 µm  
178 core optical fiber and a corrector cosine (4.9 mm diameter)). The solutions were  
179 irradiated by a home-made device consisting of four Phillips TL UV 8W 30 cm  
180 fluorescent lamps. The device was placed on top of the solutions and the height was  
181 adjusted until obtaining the desired irradiance. The lamp was preheated for more than  
182 15 min before disinfection. 250 mL of wastewater was treated each time in the  
183 specific glass beaker. The exposure time was calculated by the desired dose,  
184 irradiance rate and transmittance value of the wastewater sample.

### 185 2.3 Microbial analysis

186 The *Escherichia coli* (*E. coli*) counts were determined by a standard  
187 beta-glucuronidase positive method (APHA, 2005).

188 The water samples were filtered by sterile disposable filtration units (MicroFunnel,  
189 Pall Corporation, USA) with 0.2 µm Supor membrane to concentrate the microbial  
190 biomass. The iQ-Check Free DNA Removal Solution (FDRS, Bio-Rad Laboratories,  
191 USA), which degrade free DNA by enzymatic treatment, was used to ensure that the  
192 samples are devoid of free DNA prior to DNA extraction. For the free DNA  
193 elimination, we followed standard protocol. In short, the membranes were folded into  
194 cones and transferred into Eppendorf tubes containing 40 µl FDRS and 460 µl  
195 distilled Milli-Q water, followed by 30 min incubation at 37 °C. To inactivate the  
196 FDRS and for DNA extraction, 1 mL lysis solution (R1) from Aquadien™ Bacterial  
197 DNA Extraction and Purification Kit (Bio-Rad Laboratories, USA) were then added  
198 and incubated for 15 min at 95 °C with 1,300 rpm in a shaking heating block  
199 (Eppendorf ThermoMixer C, Eppendorf, Germany). We followed the standard  
200 protocol for the Aquadien™ Bacterial DNA Extraction and Purification Kit for the  
201 following steps.

202 The V3-V4 region of 16S rRNA gene was amplified using the primer pair 341F  
203 (5' - CCTAYGGGRBGCASCAG - 3') and 806R (5' -  
204 GGACTACNNGGGTATCTAAT - 3') (Gao et al., 2020) connecting with barcodes.  
205 PCR amplification, library preparation and sequencing were conducted by Novogene  
206 Europe (Cambridge, UK) using their proprietary methods. To ensure an accurate  
207 recognition of the samples, the index pairs were specific for each sample. Quality  
208 control was performed at each step of the procedure. The PCR products were purified  
209 using AMPureXP magnetic beads, pooled in equimolar and the libraries generated  
210 with NEBNext® Ultra™ DNA Library Prep Kit for Illumina and quantified via  
211 Qubit and Q-PCR. Paired-end 250 bp sequencing was then performed on Illumina  
212 NovaSeq 6000 sequencer (Illumina, San Diego, CA, USA).



## 213 2.4 Bioinformatics analysis and visualization

214 Paired-end reads were assigned to samples based on the sample-specific barcode  
215 and truncated by cutting off the barcode and primer sequences, and were merged  
216 using FLASH (V1.2.7) (Magoc and Salzberg, 2011). Qiime2 (v 2019.4) was used for  
217 the data processing and analysis. The sequence quality control and amplicon  
218 sequencing variants (ASVs) assignment were performed using DADA2 plugin.  
219 Taxonomy assignment was completed using the q2-feature-classifier plugin against  
220 SILVA database (v132) with a clustering threshold of 97% similarity. The ASVs  
221 assigned to Eukaryota, Chloroplast, Mitochondria and unassigned ASVs were  
222 removed and the filtered dataset was then rarefied at the depth of 71673 using  
223 *alpha-rarefaction.py* script. The rarefied data were then used for the following  
224 analyses without threshold. The complete dataset was deposited in the Sequence Read  
225 Archive (SRA) of the National Center for Biotechnology Information (NCBI) and is  
226 available under the Bioproject ID PRJNA907723.

227 For the alpha-diversity, the Shannon, Simpson, observed features, Chao1, ace  
228 indices were analyzed using *alpha\_diversity.py* in Qiime2. Statistical analysis  
229 (ANOVA, Tukey HSD) and visualization were performed in GraphPad Prism.  
230 Beta-diversity was calculated in Qiime2 and R based on weighted Unifrac and  
231 Bray-Curtis dissimilarity. Permutational multivariate analysis of variance  
232 (PERMANOVA) was performed with 999 times permutations using the  
233 *beta\_significance.py* script in Qiime2 and the “adonis” function in the vegan package  
234 in R. Principal coordinate analyses (PCoA) were performed and visualized by  
235 EMPERor (Vazquez-Baeza et al., 2013) and the Ape and ggplot2 packages in R. The  
236 significances of physical-chemical parameters associated with microbial community  
237 were identified by “envfit” function in the “vegan” package in R.

238 Linear discriminant analysis Effect Size (LEfSe, v 1.0) (Segata et al., 2011) was  
239 performed to identify the biomarkers between the groups, which emphasizes both  
240 statistical significance and biological relevance. LEfSe analysis was performed using  
241 the ASV table, at the ASV level spanning all the taxonomic levels. The

242 non-parametric factorial Kruskal-Wallis sum-rank test was performed first to detect  
243 features with significant differential abundance between the classes (groups). The  
244 pairwise Wilcoxon test was then applied to check if the subclasses agree with the  
245 class level trend, which was performed only among subclasses in different classes  
246 with the same name. The resulting subset of vectors was then used to build a Linear  
247 Discriminant Analysis model. The alpha-value for both Kruskal-Wallis and Wilcoxon  
248 tests were at 0.05 and the logarithmic LDA threshold score for discriminative features  
249 was 2.

250 The PICRUSt2 was used for the prediction of microbial community functions  
251 following the following 4 steps: (1) sequence placement, (2) hidden-state prediction  
252 of genomes, (3) metagenome prediction, (4) pathway-level predictions. The  
253 pathway-level predictions were performed using a customized mapping file based on  
254 the KEGG database (KEGG Brite orthologs to pathways, updated on 22 Nov. 2020,  
255 Table S2). The descriptions were then added to the Enzyme Classification (EC)  
256 numbers, KEGG orthologs (K) with default mapping table, and a customized mapping  
257 table including descriptions of higher hierarchical levels was used for the KEGG  
258 pathways (ko, Table S3). STAMP v 2.1.3 was used for visualizing PICRUSt2 output  
259 data. ANOVA and Kruskal-Wallis H-test were used for the multiple groups' statistical  
260 analysis. Tukey-Kramer and Welch's at 0.95 were performed as Post-hoc tests.

### 261 3. Results and discussion

#### 262 3.1 Disinfection treatment efficiency

263 The disinfection efficiency of PFA, UV and chlorine was determined for the three  
264 different wastewater treatment plants by determining the reduction of the *E. coli*  
265 content, which represents a key parameter for characterizing reclaimed water quality  
266 in wastewater reuse (EC, 2018; EPA, 2004). The initial *E. coli* cell concentration  
267 (before treatment BT, Fig. 1) was  $2.8 \times 10^4$ ,  $2.5 \times 10^4$  and  $5.8 \times 10^3$  CFU/100 mL for  
268 WWTP1, 2, and 3, respectively. In WWTP1 and WWTP2, the *E. coli* cell  
269 concentration significantly decreased in all the treated wastewaters ( $p < 0.001$ , Fig. 1).

270 Indeed, the WWTPs' effluents showed different physical-chemical characteristics  
271 (Table S1). The conductivity was lower in WWTP1 effluent (718  $\mu\text{S}/\text{cm}$ ) than in  
272 WWTP2 (1000  $\mu\text{S}/\text{cm}$ ) and WWTP3 (1254  $\mu\text{S}/\text{cm}$ ) effluents (Table S1). Consistently,  
273 WWTP1 effluent exhibited the lower contents of COD (36 mg/L), TSS (2 mg/L),  
274 TOC (18 mg/L), and TN (3.5 mg/L), while WWTP2 effluent showed the higher COD  
275 (80 mg/L) and NT (60.6 mg/L) contents (Table S1).

276 Noteworthy, the PFA treatment was more efficient with WWTP1, which exhibited  
277 the lowest TSS content (Table S1, Fig. 1). This result is consistent with previous  
278 studies showing that the disinfection efficiency of UV and peracetic acid increased  
279 with low TSS (Dominguez Henao et al., 2018; Walters et al., 2014).

280 The Chl and both low and high doses of PFA (PFA\_L and PFA\_H) treatments  
281 showed similar *E. coli* content reduction for WWTP1 and WWTP2 ( $p > 0.05$ ),  
282 although the *E. coli* concentration (277 CFU/100 mL) was higher for the PFA\_L  
283 treatment of WWTP2. All these three treatments were significantly more efficient in  
284 decreasing the *E. coli* concentration than the UV treatment ( $p < 0.001$ ). The Chl and  
285 PFA\_H treatments had the best efficiency, reducing the *E. coli* concentration below  
286 40 CFU/100 mL. A reduction of *E. coli* counts by 3-4  $\log_{10}$  magnitude was observed  
287 with the PFA\_H treatment for WWTP1 and WWTP2, while for WWTP3 the  
288 reduction of *E. coli* counts was 1  $\log_{10}$  magnitude (Fig. 1). The treatment of WWTP3  
289 showed different trends, except for the PFA\_H treatment that was also the most  
290 efficient for reducing the *E. coli* content. These observations suggested that the  
291 disinfection efficiency, evaluated by the decrease of *E. coli* concentration, was  
292 dependent on the water physical-chemical parameters. The treatment with the high  
293 dose of PFA (PFA\_H) provided better performance than chlorine and UV,  
294 irrespective of the wastewater origin, observation that was in agreement with a  
295 previous report (Ragazzo et al., 2020).

296 The UV and PFA treatments were less efficient in decreasing the *E. coli*  
297 concentration for the WWTP3 wastewater than for WWTP1 and WWTP2, even if the  
298 TSS of WWTP2 and WWTP3 were at a similar level (16 and 18 mg/L, respectively).  
299 It is likely that the high TSS content of WWTP3 might relate high *E. coli*

300 concentration since WWTP3 exhibited also the highest conductivity (1254  $\mu\text{S}/\text{cm}$ ,  
301 Table S1), which has been shown to be related to the *E. coli* concentration (Foschi et  
302 al., 2021). The inactivation efficiency of *E. coli* has been shown to be dependent on  
303 the WWTP treatment processes (Biswal et al., 2014). Although UV disinfection  
304 significantly decreased *E. coli* concentration, it was not efficient as Chl and PFA in  
305 WWTP1 and WWTP2. Among the disinfection treatments, only the PFA\_H was  
306 effective for reducing the *E. coli* concentration. Such observation was in agreement  
307 with previous studies showing that PFA was more efficient in reducing *E. coli*  
308 concentration than the disinfectants commonly used for municipal wastewater  
309 treatment (Maffettone et al., 2020; Wang et al., 2023).

### 310 3.2 Effect of disinfection treatment on microbial community

311 A total of 5,724,372 raw reads was obtained and assigned to 17,151 amplicon  
312 sequence variants (ASVs). The sequences were then rarefied at the minimum 71,673  
313 reads per sample. The final dataset, including 3,153,612 reads dispatched within  
314 14,838 ASVs, was used for the microbial community analysis.

#### 315 3.2.1 Impact of disinfection treatments on microbial community diversity

316 The diversity indices (Shannon, Simpson, observed richness, Chao1 and ace) were  
317 significantly different (two-way ANOVA,  $p < 0.001$ ) according to the WWTP origin  
318 and the treatment, WWTP origin explaining most of the variation (62.8 %,  $p < 0.001$ ;  
319 Table S4). The microbial community of the wastewater from WWTP2 exhibited  
320 significantly higher diversity indices than those from WWTP1 and WWTP3 (two-way  
321 ANOVA,  $p < 0.001$ ; Fig. S1). The microbial community of wastewater from WWTP3  
322 had significantly higher Shannon index than that from WWTP1 (two-way ANOVA,  $p$   
323  $< 0.001$ ; Fig. S1).

324 Noteworthy, the treatments for a given WWTP decreased the microbial diversity  
325 (Shannon index and the observed richness, two-way ANOVA,  $p < 0.05$ ; Fig. S2),  
326 except for WWTP2 (Fig. S2). Interestingly, the WWTP2, lagoon and reed beds  
327 process, showed the higher COD, TOC and TN contents (Table S1). It has been

328 shown that the efficiency of a treatment **in decreasing** the microbial diversity is  
329 dependent on the wastewater physical-chemical characteristics such as COD, TOC  
330 and TN (Tian and Wang, 2020; Wu et al., 2022), the treatment processes (Sauter et al.,  
331 2023), and even on the different type of reed beds systems (Kowal et al., 2021). Thus,  
332 it is likely that the high content of organic matter (COD, TOC and TN) might explain  
333 the fact that the microbial diversity was not decreased by the treatments in WWTP2.

334 The WWTP3 showed higher diversity (Shannon and observed richness) indices  
335 before treatment (BT) than the two other WWTPs (**two-way ANOVA,  $p < 0.05$** ; Fig.  
336 S2), which may explain the lower *E. coli* inactivation observed with WWTP3  
337 wastewater (Fig 1) despite that it showed the lowest *E. coli* concentration. Such  
338 observation was consistent with a previous report showing that the *E. coli* inactivation  
339 is dependent on the microbial diversity (Qian et al., 2022).

340 For the WWTP1, the Shannon index was significantly lower (**two-way ANOVA,**  
341  **$p < 0.001$** ; Fig. S2) for PFA\_H than for before treatment (BT), and for the three other  
342 treatments. Similarly, the observed richness for PFA\_H was significantly lower than  
343 for Chl (**two-way ANOVA,  $p < 0.05$** ; Fig. S2). The WWTP3 treated by PFA\_H and  
344 Chl showed significantly lower Shannon index and observed richness than BT  
345 (**two-way ANOVA,  $p < 0.05$** ; Fig. S2).

### 346 **3.2.2 Impact of disinfection on microbial community structure**

347 The principal coordinate analysis (PCoA), based on either the weighted UniFrac  
348 or Bray-Curtis dissimilarity matrices (Fig. 2), further confirmed that the microbial  
349 communities of the wastewaters were different according to the WWTPs, revealing  
350 also the treatments effects. The PCoA clustered the microbial communities according  
351 to their origin (i.e. WWTP), separating the different WWTPs along the axis 1, which  
352 explained 66.7% of the variation (Fig. 2a). This observation was supported by the  
353 permutational multivariate ANOVA (PERMANOVA) indicating that the WWTP  
354 origin was the main factor explaining the differences between the microbial  
355 communities (pseudo-F = 79.68,  $p = 0.001$ ). Within a WWTP cluster, the microbial  
356 communities clustered by treatment (Fig. 2a). Thus, in order to reveal the effect of the

357 disinfection treatments on the microbial community, PCoA were performed for each  
358 WWTP (Fig. 2b, 2c, 2d). For WWTP2 and WWTP3, wastewater samples from the  
359 same treatment tightly clustered together (Fig. 2c, 2d). Envfit (Fits an Environmental  
360 Vector or Factor onto an Ordination) analysis also revealed that the composition of  
361 wastewater microbial communities significantly varied by treatment in WWTP2 ( $r^2 =$   
362  $0.94, p < 0.001$ ) and WWTP3 ( $r^2 = 0.89, p < 0.001$ ), but not in WWTP1.

363 Before the treatments, COD, TOC and TN were identified as the main factors  
364 driving the microbial communities of WWTP2 (Envfit; COD:  $r^2 = 0.9712, p = 0.004$ ;  
365 TOC:  $r^2 = 0.9688, p = 0.004$ ; NT:  $r^2 = 0.9682, p = 0.009$ ), while the conductivity  
366 (Envfit;  $r^2 = 0.9930, p = 0.001$ ) and TSS (Envfit;  $r^2 = 0.9844, p = 0.001$ ) were the  
367 main factors driving the microbial communities from WWTP3. After the disinfection  
368 treatments, the same factors were found to drive the microbial communities from  
369 WWTP2 and WWTP3 irrespective of the treatment: COD (Chl:  $r^2 = 0.9568, p = 0.004$ ;  
370 UV:  $r^2 = 0.9955, p = 0.004$ ; PFA\_L:  $r^2 = 0.9664, p = 0.003$ ; PFA\_H:  $r^2 = 0.9251, p =$   
371  $0.003$ ), TOC (Chl:  $r^2 = 0.9522, p = 0.004$ ; UV:  $r^2 = 0.9946, p = 0.004$ ; PFA\_L:  $r^2 =$   
372  $0.9601, p = 0.003$ ; PFA\_H:  $r^2 = 0.9139, p = 0.004$ ) and NT (Chl:  $r^2 = 0.9503, p =$   
373  $0.020$ ; UV:  $r^2 = 0.9927, p = 0.004$ ; PFA\_L:  $r^2 = 0.9547, p = 0.009$ ; PFA\_H:  $r^2 =$   
374  $0.9051, p = 0.026$ ) for WWTP2; conductivity (Chl:  $r^2 = 0.9936, p = 0.001$ ; UV:  $r^2 =$   
375  $0.9907, p = 0.008$ ; PFA\_L:  $r^2 = 0.9968, p = 0.001$ ; PFA\_H:  $r^2 = 0.9866, p = 0.002$ )  
376 and TSS (Chl:  $r^2 = 0.9798, p = 0.006$ ; UV:  $r^2 = 0.9943, p = 0.009$ ; PFA\_L:  $r^2 = 0.9883,$   
377  $p = 0.006$ ; PFA\_H:  $r^2 = 0.9678, p = 0.006$ ) for WWTP3.

378 These results suggested that the origin (i.e. WWTP) was the main factor  
379 determining the composition of the microbial community. The different disinfection  
380 treatments resulted in specific microbial communities, which effects were different  
381 according to the wastewater origin (WWTP).

### 382 **3.2.3 Microbial composition differences between WWTPs: identification of** 383 **specific biomarkers**

384 The distinction of the microbial communities according to their origin (i.e.  
385 WWTP) was confirmed by the presence of different dominant phyla (Fig. 3).

386 Proteobacteria (60.5%) was the most dominant bacterial phylum in all three WWTPs,  
387 consistent with previous studies in both domestic and industrial WWTPs (Sharma et  
388 al., 2021; Tang et al., 2020a). The relative abundance of the Proteobacteria  
389 significantly decreased from WWTP2 (70.7%) to WWTP3 (61.9%), and to WWTP1  
390 (48.9%) (ANOVA,  $p < 0.001$ ).

391 The second dominant phylum in the three WWTPs was Bacteroidetes (11.4%),  
392 followed then by Actinobacteria (8.1%), Nitrospirae (4.3%) and Nanoarchaeaeota  
393 (3.7%). The relative abundance of the Actinobacteria significantly decreased from  
394 WWTP1 (15.1%) to WWTP2 (7.1%), and to WWTP3 (2.3%) (ANOVA,  $p < 0.01$ ).  
395 Nitrospirae was significantly more abundant in WWTP3 (11.9%) than in WWTP1  
396 (0.6%) and WWTP2 (0.6%) (ANOVA,  $p < 0.001$ ). Nitrospirae includes nitrifying  
397 taxa, nitrite-oxidizing bacteria (NOB) and comammox bacteria (Koch et al., 2019).  
398 Nitrospira, the largest class, have been found to be the dominant nitrifiers in  
399 wastewater treatment plants (Forrez et al., 2011). Nitrospira, an aerobic  
400 chemolithoautotrophic nitrite-oxidizing bacterium, plays important roles in  
401 nitrification and oxidizes nitrite to nitrate (Daims et al., 2015; Daims and Wagner,  
402 2018). It has been identified as keystone taxon, an ideal partner for establishing  
403 microbial relationships under various conditions, in aquatic environment (Veloso et  
404 al., 2023).

405 The linear discriminant effect size (LEfSe) analysis revealed microbial taxa  
406 specifically more abundant according to the origin of the WWTP. Such taxa found  
407 significantly more abundant to a specific condition by LEfSe analysis has been  
408 proposed to serve as biomarkers (Segata et al., 2011). The LEfSe comparing the  
409 microbial communities before treatment (BT) revealed biomarkers for each WWTP  
410 (Fig. 4). For WWTP1, Actinobacteria, Elusimicrobia, Epsilonbacteraeota,  
411 Latescibacteria, Omnitrophicaeota and Spirochaetes phyla, and Parcubacteria and  
412 Campylobacteria class were identified as biomarkers (Fig. 4). Actinobacteria, known  
413 for carrying antibiotic resistant genes (ARGs) (Tang et al., 2020b), have been reported  
414 dominant in activated sludge processed municipal wastewaters (Kang et al., 2020; Xu  
415 et al., 2017). Bacteria affiliated to Parcubacteria have been reported to be involved in

416 sulphamethoxazole antibiotic degradation (Song et al., 2019). *Campylobacter* species  
417 are known as bacterial indicators for faecal contamination by animals (Ogden et al.,  
418 2009).

419 For WWTP2, the LEfSe analysis revealed Proteobacteria, Cyanobacteria phyla  
420 and Oxyphotobacteria class as biomarkers (Fig. 4). Cyanobacteria are considered to  
421 be well adapted to high organic and low oxygen contents (Saqrane et al., 2020).  
422 WWTP2 process is based on lagoon system and reed beds, providing probably  
423 appropriate growth condition for Cyanobacteria. Cyanobacteria are known to release  
424 cyanotoxins (Saqrane et al., 2020). Nevertheless, after disinfection treatments, the  
425 relative abundance of Cyanobacteria in WWTP2 decreased to the same level of that  
426 found in WWTP1 and WWTP3 (Fig. S3).

427 For WWTP3, the biofiltration based process, the LEfSe analysis revealed  
428 Armatimonadetes, Verrucomicrobia, Acidobacteria, Nitrospirae and  
429 Gemmatimonadetes phyla, and the Acidobacteria, Nitrospira and Planctomycetacia  
430 classes as biomarkers (Fig. 4). Such observation was consistent with previous studies  
431 showing that these taxa were found dominant in three different types of biofilters  
432 (Gulay et al., 2019; Lautenschlager et al., 2014; Li et al., 2020). Nitrospirae and  
433 Verrucomicrobia have been demonstrated to be involved in the transformation of  
434 organic nitrogen (Tang et al., 2021).

435 After the disinfection treatments, most of the detected biomarkers differed from  
436 those of the non-treated WWTPs (Fig. S3). However, the Actinobacteria,  
437 Alphaproteobacteria and Acidobacteria classes were still identified as biomarkers for  
438 WWTP1, WWTP2 and WWTP3 respectively, irrespective of the disinfection  
439 treatment (Fig. 4, Fig. S3). Such result further confirmed that the origin of  
440 wastewaters is an important factor shaping the microbial community (habitat filtering).  
441 Thus, it is likely that the WWTP specific biomarkers are reflecting not only the  
442 differences on wastewater composition, but also the processing methods involved in  
443 the wastewater treatment systems (activated sludge, lagoon and reed beds, and  
444 biofilter, for WWTP1, WWTP2 and WWTP3 respectively).



### 445 3.3 Identification of biomarkers sensitive and resistant to the disinfection 446 treatments

447 The LEfSe analysis comparing the bacterial communities before (BT) and after  
448 each treatment revealed biomarkers, taxa significantly more abundant in a specific  
449 condition. Such biomarkers reflect thus the disinfection efficiency of each treatment  
450 towards microbial taxa (Fig. 5). Two groups of biomarkers were detected: i)  
451 treatment-sensitive biomarkers corresponding to taxa significantly found more  
452 abundant in BT (i.e. depleted in the wastewater treated by a disinfectant), and ii)  
453 treatment-resistant biomarkers corresponding to taxa significantly found more  
454 abundant in treated water.

455 The numbers of treatment-sensitive biomarkers decreased as follow PFA\_H,  
456 PFA\_L, UV, and chlorine. For PFA\_H, 39 treatment-sensitive biomarkers were  
457 detected including ASVs related to Flavobacteriaceae, Chromobacteriaceae,  
458 *Chromobacterium* and *Escherichia\_Shigella* (Fig. 5), members of which have been  
459 reported as dominant taxa in sewage and WWTP (Ahmed et al., 2017; Leroy-Freitas et  
460 al., 2022; Yousra et al., 2017). For the PFA\_L, 11 treatment-sensitive biomarkers  
461 including the previous discussed taxa Chromobacteriaceae, *Chromobacterium* and  
462 *Escherichia\_Shigella* were revealed. For chlorine and UV, only 2 and 4  
463 treatment-sensitive biomarkers were detected, respectively. Additionally, 11, 3, 3 and  
464 1 treatment-resistant biomarkers were detected for the UV, chlorine, PFA\_H and  
465 PFA\_L, respectively (Fig. 5). Paracaedibacteraceae, identified as treatment-resistant  
466 biomarker for both Chl and UV, has been reported to be significantly enriched after  
467 hydrogen peroxide treatment (Piel et al., 2021). *Acetoanaerobium* and *Acetobacterium*  
468 were identified as treatment-resistant biomarker of Chl and UV, respectively. *Ottowia*,  
469 identified as UV resistant biomarker, was isolated from different types of wastewater  
470 treated by activated sludge (Spring et al., 2004). Obscuribacterales, identified as  
471 treatment-resistant biomarkers for both PFA\_L and PFA\_H, have been found in  
472 activated sludge of aerobic reactor, aquifer, soil and animal (McGorum et al., 2015;  
473 Salmaso et al., 2018; Soo et al., 2014). The genome analyses of bacteria belonging to

474 Obscuribacterales revealed their ability to perform both aerobic and anaerobic  
475 respiration, store polyphosphate, utilize nitrate, as well as their potential to use  
476 carbohydrates and/or fatty acids, but their harmful potential has not been reported so  
477 far (Dueholm et al., 2022; Salmaso et al., 2018).

478 Such results, showing more sensitive biomarkers and less resistant biomarkers for  
479 PFA than for the other treatments, demonstrated a more efficient disinfection of  
480 wastewater by the PFA treatment than the treatments involving UV and chlorine  
481 disinfectants.

482 In order to better understand the differences in effectiveness of disinfectants, we  
483 further performed LEfSe analysis comparing all pairs of two treatments (Fig. 6).  
484 Twelve taxa were sensitive to PFA\_L and resistant to chlorine, including  
485 microorganisms affiliated to i) Aeromonadales, Aeromonadaceae and *Aeromonas* that  
486 have been reported as typical taxa in WWTP (Gao et al., 2023; VandeWalle et al.,  
487 2012), and ii) Enterobacteriales and Enterobacteriaceae (Fig. 6, Fig. S4a), members of  
488 which represent faecal indicators (Kausar et al., 2019). Also, 10 taxa were sensitive to  
489 PFA\_L and resistant to UV corresponding, in addition to the above-mentioned taxa, to  
490 *Chromobacterium* and Fusobacteriaceae (Fig. 6, Fig. S4b), also known as faecal  
491 indicators (Hutinel et al., 2022). Thus, PFA exhibits superior disinfection efficacy over  
492 chlorine and UV because taxa related to faecal bacteria have shown increased  
493 sensitivity to PFA.

494 Additionally, biomarkers sensitive to the PFA\_H treatment were found to be  
495 resistant to either Chl (18 biomarkers) or UV (26 biomarkers) treatments (Fig. S4e,f).  
496 The 18 PFA\_H treatment-sensitive biomarkers resistant to Chl included taxa affiliated  
497 to faecal indicators such as Chromobacteriaceae, *Chromobacterium*,  
498 Flavobacteriaceae and *Flavobacterium* (Fig. S4e). Similarly, the 26 PFA\_H  
499 treatment-sensitive biomarkers resistant to UV included faecal indicators such as  
500 Aeromonadales and Aeromonadaceae (Fig. S4f). These observations further  
501 confirmed the higher disinfection efficiency of PFA. Moreover, PFA was also found  
502 to be more efficient when increasing the PFA dose since 11 biomarkers sensitive to  
503 PFA\_H were found resistant to PFA\_L (Fig. 6, Fig. S4c).

504 Previous studies have noted that the reaction pathways of *E. coli* inactivation by  
505 PFA included both direct and indirect free radical reactions, in which the PFA,  $\bullet$  OH  
506 and peroxide radicals accounted for 73 %, 20 % and 6 %, respectively (Ding et al.,  
507 2023). Thus, PFA exhibit higher efficiency and effectiveness than the free radicals. It  
508 has been shown that PFA is predominantly responsible of cell inactivation by  
509 penetrating the microbial cell walls and membranes to damage functional proteins. In  
510 contrast, the  $\bullet$  OH and peroxide radicals effectively oxidize the cell interior  
511 (Koivunen and Heinonen-Tanski, 2005; Luukkonen and Pehkonen, 2017). PFA has  
512 been shown to exhibit high reactivity with cysteine and methionine residues through  
513 an oxygen transfer pathway (Du et al., 2018; Wang et al., 2023). It has been shown  
514 that the number of broken and cross-linked bacterial cells increases after PFA  
515 disinfection causing cell damage like wrinkles, chapped and rough surface on *E. coli*,  
516 *S. aureus*, and *B. subtilis* (Ding et al., 2023). The intracellular oxidative stress has  
517 been shown to be higher after PFA treatment (Wang et al., 2023). Thus, the PFA  
518 destroys the cell walls and membranes of microorganisms, resulting in the release of  
519 the cellular content.

520 The regrowth and recovery of pathogenic bacteria and antibiotic resistant bacteria  
521 (ARBs) carrying antibiotic resistance genes (ARGs) can potentially lead to  
522 subsequent contamination of the surface and ground water reservoirs (Shekhawat et  
523 al., 2020). Previous studies showed that bacteria were not able to regrow 24 hours  
524 after PFA disinfection of wastewater without irradiation (Ragazzo et al., 2013).  
525 Similarly, *E. coli* and *enterococci* cells showed irreversible inactivation (Rocher et al.,  
526 2021). Additionally, PFA has been shown to be beneficial in controlling antibiotic  
527 resistance using antibiotic resistant *E. coli* as typical indicator (Campo et al., 2020).

### 528 3.4 Functional predictions

529 In order to estimate the functional capabilities of the microbial communities,  
530 functional profiles were determined by PICRUSt2. The nearest sequenced taxon  
531 indices (NSTI) indicated that PICRUSt2 prediction was accurate (mean NSTI = 0.11  
532  $\pm$  0.05 SD). Although predictive, with the limitation that no direct evidence of a

533 function is provided, such approach provides an overview of the microbial processes  
534 (Menendez-Serra et al., 2019; Bourhane et al., 2022, 2023). The comparison of the  
535 PICRUST2 predicted functions of bacterial communities showed that the wastewater  
536 samples from the same WWTP clustered together at both low and high KEGG  
537 hierarchy levels (Fig. 7a, e). Most of the KEGG orthologs at level 2 had  
538 enrichment/depletion variations among the three WWTPs. They were more abundant  
539 in WWTP2 and WWTP3 than in WWTP1 (Fig. 7e).

540 PCA analysis considering each WWTP revealed that the bacterial communities  
541 clustered according to the disinfectant used for the treatment (Fig. 7b, c, d), showing  
542 the functional shifts within the bacterial communities caused by the different  
543 disinfectants. The heatmap of the abundant functional patterns demonstrated that the  
544 wastewater owns a specific functional pattern according to the origin (Fig. 7e).  
545 Noteworthy, the functional pattern was also modified by the treatment depending on  
546 the disinfectant used. Consistently with the microbial community differences  
547 observed (Fig. 2), the physical-chemical characteristics prevailing at the WWTP are  
548 the main factors shaping the microbial community structure, composition and  
549 functions. Such observation was in agreement with previous studies reporting that  
550 microbial changes are linked with effluent quality and system performance (Cao et al.,  
551 2020; ElNaker et al., 2019).

552 The relative abundance of genes involved in cell cycle pathway of *Caulobacter*  
553 [PATH: ko04112] significantly decreased in PFA\_H and Chl compared with BT  
554 (ANOVA,  $p < 0.001$ ; Fig. S5). It demonstrates the effects of PFA and chlorine on  
555 *Caulobacter* life cycle, which was consistent with a previous study showing that cell  
556 damages (e.g. discharge of cellular materials) were observed after chlorination in  
557 *Caulobacter* (Kim et al., 1982). Similarly, the relative abundance of genes associated  
558 to cell motility pathway significantly decreased in PFA\_H compared with BT, being  
559 at lower abundance in PFA\_H and PFA\_L than in UV and Chl (ANOVA,  $p < 0.001$ ;  
560 Fig. S6). This result demonstrated the effect of PFA on decreasing the motility and  
561 activity of microorganisms, which was consistent with the reduction of the microbial  
562 diversity and the reduction of *E. coli* and other faecal microorganisms counts

563 observed in PFA treatment (Fig. 1, Fig. 5, Fig. 6). Such observation further confirmed  
564 the effect of PFA on microbial community, which likely relies on the disinfection  
565 efficiency. It has been shown that the application of 4 mg/L PFA for 16 h has not  
566 toxicological effect using the *V. fischeri* assay (Chhetri et al., 2014), suggesting that  
567 PFA has a short decomposition time. PFA was also reported causing less disinfection  
568 by-products than other commonly used peracids (Luukkonen and Pehkonen, 2017). In  
569 this study, the disinfection was performed with 1 and 4 mg/L PFA for 15 min  
570 followed by a quenching step to stop the reaction. Although the residual toxicity test  
571 was not conducted in this study, PFA achieved high disinfection efficacy while  
572 producing very low residual toxicity according to previous reports (Rocher et al.,  
573 2021).

574 Regarding the metabolism associated pathways, the relative abundance of genes  
575 involved in the biosynthesis of secondary metabolites and metabolism of amino acids  
576 was significantly different (ANOVA,  $p < 0.05$ ) according to the disinfection treatment  
577 among all three WWTPs (Fig. 7e, S7-9). Previous studies have also reported that  
578 enzymes from the same species may have different activities depending on the  
579 treatment and conditions (Delforno et al., 2017; Emmanuel et al., 2019; Yang et al.,  
580 2020).

581 The genes involved in penicillin and cephalosporin biosynthesis pathway [PATH:  
582 ko00311] were significantly depleted on UV and Chl in comparison with PFA\_H and  
583 PFA\_L treatments (ANOVA,  $p < 0.05$ ; Fig. S7). Similarly, the abundance of genes  
584 involved in betalain biosynthesis pathway [PATH: ko00965] was significantly more  
585 abundant in PFA\_H than UV (ANOVA,  $p < 0.05$ ; Fig. S8). Betalain has been reported  
586 for its antimicrobial activity on a broad spectrum of microbes such as  
587 multidrug-resistant bacteria, single-species and dual-species biofilm-producing  
588 bacteria, dengue virus and fungi (Wijesinghe and Choo, 2022). Betalains primarily  
589 occur in Caryophyllales order plants. The enzymes involved in betalain biosynthesis  
590 have been reported in fungi, *Gluconacetobacter diazotrophicus* and Cyanobacteria  
591 (Contreras-Llano et al., 2019; Guerrero-Rubio et al., 2020). However, LEfSe did not  
592 identified Cyanobacteria as PFA-resistant biomarkers (Fig. S4), suggesting that

593 bacteria producing antimicrobial compounds (betalains), likely present in treated  
594 wastewater, were more sensitive to UV and chlorine than to PFA. Since UV and  
595 chlorination have been reported for their strong elimination efficiency of antibiotic by  
596 photolysis (Ding et al., 2020; Serna-Galvis et al., 2017), such a slight deficiency does  
597 not indicate that PFA is incompetent. It nevertheless alerts the risk of antibiotics  
598 release from wastewater into water system that potentially alters microbial ecosystems,  
599 that in turn represents a sanitary issue for human health (Baquero et al., 2008). The  
600 genes involved in glutathione metabolism pathway [PATH: ko00480] were  
601 significantly more abundant in PFA\_H (ANOVA,  $p < 0.05$ ; Fig. S9). The glutathione  
602 metabolism has been suggested to play important role in bacterial defence against  
603 chlorine-based disinfection (Du et al., 2015), demonstrating that PFA has likely  
604 similar effect on bacteria.

605 These results suggested that PFA\_H, even efficient on disinfecting most of the  
606 microorganisms (including pathogens), was not effective on inactivating the  
607 microorganisms carrying penicillin and cephalosporin, and betalain biosynthesis  
608 pathways. However, further analyses determining direct functions, such as shotgun  
609 metagenomics, are needed in order to describe in depth the effect of disinfection on  
610 the functional capabilities of microbial communities.

#### 611 4. Conclusions

612 Although the operational cost of PFA treatment has been estimated to be  
613 approximately 2.5 times more expensive than that of chlorine (Luukkonen et al.,  
614 2015), the PFA treatment owns several assets to be used as treatment for safe reuse of  
615 wastewater as it does not produce by-product. Also it has been shown that its cost can  
616 be drastically reduced for treatment plants with larger capacity (Ragazzo et al., 2013).  
617 But, even if further analyses are still required to comprehensively evaluate the costs, it  
618 is also necessary to evaluate the disinfection efficiency of PFA treatment in  
619 comparison to other commonly used treatments.

620 Our study elucidated the effect of PFA, UV and chlorine on microorganism  
621 disinfection, microbial community diversity, structure and functions crossing three

622 different WWTPs. It provides compelling evidence that different disinfectants  
623 resulted in specific microbial communities and functions, which effects differed  
624 according to the physical-chemical characteristic of the wastewater origin (WWTP).  
625 Our findings indicated that PFA was efficient in *E. coli* disinfection of wastewater,  
626 being able to decrease the microbial community diversity. Additionally, specific  
627 biomarkers of each WWTP were revealed, which likely relies on the processing  
628 treatments methods. Also, our study detected sensitive/resistant biomarkers for each  
629 disinfectant, PFA exhibiting higher number of sensitive biomarkers than chlorine and  
630 UV, PHA\_H having the highest number. Our findings underline the fact that PFA is  
631 efficient on wastewater disinfection in comparison with the traditional treatments  
632 involving UV and chlorine disinfectants. Because PFA was particularly effective in  
633 removing known faecal microorganisms, it has high potential for safe reuse of  
634 wastewater.

635

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## References

- 655
- 656
- 657 Ahmed, W., Staley, C., Sidhu, J., Sadowsky, M., Toze, S., 2017. Amplicon-based profiling of bacteria in  
 658 raw and secondary treated wastewater from treatment plants across Australia. *Applied Microbiology  
 659 and Biotechnology* 101, 1253-1266. 10.1007/s00253-016-7959-9.
- 660 Anastasi, E.M., Wohlsen, T.D., Stratton, H.M., Katouli, M., 2013. Survival of *Escherichia coli* in two  
 661 sewage treatment plants using UV irradiation and chlorination for disinfection. *Water research* 47,  
 662 6670-6679. 10.1016/j.watres.2013.09.008.
- 663 Andreadakis, A., Mamais, D., Christoulas, D., Kabylafka, S., 1999. Ultraviolet Disinfection of Secondary  
 664 and Tertiary Effluent in the Mediterranean Region. *Water Science and Technology* 40, 253-260.  
 665 10.2166/wst.1999.0598.
- 666 APHA, A.W., 2005. *Standard Methods for the Examination of Water and Wastewater*, 21st Edition ed.  
 667 American Public Health Association, American Water Works Association and Water Environment  
 668 Federation, Washington, DC, USA
- 669 Baquero, F., Martinez, J.L., Canton, R., 2008. Antibiotics and antibiotic resistance in water  
 670 environments. *Current opinion in biotechnology* 19, 260-265. 10.1016/j.copbio.2008.05.006.
- 671 Behera, P., Mahapatra, S., Mohapatra, M., Kim, J.Y., Adhya, T.K., Raina, V., Suar, M., Pattnaik, A.K.,  
 672 Rastogi, G., 2017. Salinity and macrophyte drive the biogeography of the sedimentary bacterial  
 673 communities in a brackish water tropical coastal lagoon. *Science of the Total Environment* 595,  
 674 472-485. 10.1016/j.scitotenv.2017.03.271.
- 675 Biswal, B.K., Khairallah, R., Bibi, K., Mazza, A., Gehr, R., Masson, L., Frigon, D., 2014. Impact of UV and  
 676 peracetic acid disinfection on the prevalence of virulence and antimicrobial resistance genes in  
 677 uropathogenic *Escherichia coli* in wastewater effluents. *Appl Environ Microb* 80, 3656-3666
- 678 Bourhane, Z., Cagnon, C., Castañeda, C., Rodriguez-Ochoa, R., Alvaro-Fuentes, J., Cravo-Laureau, C.,  
 679 Duran, R., 2023. Vertical organisation of microbial communities in Salineta hypersaline wetland, Spain.  
 680 *Front Microbiol* 14: 869907.
- 681 Bourhane, Z., Lanzen, A., Cagnon, C., Ben Said, O., Mahmoudi, E., Coulon, F., Atai, E., Borja, A.,  
 682 Cravo-Laureau, C., Duran, R., 2022. Microbial diversity alteration reveals biomarkers of contamination  
 683 in soil-river-lake continuum. *J Haz Mat* 421, 126789.
- 684 Bull, R.J., Birnbaum, L.S., Cantor, K.P., Rose, J.B., Butterworth, B.E., Pegram, R., Tuomisto, J., 1995.  
 685 Water chlorination: essential process or cancer hazard? *Fundamental and applied toxicology : official  
 686 journal of the Society of Toxicology* 28, 155-166. 10.1006/faat.1995.1156.
- 687 Campo, N., De Flora, C., Maffettone, R., Manoli, K., Sarathy, S., Santoro, D., Gonzalez-Olmos, R., Auset,  
 688 M., 2020. Inactivation kinetics of antibiotic resistant *Escherichia coli* in secondary wastewater  
 689 effluents by peracetic and performic acids. *Water research* 169, 115227.  
 690 10.1016/j.watres.2019.115227.
- 691 Cao, J., Zhang, T., Wu, Y., Sun, Y., Zhang, Y., Huang, B., Fu, B., Yang, E., Zhang, Q., Luo, J., 2020.  
 692 Correlations of nitrogen removal and core functional genera in full-scale wastewater treatment plants:  
 693 Influences of different treatment processes and influent characteristics. *Bioresource technology* 297,  
 694 122455. 10.1016/j.biortech.2019.122455.
- 695 Chhetri, R.K., Thornberg, D., Berner, J., Gramstad, R., Ojstedt, U., Sharma, A.K., Andersen, H.R., 2014.  
 696 Chemical disinfection of combined sewer overflow waters using performic acid or peracetic acids. *The  
 697 Science of the total environment* 490, 1065-1072. 10.1016/j.scitotenv.2014.05.079.
- 698 Collivignarelli, M.C., Abbà, A., Alloisio, G., Gozio, E., Benigna, I., 2017. Disinfection in Wastewater

699 Treatment Plants: Evaluation of Effectiveness and Acute Toxicity Effects. Sustainability 9  
700 Contreras-Llano, L.E., Guerrero-Rubio, M.A., Lozada-Ramirez, J.D., Garcia-Carmona, F., Gandia-Herrero,  
701 F., 2019. First Betalain-Producing Bacteria Break the Exclusive Presence of the Pigments in the Plant  
702 Kingdom. *Mbio* 10. 10.1128/mBio.00345-19.

703 Daims, H., Lebedeva, E.V., Pjevac, P., Han, P., Herbold, C., Albertsen, M., Jehmlich, N., Palatinszky, M.,  
704 Vierheilig, J., Bulaev, A., Kirkegaard, R.H., von Bergen, M., Rattei, T., Bendinger, B., Nielsen, P.H.,  
705 Wagner, M., 2015. Complete nitrification by *Nitrospira* bacteria. *Nature* 528, 504-509.  
706 10.1038/nature16461.

707 Daims, H., Wagner, M., 2018. *Nitrospira*. *Trends Microbiol* 26, 462-463. 10.1016/j.tim.2018.02.001.

708 Delforno, T.P., Lacerda, G.V., Jr., Sierra-Garcia, I.N., Okada, D.Y., Macedo, T.Z., Varesche, M.B., Oliveira,  
709 V.M., 2017. Metagenomic analysis of the microbiome in three different bioreactor configurations  
710 applied to commercial laundry wastewater treatment. *The Science of the total environment* 587-588,  
711 389-398. 10.1016/j.scitotenv.2017.02.170.

712 Diamond, M.B., Keshaviah, A., Bento, A.I., Conroy-Ben, O., Driver, E.M., Ensor, K.B., Halden, R.U.,  
713 Hopkins, L.P., Kuhn, K.G., Moe, C.L., Rouchka, E.C., Smith, T., Stevenson, B.S., Susswein, Z., Vogel, J.R.,  
714 Wolfe, M.K., Stadler, L.B., Scarpino, S.V., 2022. Wastewater surveillance of pathogens can inform public  
715 health responses. *Nat Med* 28, 1992-1995. 10.1038/s41591-022-01940-x.

716 Ding, N., Li, Z., Jiang, L., Liu, H., Zhang, Y., Sun, Y., 2023. Kinetics and mechanisms of bacteria  
717 disinfection by performic acid in wastewater: In comparison with peracetic acid and sodium  
718 hypochlorite. *The Science of the total environment* 878, 162606. 10.1016/j.scitotenv.2023.162606.

719 Ding, Y., Jiang, W., Liang, B., Han, J., Cheng, H., Haider, M.R., Wang, H., Liu, W., Liu, S., Wang, A., 2020.  
720 UV photolysis as an efficient pretreatment method for antibiotics decomposition and their  
721 antibacterial activity elimination. *Journal of hazardous materials* 392, 122321.  
722 10.1016/j.jhazmat.2020.122321.

723 Dominguez Henao, L., Cascio, M., Turolla, A., Antonelli, M., 2018. Effect of suspended solids on  
724 peracetic acid decay and bacterial inactivation kinetics: Experimental assessment and definition of  
725 predictive models. *The Science of the total environment* 643, 936-945.  
726 10.1016/j.scitotenv.2018.06.219.

727 Du, P., Liu, W., Cao, H., Zhao, H., Huang, C.H., 2018. Oxidation of amino acids by peracetic acid:  
728 Reaction kinetics, pathways and theoretical calculations. *Water research X* 1, 100002.  
729 10.1016/j.wroa.2018.09.002.

730 Du, Z., Nandakumar, R., Nickerson, K.W., Li, X., 2015. Proteomic adaptations to starvation prepare  
731 *Escherichia coli* for disinfection tolerance. *Water research* 69, 110-119. 10.1016/j.watres.2014.11.016.

732 Dueholm, M.K.D., Nierychlo, M., Andersen, K.S., Rudkjøbing, V., Knutsson, S., Mi, D.A.S.G.C., Albertsen,  
733 M., Nielsen, P.H., 2022. MiDAS 4: A global catalogue of full-length 16S rRNA gene sequences and  
734 taxonomy for studies of bacterial communities in wastewater treatment plants. *Nat Commun* 13, 1908.  
735 10.1038/s41467-022-29438-7.

736 EC, 2018. Proposal for a Regulation of the European Parliament and of the Council on Minimum  
737 Requirements for Water Reuse, in: Commission, E. (Ed.), COM, Brussels

738 EC, 2022. Water Reuse Fact Sheet-Water is too precious to waste. European Commission

739 ElNaker, N.A., Hasan, S.W., Yousef, A.F., 2019. Impact of current density on the function and microbial  
740 community structure in electro-bioreactors. *Journal of hazardous materials* 368, 877-884.  
741 10.1016/j.jhazmat.2018.09.016.

742 Emmanuel, S.A., Sul, W.J., Seong, H.J., Rhee, C., Ekpheghere, K.I., Kim, I.S., Kim, H.G., Koh, S.C., 2019.

743 Metagenomic analysis of relationships between the denitrification process and carbon metabolism in  
744 a bioaugmented full-scale tannery wastewater treatment plant. *World journal of microbiology &*  
745 *biotechnology* 35, 149. 10.1007/s11274-019-2716-8.

746 EPA, I., 2011. *Water Treatment Manual: Disinfection*. Environmental Protection Agency Wexford,  
747 Ireland

748 EPA, U., 2004. *Guidelines for water reuse*. Washington, DC 450

749 Forrez, I., Boon, N., Verstraete, W., Carballa, M., 2011. 6.38 - Biodegradation of Micropollutants and  
750 Prospects for Water and Wastewater Biotreatment, in: Moo-Young, M. (Ed.), *Comprehensive*  
751 *Biotechnology* (Second Edition). Academic Press, Burlington, pp. 485-494.

752 Foschi, J., Turolla, A., Antonelli, M., 2021. Soft sensor predictor of *E. coli* concentration based on  
753 conventional monitoring parameters for wastewater disinfection control. *Water research* 191, 116806.  
754 10.1016/j.watres.2021.116806.

755 Furst, K.E., Pecson, B.M., Webber, B.D., Mitch, W.A., 2018. Tradeoffs between pathogen inactivation  
756 and disinfection byproduct formation during sequential chlorine and chloramine disinfection for  
757 wastewater reuse. *Water research* 143, 579-588. 10.1016/j.watres.2018.05.050.

758 Gao, F.Z., He, L.Y., He, L.X., Zou, H.Y., Zhang, M., Wu, D.L., Liu, Y.S., Shi, Y.J., Bai, H., Ying, G.G., 2020.  
759 Untreated swine wastes changed antibiotic resistance and microbial community in the soils and  
760 impacted abundances of antibiotic resistance genes in the vegetables. *The Science of the total*  
761 *environment* 741, 140482. 10.1016/j.scitotenv.2020.140482.

762 Gao, Y., Shi, X., Jin, X., Wang, X.C., Jin, P., 2023. A critical review of wastewater quality variation and  
763 in-sewer processes during conveyance in sewer systems. *Water research* 228, 119398.  
764 10.1016/j.watres.2022.119398.

765 Gehr, R., Chen, D., Moreau, M., 2009. Performic acid (PFA): tests on an advanced primary effluent  
766 show promising disinfection performance. *Water science and technology : a journal of the*  
767 *International Association on Water Pollution Research* 59, 89-96. 10.2166/wst.2009.761.

768 Gerba, C.P., Pepper, I.L., 2019. Chapter 22 - Municipal Wastewater Treatment, in: Brusseau, M.L.,  
769 Pepper, I.L., Gerba, C.P. (Eds.), *Environmental and Pollution Science* (Third Edition). Academic Press, pp.  
770 393-418.

771 Guerrero-Rubio, M.A., Garcia-Carmona, F., Gandia-Herrero, F., 2020. First description of betalains  
772 biosynthesis in an aquatic organism: characterization of 4,5-DOPA-extradial-dioxygenase activity in the  
773 cyanobacteria *Anabaena cylindrica*. *Microbial biotechnology* 13, 1948-1959.  
774 10.1111/1751-7915.13641.

775 Gulay, A., Fowler, S.J., Tatari, K., Thamdrup, B., Albrechtsen, H.J., Al-Soud, W.A., Sorensen, S.J., Smets,  
776 B.F., 2019. DNA- and RNA-SIP Reveal *Nitrospira* spp. as Key Drivers of Nitrification in Groundwater-Fed  
777 Biofilters. *Mbio* 10, e01870-01819. 10.1128/mBio.01870-19.

778 Hutinel, M., Larsson, D.G.J., Flach, C.F., 2022. Antibiotic resistance genes of emerging concern in  
779 municipal and hospital wastewater from a major Swedish city. *The Science of the total environment*  
780 812, 151433. 10.1016/j.scitotenv.2021.151433.

781 Kang, X.H., Leng, Y., O, M.M., Zeng, X.Y., Li, S.W., 2020. The seasonal changes of core bacterial  
782 community decide sewage purification in sub-plateau municipal sewage treatment plants. *Bioprocess*  
783 *and biosystems engineering* 43, 1609-1617. 10.1007/s00449-020-02352-2.

784 Kantor, R.S., Miller, S.E., Nelson, K.L., 2019. The Water Microbiome Through a Pilot Scale Advanced  
785 Treatment Facility for Direct Potable Reuse. *Front Microbiol* 10, 993. 10.3389/fmicb.2019.00993.

786 Kauser, I., Ciesielski, M., Poretsky, R.S., 2019. Ultraviolet disinfection impacts the microbial community

787 composition and function of treated wastewater effluent and the receiving urban river. PeerJ 7, e7455.  
788 10.7717/peerj.7455.

789 Kim, C.-K., Park, M.-K., Yum, K., 1982. Effects of chlorine and chlorine compounds on morphology and  
790 function of *Caulobacter* cells. *Applied Microscopy* 12, 23-32

791 Koch, H., van Kessel, M., Lucker, S., 2019. Complete nitrification: insights into the ecophysiology of  
792 comammox *Nitrospira*. *Appl Microbiol Biotechnol* 103, 177-189. 10.1007/s00253-018-9486-3.

793 Koivunen, J., Heinonen-Tanski, H., 2005. Inactivation of enteric microorganisms with chemical  
794 disinfectants, UV irradiation and combined chemical/UV treatments. *Water research* 39, 1519-1526.  
795 10.1016/j.watres.2005.01.021.

796 Kowal, P., Ciesielski, S., Godzieba, M., Fitobor, K., Gajewska, M., Kolecka, K., 2021. Assessment of  
797 diversity and composition of bacterial community in sludge treatment reed bed systems. *The Science*  
798 *of the total environment* 756, 144060. 10.1016/j.scitotenv.2020.144060.

799 Kulkarni, P., Olson, N.D., Paulson, J.N., Pop, M., Maddox, C., Claye, E., Rosenberg Goldstein, R.E.,  
800 Sharma, M., Gibbs, S.G., Mongodin, E.F., Sapkota, A.R., 2018. Conventional wastewater treatment and  
801 reuse site practices modify bacterial community structure but do not eliminate some opportunistic  
802 pathogens in reclaimed water. *The Science of the total environment* 639, 1126-1137.  
803 10.1016/j.scitotenv.2018.05.178.

804 Lautenschlager, K., Hwang, C., Ling, F., Liu, W.T., Boon, N., Koster, O., Egli, T., Hammes, F., 2014.  
805 Abundance and composition of indigenous bacterial communities in a multi-step biofiltration-based  
806 drinking water treatment plant. *Water research* 62, 40-52. 10.1016/j.watres.2014.05.035.

807 Leroy-Freitas, D., Machado, E.C., Torres-Franco, A.F., Dias, M.F., Leal, C.D., Araujo, J.C., 2022. Exploring  
808 the microbiome, antibiotic resistance genes, mobile genetic element, and potential resistant  
809 pathogens in municipal wastewater treatment plants in Brazil. *The Science of the total environment*  
810 842, 156773. 10.1016/j.scitotenv.2022.156773.

811 Li, Y., Wang, Y., Wan, D., Li, B., Zhang, P., Wang, H., 2020. Pilot-scale application of sulfur-limestone  
812 autotrophic denitrification biofilter for municipal tailwater treatment: Performance and microbial  
813 community structure. *Bioresource technology* 300, 122682. 10.1016/j.biortech.2019.122682.

814 Luukkonen, T., Heynink, T., Ramo, J., Lassi, U., 2015. Comparison of organic peracids in wastewater  
815 treatment: Disinfection, oxidation and corrosion. *Water research* 85, 275-285.  
816 10.1016/j.watres.2015.08.037.

817 Luukkonen, T., Pehkonen, S.O., 2017. Peracids in water treatment: A critical review. *Critical Reviews in*  
818 *Environmental Science and Technology* 47, 1-39. 10.1080/10643389.2016.1272343.

819 Maffettone, R., Manoli, K., Santoro, D., Passalacqua, K.D., Wobus, C.E., Sarathy, S., 2020. Performic  
820 Acid Disinfection of Municipal Secondary Effluent Wastewater: Inactivation of Murine Norovirus, Fecal  
821 Coliforms, and Enterococci. *Environ Sci Technol* 54, 12761-12770. 10.1021/acs.est.0c05144.

822 Magoc, T., Salzberg, S.L., 2011. FLASH: fast length adjustment of short reads to improve genome  
823 assemblies. *Bioinformatics* 27, 2957-2963. 10.1093/bioinformatics/btr507.

824 McCall, C., Wu, H., Miyani, B., Xagorarakis, I., 2020. Identification of multiple potential viral diseases in  
825 a large urban center using wastewater surveillance. *Water research* 184, 116160.  
826 10.1016/j.watres.2020.116160.

827 McGorum, B.C., Pirie, R.S., Glendinning, L., McLachlan, G., Metcalf, J.S., Banack, S.A., Cox, P.A., Codd,  
828 G.A., 2015. Grazing livestock are exposed to terrestrial cyanobacteria. *Veterinary research* 46, 16.  
829 10.1186/s13567-015-0143-x.

830 Menendez-Serra, M., Triado-Margarit, X., Castaneda, C., Herrero, J., Casamayor, E.O., 2019. Microbial

831 composition, potential functional roles and genetic novelty in gypsum-rich and hypersaline soils of  
832 Monegros and Gallocanta (Spain). *The Science of the total environment* 650, 343-353.  
833 10.1016/j.scitotenv.2018.09.050.

834 Ng, C., Tan, B., Jiang, X.T., Gu, X., Chen, H., Schmitz, B.W., Haller, L., Charles, F.R., Zhang, T., Gin, K.,  
835 2019. Metagenomic and Resistome Analysis of a Full-Scale Municipal Wastewater Treatment Plant in  
836 Singapore Containing Membrane Bioreactors. *Front Microbiol* 10, 172. 10.3389/fmicb.2019.00172.

837 Nieuwenhuijsen, M.J., Toledano, M.B., Eaton, N.E., Fawell, J., Elliott, P., 2000. Chlorination disinfection  
838 byproducts in water and their association with adverse reproductive outcomes: a review. *Occup*  
839 *Environ Med* 57, 73-85. 10.1136/oem.57.2.73.

840 Numberger, D., Ganzert, L., Zoccarato, L., Muhldorfer, K., Sauer, S., Grossart, H.P., Greenwood, A.D.,  
841 2019. Characterization of bacterial communities in wastewater with enhanced taxonomic resolution  
842 by full-length 16S rRNA sequencing. *Sci Rep* 9, 9673. 10.1038/s41598-019-46015-z.

843 Ogden, I.D., Dallas, J.F., MacRae, M., Rotariu, O., Reay, K.W., Leitch, M., Thomson, A.P., Sheppard, S.K.,  
844 Maiden, M., Forbes, K.J., Strachan, N.J., 2009. *Campylobacter* excreted into the environment by  
845 animal sources: prevalence, concentration shed, and host association. *Foodborne Pathog Dis* 6,  
846 1161-1170. 10.1089/fpd.2009.0327.

847 Oguma, K., Katayama, H., Mitani, H., Morita, S., Hirata, T., Ohgaki, S., 2001. Determination of  
848 pyrimidine dimers in *Escherichia coli* and *Cryptosporidium parvum* during UV light inactivation,  
849 photoreactivation, and dark repair. *Applied and environmental microbiology* 67, 4630-4637.  
850 10.1128/AEM.67.10.4630-4637.2001.

851 Pei, M., Zhang, B., He, Y., Su, J., Gin, K., Lev, O., Shen, G., Hu, S., 2019. State of the art of tertiary  
852 treatment technologies for controlling antibiotic resistance in wastewater treatment plants. *Environ*  
853 *Int* 131, 105026. 10.1016/j.envint.2019.105026.

854 Piel, T., Sandrini, G., Muyzer, G., Brussaard, C.P.D., Slot, P.C., van Herk, M.J., Huisman, J., Visser, P.M.,  
855 2021. Resilience of Microbial Communities after Hydrogen Peroxide Treatment of a Eutrophic Lake to  
856 Suppress Harmful Cyanobacterial Blooms. *Microorganisms* 9. 10.3390/microorganisms9071495.

857 Qian, S., Hou, R., Yuan, R., Zhou, B., Chen, Z., Chen, H., 2022. Removal of *Escherichia coli* from  
858 domestic sewage using biological sand filters: Reduction effect and microbial community analysis.  
859 *Environmental research* 209, 112908. 10.1016/j.envres.2022.112908.

860 Ragazzo, P., Chiuccini, N., Piccolo, V., Ostoich, M., 2013. A new disinfection system for wastewater  
861 treatment: performic acid full-scale trial evaluations. *Water science and technology : a journal of the*  
862 *International Association on Water Pollution Research* 67, 2476-2487. 10.2166/wst.2013.137.

863 Ragazzo, P., Chiuccini, N., Piccolo, V., Spadolini, M., Carrer, S., Zanon, F., Gehr, R., 2020. Wastewater  
864 disinfection: long-term laboratory and full-scale studies on performic acid in comparison with  
865 peracetic acid and chlorine. *Water research* 184, 116169. 10.1016/j.watres.2020.116169.

866 Rocher, V., Azimi, S., Mailler, R., Rechdaoui-Guérin, S., Mèche, P., Pichon, S., Goffin, A., Bernier, J., Roy,  
867 J., Varrault, G., 2021. Effectiveness of Disinfecting Wastewater Treatment Plant Discharges: Case of  
868 chemical disinfection using performic acid. IWA Publishing.

869 Salmaso, N., Albanese, D., Capelli, C., Boscaini, A., Pindo, M., Donati, C., 2018. Diversity and Cyclical  
870 Seasonal Transitions in the Bacterial Community in a Large and Deep Perialpine Lake. *Microb Ecol* 76,  
871 125-143. 10.1007/s00248-017-1120-x.

872 Saqrane, S., Oudra, B., El Mhammedi, M.A., 2020. Remediation of Freshwaters Contaminated by  
873 Cyanobacteria, in: Inamuddin, Ahamed, M.I., Lichtfouse, E., Asiri, A.M. (Eds.), *Methods for*  
874 *Bioremediation of Water and Wastewater Pollution*. Springer International Publishing, Cham, pp.

875 161-180.

876 Sauter, D., Steuer, A., Wasmund, K., Hausmann, B., Szewzyk, U., Sperlich, A., Gnirss, R., Cooper, M.,  
877 Wintgens, T., 2023. Microbial communities and processes in biofilters for post-treatment of ozonated  
878 wastewater treatment plant effluent. *The Science of the total environment* 856, 159265.  
879 10.1016/j.scitotenv.2022.159265.

880 Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S., Huttenhower, C., 2011.  
881 Metagenomic biomarker discovery and explanation. *Genome biology* 12, R60.  
882 10.1186/gb-2011-12-6-r60.

883 Serna-Galvis, E.A., Ferraro, F., Silva-Agredo, J., Torres-Palma, R.A., 2017. Degradation of highly  
884 consumed fluoroquinolones, penicillins and cephalosporins in distilled water and simulated hospital  
885 wastewater by UV(254) and UV(254)/persulfate processes. *Water research* 122, 128-138.  
886 10.1016/j.watres.2017.05.065.

887 Sharma, P., Tripathi, S., Chandra, R., 2021. Metagenomic analysis for profiling of microbial  
888 communities and tolerance in metal-polluted pulp and paper industry wastewater. *Bioresource  
889 technology* 324, 124681. 10.1016/j.biortech.2021.124681.

890 Shekhawat, S.S., Kulshreshtha, N.M., Gupta, A.B., 2020. Investigation of chlorine tolerance profile of  
891 dominant gram negative bacteria recovered from secondary treated wastewater in Jaipur, India.  
892 *Journal of environmental management* 255, 109827. 10.1016/j.jenvman.2019.109827.

893 Song, M., Luo, C., Jiang, L., Peng, K., Zhang, D., Zhang, R., Li, Y., Zhang, G., 2019. The presence of in situ  
894 sulphamethoxazole degraders and their interactions with other microbes in activated sludge as  
895 revealed by DNA stable isotope probing and molecular ecological network analysis. *Environ Int* 124,  
896 121-129. 10.1016/j.envint.2018.12.039.

897 Soo, R.M., Skennerton, C.T., Sekiguchi, Y., Imelfort, M., Paech, S.J., Dennis, P.G., Steen, J.A., Parks, D.H.,  
898 Tyson, G.W., Hugenholtz, P., 2014. An expanded genomic representation of the phylum cyanobacteria.  
899 *Genome biology and evolution* 6, 1031-1045. 10.1093/gbe/evu073.

900 Spring, S., Jackel, U., Wagner, M., Kampfer, P., 2004. *Ottowia thiooxydans* gen. nov., sp. nov., a novel  
901 facultatively anaerobic, N<sub>2</sub>O-producing bacterium isolated from activated sludge, and transfer of  
902 *Aquaspirillum gracile* to *Hylemonella gracilis* gen. nov., comb. nov. *Int J Syst Evol Microbiol* 54, 99-106.  
903 10.1099/ijs.0.02727-0.

904 Stamps, B.W., Leddy, M.B., Plumlee, M.H., Hasan, N.A., Colwell, R.R., Spear, J.R., 2018. Characterization  
905 of the Microbiome at the World's Largest Potable Water Reuse Facility. *Front Microbiol* 9, 2435.  
906 10.3389/fmicb.2018.02435.

907 Tang, G., Li, B., Zhang, B., Wang, C., Zeng, G., Zheng, X., Liu, C., 2021. Dynamics of dissolved organic  
908 matter and dissolved organic nitrogen during anaerobic/anoxic/oxic treatment processes. *Bioresource  
909 technology* 331, 125026. <https://doi.org/10.1016/j.biortech.2021.125026>.

910 Tang, G., Zheng, X., Li, X., Liu, T., Wang, Y., Ma, Y., Ji, Y., Qiu, X., Wan, Y., Pan, B., 2020a. Variation of  
911 effluent organic matter (EfOM) during anaerobic/anoxic/oxic (A(2)O) wastewater treatment processes.  
912 *Water research* 178, 115830. 10.1016/j.watres.2020.115830.

913 Tang, Z., Xi, B., Huang, C., Tan, W., Li, W., Zhao, X., Liu, K., Xia, X., 2020b. Mobile genetic elements in  
914 potential host microorganisms are the key hindrance for the removal of antibiotic resistance genes in  
915 industrial-scale composting with municipal solid waste. *Bioresource technology* 301, 122723.  
916 10.1016/j.biortech.2019.122723.

917 Tian, L., Wang, L., 2020. A meta-analysis of microbial community structures and associated metabolic  
918 potential of municipal wastewater treatment plants in global scope. *Environmental Pollution* 263,

919 114598. 10.1016/j.envpol.2020.114598.  
920 USEPA, 2003. Wastewater Technology Fact Sheet-Disinfection for Small Systems. United States  
921 Environmental Protection Agency  
922 USEPA, 2004. Primer for municipal wastewater treatment systems, in: USEPA (Ed.). United States  
923 Environmental Protection Agency, Washington, D.C., United States, p. 29 p.  
924 Vällitalo, P., Massei, R., Heiskanen, I., Behnisch, P., Brack, W., Tindall, A.J., Du Pasquier, D., Küster, E.,  
925 Mikola, A., Schulze, T., Sillanpää, M., 2017. Effect-based assessment of toxicity removal during  
926 wastewater treatment. *Water research* 126, 153-163. 10.1016/j.watres.2017.09.014.  
927 VandeWalle, J.L., Goetz, G.W., Huse, S.M., Morrison, H.G., Sogin, M.L., Hoffmann, R.G., Yan, K.,  
928 McLellan, S.L., 2012. *Acinetobacter*, *Aeromonas* and *Trichococcus* populations dominate the microbial  
929 community within urban sewer infrastructure. *Environmental microbiology* 14, 2538-2552.  
930 <https://doi.org/10.1111/j.1462-2920.2012.02757.x>.  
931 Vazquez-Baeza, Y., Pirrung, M., Gonzalez, A., Knight, R., 2013. EMPeRor: a tool for visualizing  
932 high-throughput microbial community data. *GigaScience* 2, 16. 10.1186/2047-217X-2-16.  
933 Veloso, S., Amouroux, D., Lancelot, L., Cagnon, C., Monperrus, M., Deborde, J., Laureau, C.C., Duran,  
934 R., 2023. Keystone microbial taxa organize micropollutant-related modules shaping the microbial  
935 community structure in estuarine sediments. *Journal of hazardous materials* 448, 130858.  
936 10.1016/j.jhazmat.2023.130858.  
937 Victoria, E., 2002. Disinfection of Treated Wastewater-Guidelines for Environmental Management.  
938 Environmental Protection Authority, Australia.  
939 Walters, E., Graml, M., Behle, C., Müller, E., Horn, H., 2014. Influence of particle association and  
940 suspended solids on UV inactivation of fecal indicator bacteria in an urban river. *Water, Air, & Soil*  
941 *Pollution* 225, 1-9  
942 Wang, J., Chen, W., Wang, T., Reid, E., Krall, C., Kim, J., Zhang, T., Xie, X., Huang, C.H., 2023. Bacteria  
943 and Virus Inactivation: Relative Efficacy and Mechanisms of Peroxyacids and Chlor(am)ine. *Environ Sci*  
944 *Technol.* 10.1021/acs.est.2c09824.  
945 Wijesinghe, V.N., Choo, W.S., 2022. Antimicrobial betalains. *Journal of applied microbiology* 133,  
946 3347-3367. 10.1111/jam.15798.  
947 Wu, Q., Ma, H., Su, Z., Lu, W., Ma, B., 2022. Impact of marine aquaculture wastewater discharge on  
948 microbial diversity in coastal waters. *Regional Studies in Marine Science* 56, 102702.  
949 <https://doi.org/10.1016/j.rsma.2022.102702>.  
950 Xu, D., Liu, S., Chen, Q., Ni, J., 2017. Microbial community compositions in different functional zones of  
951 Carrousel oxidation ditch system for domestic wastewater treatment. *AMB Express* 7, 40.  
952 10.1186/s13568-017-0336-y.  
953 Yang, Y., Wang, L., Xiang, F., Zhao, L., Qiao, Z., 2020. Activated Sludge Microbial Community and  
954 Treatment Performance of Wastewater Treatment Plants in Industrial and Municipal Zones.  
955 *International journal of environmental research and public health* 17. 10.3390/ijerph17020436.  
956 Yousra, T., Mehri, I., Lajnef, R., Rejab, A.B., Khessairi, A., Cherif, H., Ouzari, H., Hassen, A., 2017.  
957 Biofilms in bioremediation and wastewater treatment: characterization of bacterial community  
958 structure and diversity during seasons in municipal wastewater treatment process. *Environmental*  
959 *science and pollution research international* 24, 3519-3530. 10.1007/s11356-016-8090-2.  
960 Zaouri, N., Jumat, M.R., Cheema, T., Hong, P.Y., 2020. Metagenomics-based evaluation of groundwater  
961 microbial profiles in response to treated wastewater discharge. *Environmental research* 180, 108835.  
962 10.1016/j.envres.2019.108835.

963 Zimmer, J.L., Slawson, R.M., 2002. Potential repair of Escherichia coli DNA following exposure to UV  
964 radiation from both medium- and low-pressure UV sources used in drinking water treatment. Applied  
965 and environmental microbiology 68, 3293-3299. 10.1128/AEM.68.7.3293-3299.2002.  
966 Zyara, A.M., Torvinen, E., Veijalainen, A.M., Heinonen-Tanski, H., 2016. The effect of chlorine and  
967 combined chlorine/UV treatment on coliphages in drinking water disinfection. J Water Health 14,  
968 640-649. 10.2166/wh.2016.144.

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970



971 **Figure captions**

972 Fig. 1. Efficiency of the disinfection treatments on the different WWTPs. The  
973 efficiency of the disinfection treatments was estimated by *E. coli* counts. BT, before  
974 treatment; The treatments are UV, chlorine, performic acid at low (PFA\_L) and high  
975 (PFA\_H) concentrations.

976

977 Fig. 2. Comparison of microbial communities by Principal Coordinates Analysis  
978 (PCoA) based on weighted UniFrac distances including the three WWTPs (a); PCoA  
979 based on Bray-curtis distances for WWTP1 (b), WWTP2 (c), and WWTP3 (d). The  
980 arrows indicate the significant impact of the parameter (*E. coli* counts) on the  
981 microbial community composition (\*  $p < 0.05$ , \*\*  $p < 0.01$ , Permutations = 999).  
982 Circles: WWTP1; diamond: WWTP2; triangles: WWTP3. Red: chlorine; blue:  
983 performic acid at low concentration (PFA\_L); orange: performic acid at high  
984 concentration (PFA\_H); green: before treatment (BT); purple: UV.

985

986 Fig. 3. Composition of microbial communities at the phyla level in each treatment and  
987 WWTP. BT, before treatment; The treatments are UV, chlorine, performic acid at low  
988 (PFA\_L) and high (PFA\_H) concentrations.

989

990 Fig. 4. Bacterial biomarkers (bacterial taxa) for the different WWTPs before treatment  
991 (BT). The Cladogram shows the bacterial taxa identified by linear discriminant  
992 analysis effect size (LEfSe). The biomarkers are colored according to their WWTPs'  
993 specificity indicating that they are significantly more abundant in a WWTP than the  
994 others. The wastewater samples without any treatment were used in the analysis. D1:  
995 Phylum; D2: Class.

996

997 Fig. 5. Linear discriminant analysis effect size (LEfSe) identifying treatment-sensitive  
998 (external) and treatment-resistant (inner) biomarkers according to treatment PFA\_H,  
999 PFA\_L, Chlorine and UV. The comparisons were performed between the wastewater

1000 before (BT) and after each treatment from the three WWTPs. The length of the bars  
1001 corresponds to the LDA score. The known pathogens are represented in red. D1:  
1002 Phylum; D2: Class; D3: Order; D4: Family; D5: Genus; D6: Species.

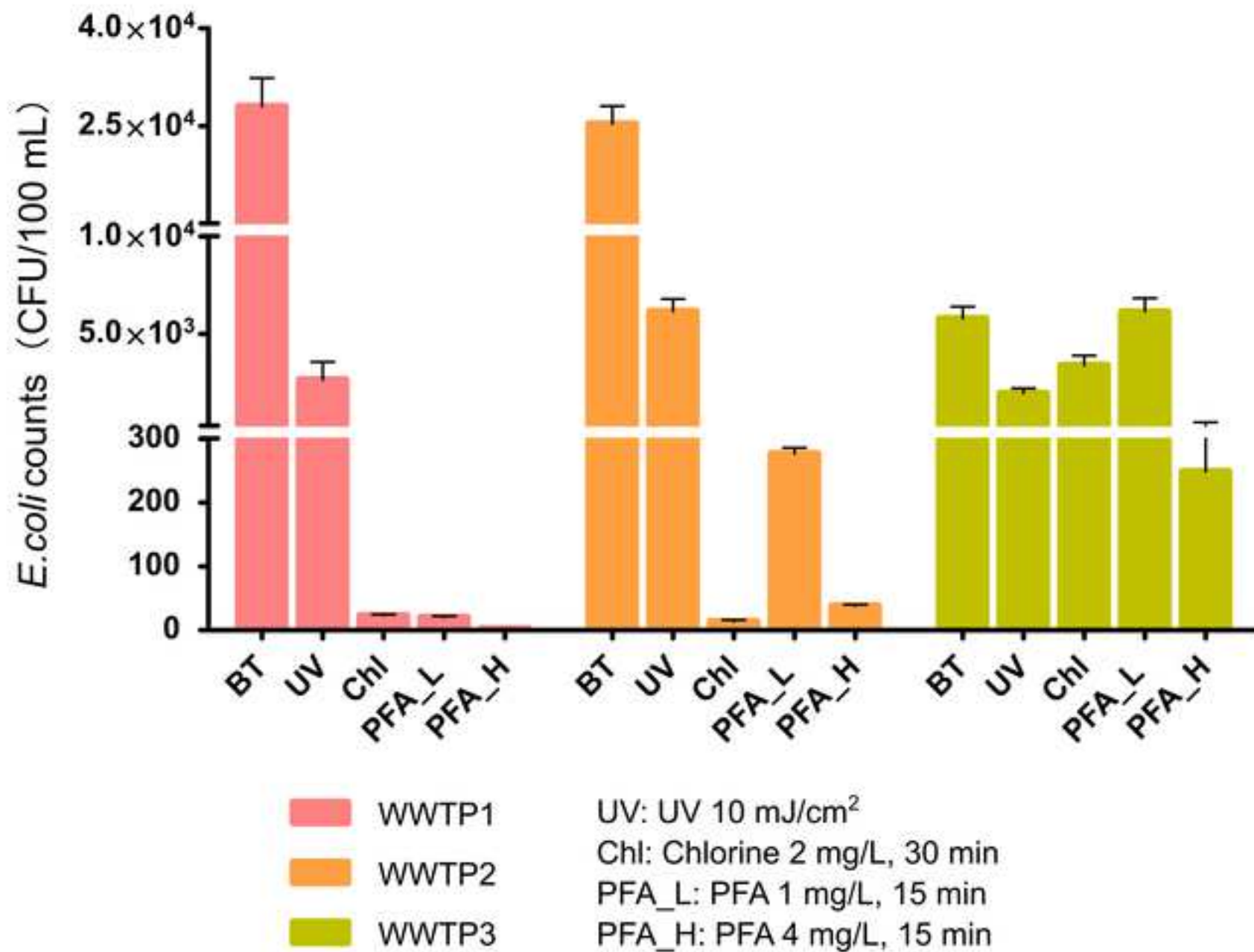
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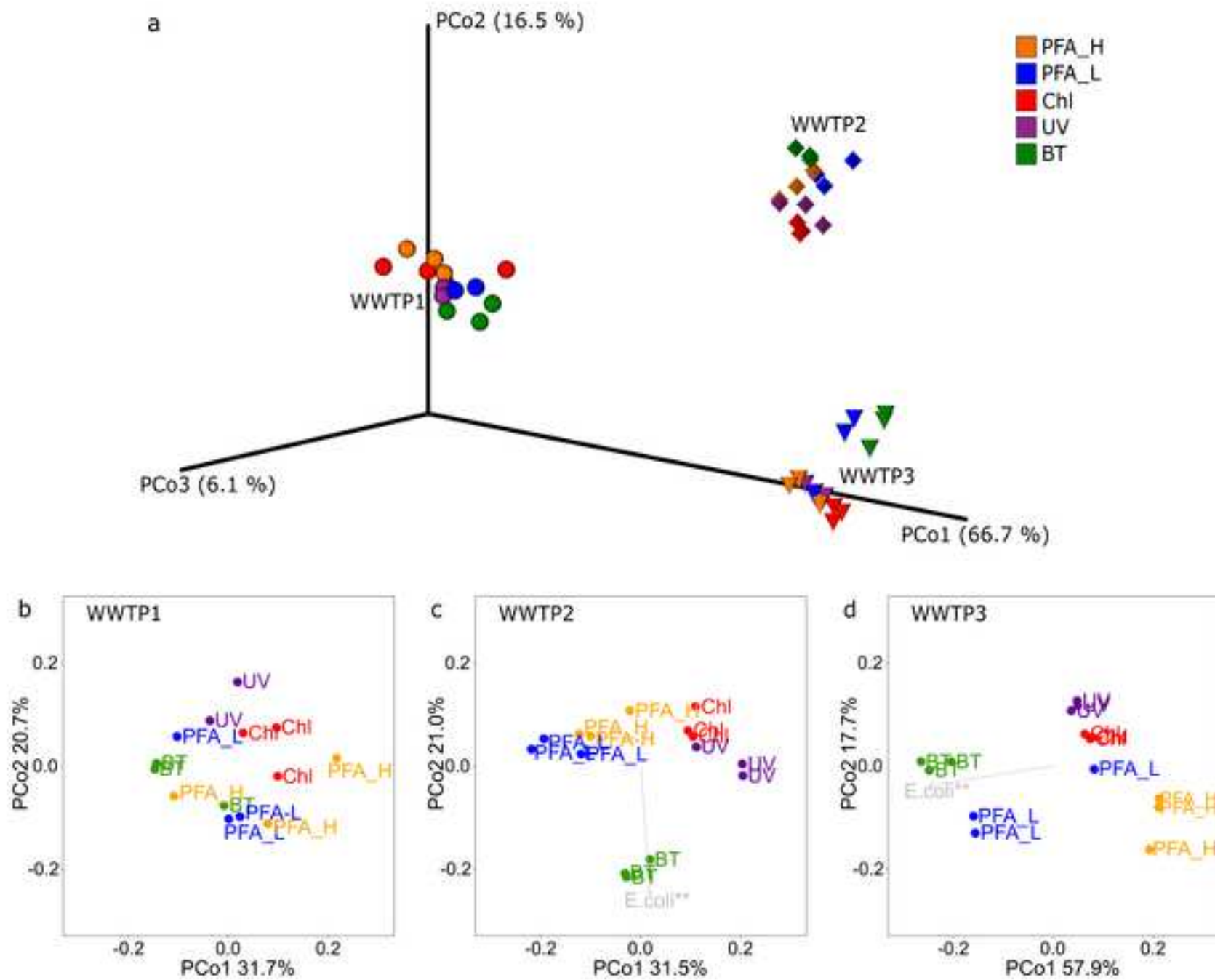
1004 Fig. 6. Disinfection efficiency on pathogen microorganisms according to the different  
1005 treatments. The comparisons were performed between each pair of the treatments  
1006 from all three WWTPs by linear discriminant analysis effect size (LEfSe) identifying  
1007 treatment-sensitive biomarkers. The colored dots correspond to microbial genera that  
1008 sensitive to PFA\_H (red), PFA\_L (orange), Chlorine (purple) and UV (blue)  
1009 treatment respectively.

1010

1011 Fig. 7. Functional comparison of bacterial communities predicted by PICRUSt2. The  
1012 PCAs show the predicted functions, based on KEGG categories (level 3), of the three  
1013 WWTPs (a), and for the different disinfectants treatments in WWTP1 (b), WWTP2  
1014 (c), WWTP3 (d). (e) Heatmap of the standardized abundance of predicted functions  
1015 based on KEGG categories (level 2). BT, before treatment; the treatments are UV,  
1016 chlorine, performic acid at low (PFA\_L) and high (PFA\_H) concentrations.

1017





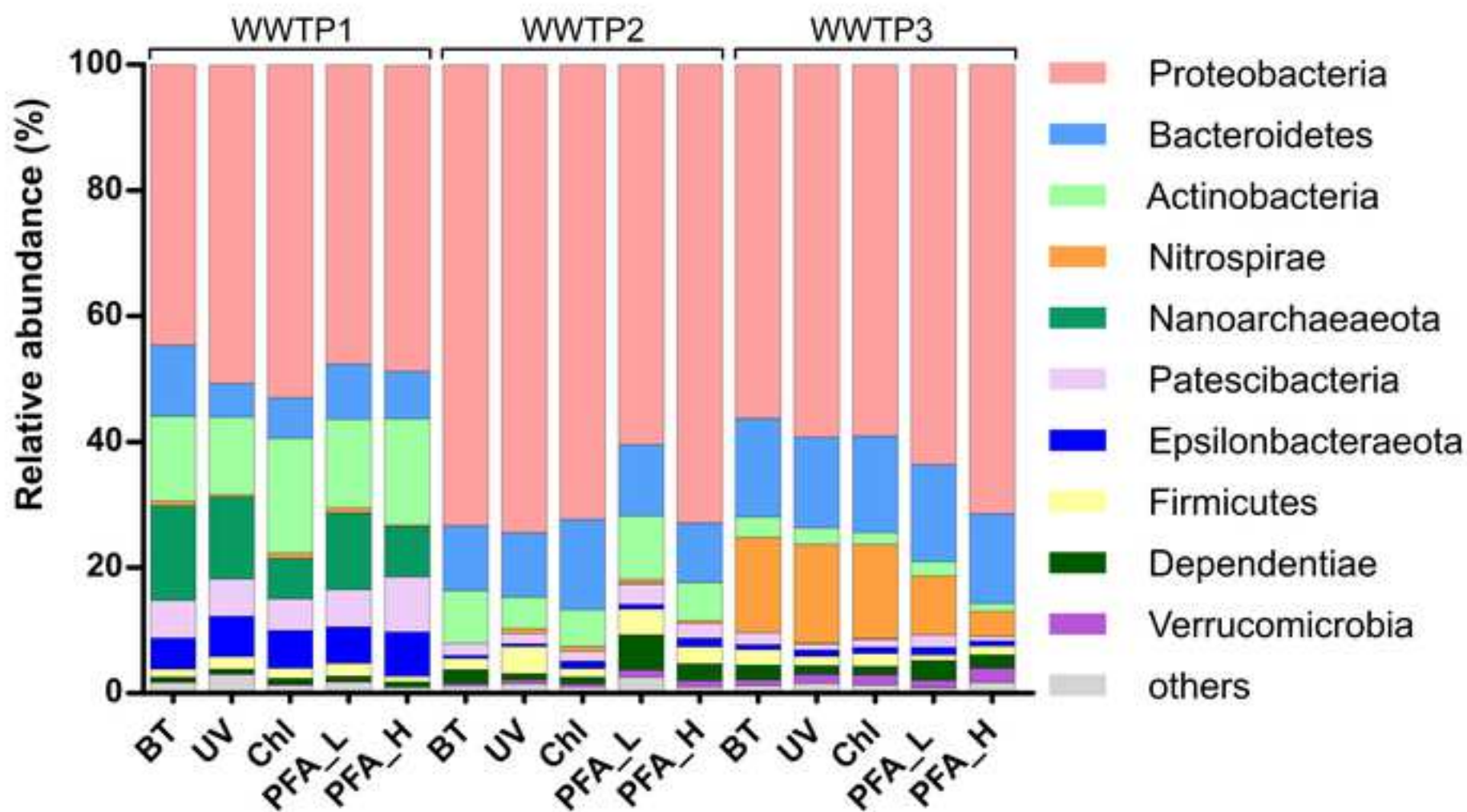
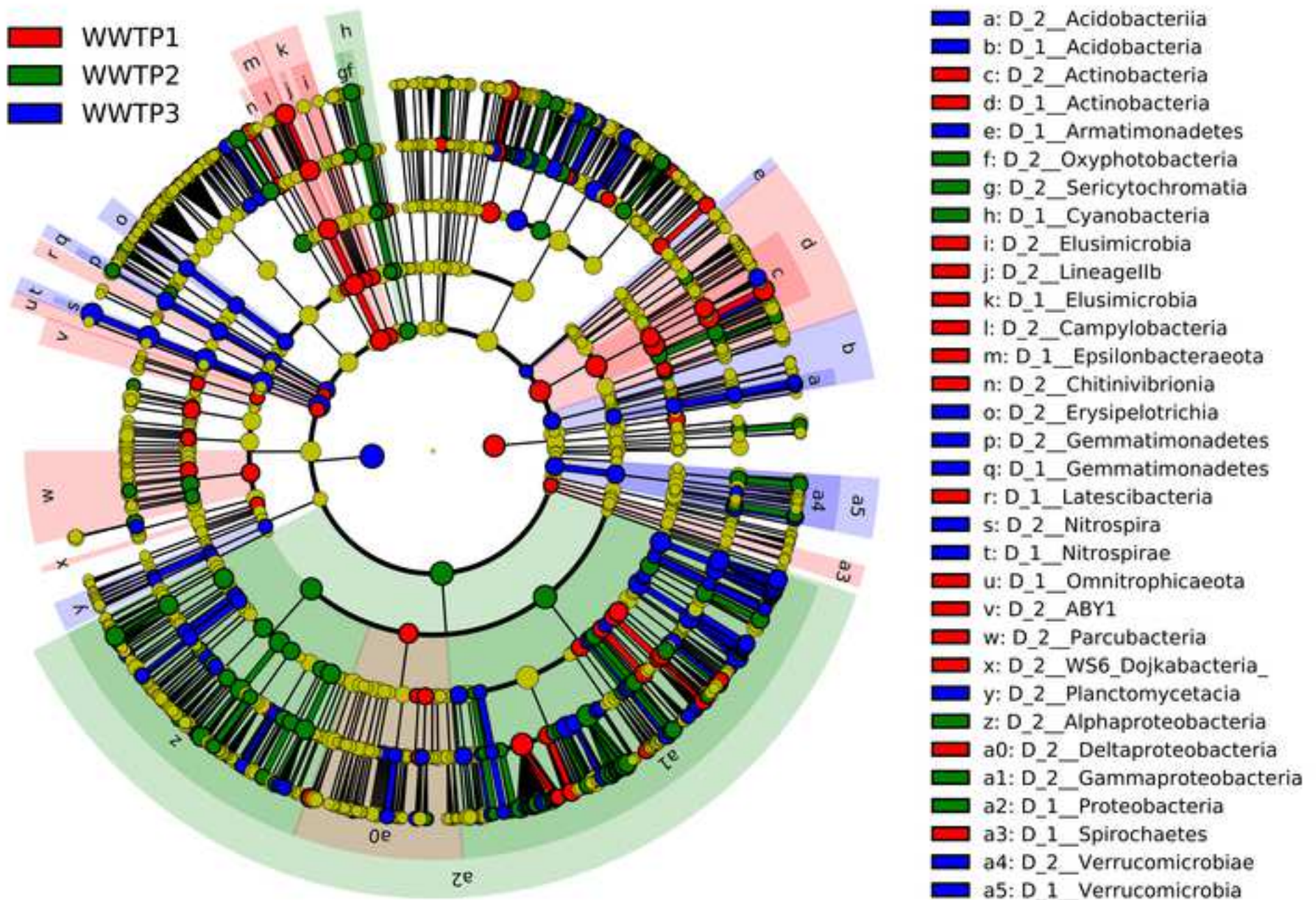
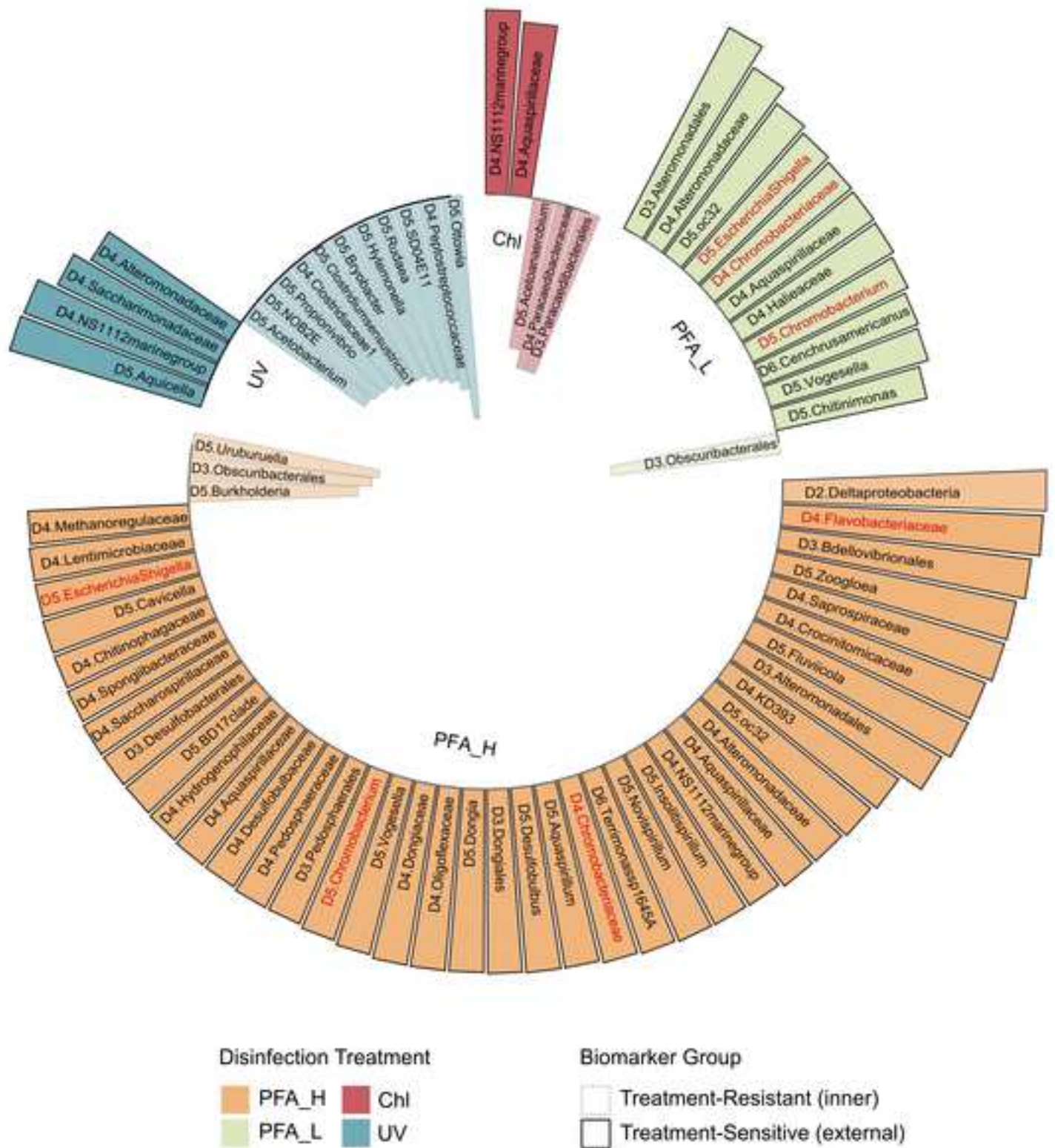
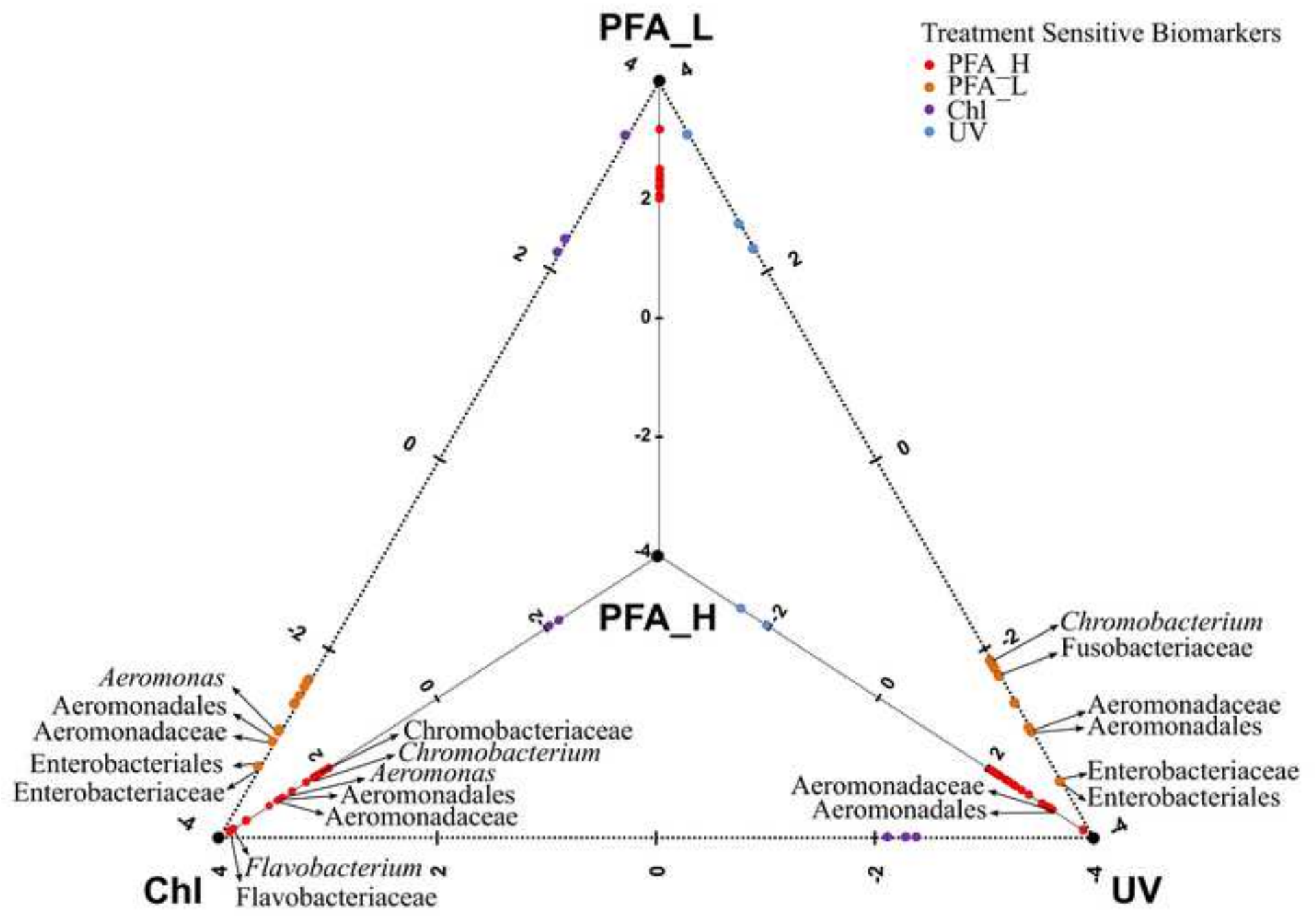


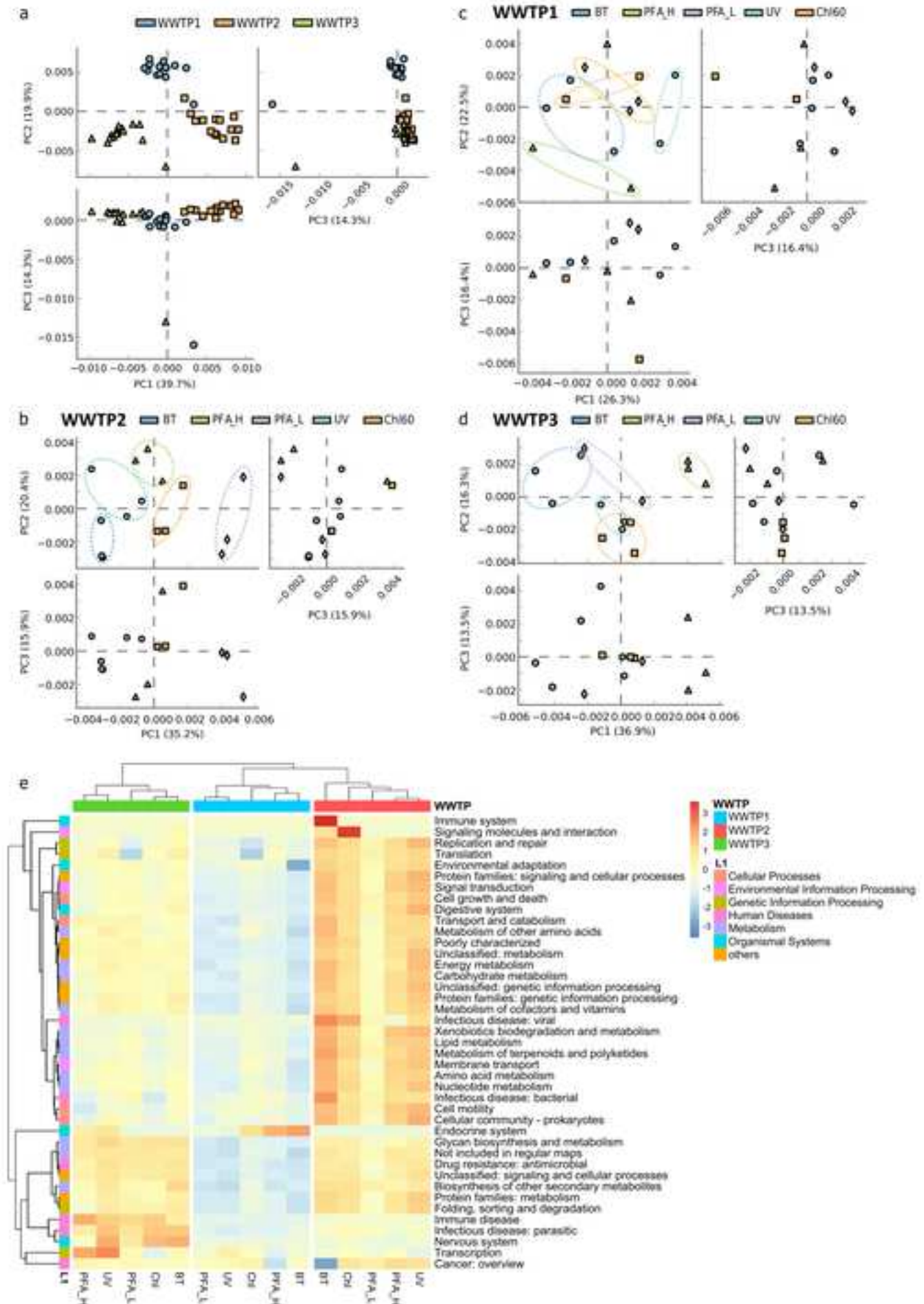
Figure 4

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**Authors Contributions:** **BW:** Conceptualisation, Investigation, Resources, Formal analysis, Writing – original draft. **RD:** Conceptualisation, Investigation, Resources, Formal analysis, Writing – original draft, Supervision, Project administration, Funding acquisition. **TG:** Conceptualisation, Writing – original draft, Supervision, Project administration, Funding acquisition. **CCL:** Conceptualisation, Investigation, Resources, Formal analysis, Writing – original draft, Supervision, Project administration, Funding acquisition.

**Declaration of interests**

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.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: