

1    **Physico-chemical and biological aspects of a serially connected lab-scale**  
2    **constructed wetland-stabilization tank-GAC slow sand filtration system during**  
3    **removal of selected PPCPs**

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12    **Abstract**

13        A serially connected lab-scale Greater duckweed constructed wetland (CW)-  
14    stabilization tank (ST)-GAC sandwich slow sand filtration system was tested to  
15    remove four widely detected pharmaceuticals and personal care products (PPCPs)  
16    compounds from natural water with a spiked concentration of 25 µg/L. High removals  
17    were achieved rapidly (93.5~100%), being on average 95.9%, 99.1%, 98.1% and  
18    97.4% for DEET, paracetamol, caffeine and triclosan (n=3), respectively. Except for  
19    DEET, no significant difference was observed between overall removals with and  
20    without artificial aeration in CW tank ( $p>0.05$ ), showing good stability of the system.  
21    COD was considerably removed under aeration and final TOC removal was 64.7%.  
22    No nitrite, nitrate, ammonia and phosphate were detected at the test end (day 26). The  
23    microbial community structure in three connected units of the tested system showed  
24    differences and good stability after the aerators were removed. *Proteobacteria* was the  
25    most dominant phylum among the 47 phyla found. Microbes attaching to the Greater  
26    duckweed contributed more to the microbial community structure in CW and ST than

original natural water. However, at the end of the run, the structural differences among three units decreased. After aeration stopped, phylum composition became more stable in ST tank while CW tank showed small structural variation throughout the test. Various correlations were found between detected phyla, among which *Proteobacteria* and *Bacteroidetes* showed a significant negative correlation ( $R=-0.73$ ,  $p<0.001$ , FDR corrected). Good removal of target PPCPs and stability of the system show the potential applicability of this combined treatment process.

**Keywords:** PPCPs; Constructed wetland; GAC sandwich SSF; Water treatment; Microbial community

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## 1. Introduction

In recent years, concern about the presence of PPCPs in water has risen, resulting more research in this area [1,2]. Usually, concentrations of PPCP compounds in the environment are in the low range (ng/L- $\mu$ g/L), but their persistence and toxicity may cause potential risks to both humans and the environment [3,4]. Furthermore, PPCPs in the environment can bring other negative consequences, such as antibiotic resistance genes [5]. Generally, wastewater treatment plant (WWTP) effluents are considered as important sources of PPCPs in the environment [6]. To tackle this issue, various techniques (e.g. biological treatment, membrane bioreactor, UV light, ozonation) have been studied, but the removal of PPCPs varies substantially and these techniques are usually expensive [7–10].

Constructed wetlands (CW), as an eco- and cost-friendly technique, can be used both as WWTP tertiary process and single decentralized system in rural locations [11,12]. In the last two decades, PPCP removal has also been investigated by various CW systems but their removal varied considerably (<30% to >95%) [13–15]. During the CW treatment, microbial biodegradation, photodegradation and plant effect are regarded as some of the mechanisms involved in the removal of PPCPs [16]. Among various types of CWs, surface free water constructed wetlands (SFCW) are becoming popular to treat wastewater and polluted surface water because they are easy to run and clean [17–19].

Another eco-friendly and low-cost technique, slow sand filtration (SSF, one of the earliest water treatment technologies) has also gained more attention because it has many advantages including: no chemical coagulation requirement, low electricity costs, and simplicity in maintenance and operation [20]. Some studies on conventional SSF have been conducted to treat various PPCP compounds but was not shown to be highly effective [21–23], indicating that microbial degradation and sorption were not enough to remove their target compounds. In recent years, PPCP removal was also investigated by SSF modified with the addition of granular activated carbon (GAC) – i.e. dual- or sandwich media - and removals were significantly enhanced [24,25].

As tertiary treatment processes in WWTP, CW and SSF are normally used separately and rarely connected. Gunes and Tuncsiper [26] investigated a serially connected sand filter with a subsurface flow constructed wetland system for small community wastewater treatment, achieving average removals of the BOD, total nitrogen and total phosphorus at 97%, 85% and 69%, respectively, suggesting good applicability of this combination. However, SSF-CW systems have some potential limitations if SSF is placed first since it can only receive influent with a certain

quality [27]. The service life may also be negatively affected by suspended solids increasing rapidly the headloss [28]. In contrast, the reverse CW-SSF system has rarely been studied. In our previous studies, modified Greater duckweed (*Spirodela polyrhiza*) based lab-scale continuous SFCW system (CW-stabilization tank) was tested and proposed [29]. In addition, GAC sandwich SSF using coarse sand (effective size at 0.6 mm) was found to be effective for the removal of selected PPCPs [25]. Besides, although investigation of microbial community during treatment by CW and SSF separately has been reported before [22,30], no study has been carried out using the combined system. By continuous sampling, the knowledge of temporal changes of microbial community can provide a deeper insight of its development and better understanding of the system performance. Thus, in the present study, a lab-scale CW-ST (stabilization tank)-GAC sandwich SSF system was built and explored to remove four widely detected PPCP compounds (i.e. DEET, paracetamol, caffeine and triclosan) from natural water (description of the four target compounds can be found in Li et al. [29]). Microbial communities in this combined system were monitored using bacterial 16S rRNA gene sequencing to gain deeper insight into structural changes during the treatment process. To our knowledge, this is the first study investigating PPCP removal using CW-ST followed by GAC sandwich SSF system.

## **2. Materials and methods**

### **2.1 Chemicals and materials**

Standards and chemicals of DEET (purity  $\geq 97.0\%$ ), paracetamol (PAR, purity  $\geq 98.0\%$ ), caffeine (CAF, purity  $\geq 99.0\%$ ) and triclosan (TCS, purity  $\geq 99.0\%$ ) were purchased from Sigma-Aldrich (UK). Characteristics of compounds are shown in Table S1. Stock solution of 1 mg/mL mixed target compounds prepared in methanol

was stored at -20 °C and added into the natural water to reach a spiked concentration of 25 µg/L. Methanol and acetonitrile (HPLC grade) were purchased from Fisher Scientific (UK). In this study, natural water was collected from the Regent's Park Lake, London, UK, which has average turbidity < 2 NTU and pH around 7.6~8.0.

Greater duckweed (*Spirodela polyrhiza*) was purchased from Claremontaquatic Leyland (UK). Acrylic column with an internal diameter of 34 mm was purchased from Plastic Shop (UK). Filter sand (Mineral Marketing, UK) had an effective size of 0.6 mm and a uniform coefficient of 1.4. The surface area of GAC (Chemviron Carbon, UK) was about 556 m<sup>2</sup>/g. Washing, treatment and properties of Greater duckweed, coarse sand, GAC and gravel can be found in Li et al. [25,29].

## 2.2 Experimental design and description

The experiment was conducted during September and October 2017. A schematic representation of the experiment system is shown in Fig.1. The system consisted of one influent tank, one constructed wetland tank (CW, 32×22×17 cm), one stabilization tank (ST, 32×22×17 cm), one GAC sandwich filter and one outflow tank, successively connected by peristaltic pumps. The area above the CW and ST tanks was covered by reflective fabric. Experimental conditions (full aeration in the CW tank, 240 µmol·m<sup>-2</sup>·s<sup>-1</sup> light intensity, 1.00 kg/m<sup>2</sup> plant density) were the optimised parameters from our previous study [29]. Lights (NARVA LT 15W/077) which were left on for 14 hours and off for 10 hours each day were placed over the CW-ST area in order to simulate the day and night. 70g fresh and washed Greater duckweed was placed in the CW tank. Four aerators (3.2 L/min output each) were evenly placed at the CW tank bottom and room temperature was maintained at 23±2 °C. Seven litres of the natural water spiked with 25 µg/L target PPCP compounds were put in CW and

ST tanks separately at the beginning of the test. The pump flow rate was set at 1.38 mL/min (equivalent to a hydraulic retention time of 7 days).

The GAC sandwich column was connected after the ST tank receiving CW-ST system effluent. The flow rate into the SSF was set at 3.00 mL/min. Excess water flowed back to the ST tank via an overflow pipe (Fig.1) and the water level was maintained at 5 cm above the media constantly. Column height was 65 cm with 10 cm sand/20 cm GAC/20 cm sand/3 cm gravel from top to bottom. The effluent pipe, which had one valve controlling the filtration rate, was located 1 cm above the column base. The filtration rate was set at 10 cm/h. Before the start of the experiment, water was filtered through sandwich filter for maturation, as detailed in Li et al. [25]. After maturation, the SSF was connected to CW-ST system and lake water (system inlet water) spiked with 25 µg/L of each of the selected PPCPs was added to the CW-ST unit and pumped into the system.

The duration of the test was 4 weeks. To explore the system performance without artificial aeration, all aerators were removed from the CW system at day 14. The filtration rate of GAC sandwich filter was monitored and adjusted twice daily if needed. In order not to disturb treatment performance in each unit of the system, water samples were only collected three times a week on Mondays, Wednesdays and Fridays from the system effluents to determine concentrations of PPCPs, nitrate, phosphate, nitrite, ammonium, COD (chemical oxygen demand) and TOC (total organic carbon). General water quality parameters (i.e. pH, conductivity and redox potential) of effluent samples were also determined. On each sampling day, dissolved oxygen (DO) concentrations in both CW and ST tanks were determined. Total headloss of the GAC-SSF was measured at the end of the test.

## 2.3 Determination of selected PPCP compounds and general water quality parameters

The extraction method for the target PPCP compounds from water and the PPCP quantification method are shown in Text S1. Determination of other general water quality parameters, including concentrations of COD, TOC, nitrate, phosphate, nitrite, ammonium, DO, pH, conductivity and redox potential was performed as described previously [29]. Samples were run in triplicate. Removal (%) of the target PPCP compounds was calculated using:

$$\text{Removal (\%)} = \frac{C_i + C_a - C_e}{C_i + C_a} \times 100\%$$

Where  $C_i$  (µg/L) is the influent concentration of target compounds from lake water.  $C_a$  is the added concentration (25 µg/L each compound) and  $C_e$  (µg/L) is the concentration in the final effluent.

## 2.4 Microbial community analysis

Water samples of 200 mL were collected from CW and ST tanks respectively on days 5, 12, 19 and 26 for microbial analysis. System inlet water without spiked PPCPs was also collected (Day1) when test began. Water samples were filtered through a 0.22 µm cellulose acetate membrane (Whatman, UK) to retain microorganisms. Filtered water was recirculated to the CW/ST tank. Sand samples of 1.0 g were collected from the top of the SSF after filter maturation (SI) and at day 26 (SF). To explore the influence of microbes attaching to Greater duckweed on the experimental system, 10 g plants were collected randomly from washed Greater duckweed before test began and rinsed with 500 mL ultrapure water three times. The rinsed combined water sample (WP, total 1500 mL) of the plants was collected onto

membranes as described above. The summarized sample description is shown in Table S2.

DNA extraction was done using FastDNA™ SPIN Kit for Soil (MP Biomedicals, France) and subsequent tests were conducted in triplicate. DNA quality check was carried out using the Qubit® dsDNA BR Assay Kit (ThermoFisher, USA). 16S rDNA amplicon PCR and library construction and next generation sequencing were carried by the BGI Company (Hong Kong). Briefly, DNA was normalized to 30 ng per reaction. A polymerase chain reaction (PCR) of the bacterial 16S rRNA gene was conducted with the primer pair targeting the V4 region (forward: 5'-GTGCCAGCMGCCGCGGTAA-3'; reverse: 5'-GGACTACHVGGGTWTCTAAT-3'), used widely in microbial community analysis of environmental samples [31–33]. The post-PCR clean-up step was completed using Agencourt AMPure XP (Beckman Coulter, High Wycombe, USA) and PCR products were quantified. Next, pooled library quantification and DNA quality check were further performed. The pooled library was run on the HiSeq 2500 PE250 Dual Index platform (Illumina, San Diego, USA). Sequencing results were further filtered. First reads with adaptors and/or more than 3% unknown bases (N) were removed. Then reads with larger than 40% of the basecalls with a quality lower than 20 were also removed. Lastly, the filtered data were clustered into operational taxonomic units (OTUs) at 97% similarity. Processing of the cleaned 16S rDNA sequences was performed according to Koopman et al. [34] with two differences: 25 mismatches in the overlap was used (as 10% mismatches translates to 25 mismatches) and SILVA v132 was used. Further analyses were conducted based on the relative abundance of OTU results.

## 2.5 Statistical analysis



The data processing was conducted by Microsoft Excel 2013. ANOVA tests were carried out to assess the differences between sample concentrations and  $p$ -value  $< 0.05$  was considered statistically significant. Non-metric multidimensional scaling (NMDS) using Wisconsin scaling and Bray-Curtis dissimilarity [35] was employed to further look into the dissimilarity of the microbial community structures [36] based on relative abundances. To allow robust comparisons among samples, alpha diversity indices were calculated by the rarefied OTUs (530,000 reads/sample). Dynamic changes of *Proteobacteria* were based on numbers of (*Proteobacteria*) OTUs. Spearman correlation analysis was carried out to assess correlations among detected phyla and  $p$  value was corrected by false discovery rate (FDR). OriginPro 9.1, SankeyMATIC and Rstudio v1.1.447 (R v3.4.4) with packages ggplot2 v3.1.0, reshape2 v1.4.3, gplots v3.0.1, corplots v0.84, phyloseq v1.22.3, microbiome v1.0.2, and vegan v2.5-3 were used to develop all graphs.

### 3. Results and discussion

#### 3.1 Removal of target PPCP compounds in CW-ST-SSF system

The concentrations and removals of four target PPCP compounds are shown in Table 1. Fig. S1 shows the concentration change trend. All four compounds were detected in lake water, with concentrations of DEET at  $0.88 \pm 0.32$   $\mu\text{g/L}$ , PAR at  $1.26 \pm 0.21$   $\mu\text{g/L}$ , CAF at  $0.72 \pm 0.37$   $\mu\text{g/L}$  and TCS at  $3.54 \pm 1.84$   $\mu\text{g/L}$ . As DEET and TCS are synthetic organic compounds [37,38], occurrence of these two compounds in the water sampling area indicates that the pollution is from human activities.

Table 1 shows that by using the CW-ST-SSF system, good removal, of above 90%, was achieved on all compounds, with average removals at 95.9%, 99.1%, 98.1% and 97.4% for DEET, PAR, CAF and TCS, respectively, and high removal ( $>90\%$ )

was achieved as soon as the test started, which may be attributed to the effects of different mechanisms (e.g. biodegradation, adsorption) [16,24]. In a previous study using the continuous CW-ST system only [29], the average removals of the PPCPs from the synthetic wastewater (COD at 300 mg/L) were 27.1%, 92.2%, 65.8% and 99.3% (calculated by authors) for DEET, PAR, CAF and TCS, respectively. Biodegradation, photodegradation and plant effect were proved to play roles in target PPCP removal [29]. As for the GAC sandwich SSF, under the filtration rate of 10 cm/h, average removals of DEET, PAR, CAF and TCS from synthetic wastewater (COD at 40 mg/L) were 98.0%, 100%, 100% and 94.8%, respectively. Adsorption and biodegradation were regarded as the main mechanisms [25]. As for other techniques, removals of DEET, CAF, PAR and TCS at 1.4%, 76.9%, 58.1% and 90.2% were found using anaerobic membrane bioreactors [39] and all below 25% (except for CAF) were found in the activated sludge tank-plate and frame/hollow-fibre membrane system [40]. In this study, natural water was used. A much more complex matrix (e.g. humic substance) existing in natural water may lead to competitive adsorption between target compounds and other substances onto adsorbents (GAC in current study) [41,42]. Besides, the carbon resource in the synthetic wastewater used was glucose (300 mg/L), which is highly degradable [43]. High concentration of glucose could favour microbial growth and activity, probably including PPCP-degradation microorganisms, accelerating target PPCP elimination. In contrast, natural water harbours less nutrients. Therefore, it was assumed that a relatively lower intensity of bioactivity in the current natural water system might limit PPCP removal. However, high removal of the four PPCP compounds (>90%) indicates the good performance of present system treating natural water contaminated with target PPCPs.

Oxygen is an essential factor influencing plants and microbial activity in CW systems, thus affecting relevant biodegradation and plant effect. Roles of plants include PPCP direct uptake and creation of favourable conditions for microbial removal [44–46]. At day 14, aerators from the CW tank were removed. Except for DEET ( $p<0.05$ ), no significant difference was observed between removals with (DO above 7.98 mg/L in CW tank) and without aeration (DO at 5.32~6.26 mg/L in CW tank) ( $p>0.05$ ) for the other three compounds. DEET is usually regarded as a recalcitrant compound [47,48]. Higher DO concentration in the CW significantly favoured DEET elimination and removal of aerators led to higher concentration of DEET in effluents [29]. In the present study, although a significant difference was found ( $p<0.05$ ), removal of DEET with aeration (96.1% on average) was only slightly higher than without aeration (95.7% on average) (Table 1). From Table S4, after switching off the aerators, DO concentrations dropped from above 8.20 mg/L to 5.32 mg/L after one day (day 15) in the CW tank and then increased again, while in the ST tank DO was more stable. Apart from natural oxygen diffusion from air into water, many aquatic plants are thought to be able to transport oxygen from leaves to roots and Greater duckweed may also have this ability [16,29]. A sudden change of DO could affect CW plants and microbial activity, leading to PPCP removal fluctuations. However, diffusion of oxygen in the ST tank for biodegradation and GAC adsorption in the filter which was not influenced by DO changes ensured the continuous removal of the target PPCPs. The good removal in the present study indicates the stability of the combined system for PPCP elimination. Nevertheless, this research only investigated the PPCP removal efficiency. Degradation pathways and metabolites could be further studied to provide a deeper insight into the PPCP degradation mechanisms.

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### 275 **3.2 General water quality parameters**

276 Table S3 summarizes the monitored concentrations of COD, TOC, nitrate,  
277 phosphate and ammonium in raw lake water and final effluents. While nitrite was not  
278 found, nitrate and phosphate were detected in lake water at concentrations of  
279  $0.07\pm0.01$  and  $0.12\pm0.02$  mg/L, respectively. However, high ammonium  
280 concentration of  $19.56\pm0.18$  mg/L was detected, which can be attributed to the fact  
281 that Regent's Park is a natural habitat for a variety of waterfowls and biologic  
282 excretion leads to high ammonium concentration. Analysis also shows that the lake  
283 water had COD level at  $20\pm5$  mg/L and TOC at  $1.67\pm0.18$  mg/L.

284 During the treatment process, COD concentrations of effluent kept below 1  
285 mg/L until at day 22 its concentration increased to 21 mg/L (possible effect of DO  
286 decrease in CW tank exhibited several days later) and then declined, whilst TOC had  
287 the same trend. The phenomenon that COD and TOC concentrations increased first  
288 and then dropped after aeration stopped was also observed in the continuous CW-ST  
289 system [29]. Generally, sharp DO concentration decrease would influence  
290 dynamically stable aerobic microbial community and break ecological balance [49],  
291 hence affecting nutrients removal. However, in the present study, re-decrease of COD  
292 and TOC demonstrated stability of the combined system.

293 No nitrite was found in the final treated water. Nitrate was only found in several  
294 sampling days at very low concentrations (below 0.1 mg/L) after the aeration stopped  
295 and was totally removed afterward. Phosphate was only detected at day 17 at 0.33  
296 mg/L. Previous research by Gunes and Tuncsiper [26] observed that total phosphorus  
297 was removed 69% under the raw wastewater concentration of  $8.94\pm3.97$  mg/L by the  
298 serially connected SSF-CW system. Using GAC sandwich SSF alone, only around 10%

of phosphate could be removed [25], while 30~50% of phosphate was removed in continuous CW-ST system [29]. In the present study, the no occurrence of phosphate in final treated water of the combined system can be attributed to both the good treatment performance and low phosphate load from the raw lake water. Ammonium was thoroughly removed under aeration condition but appeared at  $15.53 \pm 0.23$  mg/L at day 19 after switching off the aerators, then gradually decreased to zero again (day 26). Nitrate and nitrite concentrations in the effluents were within the range of standards for drinking water quality (50 mg/L for nitrate and 0.5 mg/L for nitrite, EU Directive 98/83/EC).

Other general parameters (e.g. pH, DO) monitored are shown in Table S4. Final treated effluent pH was around 8.2~8.5, lying within the range (6.5~8.5) of discharge standards suggested by WHO-EM/CEH/142/E. Total headloss of the filter was smaller than one centimetre.

### 3.3 Microbial community structural changes during test operation

A total of 47 bacterial phyla were found in all 12 tested samples, among which relative abundance of top 7 predominant phyla are shown in Fig.2-a. The alpha diversity indices of samples are displayed in Table S5. Top 10 dominant OTUs are shown in Table S6. *Proteobacteria* was the most dominant phylum in almost all samples, accounting for more than 50% of the total detected reads (except Day1 and SF), followed by *Bacteroidetes*, *Actinobacteria*, *Verrucomicrobia*, *Planctomycetes*, *Patescibacteria* and *Cyanobacteria*. These phyla were also reported as dominant phylum in other CW systems [50,51]. Besides, more than half of the *Proteobacteria* were from class of *Gammaproteobacteria*. A high proportion of *Proteobacteria* was also reported by Haig et al. [20] who found *Proteobacteria*, *Bacteroidetes*,

324 *Acidobacteria* and *Verrucomicrobia* representing 60.80%, 9.27%, 5.17%, and 1.78%  
325 of the total community in the sand filtration system, respectively, and  
326 *Gammaproteobacteria* was one dominant class in phylum *Proteobacteria*. In the  
327 present study, although there were 47 phyla determined, the abundances of the OTUs  
328 in these 7 phyla occupied more than 97% of all sequences. The relative abundance  
329 comparison (scaled among each phylum) among all detected phyla is shown Fig.2-b.  
330 The graph shows that there was a great change from inlet water (Day1) to the  
331 subsequent system samples and abundance of two-third of the detected phyla were  
332 declining (e.g. *Epsilonbacteraeota*, *Fibrobacteres*) when test began, while some  
333 microbes (e.g. *Dependentiae*, *Hydrogenedentes*) thrived during the experiment. As the  
334 target PPCP compounds were spiked into the water, such obvious changes could be  
335 ascribed to the influence of added PPCPs [52], or the environmental change (i.e.  
336 temperature) from natural environment to the laboratory [41]. Compared to the Day1,  
337 the phylum relative abundance of microbes attached to the plants (WP) was similar to  
338 those in the CW and ST tanks samples (Fig.2-a). This phenomenon indicated that  
339 microbial structure from the plants played a more important role than the CW tank  
340 inlet water. It can be assumed that though the external environment changed,  
341 microorganisms in the CW tank, especially around roots, are important to plants  
342 [53,54] and interaction between plants and microbes existed during the tested period,  
343 helping maintaining the original structure. As for the GAC sandwich SSF, the  
344 microbial community at the final sampling day (SF) was quite distinct from that at test  
345 beginning (SI) (Fig.2-b). The dominant *Proteobacteria* phylum (accounts for more  
346 50% in other samples of system) decreased from around 76% to 40% but the  
347 *Alphaproteobacteriac* lass increased from 12% to 17%, while some other  
348 subdominant phyla (e.g. *Bacteroidetes*, *Planctomycetes* and *Verrucomicrobia*)

increased, showing higher alpha diversity (Table S5). This result agrees well with the finding of Haig et al. [20] that diversity of microbial community structure increased over time in a lab-scale slow sand filter. Since effluent of the ST tank was pumped directly into the GAC sandwich filter, it was expected that microbial community of ST26 and SF were similar at phylum level. However, the difference observed (Fig.2) suggests the filter formed microbial community independently.

The result of NMDS analysis is shown in Fig.3. At day 5, the community structure of both CW5 and ST5 clearly differed from initial Day1, while WP was similar to CW5, showing the same assumption that WP contributed more to the CW tank than the raw water. Afterwards, microbial community in the two tanks developed independently and CW tank showed small variation throughout the test. At day19, after the aerators were removed, the largest difference was observed between the two tanks (Fig. 3), indicating the shutdown of the artificial aeration led to change in the microbial community. However, at day26, the microbial communities in the two tanks become similar again. Compared with the ST tank, microbes in the CW tank showed more similarity and both of them developed its own bacterial community, although water flowed from the CW to the ST tank continuously. As for the filter, after the maturation stage, SI was quite different from Day1, and changed noticeably to SF. Interestingly, even though three units of the system presented independent microbial community development, at the end of the test, the microbial structure differences were smaller. The same trend was also observed by Haig et al. [20] who treated a water by different sand filters and found that the differences of microbial community composition between filters increased first and gradually decreased..

The sudden decrease of DO in the CW tank after aeration shutdown might have changed the biotope of the system [45,49], thus potentially influencing the subsequent

ST and GAC sandwich filter units. NMDS analysis showed that the microbial community structure in the CW tank changed less than in the ST tank (Fig.3). However, phylum composition in the ST tank changed less than in the CW tank (Fig.2). Besides, although the microbial diversity (e.g. Shannon index) dropped sharply, the CW tank kept higher microbial richness (e.g. Chao1 index) than that in the ST tank (Table S5). As discussed above, at day 26, OTUs relative abundance of the two tanks became similar again after showing difference at day 19. This phenomenon shows the stability of the combined system, in accordance with the former finding that the system was stable in removing target PPCPs. Albeit changes of DO concentration can affect activities of microorganisms, CW plant roots enhance the oxygen condition [55], helping maintaining aerobic bacterial community. The stability of a CW system facing various condition changes were also observed [56,57]. In the current system, although three process units were serially connected and water flowed from the CW tank to the GAC sandwich filter, each part gradually formed different dynamically stable microbial community.

### **3.4 Dynamic OTUs changes of *Proteobacteria***

Proteobacterial microorganisms were commonly found in CW and sand filtration systems [50,58–60] and considered playing important roles in the biodegradation or biotransformation of organic compounds in surface water CW systems or filtration systems [59,61]. Therefore, *Proteobacteria* might have helped to biodegrade the target PPCP compounds and some microorganisms e.g. *Novosphingobium* and *Hydrogenophaga*, which are considered to have ability of degrading environmental organic pollutants [62], have been found in this study (Table S6). Hence, temporal variations in OTU numbers (richness) from *Proteobacteria* in



different units were further investigated. Fig.4 shows the temporal dynamic OTUs changes and occurrence of new OTUs in each unit. More than half of OTUs disappeared from Day1 to Day5 in both CW and ST tanks. Then OTUs disappeared and reappeared in the following sampling days. In addition, new OTUs also appeared in each week, but the proportion of new-coming OTUs gradually reduced and more than half of new OTUs were found in the following sampling day, showing new OTUs kept on adapting to the system. As the system was fed with natural water continuously, this phenomenon indicates the microbial community in the two tanks were in status of temporal dynamic changes [63], and this change can be attributed to different operating conditions in laboratory, competitions among microbes [64], and sensitivity to spiked PPCP compounds [37,52]. In addition, there were less OTUs in the ST tank than in the CW tank during the whole experiment period (Fig.4). The aquatic plants can provide an adherent substrate and habitat for microorganisms [65]. Some bacteria have mutualistic interactions with plants (e.g. providing nitrogen and phosphorus in exchange of carbon) [66]. Hence, the absence of plants in the ST tank may have led to a decrease in microbial richness (Table S5) when water flew into.

Compared to the Day1, the number of new OTUs accounted for nearly half of the detected *Proteobacteria* in the filter. It is worth noting that, although the relative abundance of *Proteobacteria* decreased in the GAC sandwich filter (Fig.2), the number of OTUs increased at the end of the test, demonstrating a more diverse microbial biotope.

### 3.5 Correlations among different microbial phyla

Spearman correlation analysis of relative abundance was conducted among 19 phyla, OTUs of which were detected in all samples. The results are shown in Fig.5.

Various statistically significant correlations have been found among tested phyla. The most significant positive and negative correlations were between *Planctomycetes* and *Chloroflexi* ( $R=0.92$ ,  $p<0.001$ , FDR corrected, similarly hereinafter) and between *Verrucomicrobia/Bacteroidetes* and *Proteobacteria* ( $R=-0.73$ ,  $p<0.001$ ), respectively. As *Bacteroidetes* and *Proteobacteria* were two top dominant phyla in the present study, a competitive relationship between them may exist, since DO concentration indicated aerobic environment in present study and microbes in both two phyla can aerobically biodegrade organic matters [67], probably due to the competition of nutrients [68] and/or different toxicity tolerance to PPCPs [52], which can be further studied. In addition, other correlations were found among 7 dominant phyla. *Proteobacteria* was found to be negatively correlated with *Patescibacteria* and *Cyanobacteria* ( $p<0.01$ ). Positive correlation between *Patescibacteria* and *Verrucomicrobia* ( $p<0.001$ ) was also observed. Interestingly, some phyla (e.g. *Acidobacteria*, *Gemmatimonadetes*) negatively correlated with *Cyanobacteria* ( $p<0.01$ ), but positively correlated with *Planctomycetes* and *Chloroflexi* ( $p<0.01$ ), showing a possible direct or indirect microbial cooperation [68,69]. Other noteworthy positive correlations ( $p<0.01$ ) mainly occurred between *Acidobacteria/Armatimonadetes/Hydrogenedentes* and other phyla. In comparison, fewer significant negative correlations ( $p<0.05$ ) were found, the majority of which lay between *Proteobacteria/Cyanobacteria* with others (Fig. 5).

In the present study, microbial community structure in the initial influent changed considerably when system began to operate, one reason of which can be ascribed to the influence of spiked PPCPs [52,70]. Table S6 shows that classes of *Gammaproteobacteria* and *Alphaproteobacteria* (*Proteobacteria*) existed in top 10 most abundant OTUs of the CW and ST tanks all the time. In the later part of the

experiment, *Bacteroidia* from *Bacteroidetes* also thrived in the system. These three types of microorganisms may have potential ability to degrade the target PPCP compounds. However, as the concentration of target PPCP compounds were only determined in the effluents, it is difficult to state the real connections between them and community variations. Hence, this aspect can be investigated in the future. Besides, (shotgun) metagenomic sequencing will also shed more light on the dominant genes from specific groups of bacteria involved in PPCP degradation.

#### 4. Conclusions

In the present study, Greater duckweed constructed wetland, stabilization tank and GAC sandwich slow sand filtration system was connected in series and tested to remove DEET, PAR, CAF and TCS at spiked 25 µg/L from natural water at lab-scale. The microbial community composition was comprehensively studied in different units of the system during the treatment process. Main conclusions are:

- DEET, PAR, CAF and TCS were removed at 95.9%, 99.1%, 98.1% and 97.4% on average, respectively. After aerators were stopped, no significant PPCP removal difference compared to with aeration was observed ( $p>0.05$ ), except for DEET, showing good stability of the test system in removing these compounds.
- The tested system achieved good treatment of nitrogen and phosphate. No nitrite, nitrate, phosphate and ammonium were detected at end of the experiment.
- Diversity of microbial community in water decreased when system began to operate, potentially because of influence of spiked PPCP compounds. *Proteobacteria* was the most dominant phylum among 47 phyla detected.

Bacteria attached to the plants influenced more to the microbial composition in the CW and ST tanks than that in the initial inlet water.

- Although water flowed into the combined system successively, microbial community structure in three units of the tested system displayed difference and system showed stability when aerators were removed. After aeration stopped, microbial community structure in CW tank changed less and maintained a higher richness but diversity dropped sharper than ST tank. At the end of the test, the structural differences among three units decreased.
- Temporal changes in the number of different OTUs of the predominant phylum *Proteobacteria* in the three process units presented a dynamic development. Various correlations were found between different phyla. *Bacteroidetes* and *Proteobacteria* had a significant Spearman negative correlation ( $R=-0.73$ ,  $p<0.001$ , FDR corrected), probably due to microbial competition.

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## References

- 499 [1] E. Archer, B. Petrie, B. Kasprzyk-Hordern, G.M. Wolfaardt, The fate of  
500 pharmaceuticals and personal care products (PPCPs), endocrine disrupting  
501 contaminants (EDCs), metabolites and illicit drugs in a WWTW and  
502 environmental waters, *Chemosphere*. 174 (2017) 437–446.
- 503 [2] A.J. Ebele, M.A.-E. Abdallah, S. Harrad, Pharmaceuticals and personal care  
504 products (PPCPs) in the freshwater aquatic environment, *Emerg. Contam.*  
505 (2017).
- 506 [3] S. Zhu, H. Chen, J. Li, Sources, distribution and potential risks of  
507 pharmaceuticals and personal care products in Qingshan Lake basin, Eastern  
508 China, *Ecotoxicol. Environ. Saf.* 96 (2013) 154–159.  
509 doi:10.1016/j.ecoenv.2013.06.033.
- 510 [4] B.J. Richardson, P.K. Lam, M. Martin, Emerging chemicals of concern:  
511 pharmaceuticals and personal care products (PPCPs) in Asia, with particular  
512 reference to Southern China, *Mar Pollut Bull.* 50 (2005) 913–920.  
513 doi:10.1016/j.marpolbul.2005.06.034.
- 514 [5] J. Li, W. Cheng, L. Xu, P.J. Strong, H. Chen, Antibiotic-resistant genes and  
515 antibiotic-resistant bacteria in the effluent of urban residential areas, hospitals,  
516 and a municipal wastewater treatment plant system, *Environ. Sci. Pollut. Res.*  
517 22 (2015) 4587–4596. doi:10.1007/s11356-014-3665-2.
- 518 [6] H. Chen, X. Li, S. Zhu, Occurrence and distribution of selected  
519 pharmaceuticals and personal care products in aquatic environments: A  
520 comparative study of regions in China with different urbanization levels,  
521 *Environ. Sci. Pollut. Res.* 19 (2012) 2381–2389. doi:10.1007/s11356-012-  
522 0750-2.

- 523 [7] M. Carballa, F. Omil, J.M. Lema, M. Llompарт, C. Garcia-Jares, I. Rodriguez,  
524 M. Gomez, T. Ternes, Behavior of pharmaceuticals, cosmetics and hormones in  
525 a sewage treatment plant, *Water Res.* 38 (2004) 2918–2926.  
526 doi:10.1016/j.watres.2004.03.029.
- 527 [8] Q. Sui, J. Huang, S. Deng, W. Chen, G. Yu, Seasonal variation in the  
528 occurrence and removal of pharmaceuticals and personal care products in  
529 different biological wastewater treatment processes, *Environ. Sci. Technol.* 45  
530 (2011) 3341–3348. doi:10.1021/es200248d.
- 531 [9] I. Kim, N. Yamashita, H. Tanaka, Chemosphere Photodegradation of  
532 pharmaceuticals and personal care products during UV and UV / H<sub>2</sub>O<sub>2</sub>  
533 treatments, *Chemosphere.* 77 (2009) 518–525.  
534 doi:10.1016/j.chemosphere.2009.07.041.
- 535 [10] S. Zheng, C. Cui, Q. Liang, X. Xia, F. Yang, Ozonation performance of  
536 WWTP secondary effluent of antibiotic manufacturing wastewater,  
537 *Chemosphere.* 81 (2010) 1159–1163. doi:10.1016/j.chemosphere.2010.08.058.
- 538 [11] J. Zhai, H.W. Xiao, K. Kujawa-Roeleveld, Q. He, S.M. Kerstens, Experimental  
539 study of a novel hybrid constructed wetland for water reuse and its application  
540 in Southern China, *Water Sci Technol.* 64 (2011) 2177–2184.  
541 doi:10.2166/wst.2011.790.
- 542 [12] S.A. Baig, Q. Mahmood, B. Nawab, M.N. Shafqat, A. Pervez, Improvement of  
543 drinking water quality by using plant biomass through household biosand filter  
544 – A decentralized approach, *Ecol. Eng.* 37 (2011) 1842–1848.  
545 doi:10.1016/j.ecoleng.2011.06.011.
- 546 [13] V. Matamoros, C. Arias, H. Brix, J.M. Bayona, Removal of pharmaceuticals  
547 and personal care products (PPCPs) from urban wastewater in a pilot vertical

548 flow constructed wetland and a sand filter, *Environ. Sci. Technol.* 41 (2007)  
549 8171–8177. doi:10.1021/es071594+.

550 [14] C. Reyes-Contreras, M. Hijosa-Valsero, R. Sidrach-Cardona, J.M. Bayona, E.  
551 Bécares, Temporal evolution in PPCP removal from urban wastewater by  
552 constructed wetlands of different configuration: A medium-term study,  
553 *Chemosphere*. 88 (2012) 161–167. doi:10.1016/j.chemosphere.2012.02.064.

554 [15] L. Zhang, T. Lv, Y. Zhang, O.R. Stein, C.A. Arias, H. Brix, P.N. Carvalho,  
555 Effects of constructed wetland design on ibuprofen removal—A mesocosm scale  
556 study, *Sci. Total Environ.* 609 (2017) 38–45.

557 [16] D. Zhang, R.M. Gersberg, W.J. Ng, S.K. Tan, Removal of pharmaceuticals and  
558 personal care products in aquatic plant-based systems: A review, *Environ.*  
559 *Pollut.* 184 (2014) 620–639. doi:10.1016/j.envpol.2013.09.009.

560 [17] M. Breitholtz, M. Näslund, D. Stråe, H. Borg, R. Grabic, J. Fick, An evaluation  
561 of free water surface wetlands as tertiary sewage water treatment of micro-  
562 pollutants, *Ecotoxicol. Environ. Saf.* 78 (2012) 63–71.  
563 doi:10.1016/j.ecoenv.2011.11.014.

564 [18] S. Toet, R.S.P. Van Logtestijn, M. Schreijer, R. Kampfb, J.T.A. Verhoeven,  
565 The functioning of a wetland system used for polishing effluent from a  
566 sewage treatment plant, *Ecol. Eng.* 25 (2005) 101–124.

567 [19] M. Martín, N. Oliver, C. Hernández-Crespo, S. Gargallo, M.C. Regidor, The  
568 use of free water surface constructed wetland to treat the eutrophicated waters  
569 of lake L'Albufera de Valencia (Spain), *Ecol. Eng.* 50 (2013) 52–61.

570 [20] S.-J. Haig, C. Quince, R.L. Davies, C.C. Dorea, G. Collins, Replicating the  
571 microbial community and water quality performance of full-scale slow sand

572 filters in laboratory-scale filters, *Water Res.* 61 (2014) 141–151.  
 573 doi:10.1016/j.watres.2014.05.008.

574 [21] N. Nakada, H. Shinohara, A. Murata, K. Kiri, S. Managaki, N. Sato, H. Takada,  
 575 Removal of selected pharmaceuticals and personal care products (PPCPs) and  
 576 endocrine-disrupting chemicals (EDCs) during sand filtration and ozonation at  
 577 a municipal sewage treatment plant, *Water Res.* 41 (2007) 4373–4382.  
 578 doi:10.1016/j.watres.2007.06.038.

579 [22] C.M.E. Pompei, L. Ciric, M. Canales, K. Karu, E.M. Vieira, L.C. Campos,  
 580 Influence of PPCPs on the performance of intermittently operated slow sand  
 581 filters for household water purification, *Sci. Total Environ.* (2016).

582 [23] T.J. Kennedy, T. a Anderson, E.A. Hernandez, A.N. Morse, Assessing an  
 583 intermittently operated household scale slow sand filter paired with household  
 584 bleach for the removal of endocrine disrupting compounds., *J. Environ. Sci.*  
 585 *Health. A. Tox. Hazard. Subst. Environ. Eng.* 48 (2013) 753–9.  
 586 doi:10.1080/10934529.2013.744616.

587 [24] J. Altmann, D. Rehfeld, K. Träder, A. Sperlich, M. Jekel, Combination of  
 588 granular activated carbon adsorption and deep-bed filtration as a single  
 589 advanced wastewater treatment step for organic micropollutant and phosphorus  
 590 removal, *Water Res.* 92 (2016) 131–139.

591 [25] J. Li, Q. Zhou, L.C. Campos, The application of GAC sandwich slow sand  
 592 filtration to remove pharmaceutical and personal care products, *Sci. Total*  
 593 *Environ.* 635 (2018) 1182–1190.

594 [26] K. Gunes, B. Tuncsiper, A serially connected sand filtration and constructed  
 595 wetland system for small community wastewater treatment, *Ecol. Eng.* 35  
 596 (2009) 1208–1215.



- 597 [27] S.J. Haig, G. Collins, R.L.D.C.C. Dorea, C. Quince, Biological Aspects of  
598 Slow Sand Filtration : Past , Present and Future, Water Sci. Technol. Water  
599 Supply. 11 (2011) 468–472. doi:10.2166/ws.2011.076.
- 600 [28] L.C. Campos, M.F.J. Su, N.J.D. Graham, S.R. Smith, Biomass development in  
601 slow sand filters, Water Res. 36 (2002) 4543–4551. doi:10.1016/S0043-  
602 1354(02)00167-7.
- 603 [29] J. Li, Q. Zhou, L.C. Campos, Removal of selected emerging PPCP compounds  
604 using greater duckweed ( *Spirodela polyrhiza* ) based lab-scale free water  
605 constructed wetland, Water Res. 126 (2017) 252–261.  
606 doi:10.1016/j.watres.2017.09.002.
- 607 [30] X. Huang, J. Zheng, C. Liu, L. Liu, Y. Liu, H. Fan, T. Zhang, Performance and  
608 bacterial community dynamics of vertical flow constructed wetlands during the  
609 treatment of antibiotics-enriched swine wastewater, Chem. Eng. J. 316 (2017)  
610 727–735.
- 611 [31] R.J. Olson, S.K. Holladay, R.B. Cook, E. Falge, D. Baldocchi, L. Gu,  
612 FLUXNET: Database of fluxes, site characteristics, and flux-community  
613 information, Ornl/Tm-2003/204. (2004). doi:10.1073/pnas.1000080107/-  
614 /DCSupplemental.www.pnas.org/cgi/doi/10.1073/pnas.1000080107.
- 615 [32] T. Inaba, T. Hori, R.R. Navarro, A. Ogata, D. Hanajima, H. Habe, Revealing  
616 sludge and biofilm microbiomes in membrane bioreactor treating piggery  
617 wastewater by non-destructive microscopy and 16S rRNA gene sequencing,  
618 Chem. Eng. J. 331 (2018) 75–83. doi:10.1016/j.cej.2017.08.095.
- 619 [33] F. Guo, T. Zhang, Profiling bulking and foaming bacteria in activated sludge  
620 by high throughput sequencing, Water Res. 46 (2012) 2772–2782.  
621 doi:10.1016/j.watres.2012.02.039.

- 622 [34] J.E. Koopman, M.J. Buijs, B.W. Brandt, B.J.F. Keijser, W. Crielaard, E. Zaura,  
623 Nitrate and the origin of saliva influence composition and short chain fatty acid  
624 production of oral microcosms, *Microb. Ecol.* 72 (2016) 479–492.
- 625 [35] J.R. Bray, J.T. Curtis, An ordination of the upland forest communities of  
626 southern Wisconsin, *Ecol. Monogr.* 27 (1957) 325–349.
- 627 [36] R. Menon, C.R. Jackson, M.M. Holland, The influence of vegetation on  
628 microbial enzyme activity and bacterial community structure in freshwater  
629 constructed wetland sediments, *Wetlands.* 33 (2013) 365–378.
- 630 [37] B. Drury, J. Scott, E.J. Rosi-Marshall, J.J. Kelly, Triclosan exposure increases  
631 triclosan resistance and influences taxonomic composition of benthic bacterial  
632 communities, *Env. Sci Technol.* 47 (2013) 8923–8930. doi:10.1021/es401919k.
- 633 [38] J.A. Pickett, M.A. Birkett, J.G. Logan, DEET repels ORNery mosquitoes, *Proc.*  
634 *Natl. Acad. Sci.* 105 (2008) 13195–13196.
- 635 [39] V.M. Monsalvo, J.A. McDonald, S.J. Khan, P. Le-Clech, Removal of trace  
636 organics by anaerobic membrane bioreactors, *Water Res.* 49 (2014) 103–112.
- 637 [40] S.D. Kim, J. Cho, I.S. Kim, B.J. Vanderford, S.A. Snyder, Occurrence and  
638 removal of pharmaceuticals and endocrine disruptors in South Korean surface,  
639 drinking, and waste waters, *Water Res.* 41 (2007) 1013–1021.
- 640 [41] Y. Matsui, T. Yoshida, S. Nakao, D.R.U. Knappe, T. Matsushita,  
641 Characteristics of competitive adsorption between 2-methylisoborneol and  
642 natural organic matter on superfine and conventionally sized powdered  
643 activated carbons, *Water Res.* 46 (2012) 4741–4749.
- 644 [42] D.J. De Ridder, A.R.D. Verliefde, S.G.J. Heijman, J. Verberk, L.C. Rietveld,  
645 L.T.J. Van Der Aa, G.L. Amy, J.C. Van Dijk, Influence of natural organic

646 matter on equilibrium adsorption of neutral and charged pharmaceuticals onto  
 647 activated carbon, *Water Sci. Technol.* 63 (2011) 416–423.

648 [43] G. Zeng, H. Fu, H. Zhong, X. Yuan, M. Fu, W. Wang, G. Huang, Co-  
 649 degradation with glucose of four surfactants, CTAB, Triton X-100, SDS and  
 650 Rhamnolipid, in liquid culture media and compost matrix, *Biodegradation*. 18  
 651 (2007) 303–310.

652 [44] A. Caselles-Osorio, J. García, Impact of different feeding strategies and plant  
 653 presence on the performance of shallow horizontal subsurface-flow constructed  
 654 wetlands, *Sci. Total Environ.* 378 (2007) 253–262.  
 655 doi:10.1016/j.scitotenv.2007.02.031.

656 [45] Y. Li, G. Zhu, W.J. Ng, S.K. Tan, A review on removing pharmaceutical  
 657 contaminants from wastewater by constructed wetlands: Design, performance  
 658 and mechanism, *Sci. Total Environ.* 468–469 (2014) 908–932.  
 659 doi:10.1016/j.scitotenv.2013.09.018.

660 [46] P. Verlicchi, E. Zambello, How efficient are constructed wetlands in removing  
 661 pharmaceuticals from untreated and treated urban wastewaters? A review, *Sci.*  
 662 *Total Environ.* 470–471 (2014) 1281–1306.  
 663 doi:10.1016/j.scitotenv.2013.10.085.

664 [47] S. Zhu, H. Chen, The fate and risk of selected pharmaceutical and personal care  
 665 products in wastewater treatment plants and a pilot-scale multistage constructed  
 666 wetland system, *Environ. Sci. Pollut. Res.* 21 (2014) 1466–1479.  
 667 doi:10.1007/s11356-013-2025-y.

668 [48] C. Ávila, C. Pelissari, P.H. Sezerino, M. Sgroi, P. Roccaro, J. García,  
 669 Enhancement of total nitrogen removal through effluent recirculation and fate

670 of PPCPs in a hybrid constructed wetland system treating urban wastewater,  
 671 Sci. Total Environ. 584 (2017) 414–425.

672 [49] T.C. Yadav, A.A. Khardenavis, A. Kapley, Shifts in microbial community in  
 673 response to dissolved oxygen levels in activated sludge, Bioresour. Technol.  
 674 165 (2014) 257–264.

675 [50] W. Guan, M. Yin, T. He, S. Xie, Influence of substrate type on microbial  
 676 community structure in vertical-flow constructed wetland treating polluted  
 677 river water, Environ. Sci. Pollut. Res. 22 (2015) 16202–16209.

678 [51] Q. Wang, H. Xie, H.H. Ngo, W. Guo, J. Zhang, C. Liu, S. Liang, Z. Hu, Z.  
 679 Yang, C. Zhao, Microbial abundance and community in subsurface flow  
 680 constructed wetland microcosms: role of plant presence, Environ. Sci. Pollut.  
 681 Res. 23 (2016) 4036–4045.

682 [52] C. Zhao, H. Xie, J. Xu, X. Xu, J. Zhang, Z. Hu, C. Liu, S. Liang, Q. Wang, J.  
 683 Wang, Bacterial community variation and microbial mechanism of triclosan (  
 684 TCS ) removal by constructed wetlands with different types of plants, Sci.  
 685 Total Environ. 505 (2015) 633–639. doi:10.1016/j.scitotenv.2014.10.053.

686 [53] E.J. Joner, A. Johansen, A.P. Loibner, M.A. dela Cruz, O.H.J. Szolar, J.-M.  
 687 Portal, C. Leyval, Rhizosphere effects on microbial community structure and  
 688 dissipation and toxicity of polycyclic aromatic hydrocarbons (PAHs) in spiked  
 689 soil, Environ. Sci. Technol. 35 (2001) 2773–2777.

690 [54] D.Q. Zhang, R.M. Gersberg, T. Hua, J. Zhu, N.A. Tuan, S.K. Tan,  
 691 Pharmaceutical removal in tropical subsurface flow constructed wetlands at  
 692 varying hydraulic loading rates, Chemosphere. 87 (2012) 273–277.  
 693 doi:10.1016/j.chemosphere.2011.12.067.

- 694 [55] C. Sundberg, K. Tonderski, P.-E. Lindgren, Potential nitrification and  
695 denitrification and the corresponding composition of the bacterial communities  
696 in a compact constructed wetland treating landfill leachates, *Water Sci.*  
697 *Technol.* 56 (2007) 159–166.
- 698 [56] M. Truu, J. Juhanson, J. Truu, Microbial biomass, activity and community  
699 composition in constructed wetlands, *Sci. Total Environ.* 407 (2009) 3958–  
700 3971.
- 701 [57] K.P. Weber, M. Gehder, R.L. Legge, Assessment of changes in the microbial  
702 community of constructed wetland mesocosms in response to acid mine  
703 drainage exposure, *Water Res.* 42 (2008) 180–188.
- 704 [58] W. Zhi, G. Ji, Constructed wetlands, 1991–2011: a review of research  
705 development, current trends, and future directions, *Sci. Total Environ.* 441  
706 (2012) 19–27.
- 707 [59] T. He, W. Guan, Z. Luan, S. Xie, Spatiotemporal variation of bacterial and  
708 archaeal communities in a pilot-scale constructed wetland for surface water  
709 treatment, *Appl. Microbiol. Biotechnol.* 100 (2016) 1479–1488.
- 710 [60] S.-J. Haig, C. Quince, R.L. Davies, C.C. Dorea, G. Collins, The relationship  
711 between microbial community evenness and function in slow sand filters,  
712 *MBio.* 6 (2015) e00729-15.
- 713 [61] X. Liao, C. Chen, J. Zhang, Y. Dai, X. Zhang, S. Xie, Operational  
714 performance, biomass and microbial community structure: impacts of  
715 backwashing on drinking water biofilter, *Environ. Sci. Pollut. Res.* 22 (2015)  
716 546–554.
- 717 [62] W. Cheng, J. Zhang, Z. Wang, M. Wang, S. Xie, Bacterial communities in  
718 sediments of a drinking water reservoir, *Ann. Microbiol.* 64 (2014) 875–878.

- 719 [63] S.A. Smits, J. Leach, E.D. Sonnenburg, C.G. Gonzalez, J.S. Lichtman, G. Reid,  
720 R. Knight, A. Manjurano, J. Chagalucha, J.E. Elias, Seasonal cycling in the  
721 gut microbiome of the Hadza hunter-gatherers of Tanzania, *Science* (80-. ). 357  
722 (2017) 802–806.
- 723 [64] S.D. Bridgman, H. Cadillo - Quiroz, J.K. Keller, Q. Zhuang, Methane  
724 emissions from wetlands: biogeochemical, microbial, and modeling  
725 perspectives from local to global scales, *Glob. Chang. Biol.* 19 (2013) 1325–  
726 1346.
- 727 [65] C. Wang, S.S. Zheng, P.F. Wang, J. Qian, Effects of vegetations on the  
728 removal of contaminants in aquatic environments: A review, *J. Hydrodyn.* 26  
729 (2014) 497–511. doi:10.1016/S1001-6058(14)60057-3.
- 730 [66] L. Philippot, J.M. Raaijmakers, P. Lemanceau, W.H. Van Der Putten, Going  
731 back to the roots: the microbial ecology of the rhizosphere, *Nat. Rev.*  
732 *Microbiol.* 11 (2013) 789.
- 733 [67] G. Fuchs, M. Boll, J. Heider, Microbial degradation of aromatic compounds—  
734 from one strategy to four, *Nat. Rev. Microbiol.* 9 (2011) 803.
- 735 [68] K.Z. Coyte, H. Tabuteau, E.A. Gaffney, K.R. Foster, W.M. Durham, Microbial  
736 competition in porous environments can select against rapid biofilm growth,  
737 *Proc. Natl. Acad. Sci.* 114 (2017) E161–E170.
- 738 [69] A. Sauvetre, P. Schroder, Uptake of carbamazepine by rhizomes and  
739 endophytic bacteria of *Phragmites australis*, *Front Plant Sci.* 6 (2015) 83.  
740 doi:10.3389/fpls.2015.00083.
- 741 [70] Z. Xia, W. Xiao-chun, C. Zhong-lin, X. Hao, Z. Qing-fang, Microbial  
742 community structure and pharmaceuticals and personal care products removal

743 in a membrane bioreactor seeded with aerobic granular sludge, Appl.  
744 Microbiol. Biotechnol. 99 (2015) 425–433.  
745

Table caption

Table 1 Concentrations and removals of the target PPCP compounds in the untreated and treated water



Table 1 Concentrations and removals of the target PPCP compounds in the untreated and treated water

Day	DEET		PAR		CAF		TCS	
	Concentration (µg/L)	Removal (%)	Concentration (µg/L)	Removal (%)	Concentration (µg/L)	Removal (%)	Concentration (µg/L)	Removal (%)
Untreated water	0.88±0.32	n.a.**	0.26±0.21	n.a.	0.72±0.37	n.a.	3.54±1.84	n.a
1*	0.97±0.00	96.3	0.26±0.01	99.0	0.66±0.04	97.4	1.63±0.03	93.5
3	1.05±0.03	96.0	0.03±0.05	99.9	0.51±0.01	97.9	0.56±0.31	97.8
5	0.86±0.01	96.7	0.01±0.01	99.9	0.58±0.06	97.7	0.65±0.06	97.4
8	1.05±0.02	95.9	1.08±0.11	95.7	0.54±0.09	97.8	0.35±0.01	98.6
10	1.03±0.02	96.0	0.43±0.05	98.3	0.67±0.09	97.3	0.34±0.01	98.6
12	1.05±0.06	95.9	n.d.***	100.0	0.54±0.01	97.9	0.42±0.10	98.5
15	1.05±0.06	95.9	n.d.	100.0	n.d.	100.0	0.29±0.02	99.0
17	1.16±0.09	95.5	n.d.	100.0	0.55±0.02	97.8	1.75±0.04	93.9
19	1.05±0.09	96.0	n.d.	100.0	0.56±0.03	97.8	0.60±0.02	97.9
22	1.22±0.06	95.3	0.75±0.16	97.0	n.d.	100.0	0.14±0.00	99.5
24	1.10±0.02	95.7	0.14±0.01	99.5	0.57±0.01	97.7	0.25±0.02	99.1
26	1.13±0.00	95.6	0.11±0.02	99.6	0.55±0.01	97.8	1.25±0.03	95.6
Average	1.06±0.04	95.9	0.23±0.04	99.1	0.48±0.03	98.1	0.69±0.05	97.4

\* From day 1, mixed target PPCPs were added into the untreated water to reach a spiked concentration of 25 µg/L. The initial concentrations of DEET, PAR, CAF and TCS at day 1 in the system were 25.88, 25.21, 25.38 and 25.08 µg/L, respectively.

\*\* n.a. not available

\*\*\* n.d. not detected

Figure captions:

Fig. 1 Schematic representation of the experimental system.

(a. influent tank; b. reflective fabric; c. lights; d. Greater duckweed; e. aerator; f. peristaltic pump; g. effluent tank; h. wetland tank; i. stabilization tank; j. GAC sandwich slow sand filter; k. overflow pipe)

Fig. 2 Relative abundance of 7 predominant phyla and heatmap of all detected 47 phyla of microbial samples.

(2-a. Proteobacteria were classified into classes of alpha-, gamma- and others which accounts for less than 4%; 2-b. 47 detected phyla were scaled in row to compare relative abundance of each phylum. Colour key, relative abundance increases from red to green. Clustering method: average hierarchical clustering)

Fig. 3 Non-metric multidimensional scaling (NMDS) analysis of microbial samples based on the OTU table using Bray-Curtis dissimilarity.

(Scaling method: Wisconsin scaling. Points which are closer in the plot indicate samples with more similar microbial community structure)

Fig. 4 Dynamic changes of the number of *Proteobacteria* OTUs in the CW tank, ST tank and filter.

(Length of nodes and width of flow indicate number of OTUs. Flows indicate the links between two nodes. “New” of each sampling day indicates OTUs not detected in previous samples. Total *Proteobacteria* OTUs number of each sample is displayed below).

Fig. 5 Spearman correlations among selected phyla

(Lower triangle: numbers indicate the R value of the Spearman correlation, darker blue colour indicated stronger positive correlation, and darker red colour indicates stronger negative correlation; Upper triangle: block with slash indicates negative correlation, darker blue colour indicated stronger positive correlation, darker red colour indicates stronger negative correlation, \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , FDR corrected)

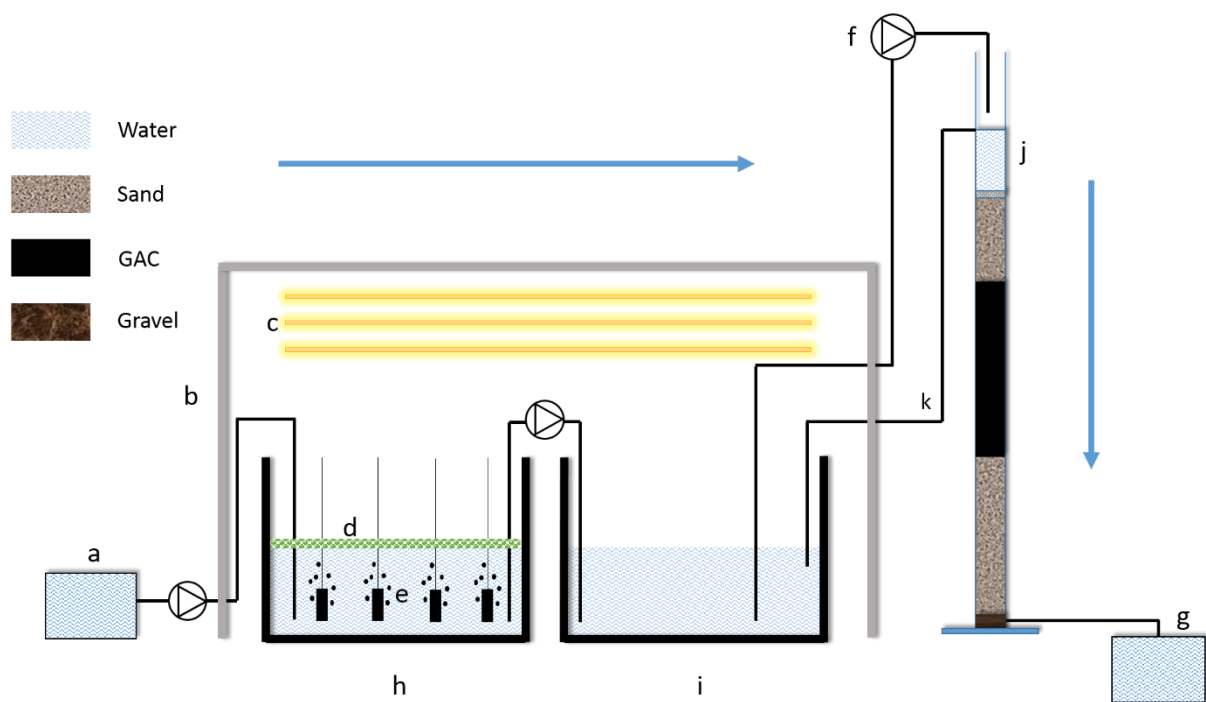


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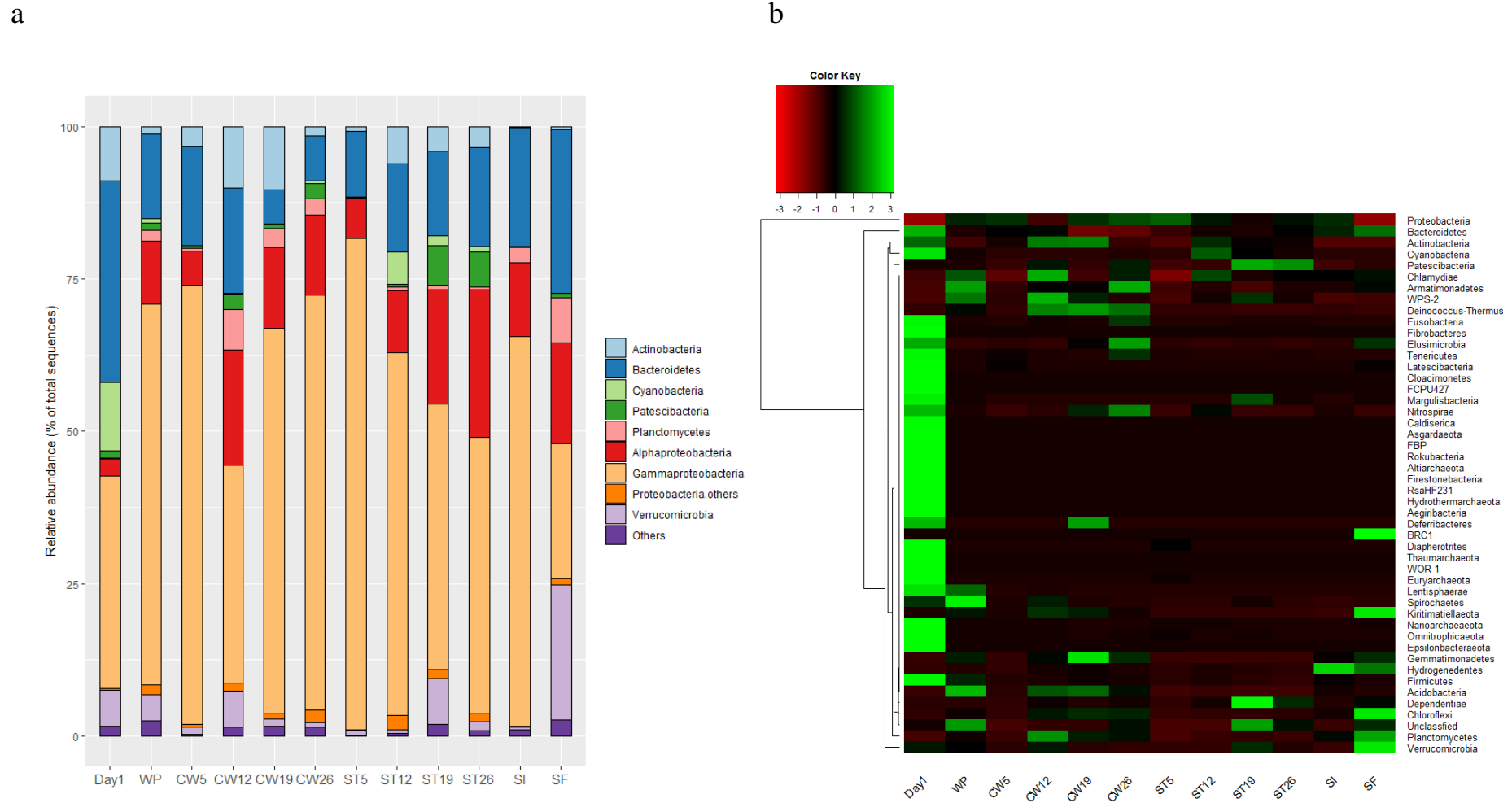


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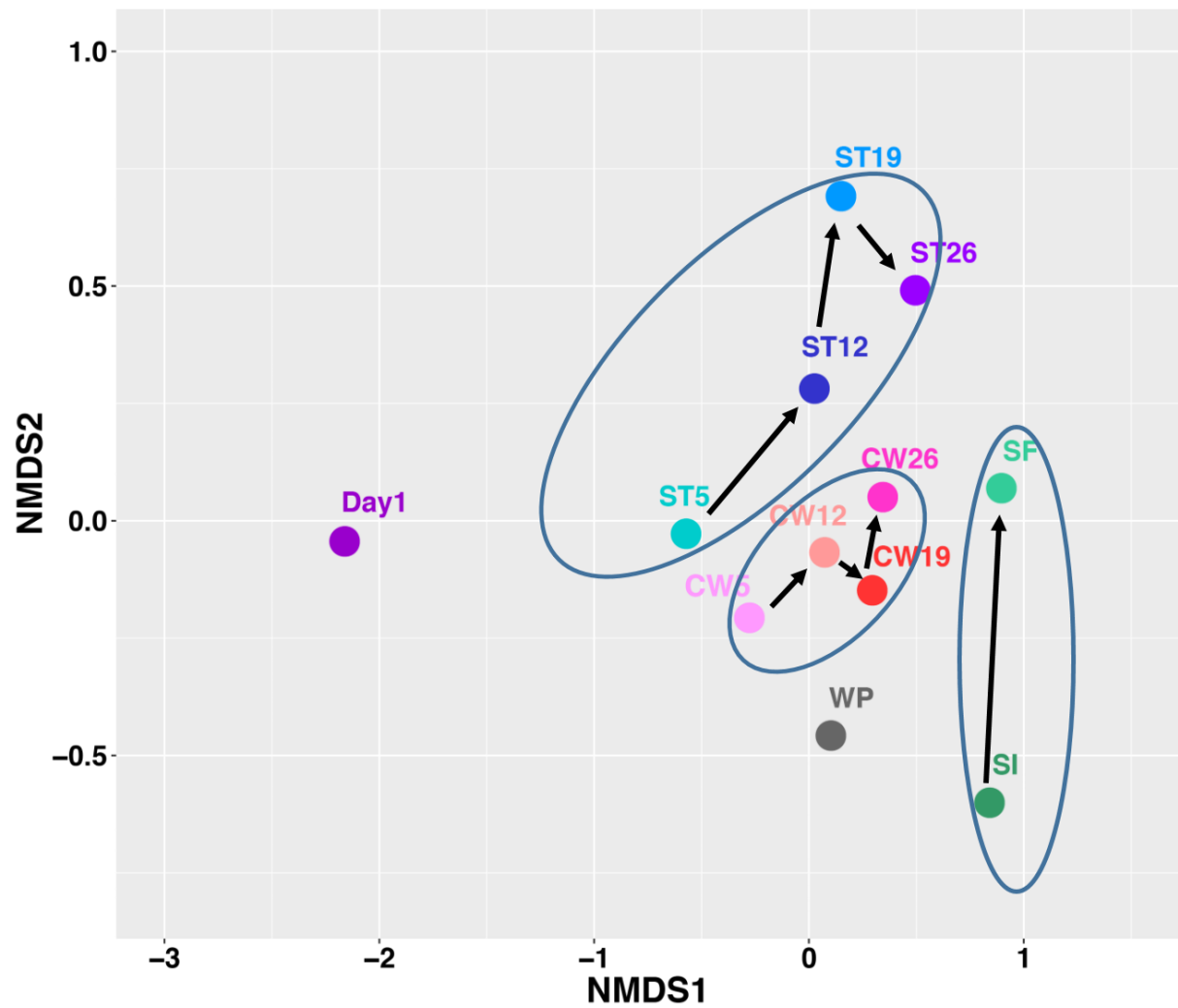


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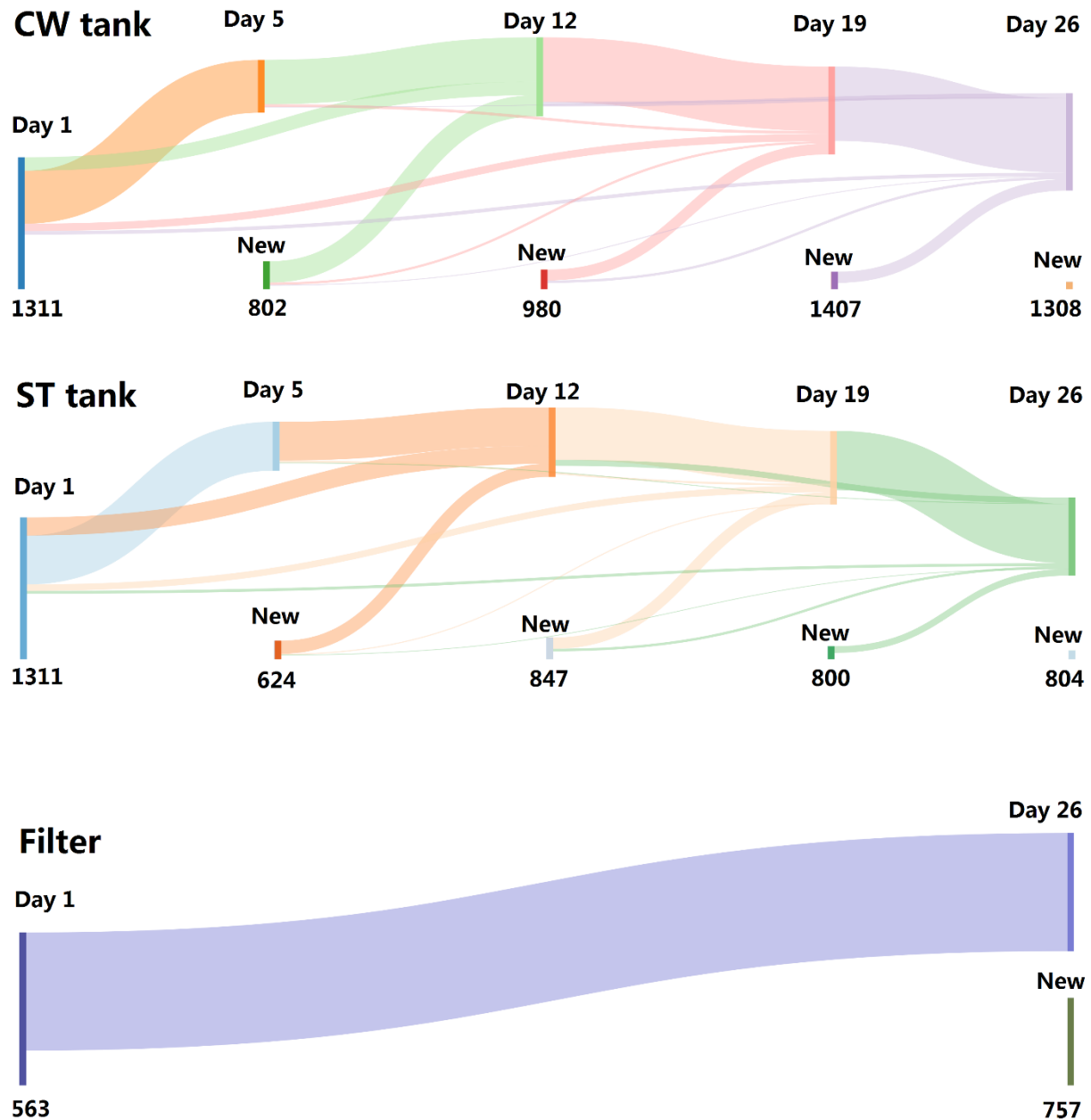


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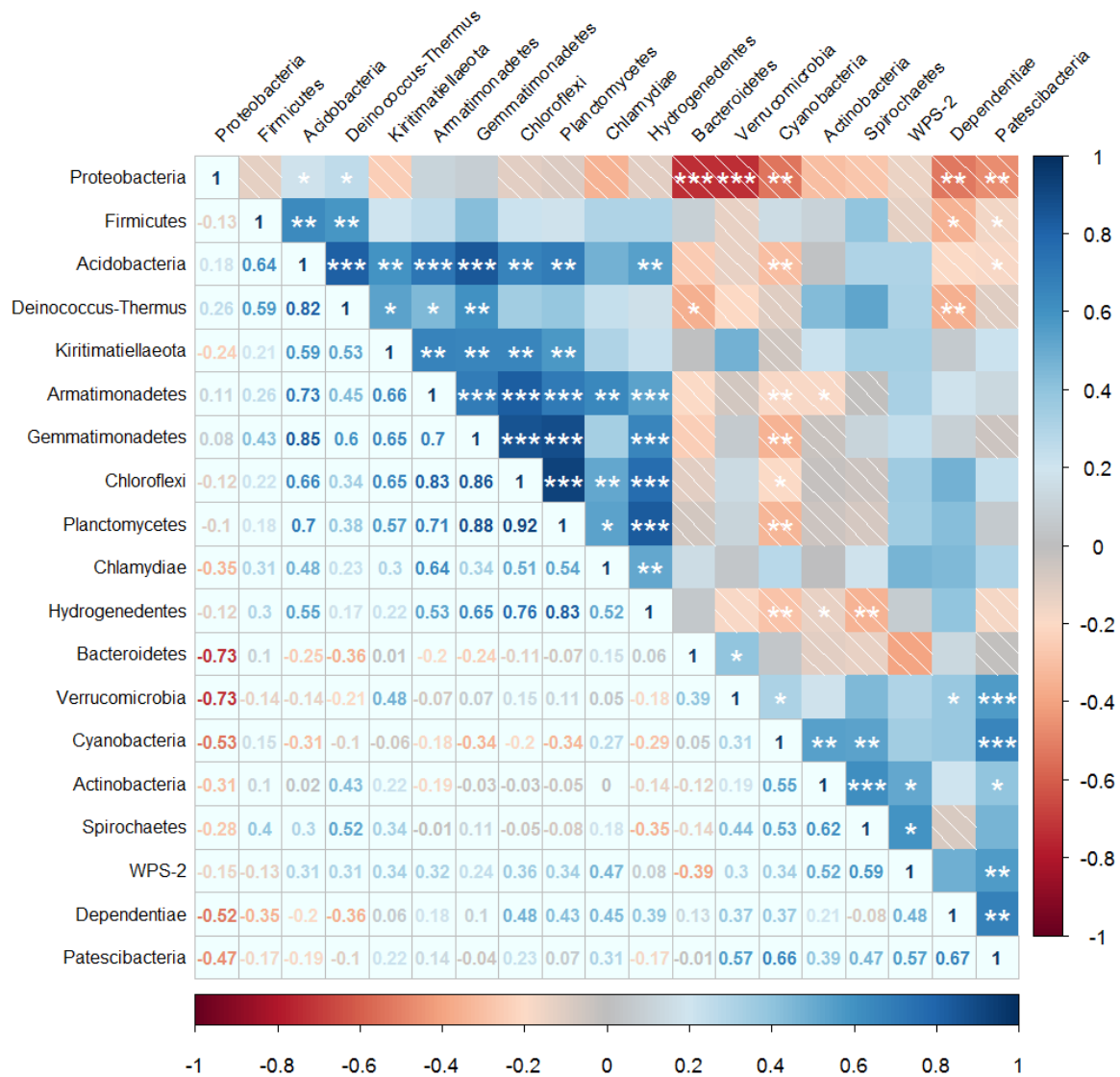


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## Supplementary Material

[Click here to download Supplementary Material: Supplementary data-R1.docx](#)