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Response of prokaryotic, eukaryotic and algal communities to heavy rainfall in a reservoir supplied with reclaimed water

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20 Abstract

The gobal climate change made the heavy rainfall happen more frequent, and the non-point source 21 22 pollution caused by it would exacerbate the risk to the water ecological environment. In this study, we took a reservoir (Shahe reservoir, Beijing, China) supplied with reclaimed water for an exapmle to 23 investigate how spatiotemporal changes in the quantity and diversity of prokaryotic, eukaryotic, and 24 algal communities response to heavy rainfall. Results showed that heavy rainfall could directly impact 25 the composition of the prokaryotic community by introducing amounts of runoff closely associated 26 bacterium especially for the human potential pathogens of Aliarcobacter, Aeromonas, and 27 Pseudomonas in the Shahe reservoir area. While the eukaryotic community was rather stable, and the 28 development and changes of algal community happened not the beginning of the heavy rainfall but 29 after it. The microbial source tracking through FEAST indicated that the Nansha river (S) was the 30 major contributor to the development of all the three concerned communities in the reservoir, where 31 32 they were all strongly and positively connected with Chl-a, pH, turbidity, COD, and TOC, but negatively correlated with NO₃-N (p<0.01). The eukaryote played a key role in the interaction network 33 among the three communities, and were more likely to interact with algae and prokaryotes. It was 34 suggested that the controlling of human potential pathogens associated with prokaryotic community 35 should be emphasized at the beginning of the heavy rainfall, but the prevention of the eutriphication 36 bloom should be another focus after the heavy rainfall. This study provided valuble information 37 concerning the role of heavy rainfall on the water ecological environment from the perspective of 38 microbial community. 39

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41 Keywords: Reservoir; Heavy rainfall; Prokaryotic community; Eukaryotic community; Algal
42 community

43 **1. Introduction**

44

wastewater recycling and reuse has been considered as a feasible and practical solution (Tram VO et 45 al., 2014). It was indicated that the amount and proportion of rivers supplied with reclaimed water 46 increased dramatically in recent years in China (Heng et al., 2021). The function and biological 47 processes of the receiving river are directly affected by the quality of reclaimed water (Chu et al., 48 49 2004), and the reclaimed water with high concentration of nitrogen, phosphorus, and other nutrients 50 would raise the danger of eutrophication and transient oxygen deficiencies (Meng et al., 2011). Shahe Reservoir with a total size of around 1.8 km² is located in Changping District, Beijing, 51 China (Sun et al., 2019). On the upper side, it is connected to the Dongsha River, Beisha River and 52 Nansha River; on the lower side, it is connected to the Wenyu River, which make it an important 53 junction in the North Canal's upper sections. The discharge effluent from the wastewater treatment 54 55 plants accounted for 70%~80% of the total inflow in Shahe Reservoir, which could be considered as a typical water area supplied with reclaimed water (Rong et al., 2021). This increases the danger of 56 eutrophication in Shahe Reservoir, and non-point source pollution produced by heavy rainfall is 57 58 expected to exacerbate the area's eutrophication potential (Zhu et al., 2018). Besides, the global climate change resulted in the changes of the frequency, intensity, duration and variability of extreme (high 59 and low) rainfall events, where the heavy rainfall happened more frequent in China (Liu et al., 2014; 60 Semenza, 2020). Extreme rainfall events could cause disturbances to water bodies by changing the 61 hydrological conditions and influencing the thermal structure (Huang et al., 2014), and large amounts 62 of particulate pollutants carried by runoff are brought into reservoirs, which cause serious exogenous 63 64 pollution. It was reported that when the abrupt disruption of aquatic ecosystem can be evoked by the

The scarcity of water resources is limiting the rapid expansion of China's modern cities, and urban

rapid changes of climate condition (such as heavy rainfall), the metabolism of the microbial community would be thereby altered, especially the overproduction of algae (Zhang et al., 2006). However, in the face of disturbance, the water body microbial community itself has a certain recovery ability to maintain the ecosystem stability. So much attention has been paid to their resilience, which raises intriguing questions about whether these ecosystems can absorb and digest extreme rainfall disturbances, and how long it takes to reach a new steady state.

Previous studies indicated that the recovery of phytoplankton in the water was later than that of 71 physical and chemical indexes in the face of extreme rainfall (Shabarova et al., 2021). According to 72 73 our previous studies, the water quality of Shahe Reservoir was not significant affected after a single heavy rainfall and could be recovered within a week (Xin et al., 2021; Zhu et al., 2018). Physical and 74 chemical indices are often one-sided when compared to microbial community indexes. As a result, it's 75 critical to comprehend the changes in the microbial population in the reservoir area following heavy 76 rainfall, which serves as a valuable reference point for studying water quality changes associate with 77 global climate change. 78

Several researches on the microbial community linked with freshwater habitats have been 79 conducted throughout the last few decades (Eckert et al., 2018). These ecosystems have also been 80 compared to large-scale studies of water microbial communities in urban water supply reservoirs 81 (Ibekwe et al., 2016), polluted urban rivers (García-Armisen et al., 2014), and wastewater treatment 82 systems (Fang et al., 2018). However, The majority of current research often focuses on a single 83 microbial community, such as prokaryotic community, phytoplankton, etc. A single community, on 84 85 the other hand, cannot always properly reflect the changes in an ecosystem. Because there could be interactions between prokaryotes, eukaryotes and algae. Thus, in this study, the Shahe Reservoir, 86

which used reclaimed water as its primary source of influent, is chosen to investigate the 87 spatiotemporal changes in the composition and abundance of the three microbial communities 88 (prokaryotes, eukaryotes, and algae) following a heavy rainfall. The aims of this study are to: 1) clarify 89 the changes of the prokaryotic, eukaryotic, and algal community response to the heavy rainfall in Shahe 90 91 reservoir; 2) identify the major tributrary influencing the development of microbial community in the reservoir during the heavy rainfall; 3) figure out the interactions among the prokaryotic, eukaryotic, 92 and algal community; 4) provide valuble information and references to the impact of the heavy rainfall 93 on the water ecological environment from the perspective of microbial community in the context of 94 95 global climate change.

96 2. Method and methods

97 2.1. Study area and sampling

The Shahe reservoir area belonged to the mild continental monsoon climate (40.114-40.148°N, 98 116.262-116.348°E), the annual average precipitation is 557.4 mm (2020), the annual average 99 100 temperature is 9-19°C, and the rainy season located between June and September each year (Liu et al., 2014). The reservoir has a dam to control the water level and daily flow, and the water level and daily 101 flow is 34.2 m (the lowest level) and 416,000 m³·d⁻¹, respectively, when the study was conducted. 102 Dongsha River (approximately 50,000 m³·d⁻¹), Beisha River (15,000-20,000 m³·d⁻¹), Nansha River 103 (100,000-200,000 m³·d⁻¹) and reclaimed water from Shahe Wastewater Treatment Plant (90,000 m³·d⁻¹) 104 constituted the water sources of the reservoir. The reservoir is designed to be a recreational landscape 105 106 water body, and its water quality should meet the requirement of surface water IV according to the Environmental Quality Standards for Surface Water (GB3838-2002) of China. 107



Figure 1. The map of sampling sites in Shahe Reservoir area, Beijing, China. E: Dongsha River, N: 111 112 Beisha River; S: Nansha River; WE: Effluent from the Shahe wastewater treatment plant; R: Shahe Reservoir, where the dam located. 113

A total of 5 sampling points was set up (Figure 1), covering 3 tributaries, WWTP effluent and 114 Shahe reservoir, and these monitoring locations belonged to Dongsha River (Eastern, E), Beisha River 115 (Northern, N), Nansha River (Sourthern, S), wastewater effluent (WE), and Shahe Reservoir (R), 116 respectively. Beijing experienced its first heavy rainfall of 2019 on July 22nd in the flood season with 117 a rainfall capacity of 51.6 mm·d⁻¹, a high intensity of 12.9 mm·h⁻¹ with short duration (Figure S1). 118 According to the national standard of Precipitation Grade released by China in 2012 (GB/T 28592-119

2012), the rainfall reached the rainstorm level (30 mm ~ 69.9 mm/12 h). The daily effluent of Shahe 120 reservoir reached to maximum of 2.171 million m³, and the water level rose 0.2 m during the heavy 121 rainfall (Table S1). After the heavy rainfall, the surface water samples were collected daily from the 5 122 sites covering the July 23 nd, 2019 to July 28 nd, 2019 (30 samples in total). There was no more 123 precipitation throughout the sampling period. The water samples were collected and stored in 124 compliance with the Manual for Quality Assurance of Environmental Water Quality Monitoring 125 standards (second edition), where an equivalent volume (1.0 L) of surface water was collected below 126 the surface of 5-10 cm as close to the river's midpoint as practicable. The samples were preserved at 127 4°C before being transported to the laboratory within 24 h, where 500 mL of water was filtered through 128 0.22 µm of mixed cellulose ester membranes (47 mm diameter, Millipore, Tianjin Experiment 129 Equipment Co. Ltd, CHN), and the filters were stored at -80 °C until DNA extraction. 130

131 **2.2. Analyses of physico-chemical parameters**

Water temperature (*T*), Dissolved oxygen (DO), pH, Chlorophyll a (Chl-a) and turbidity were measured on site. Total nitrogen (TN), ammonium nitrogen (NH₃-N), nitrate nitrogen (NO₃-N), total phosphorus (TP), phosphate ($PO_4^{3-}P$), chemical oxygen demand (COD), and total organic carbon (TOC) were analyzed according to the national standard methods (GB 17378.4-2007). Rainfall data were recorded by the rain gauge installed on site (model: QS-3000, Qingyi Electronic Technology Co.,

137 Ltd., Handan Development Zone) every 5 mins.

138 2.3. DNA extraction and Illumina sequencing

139 The filters were used to extract the genomic DNA through the FastDNA Spin Kit for Soil 140 Extraction Kit (MP Biomedicals, Inc. USA) according to the manufacturer's instructions. The DNA 141 extracts were checked on 1% agarose gel, and DNA concentration and purity were determined by NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA). DNA samples
were then sent to Majorbio Bio-pharm Technology (Shanghai, China) for quality inspection,
amplification, purification and subsequent sequencing.

The primers of 515F/806R targeting the 16S rRNA of prokaryotes, Euk1391F/EukBrDR target 145 the 18S rRNA of the eukaryotes and p23SrV f1/p23SrV r1 targeting the 23S rRNA of the algae were 146 used for the prokaryotic, eukaryotic and algal community analysis, respectively (Caporaso et al., 2011; 147 Saunders and McDonald, 2010; Sherwood and Presting, 2007). Purified amplicons pooled in 148 equimolar were paired-end sequenced on an Illumina MiSeq PE300 platform/NovaSeq PE250 149 platform (Illumina, San Diego, USA) according to the standard protocols. The raw reads were 150 deposited into the NCBI Sequencing Read Archive (SRA) database with the accession number of 151 PRJNA832875. 152

153 **2.4. Bioinformatics analysis**

An average of 51,572 16S rRNA reads, 123,000 18S rRNA reads, and 123,030 23S rRNA reads 154 were collected for each sample. The QIIME2 pipeline of the version 2021.4 was used for the following 155 bioinformations analysis (Bolyen et al., 2019). Briefly, the raw data were firstly incorporated into the 156 157 QIIME2 using the command associated with paired-ended data, and then DADA2 was used with the default parameters to remove the primers, denoise and join the reads into exact amplicon sequence 158 variants (ASVs) (Callahan et al., 2016). The taxonomy of the ASVs was assigned taxonomy with the 159 feature-classifier plugin in QIIME2 (Bokulich et al., 2018), where the RDP database (Lan et al., 2012), 160 PR2 database (Vaulot et al., 2022) and the µgreen database (Djemiel et al., 2020) were adopted for the 161 prokaryotic, eukaryotic, and algal community analysis, respectively. 162

2.5. Statistical analysis

The principal component analysis (PCA) and Redundancy analysis (RDA) were conducted with 164 165 Canoco (Version 5.0). Analysis of variance (ANOVA) and Pearson correlation were done with SPSS 26.0 (Version 12). The microbial source tracking (MST) was performed using fast expectation-166 maximization microbial source tracking (FEAST) (Shenhav et al., 2019) in R with default parameters 167 to identify the potential contribution of E, N, S and WE to the development of microbial community 168 in reservoir (R). The modules of the microbial community were identified through the co-occurrence 169 network analysis for prokaryotic, eukaryotic, and algal community separately with the spearman's 170 correlation coefficient >0.7 and p<0.001, the abundance differences of the modules were determined, 171 and then, the correlation/regression analysis between the identified modules and concerned physico-172 chemical parameters were further conducted to identify the major environmental factors influencing 173 the specific module. In order to identify the key nodes connecting the interaction among the 174 prokaryotic, eukaryotic, and algal community, the network analysis through the ASVs along with the 175 environmental factors were done and visualized by Gephi (Bastian et al., 2009), where the edges 176 177 indicate the strong correlations between nodes with a spearman's rank correlation (r>0.7, p<0.05).

178 **3. Results and discussion**

179 **3.1.** Changes of the water quality indexes response to heavy rainfall

The heavy rainfall caused some disturbance of the water quality indicators in the Shahe Reservoir area. The heavy rainfall directly led to the increase of turbidity in the reservoir area, with turbidity rising by 560.1%. At the same time, the organic pollutants of COD and TOC in the reservoir area increased, although TP decreased by 68.2%, but the inorganic pollutants like phosphate and NH₃-N also increased (Table S2-S3 and Figure S2-S3). This indicated that amounts of runoff flowed into the reservoir along with the heavy rainfall. The increase in Chl-a occurs after the heavy rainfall, and the maximum value increased from 57.45 μ g·L⁻¹ to 219.88 μ g·L⁻¹ in this area (Figure S4-S5). This is primarily due to algae development, which is influenced by *T*, flow rate, and N/P ratio after the heavy rainfall.

The peak values of DO, Chl-a, turbidity, TP, phosphate, NH₃-N and TOC appeared on the first day 189 of rainfall in E. Except for TOC, and the other indexes may recover on the second day after a rainfall. 190 The rainfall had a significant impact on the water quality in N. Except for pH and TP, most of the 191 indicators increased, such as Chl-a (105.4%), Turbidity (263.3%), TN (107.4%), NH₃-N (398.6%). 192 193 Among the three tributaries, S is the most contaminated, and the water quality has not yet met the requirement the level of surface water Grade IV (Figure S3). The majority of the concerned parameters 194 were reduced after the rainfall due to dilution effect by the rainfall for S, such as Chl-a and turbidity, 195 196 which decreased by 50.2% and 195.4%, respectively. Nonetheless, it resulted in a 295.6% increase in NH₃-N. The water quality of WE remained steady and was not significantly influenced by the rainfall. 197 In summary, the heavy rainfall has a certain impact on the Shahe reservoir area, but the overall impact 198 is not strong, and it can recover on its own soon within a week. 199

200 **3.2.** Changes of prokaryotic community response to heavy rainfall.

After the heavy rainfall, prokaryotic community showed compositional changes in habitats and time at the phylum level. The dominant phylum belonged to Proteobacteria (49.69%±15.34%), Bacteroidetes (20.50%±7.38%) and Actinobacteria (7.47%±5.54%) (Figure 2a and Figure S6a). Proteobacteria had the highest abundance in the WE (77.63%±16%), where the maximum reached to 95.32% on the first day after the rainfall. RDA analysis revealed that Proteobacteria has a significant positive correlation with NO₃-N (p<0.01), and it was significantly and negatively correlated with

207	organic pollutants COD, pH, turbidity, and Chl-a (Figure S7a). In the first two days after the heavy
208	rainfall, the abundance of Proteobacteria in R was the lowest (less than 33.0%), but it rebounded along
209	with time. Bacteroidetes were significantly and positively correlated with TP, phosphate and TOC, but
210	negatively correlated with NO ₃ -N (p <0.05). Bacteroidetes showed obvious peaks in the E and N, and
211	returned to normal after 1-2 days after the rainfall, which indicated that the increase of Bacteroidetes
212	might be associated with the runoff caused by the heavy rainfall.



Figure 2. Changes of the prokaryotic community composition response to the heavy rainfall: (a) Phylum level; (b) Heatmap showing the top 10 genus in each sample; (c) FEAST analysis; (d) PCA analysis at the genus level.

219	The <i>Flavobacterium</i> (4.46%±5.05%) are the most common genera in this area (Figure 2b). The
220	Flavobacterium is most abundant in E and N with the relative abundance of 16.85 and 17.94%,
221	respectively, and it is thought to be involved in a number of key ecological roles during algae blooms.
222	The Aliarcobacter under Campilobacterota had a considerable rise on the first day following the heavy
223	rainfall in E, N and S. The Aliarcobacter is a kind of nteric and potentially zoonotic pathogen (Hänel
224	et al., 2021), and is mainly isolated from human waste septic tanks and closely associated with the
225	urban non-point source (Ogonowski et al., 2018). The Aliarcobacter in Shahe reservoir could well
226	represent the runoff flowing into the rivers, and the Aliarcobacter was also considered to be abundant
227	in the combine sewage overflows (CSOs) in Shahe reservior as our previous study indicated (Zhang
228	et al., 2022). The S introduced much higher abundance of <i>Aliarcobacter</i> compared to other tributaries,
229	which indicated higher pollution caused by the heavy rainfall in S. Besides, the heavy rainfall also
230	introduced other human potential pathogens like Aeromonas and Pseudomonas as shown in Figure 2b.
231	The waterborne disease caused by the heavy rainfall is also one of the most concerns for the global
232	climate change, because the frequent heavy rainfall associated with the climate change would
233	introduce amounts of pathogenic microorganisms into the waters through the CSOs and runoff. The
234	waterborne diseases associated with the Aliarcobacter, Aeromonas, and Pseudomonas should be paid
235	more attention in the flood season in Shahe reservoir area. Fortunately, these human potential
236	pathogens could be effectively reduced, and the prokaryotic community could recover within a week
237	(Figure 2b).

To unravel the effect of different water sources including E, N, S and WE on the development of prokaryotic community in the reservior, the fast expectation-maximization of microbial source tracking was performed through the FEAST. The samples from the R were employed as the sinks,

while tributaries and WE samples were used as the sources (Figure 2c and Table S4). Results showed 241 that the S contributed the most (42.29±13.60%) to the development of the prokaryotic community in 242 R, followed by N ($5.60\pm3.62\%$), E ($3.47\pm2.56\%$) and WE ($0.84\pm0.89\%$). This was related to the largest 243 flow volume and high pollution status of the S, and the results also well corresponded to the PCA and 244 Alpha indices (Figure 2d and Figure S8), where the samples from S are highly similar to those from 245 the R, while the samples of E and N are closer. But interestingly, except for R, the samples of the other 246 monitoring points showed an aggregation state in the early stage after the rainfall, and gradually 247 showed this geographical difference with the change of time. 248

249 **3.3.** Changes of eukaryotic community response to heavy rainfall.

The Ciliophora (29.63±12.79%), Metazoa (15.52±7.91%), and Fungi (14.85±9.66%) were the 250 dominant phylum in eukaryotic community (Figure 3a and Figure S6b). The abundance of the 251 dominating phylum was consistent between sites, where the abundance of Ciliophora was consistently 252 above 20%. Ciliophora is often the dominating species in the effluent of wastewater treatment plants, 253 and it was recognized as the dominant species in 160/228 protozoa previously detected in activated 254 sludge plants (Zahedi et al., 2019). This was in accorandace with the high ratio of reclaimed water 255 256 from the wastewater treatment plants. The RDA analysis revealed that Ciliophora showed no significant relationship with environmental variables (Figure S7b). Fungi, as opposed to bacteria, are 257 often considered as the important primary producers and food chain drivers in reservoirs, where they 258 have the potential to prevent harmful algal growth in freshwater ecosystems (Jia et al., 2013; Zhang et 259 al., 2015). Metazoa had a substantial and positive correlation with pH. Fungi had a very significant 260 positive connection with NO₃-N (p<0.01), and a significantly negative correlation with TOC (p<0.05). 261



Figure 3. Changes of eukaryotic community composition response to the heavy rainfall: (a) Phylum level; (b) Heatmap showing the top 10 genus in each sample; (c) FEAST analysis; (d) PCA analysis at the genus level.

The major genus varied from the different monitoring sites when it comes to the eukaryotic 268 community (Figure 3b). In the R, the dominant genus was Colpodellida CHR1 X (5.66±4.4%), and it 269 was unclassified Hypotrichia (5.50±5.30% and 13.18±7.54%, respectively) for the E and N. 270 Nonetheless, the dominant genus in S was *Perkinsida XXX* (7.57±7.26%), while the dominant genus 271 in WE was *Frontoniidae 1 X* ($8.13\pm16.27\%$). No significant change trend was discovered after the 272 heavy rainfall, but heatmap clustering revealed that Rotifera XXX, Colpodellida CHR1 X, 273 Perkinsida XXX, and Oxytricha peaked on the first day following the heavy rainfall, although the 274 abundance of them was soon recovered. Rotifera is often the most important zooplankton in the 275 reservoir (Yu et al., 2021). In comparison to prokaryotic community, the eukaryotes are rather stable 276 (Figure 3b), and no discernible eukaryote closely associated with the runoff was figured out in this 277 study. 278

The contribution by the E, N, R and WE to the development of eukaryotic community in the R was also determined through the FEAST analysis, and the results were: 3.11±1.96% for E, 15.38±13.06% for N, 36.68±14.11% for S, and 12.41±21.44% for the WE (Figure 3c; Table S4). The S is still the major contributor to the development of eukaryotic community in the R. According to the PCA results and Alpha indices (Figure 3d and Figure S9), it was found that the aggregation degree of the samples at different monitoring points was high, and there was no significant difference between the samples over time.

286 **3.4. Changes of algal community response to heavy rainfall.**

The Bacillariophyta and Cyanobacteria are the two most important phylum for the algal community (Figure 4a and Figure S6c). The main phylum in E, S and R were Bacillariophyta (35.84±9.81%, 45.28±10.62% and 58.28±7.46%, respectively) and Cyanobacteria (18.32±6.93%,

31.79±5.13% and 21.00±9.15%, respectively), while that of N was Bacillariophyta (22.38±11.53%) 290 and Ochrophyta (19.46±14.86%). In WE, Ochrophyta was the main phylum (31.84±16.38%), and 291 Cyanobacteria were the secondary phylum (20.40±4.18%). The prevalence of Bacillariophyta was 292 closely linked to both TN and TP (Peng et al., 2021), and Bacillariophyta had a positive connection 293 with pH, TP, and NH₃-N, but a negative correlation with NO₃-N (*p*<0.01) (Figure S7c). Harmful algal 294 are more of Cyanophyta phytoplankton in shallow lakes, whereas Bacillariophyta is the more common 295 catastrophic bloom species in reservoirs (Dong, 2011). Bacillariophyta are eukaryotic creatures with 296 strong siliceous cell walls, big cells, and a high density, making them better suit to flowing water and 297 relatively cold water habitats (Lin and Lin, 1999). The pH, DO, Chl-a, and COD are all positively 298 correlated with Cyanobacteria (p < 0.05), while DO is strongly favorably correlated (p < 0.01). The 299 phylum is easy to develop at high-eutrophication monitoring sites, and an increase in Cyanobacteria 300 301 content will result in an increase in Chl-a content, which corresponds to the rich Cyanophyta content in S and R. For R, Ochrophyta peaked on the last day of rain, while Cyanobacteria peaked on the first 302 day of rainfall. The remaining points of the Ochrophyta peaked on the first day after the rain, whereas 303 the Bacillariophyta and Cyanobacteria's points peaked between the 3-6th days following the rain. This 304 may be due to the large amount of rainfall causes dilution of each tributary, and the non-point source 305 pollution generated by runoff is confluent into the R, where the water flow was largely reduced. 306

As for the genus level, the major algae belonged to the *Cyclotella* (24.42 \pm 3.81%) and *Nannochloropsis* (13.35 \pm 5.64%) (Figure 4b). The major genus in E was *Thalassiosira* (17.13%) followed by *Nannochloropsis* (15.29 \pm 7.01%), and it was *Nannochloropsis* (14.75 \pm 10.07% and 30.52 \pm 16.79%, respectively) for N and WE. While the major genus in S and R belonged to the *Cyclotella* (43.17 \pm 10.59% and 53.15 \pm 6.88%, respectively). Interestingly, the *Cyclotella* peaked on the

sixth day after the heavy rainfall especially for N, S and WE, where the abundance reached to the 58.48% 312 in S. The notorious Microcystis closely associated with eutrophication also showed its peak at 4.49 % 313 314 on the sixth sampling day. These could well explain the increase of concentration of Chl-a after the heavy rainfall. It seemed that heavy rainfall did not directly change the algal community like the 315 316 prokaryotic community, but the changes of the environmental conditions and nutrition after the heavy rainfall favored the changes of the algal community. That is, it is not a recovery process for the agal 317 community after heavy rainfall, instead, it triggered the following growth of the algal community. The 318 results from the FEAST analysis showed that the S was still the major contributor to the development 319 of algal community in the R with 7.54%±9.24% for E, 1.99%±3.66% for N, 13.98%±14.89% for S, 320 and 1.56%±2.71% for the WE (Figure 4c; Table S4). According to the PCA results and Alpha indices 321 (Figure 4d and Figure S10), it was found that except for R, the samples at the other monitoring points 322 323 were scattered over time. Interestingly, these samples showed a dispersed state in the early days after the rainfall, and the samples by the fifth and sixth days showed an aggregated state. 324



Figure 4. Changes of algal community composition response to heavy rainfall: (a) Phylum level; (b)
Heatmap showing the top 10 genus in each sample; (c) FEAST analysis; (d) PCA analysis at the genus
level.

331 3.5. Co-occurrence analysis showing the environmental variables affecting the microbial

332 community response to heavy rainfall

Significant correlations between ASVs in the three communities (R > 0.7, p<0.001; indicated by 333 gray lines) were visualized using co-occurrence network analysis separately (Figure 5). The modules 334 were identified, and the cumulative relative abundance in each module was calculated, and thus, the 335 responses of the primary modules to environmental conditions can be investigated. Because E and N 336 flowed into R after confluence, the E and N are combined together as EN during the analysis. As for 337 the prokaryotic community, the modules with the largest cumulative abundance of differential ASVs 338 339 were TM1 and TM2 (Figure 5a and 5d). The TM1, TM2, TM3 and TM4 could represent the EN, R, S and WE, respectively. Through the correlation analysis between the modules and environmental 340 parameters, the prokaryotic community in EN correlated significantly and negatively with DO (Figure 341 342 S11). The prokaryotic community in R have significantly positive correlations with Chl-a, pH, turbidity, COD, TOC (p < 0.01) and T (p < 0.05), but is negatively linked with NO₃-N (p < 0.01). While 343 the prokaryotic community in S correlated significantly positive with T, TP, TN, phosphate and NH₃-344 N (p<0.01), and the prokaryotic community in WE showed significantly negative with turtidity, TP 345 (p < 0.05) and TOC (p < 0.01) but positive with NO₃-N (p < 0.01) and TN (p < 0.05). 346

In eukaryotic community, the modules with the highest cumulative abundance of differential ASVs were TM3 and TM1 (Figure 5c and 5d). The S and R contained the abundant TM2 and TM3. The TM2 significantly and positively correlated with pH, *T*, turbidity, TOC, TP (p<0.01), but negatively correlated with NO₃-N and TN (p<0.05) (Figure S12). The TM3 which significantly and positively correlated with Chl-a, DO, pH, *T*, turbidity, COD, TOC, TP (p<0.01), but negatively correlated with NO₃-N (p<0.01). WE has the highest cumulative abundance in TM4, which was significantly positively correlated with NO₃-N (p<0.05), and significantly negatively correlated with Chl-a, pH, *T*, turbidity, COD, TOC, TP (p<0.05).

As for algal community, the module with the highest cumulative abundance of differential ASVs 355 was TM1 (Figure 5e and 5f). The TM1 and TM2 could represent the S and R, and the TM3 and TM4 356 could represent the EN. R followed by S contained the highest abundance of TM1, which was 357 positively correlated with Chl-a, pH, turbidity, COD, TOC and T (p < 0.05), but negatively correlated 358 with NO₃-N (p<0.01) (Figure S13). The TM2 was positively correlated with Chl-a, pH, turbidity, COD 359 and T (p < 0.05), but negatively correlated with NO₃-N (p < 0.05). The TM3 was negatively correlated 360 with DO and pH (p<0.05), and the TM4 was negatively correlated with NO₃-N (p<0.05). The TM4 361 was negatively correlated with DO, pH, Chl-a, COD, TOC and turbidity (p < 0.05). 362

According to the network co-occurrence analysis of three different communities, we discovered that the various modules have significant geographic environment differences. The modules with the highest cumulative abundance in each community, however, were primarily S and R, and this portion responded to environmental factors in the following ways: significantly and positively correlated with Chl-a, pH, turbidity, COD, and TOC (p<0.01), but negatively correlated with NO₃-N. These may be the primary environmental factors influencing the development of the three communities after the heavy rainfall.



Figure 5. The modules were identified through the co-occurrence networks based on the significantly 373 positive pairwise correlations (R>0.7, p<0.001) between ASVs for the prokaryotic (a and d), 374 eukaryotic (b and e) and algal (c and f) communities. The cumulative relative abundance was 375 calculated through the sum of the relative abundance of the ASVs in one module. 376

377 **3.6.** Network analysis showing the relationship among prokaryotic, eukaryotic and algal

378 communities

The mantel test found that the three communities are significantly and positively connected (algae 379 380 and eukaryote, p=0.0429; eukaryote and prokaryote, p=0.0445; algae and prokaryote, p=0.0427), and environmental parameters are equally essential to the dynamics of the three microbial communities 381 (prokaryote and environmental parameters, p=0.0078; eukaryote and environmental parameters, 382 p=0.0015; algae and environmental parameters, p=0.0001). The Procrustes analysis proved the 383 relevance of the three communities, and changes of any of the community had a major impact on the 384 fate of the others (Figure S14), where 45.8% of the prokaryotic community variation was explained 385 by eukaryotic community (R=0.9331 and 0.7272), 56.4% of the prokaryotic community variation was 386 explained by algal community (R=0.9089 and 0.8939), and 55% of the eukaryotic community variation 387 was explained by algal community (R=9553 and 0.7748). 388

According to the network analysis, 423 edges were captured among the 146 nodes that described 389 significant correlations between ASVs among the three communities (r>0.7, p<0.05) (Figure 6). 390 Among them, the number of positive correlations was 384, and it was 39 for the number of negative 391 392 correlations. The nodes in the network covered 29 phylums (Prokaryotes: 6; eukaryotes: 15; algae: 8) and 10 environmental parameters. Among all the connected nodes in the network, the prokaryotes 393 belonging to Proteobacteria and Bacteroidetes accounted for the highest proportion, while it was 394 Ciliophora and Fungi for the eukaryotic community, and Cyanobacteria along with Cryptophyta for 395 the algal community. 396

From the network structure, it is obvious that the nodes were clustered into 4 modules, which indicated that there was a modular structure and "small world" topology of the network. The contents

of the 4 modules were as follows: Module I, environmental factors, eukaryotic and algal community 399 interaction area; Module II, eukaryotic and prokaryotic community interaction area; Module III, 400 prokaryotic community interaction area; Module IV, pathogenic microbe interaction area. In Module 401 I, the key nodes in eukaryote were *Rotifera XXX*, *Fungi XXXX* and *Leptophryidae X*, and it was 402 Cyclotella, Nannochloropsis and Prochlorothrix for the algal community. Cyclotella is a common 403 dominant genus in the Shahe Reservoir area, while Rotifera and Leptophryidae have been 404 demonstrated to be the predatory on algae, and *Leptophryidae X* was essential obligatory algivores 405 (Hess, 2017), and Nannochloropsis is often the object of prey. The Chl-a, turbidity, TP, and TOC were 406 the most important environmental factor nodes, which were closely related to the external input 407 induced by the runoff of the heavy rainfall. These important environmental factor nodes were also the 408 leading factors of the modules with the greatest cumulative abundance in each community in the co-409 410 occurrence network analysis (Figure 6).

The Module II contained prokaryotic and eukaryotic communities, and the Halteria and 411 Paramecium are important nodes in eukaryotes, while Limnohabitans and Leptothrix are key nodes 412 413 for the prokaryote. It was indicated that the Ciliophora belong to the bacterial predators, and Zou et al., (2021) found a positive correlation between Proteobacteria and Ciliophora. These suggested that 414 they were working together in a symbiotic relationship in exchange for photosynthetic products, 415 nutrients, and/or protection, while Proteobacteria might supply nitrogen, vitamins, and/or defense to 416 Ciliophora (Bjorbækmo et al., 2020). The network structure revealed that eukaryotes were more likely 417 to interact and connect with algae and prokaryotes, while the connection between prokaryotic and algal 418 community was limited. The Module III contained prokaryotic community, and Desertimonas, 419 unclassified Micrococcales and unclassified Actinobacteria are important nodes in prokaryotes. 420

421 These prokaryotic microorganisms only interact with *Planktothrichoides*, and mainly in negative 422 correlation.

Some pathogenic bacteria and WWTP microorganisms, including Prevotella, Aliarcobacter, 423 Pseudarcobacter, Aeromonas, Tolumonas, Pseudomonas and Phocaeicola, are present in Module IV. 424 The Aliarcobacter and Pseudarcobacter were generally isolated from the human waste septic tank and 425 have been implicated in diseases such as enteritis and bacteremia (On et al., 2020). In most cases, 426 Tolumonas were isolated from anoxic freshwater sediment (Caldwell et al., 2011). Usually, 427 Phocaeicola were isolated from human feces (Wang et al., 2021). Acinetobacter, Pseudomonas, and 428 Prevotella were the main potential pathogens carrying the observed ARGs in the recreational seawater 429 (Jang et al., 2021). This module is small in scale and not closely related to other modules, which could 430 be brought about by runoff from rainfall. 431

432 In summary, the response of prokaryotic, eukaryotic and algal community to the heavy rainfall was quite different. The prokaryotic community was directly impacted by the heavy rainfall, which 433 induced amounts of human potential pathogens into the rivers especially for the Aliarcobacter, 434 Aeromonas, and Pseudomonas. Nonetheless, the prokaryotic community could recover within a week. 435 The eukaryotic community was stable, and the impact caused by the heavy rainfall was limited. 436 However, the changes of algal community happened not by the runoff directly but by the 437 environmental condition after the heavy rainfall, and algal community begined to change and grow not 438 the recovery after the heavy rainfall. The eukaryotic community not only helped the recovery of the 439 prokaryotic community on one hand, but also the contributed to the development of the algal 440 community after the heavy rainfall on the other hand. These results indicated that we should paid more 441 attention to controlling of the human potential pathogens associated with prokaryotic community at 442



Figure 6. Network analysis between prokaryotic, eukaryotic and algal community together with environmental factors. The networks established based on the genus for the three communities. Each node denotes a genus or an environmental factor, and the nodes are colored according to the type of the microbial community; each edge linking two nodes represents a significant relationship (red for positive correlation, blue for negative correlation) (p<0.05).

4 Conclusion 453

454	The response of prokaryotic, eukaryotic and algal community to the heavy rainfall was
455	investigated in detail in this study, and the proposals and conclusions were drawn as follows:
456	• Heavy rainfall could directly impact the composition of the prokaryotic community by
457	introducing amounts of runoff closely associated bacteria especially for the human potential
458	pathogens of Aliarcobacter, Aeromonas, and Pseudomonas in the Shahe reservoir area.
459	• The eukaryotic community was rather stable even for the heavy rainfall, and the development
460	and changes of algal community happened not the beginning of the heavy rainfall but after
461	it.
462	• The microbial source tracking through FEAST indicated that the Nansha river (S) was the
463	major contributor to the development of the three concerned communities in the reservoir.
464	• The modules with the highest cumulative abundance in each community showed significantly
465	and positively correlated with Chl-a, pH, turbidity, COD, and TOC (p <0.01), but negatively
466	correlated with NO ₃ -N (p <0.01). While eukaryotic community played a crucial role on the
467	interaction between the three concerned communities and were more likely to interact with
468	algae and prokaryotes.
469	• It was suggested that we should pay more attention to controlling of the human potential
470	pathogens associated with prokaryotic community at the beginning of the heavy rainfall and
471	prevent the eutriphication bloom of the reservoir after the heavy rainfall.
472	
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479	
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