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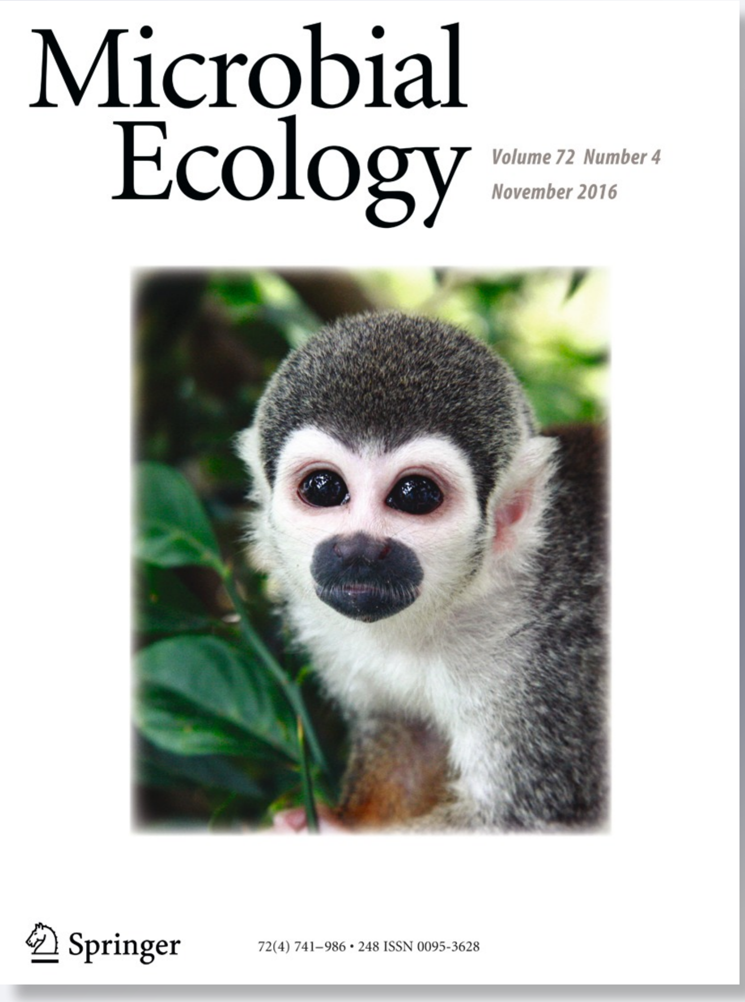
Microbial Ecology

ISSN 0095-3628
Volume 72
Number 4

Microb Ecol (2016) 72:791-801
DOI 10.1007/s00248-016-0814-9



Springer

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MICROBIOLOGY OF AQUATIC SYSTEMS

Antibiotics and Antibiotic Resistance Genes in Sediment of Honghu Lake and East Dongting Lake, China

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Received: 9 May 2016 / Accepted: 30 June 2016 /

Abstract Sediment is an ideal medium for the aggregation and dissemination of antibiotics and antibiotic resistance genes (ARGs). The levels of antibiotics and ARGs in Honghu Lake and East Dongting Lake of central China were investigated in this study. The concentrations of eight antibiotics (four sulfonamides and four tetracyclines) in Honghu Lake were in the range 90.00–437.43 μg kg^{-1} (dry weight (dw)) with mean value of 278.21 μg kg^{-1} dw, which was significantly higher than those in East Dongting Lake (60.02–321.04 μg kg^{-1} dw, mean value of 195.70 μg kg^{-1} dw). Among the tested three sulfonamide resistance genes (*sul*) and eight tetracycline resistance genes (*tet*), *sul*1, *sul*2, *tet*A, *tet*C, and *tet*M had 100 % detection frequency in sediment samples of East Dongting Lake, while only *sul*1, *sul*2, and *tet*C were observed in all samples of Honghu Lake. The relative abundance of *sul*2 was higher than that of *sul*1 at $p < 0.05$ level in both lakes. The relative abundance of *tet* genes in East Dongting Lake was in the following order: *tet*M > *tet*B > *tet*C > *tet*A. The relative abundance of *sul*1, *sul*2, and *tet*C in East Dongting Lake was significantly higher than those in Honghu Lake. The abundance of background bacteria may play an important role in the horizontal spread of *sul*2 and *tet*C genes in Honghu Lake and *sul*1 in East Dongting Lake, respectively. Redundancy analysis indicated that tetracyclines may play a more important role than sulfonamides in the abundance of *sul*1, *sul*2, and *tet*C gens in Honghu Lake and East Dongting Lake.

Keywords Sulfonamides · Tetracyclines · Antibiotic resistance gene · Honghu lake · East dongting lake

Introduction

Antibiotics are widely used in medical and agricultural activities [1, 2], leading to occurrence of antibiotics in many environmental media, such as rivers [3–5], lakes [6, 7], wastewater treatment plants [8–10], and swine farms [11]. Antibiotics discharged from hospitals, wastewater, industries, and agricultural activities finally circulated in water environments [1]. Lakes are more susceptible to the antibiotic pollution compared to rivers, which have a high water exchange. Baiyangdian Lake, the largest natural freshwater body in North China, contained 65.5–1166 μg kg^{-1} of quinolones in sediments [6]. Taihu Lake is surrounded by some highly developed cities and towns, having oxytetracycline at 52.8 μg kg^{-1} in sediment concentration [12]. The wide existence of antibiotics in the environment has been proved as a major driving force of the increasing occurrence of antibiotic resistance genes (ARGs) and the evolution of multidrug resistant bacteria [13].

ARGs in environment have been becoming an increasing worldwide concern and could be horizontally transferred to human pathogens, which increased the difficulties of infectious

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Electronic supplementary material The online version of this article (doi:10.1007/s00248-016-0814-9) contains supplementary material, which is available to authorized users.

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disease treatment and threatening public health [14]. Aquatic compartments, such as water and sediment, may be an ideal medium for the acquisition and dissemination of ARGs [15]. The human activities were important factors influencing the distribution of ARGs in the aquatic environment. The discharged wastewater has been proved as a point source of ARGs in the sediment of Lake Geneva [16]. The effect of aquaculture and/or non-aquaculture on the ARGs in bacteria has been studied in China [17], Pakistan and Tanzania [18], and Chile [19]. The influence of human activities on ARGs in environment has also been reported between the human-impacted estuary and deep ocean sediments [20], and a lake in Sweden is only lightly affected by human activities [21].

In this study, Honghu Lake and East Dongting Lake were selected to investigate the occurrence and distribution of antibiotics and ARGs because they supply water to the surrounding rural area, industrial region, and city area (Honghu City and Yueyang City, 57 and 120 km^2, respectively). Moreover, they support economical activities such as fishing (Honghu Lake), shipping, tourism, and sand dredging (East Dongting Lake). The populations around Honghu Lake and East Dongting Lake were 487,000 and 1,783,000, respectively. The gross domestic products (GDP) in Honghu City and Yueyang City were 2.9 and 43.1 billion dollars, respectively. Hence, the East Dongting Lake was affected more by human activities than the Honghu Lake. Sulfonamides and tetracyclines were widely distributed in surface water in China [22]. Hence, tetracycline and sulfonamide antibiotics with corresponding resistance genes were selected in this study. The study in both lakes not only provides information for lake management, but also expands our knowledge of abundance and distribution of antibiotics and ARGs in these specific lakes.

Materials and Methods

Reagents

Four sulfonamides (SAs) and four tetracyclines (TETs) including sulfadiazine (SD), sulfamethazine (SMZ), sulfameter (SME) and sulfamethoxazole (SMX), oxytetracycline (OTC), tetracycline (TC), chlortetracycline (CTC), and doxycycline (DC) were purchased from Sigma-Aldrich Co. (St. Louis, USA). Organic solvents used for antibiotics extraction were at chromatographical grade.

Sampling Sites and Sample Collection

East Dongting Lake (28° 59′–29° 38′ N, 112° 43′–113° 15′ E) is the largest part of Dongting Lake and naturally connected to Yangtze River, located in Yueyang City, Hunan Province. Honghu Lake (29° 39′–30° 12′ N, 113° 7′–114° 05′ E) had been cut off the relationship with Yangtze River by hydraulic projects since 1950s, located in Honghu City, Hubei Province (Fig. 1). The area and average depth of East Dongting Lake are 1328 km^2 and 6.39 m, respectively. The Honghu Lake covers an area of 348 km^2 with an average depth of 1.35 m. In each lake, 14 sediment samples were collected. Six (H2–H7) and seven samples (H8–H14) were collected at the northeastern and southwestern parts of Honghu Lake (Fig. 2a), respectively. Sample H1 in Honghu Lake was collected at the lake outlet between the main body of lake and Yangtze River. The samples in East Dongting Lake were collected from the estuary of Xiangjiang River and Miluojiang River to the lake outlet (Fig. 2b). The sampling sites D13 and D14 were very close to the two wastewater treatment plants of Yueyang City. The sediment samples (0–5 cm) were collected with a grab sampler and packed in sterile containers. Three subsamples were collected at each site and combined as one sample before analysis. All the samples were immediately placed on ice box and then transported to the laboratory and stored at −80 °C prior to analysis.

Analysis of Antibiotics and Total Organic Carbon in Sediment Samples

For quantification of antibiotics in sediments, 5 g of lyophilized and ground sediment samples was added to a solution mixture (15 ml of methanol, 5 ml of Na_2EDTA, and 10 ml of citrate buffer at pH 5.0) using ultrasonic treatment for 15 min. The mixture was centrifuged at 3500*g* for 5 min, and the supernatant was collected. The process was repeated three times. The supernatants were combined, and the volume was set at 500 ml using deionized sterile water. Then, the samples were cleaned up and concentrated by solid-phase extraction (SPE) using Strata strong anion exchanger (SAX) cartridges (3 ml/200 mg, Thermo, USA) and Oasis hydrophilic-lipophilic balance (HLB) cartridges (6 ml/500 mg, Waters, UK) in series. Detail information for extraction of antibiotics in sediment could be found in the literature [23]. High-performance liquid chromatography tandem mass spectrometry (HPLC/MS/MS) operated in positive mode with electrospray ionization (ESI) was used to separate and detect the TETs and SAs according to the literature previously published [24].

Total organic carbon (TOC) content in sediments from both lakes was determined using the $K_2Cr_2O_7$-H_2SO_4 wet oxidation method described in [25].

DNA Extraction and PCR Assays for Detection of ARGs

Exactly 0.5 g of sample from each site was used to extract DNA by FastDNA Spin Kit for Soil (QBiogene, Carlsbad, CA). All the procedures were followed according to the protocol provided by the manufacturer. Then, DNA was further

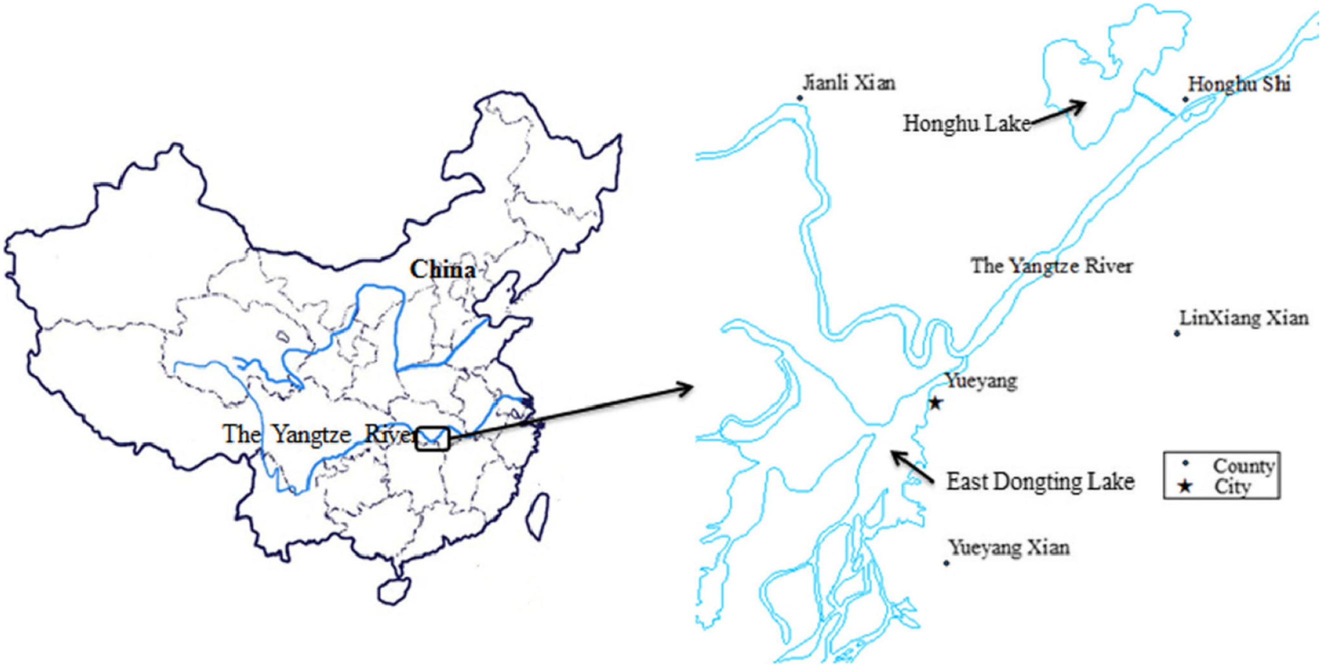


Fig. 1 The sketch map of studied area

purified by using the Geneclean Spin Kit (QBiogene, Carlsbad, CA) to minimize PCR inhibition. The concentration of DNA before and after purification was determined to calculate the recovery using spectrophotometer analysis (NanoDrop ND-2000c, Thermo) and 1.5 % agarose gel electrophoresis.

All conventional PCR assays were performed in a 25-μl volume reaction, containing 2.5 μl 10× PCR buffer (Mg^{2+} included), 2 μl 2.5-mM dNTP mixture, 1 μl of each primer, 1.5 U of Taq DNA polymerase, and 1 μl of template. The primers of tested ARGs (*sul*1, *sul*2, *sul*3, *tet*A, *tet*B, *tet*C, *tet*G, *tet*M, *tet*O, *tet*Q, and *tet*W*)* and 16S ribosomal RNA (rRNA) in this study were previously described in the literatures [23, 26–29]. Detail information on primers, annealing temperature, and gene length of ARGs and 16S rRNA is found in Table S1 (See Supplement Information). The

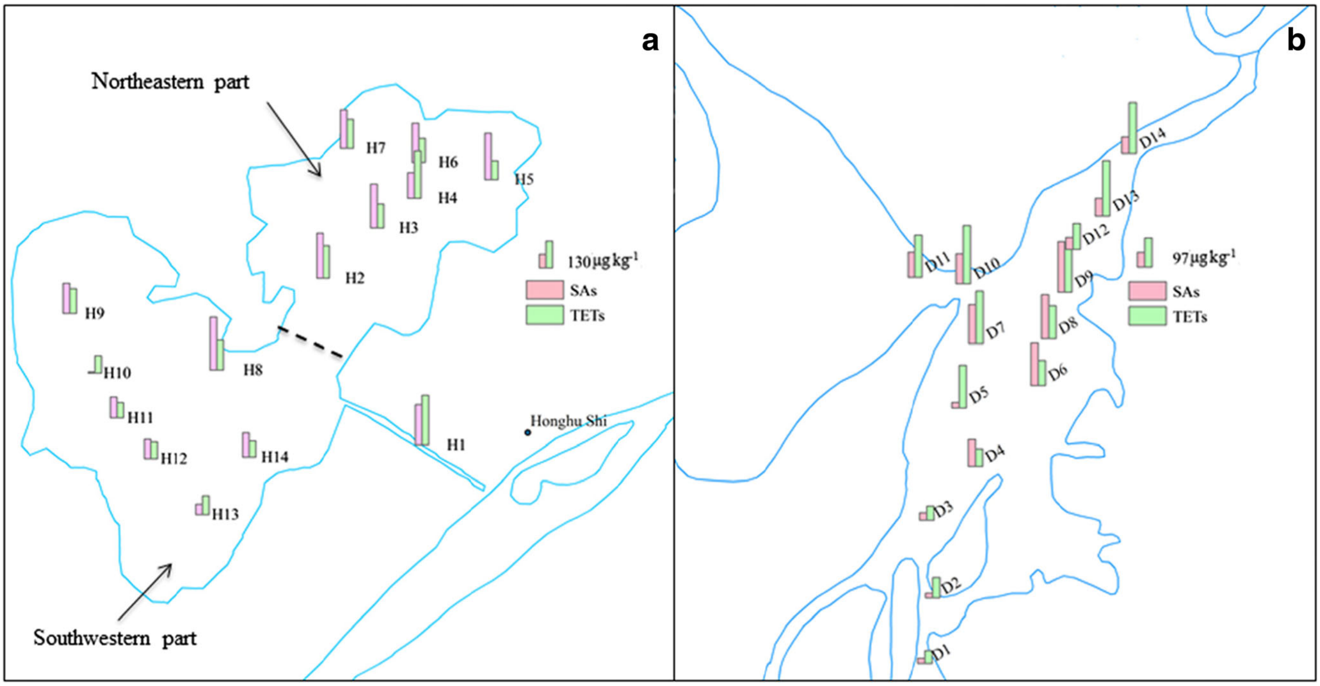


Fig. 2 The spatial distribution of SAs and TETs in northeast part and southwest part of Honghu Lake (**a**) and East Dongting Lake (**b**)

temperature program of PCR was performed as follows: initial denaturation at 95 °C for 5 min, followed by 40 cycles consisting of denaturation (15 s and 95 °C), annealing (30 s at annealing temperature shown in Table S1), and extension (30 s at 72 °C) and a final extension step at 72 °C for 10 min.

Real-Time qPCR, Standard Curves, and Quantification

Real-time qPCR was applied to quantify ARGs with 100 % detection frequencies in the conventional PCR assays. 16S rRNA was also determined to minimize the variance in the abundance of ARGs due to the DNA extraction efficiency and difference in background bacterial abundance. qPCR reactions were performed with a StepOnePlus real-time PCR system (ABI, USA) in a 25-μl reaction mixture (1× SYBR Green PCR Master Mix (Qiagen, Valencia, CA), 0.2 μM of each primer, and 1 μl of template) with a temperature program of 3 min at 95 °C, followed by 40 cycles of 15 s at 95 °C, 30 s at the annealing temperature, extension for 30 s at 72 °C, followed by a final melt curve stage with temperature ramping from 60 to 95 °C. Plasmids with targeted genes were constructed as the standards for the real-time qPCR according to the literature reported [27]. Seven-point standard curves were generated via tenfold serial dilution of the plasmid carrying targeted genes from 10^8 to 10^2 copy numbers, and the R^2 values for all standard curves were all higher than 0.99 (Table S2). The Ct values of samples were used to quantify ARGs in sediment according to the literatures published [23, 27, 30].

Statistical Analysis

T test was used to infer the difference of antibiotic concentrations and ARG abundance in both lakes. Pearson correlations and redundancy analysis (RDA) were applied to investigate the relationships between the concentrations of 16S rRNA, antibiotics, and ARG abundance by R software (Version 3.2.0) with "Vegan" package. Cluster analysis (heat map) for the log values of 16S rRNA, *sul*1, *sul*2, and *tet*C was performed by MATLAB software (Version R2009b, MathWorks, Inc., Natick, USA).

Results and Discussion

The Profiles of Antibiotics in Both Lakes

Table 1 shows the levels of antibiotics in both lakes. The total concentrations of eight antibiotics in sediments of East Dongting Lake ranged from 60.02 to 321.04 μg kg^{-1} dry weight (dw) with an average value of 195.70 μg kg^{-1} dw. The total concentrations of antibiotics in sediments of Honghu Lake were in the range 90.00–437.43 μg kg^{-1} dw with an average value of 278.21 μg kg^{-1} dw. The concentrations of antibiotics in Honghu Lake were higher than those in East Dongting Lake at $p < 0.05$ level. The total concentrations of four SAs accounted for 37.26 and 51.80 % of total measured antibiotics in East Dongting Lake and Honghu Lake, respectively. The concentrations of four individual SAs in East Dongting Lake and Honghu Lake were in the following order: SME (32.27 μg kg^{-1} dw) > SMX (30.57 μg kg^{-1} dw) > SD (8.65 μg kg^{-1} dw) > SMZ (6.24 μg kg^{-1} dw) and SMX (57.32 μg kg^{-1} dw) > SME (49.65 μg kg^{-1} dw) > SD (37.73 μg kg^{-1} dw) > SMZ (7.24 μg kg^{-1} dw), respectively. Compound SD showed 100 % detection frequency in both lakes, while SMZ, SME, and SMX were detected over 70 % of samples in both lakes. The concentrations of SME and SMX were higher than those in Jiulongjiang River (SME, not detected (nd)–1.8 μg kg^{-1} dw and SMX, 1.2–3.4 μg kg^{-1} dw) [31]. The SD and SMZ in both lakes were also higher than those in sediments from the Huangpu River (SD 0.07–0.71 μg kg^{-1} dw and SMZ 0.05–0.6 μg kg^{-1} dw) [3].

The concentrations of four individual TETs in East Dongting Lake and Honghu Lake were as follows: DC (43.84 μg kg^{-1} dw) > TC (39.85 μg kg^{-1} dw) > OTC (18.40 μg kg^{-1} dw) > CTC (15.87 μg kg^{-1} dw) and TC (48.02 μg kg^{-1} dw) > OTC (37.96 μg kg^{-1} dw) > DC (34.02 μg kg^{-1} dw) > CTC (6.27 μg kg^{-1} dw), respectively. DC, TC, and OTC showed 100 % detection frequencies in East Dongting Lake, while only TC and OTC were detected in all samples of Honghu Lake. The concentrations of TC, OTC, and CTC in both lakes were lower than TC (6.9–7614 μg kg^{-1} dw), OTC (10.2–10,364 μg kg^{-1} dw), and CTC (nd–14,667 μg kg^{-1} dw) in sediments of Jiulongjiang River [31]. The TC concentrations in both lakes were comparable to those in Pearl River (nd–72.6 μg kg^{-1} dw) [32] and Haihe River (nd–135 μg kg^{-1} dw) [33]. The OTC values in both lake were lower than those in Haihe River (nd–422 μg kg^{-1} dw) [33] and Liaohe River (nd–653 μg kg^{-1} dw) [33]. DC concentrations in both lakes were higher than those in Liaohe River (nd–2.8 μg kg^{-1} dw) [33] and Tiaoxi River (6–15.6 μg kg^{-1} dw) [34].

Distribution of Antibiotics in Sediment

SAs and TETs have been widely used in the farms of livestock and poultry and aquaculture. The aquatic products of Honghu Lake and East Dongting Lake were 255,000 and 1,285,000 t in 2014. The products of livestock and poultry are one of the important sources of income for rural area around both lakes. Hence, the agricultural and aquacultural activities and livestock breeding may be important sources for SAs and TETs in these specific lakes. The distributions of SAs and TETs in each sampling site in both lakes are shown in Fig. 2. It is interesting that most sites in East Dongting Lake had higher total concentrations of SAs compared to total concentrations of TETs, whereas the opposite trend was found in Honghu Lake.

The concentrations of antibiotics in the northeast part of Honghu Lake were higher than those in the southwest part

Table 1 The concentrations of antibiotics in East Dongting Lake and Honghu Lake (μg kg^{-1} dw)

Compounds	East Dongting Lake				Honghu Lake			
	Minimum	Maximum	Mean	DF (%)	Minimum	Maximum	Mean	DF (%)
SD	1.54	38.69	8.65	100	0.81	77.26	37.74	100
SMZ	nd	15.43	6.24	71.43	nd	29.47	7.24	78.57
SME	nd	105.29	32.27	85.71	nd	114.24	49.65	85.71
SMX	nd	115.35	30.57	78.57	nd	118.76	57.32	92.86
OTC	0.26	42.77	18.40	100	0.72	74.73	37.96	100
TC	3.07	84.35	39.85	100	5.46	114.36	48.02	100
CTC	nd	83.48	15.87	42.86	nd	55.57	6.27	28.57
DC	7.48	98.50	43.84	100	nd	74.63	34.02	92.86
SAs	15.85	167.65	77.73	100	6.13	256.42	151.94	100
TETs	42.62	193.18	117.97	100	74.80	241.33	126.27	100

DF detection frequency, *nd* not detected

(Fig. 2a). This may be due to that the northeast part of Honghu Lake was close to Honghu City and the southwest part of Honghu Lake was surrounded by rural area of Honghu City. The discharged domestic wastewater of Honghu City reached 2.1×10^7 t in 2011, and the treatment rate of wastewater was only 38 %. The disposal capacity of solid waste was also not desirable in this city. The population around the northeast part of Honghu Lake accounted for 35 % of the total population of Honghu City. Chemical oxygen demand discharges in aquaculture of Honghu Lake were 42,877 t in 2011 (http://www.honghu.gov.cn/). Domestic wastewater, aquaculture, and products of livestock and poultry may be the main sources for antibiotics in Honghu Lake.

The antibiotic concentrations in inlet from South Dongting Lake, Xiangjiang River, and Miluojiang River to East Dongting Lake (D1–D3) were lower than those in the main region of East Dongting Lake (D4–D14) (Fig. 2b). This may be due to that the water flow rate at the inlet was higher than those in the main region and the surrounding around the sites D1–D3 was rural area. The main region of East Dongting Lake was close to the intensive population and industrial region of Yueyang City, and more than 1.5×10^9 t of wastewater was discharged into the lake every year. The treatment rate of urban sewage was 84 %. Urban sewage, aquaculture, industrial source, livestock, and poultry accounted for 26, 26, 23, and 16 % of the total COD in the lake [35]. These may pay an important role in the antibiotic pollution in East Dongting Lake. Based on the analysis of antibiotics in both lakes, the sites close to the area of high intensity of human activities usually have high concentration of antibiotics in sediments.

The adsorption process of antibiotics onto sediment was influenced by many factors, such as pH, salinity, TOC, and heavy metals [36, 37]. The correlation relationship between the TOC and antibiotic concentration was investigated in this study. Pearson correlation analysis in East Dongting Lake showed that TOC had positive correlation ($r = 0.524$) with the total concentrations of TETs at $p < 0.1$ level, while no correlation relationship between the TOC and total concentrations of SAs. In Honghu Lake, the correlation relationship between TOC and the concentrations of SAs and TETs showed the similar trend with East Dongting Lake. For individual antibiotics, TOC had positive correlation ($r = 0.524$) with TC concentrations at $p < 0.05$ level and negative correlation ($r = -0.568$) with SMX concentration at $p < 0.05$ level in East Dongting Lake (Fig. 3a). In Honghu Lake, TOC had positive correlation relationships with the TC ($r = 0.629$, $p = 0.016$) and SMZ ($r = 0.537$, $p = 0.047$) levels in sediment, respectively (Fig. 3b). Hence, TOC may be an important factor influencing the distribution of TC and TETs in the both lakes. TOC has also been proved as the dominant sorption matrix for TC in sediment [38].

Profiles of ARGs in Both Lakes

Three SA resistance (*sul*) genes (*sul*1, *sul*2, and *sul*3) and two groups of TET resistance (*tet*) genes including antibiotic efflux pumps (*tet*A, *tet*B, *tet*C, and *tet*G) and ribosomal protection proteins (*tet*M, *tet*O, *tet*Q, and *tet*W) were analyzed in sediments of both lakes. Among the tested ARGs, *sul*1, *sul*2, *tet*A, *tet*C, and *tet*M had 100 % detection frequency in sediments of East Dongting Lake, followed by *tet*B (85.71 %), *tet*G (57.14 %), *sul*3 (28.57 %), and *tet*O (7.14 %). *tet*Q and *tet*W were not detected by the conventional PCR in East Dongting Lake. *sul*1, *sul*2, and *tet*C had 100 % detection frequency in sediments of Honghu Lake, followed by *sul*3 (28.57 %), *tet*B (28.57 %), *tet*A (14.28 %), *tet*M (14.28 %), and *tet*G (14.28 %). *tet*O, *tet*Q, and *tet*W were not detected in sediments of Honghu Lake. In group of *sul* genes, *sul*1 and *sul*2 had the high detection frequency except *sul*3 in both lakes. Among the *tet* genes, the gene type of antibiotic efflux pumps was more widely distributed in both lakes. Based on the detection frequency, the genes with 100 % detection

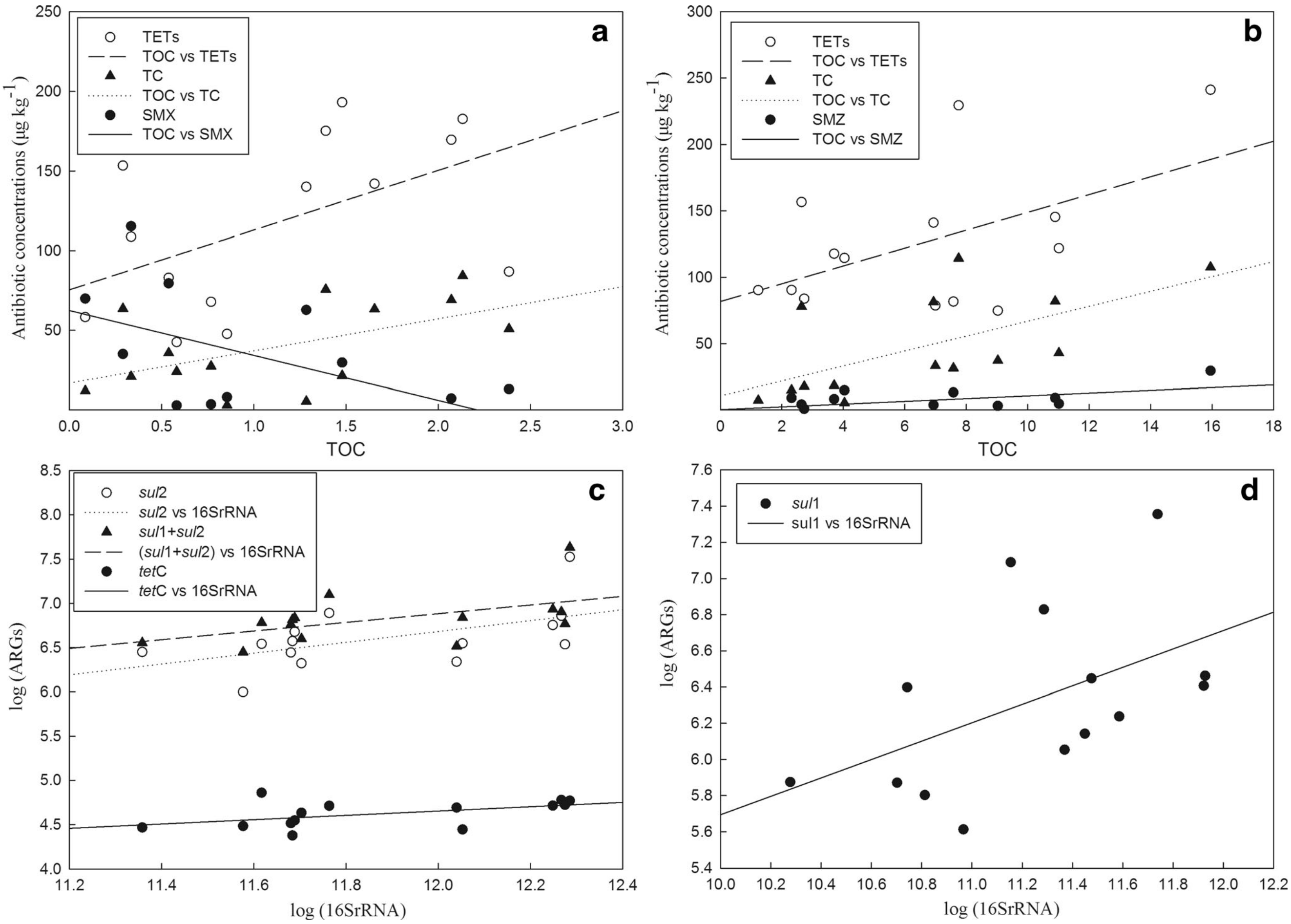


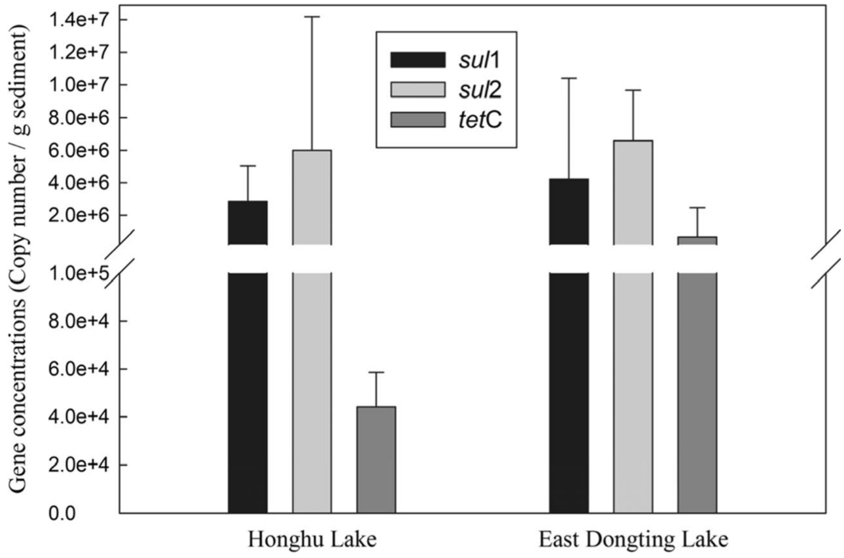
Fig. 3 The relationships between antibiotics and TOC in Honghu Lake (**a**) and East Dongting Lake (**b**), between ARGs and 16S rRNA in Honghu Lake (**c**) and East Dongting Lake (**d**)

frequency in East Dongting Lake (*sul*1, *sul*2, *tet*A, *tet*C, and *tet*M) and Honghu Lake (*sul*1, *sul*2, and *tet*C) were quantified by qPCR in this study.

Figure 4 summarizes the *sul*1, *sul*2, and *tet*C gene copies in sediments of both lakes. For *sul* genes, no significant difference was found between the *sul*1 and *sul*2 copy numbers in both lakes. Figure 5a, b shows the spatial distribution of *sul* genes and *tet*C in Honghu Lake, indicating that the northeast part of Honghu Lake suffered more serious ARG pollution compared to the southwest part. It showed similar distribution trends with the antibiotic concentrations in this lake. Figure 5c, d shows the spatial distribution of *sul* and *tet* genes in East Dongting Lake. Sites D13 and D14 adjacent to the wastewater treatment plants in Yueyang City had high concentrations of *sul* and *tet* genes. Wastewater treatment plant has been identified as an important source for ARG pollution in water environment, such as Lake Geneva [16] and Duluth-Superior Harbor [39]. Hence, wastewater treatment plant may be the important reason accounting for the difference of *sul* and *tet* genes between sites D13–14 and sites D1–D12. Heat map generated from MATLAB's clustergram function gives a visual presentation for 16S rRNA, *sul*1, *sul*2, and *tet*C in East Dongting Lake and Honghu Lake (Fig. 6). It showed that 16S rRNA and *sul*1 were clustered in one group, indicating that the bacterial abundance in the East Dongting Lake and Honghu Lake may play an important role in the distribution of *sul*1 gene. *sul*2 and *tet*C were clustered in another group, indicating that there is a coexistence possibility of *sul*2 and *tet*C in the East Dongting Lake and Honghu Lake. The figure also showed that the sampling sites in the two lakes were clustered into two main groups. The first group included 11 sampling sites in East Dongting Lake and 2 sampling sites in Honghu Lake, while 3 sampling sites of East Dongting Lake and 12 sampling sites of Honghu Lake were classified in to the second cluster. These results confirmed that there really existed difference in the bacterial and ARG abundance in the East Dongting Lake and Honghu Lake.

To avoid the DNA extraction efficiency and difference in background bacterial abundance, the relative abundance was calculated in this study. In Honghu Lake, the relative abundance of *sul*2 ($6.84 \pm 4.86 \times 10^{-6}$ copies/16S rRNA) was higher than that of *sul*1 ($3.94 \pm 2.32 \times 10^{-6}$ copies/16S rRNA) at $p < 0.05$ level. The relative abundance of *tet*C in sediments of Honghu Lake was $6.72 \pm 4.29 \times 10^{-8}$ copies/16S rRNA, which was significantly lower than that of *sul*1 and *sul*2. *sul* genes have been identified as the prevalent type of ARGs in the aquaculture environment of

Fig. 4 The gene copies of *sul*1, *sul*2, and *tet*C in sediments of Honghu Lake and East Dongting Lake



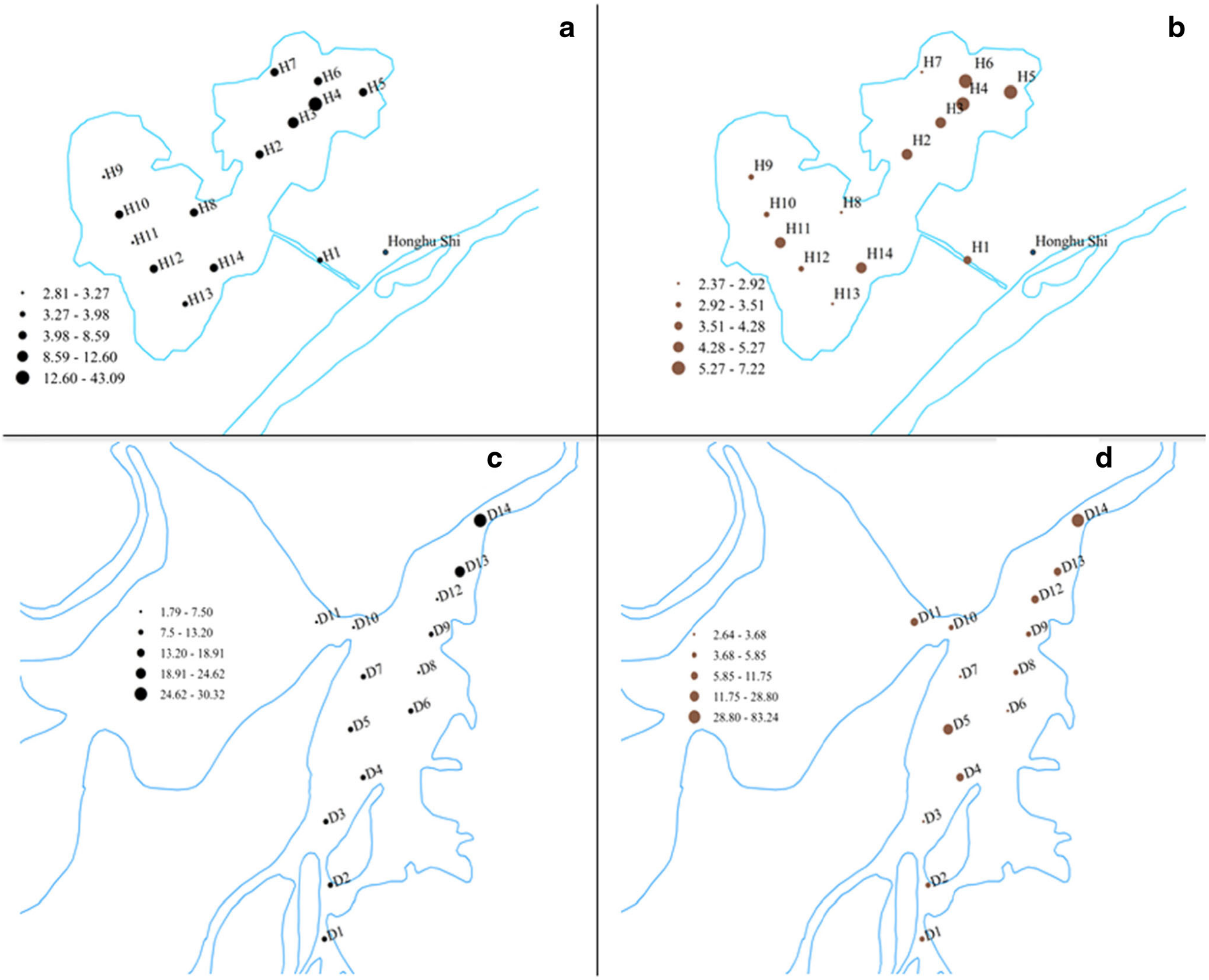


Fig. 5 The spatial distribution of *sul* genes (*sul*1 + *sul*2, ×10^6 copy number per g sediment) in Honghu (**a**) and East Dongting Lake (**c**), *tet* C (×10^4 copy number per g sediment) in sediments of Honghu Lake (**b**), and *tet* genes (*tet*C + *tet*A + *tet*M + *tet*B) (×10^5 copy number per g sediment) in sediments of East Dongting Lake (**d**)

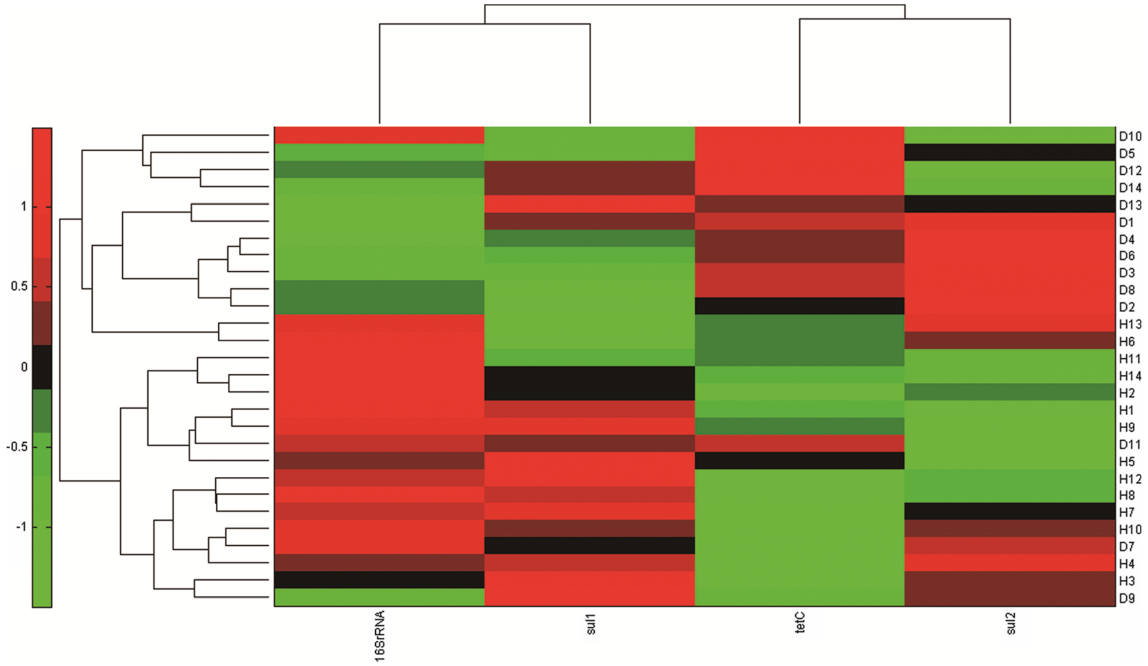


Fig. 6 Heat map constructed for the cluster of 16S rRNA, *sul*1, *sul*2, and *tet*C and sampling sites in East Dongting Lake and Honghu Lake

Tianjin [17]. In East Dongting Lake, *sul*2 also had higher relative abundance ($9.06 \pm 14.80 \times 10^{-5}$ copies/16S rRNA) than *sul*1 ($2.19 \pm 2.42 \times 10^{-5}$ copies/16S rRNA) at $p < 0.05$ level. For *tet* genes in East Dongting Lake, *tetM* had the highest relative abundance ($3.56 \pm 11.10 \times 10^{-6}$ copies/16S rRNA), followed by *tet*B ($2.67 \pm 1.94 \times 10^{-6}$ copies/16S rRNA), *tet*C ($1.79 \pm 3.32 \times 10^{-6}$ copies/16S rRNA), and *tet*A ($1.52 \pm 2.65 \times 10^{-6}$ copies/16S rRNA). In general, all the two *sul* genes were mainly detected in sediments of East Dongting Lake at 10^{-5} copies/16S rRNA level, while the *tet* genes were mostly detected at 10^{-6} copies/16S rRNA level. It is also found that the relative abundances of *sul*1, *sul*2, and *tet*C in East Dongting Lake were also significantly higher than those in Honghu Lake. This phenomenon may be due to the following reasons: firstly, the concentrations of 16S rRNA in Honghu Lake were $9.44 \pm 6.50 \times 10^{11}$ copies per gram sediment, which were significantly higher than those in East Dongting Lake ($2.89 \pm 2.76 \times 10^{11}$ copies per gram sediment). Secondly, there was no significant difference between the absolute concentrations of *sul* genes in East Dongting Lake and Honghu Lake. The *tet*C concentrations in Honghu Lake were significantly lower than those in East Dongting Lake. Thirdly, the human activities around East Dongting Lake were more intense than Honghu Lake, which may discharge more ARGs to ambient environment.

Factors Influencing the Abundance of ARGs

Many factors have been proved to influence the abundance of ARGs, such as antibiotics [23] and 16S rRNA [27]. To avoid the impact of magnitude of tested gene copies, the data was normalized with log function. In Honghu Lake, 16S rRNA had positive correlation relationship with *sul*2 ($r = 0.527$), total concentrations of *sul*1 and *sul*2 ($r = 0.504$), and *tet*C ($r = 0.521$) at $p < 0.1$ level using Pearson correlation analysis (Fig. 3c). No significant correlation relationship was found between the 16S rRNA and *sul*1. In East Dongting Lake, Pearson correlation analysis showed that 16S rRNA in sediments was only found to have a positive correction relationship with *sul*1 ($r = 0.502$) at $p < 0.1$ level (Fig. 3d), while no significant correlation relationship was observed between the 16S rRNA and other tested ARGs in East Dongting Lake. This result indicated that the abundance of background bacteria in sediments may play an important role in the horizontal spread of *sul*2 and *tet*C genes in Honghu Lake and *sul*1 in sediments of East Dongting Lake.

Antibiotic concentration has been found to have correlation with ARGs in Haihe River [23] and with no correlation with ARGs in Beijiang River [40]. In Honghu Lake, *sul*1 ($r = 0.463$) and total concentrations of *sul*1 and *sul*2 ($r = 0.514$) had positive correlation relationship with SMX concentrations at $p < 0.1$ level. *tet*C had positive correlation relationship with OTC ($r = 0.639$) and negative correlation relationship with DC concentrations ($r = -0.560$) at $p < 0.05$ level. While in East Dongting Lake, no correlation relationship was found between the SA concentrations (SD, SMZ, SMX, SME, and total concentrations of SAs) and *sul* genes (*sul*1 and *sul*2), and similar trend was also found for the TETs and *tet* genes. To sum up, SMX and OTC may play a role in the abundance of *sul*1 and *tet*C in sediments of Honghu Lake. Canonical correlation analysis was not suitable to analyze the relationship between the antibiotics and ARGs in this study, so RDA was selected to investigate the relationship. In East

Dongting Lake, Fig. 7a shows that there is no correlation relationship between the tested eight antibiotics and *sul*2 gene. TC, SD, SME, and OTC may be in low correlation with the *sul*1 gene, and similar trend was found between *tetC* gene and CTC and DC. The environmental factor (antibiotics) could explain 62.32 % of total variability of *sul*1, *sul*2, and *tet*C in East Dongting Lake, in which the four SAs, TETs, and the interaction of SAs and TETs accounted for 18.60, 34.80, and 8.92 %, respectively (Fig. 7c). Figure 7b shows that OTC, SD, and SME may have correlation relationship with the *tet*C gene in Honghu Lake and SMX was also found to have a significant correlation with *sul*1 and *sul*2 genes, which confirmed the results of Pearson correlation analysis. Figure 7d shows that the four SAs, TETs, and the interaction of SAs and TETs could explain 27.39, 34.70, and 3.98 % of total variability of *sul*1, *sul*2, and *tet*C in Honghu Lake. These results indicated that TETs may play a more important role than SAs in the abundance of *sul*1, *sul*2, and *tet*C gens in Honghu Lake and East Dongting Lake. *Sul* genes in animal manures and agricultural soils were also positively correlated with TETs, in which TC had a high positive correlation with *sul*A gene at $p < 0.1$ level [41]. The antibiotic-resistant bacteria caused by TETs may carry different kinds of ARGs. *Marinobacter litoralis* and *Stenotrophomonas maltophilia*, isolated from the aquaculture site, harbor both *tet*A and *sul*2 genes [19]. TET-SMZ-resistant bacteria were the most commonly identified combinations of antimicrobial agents in multidrug-resistant isolates [42]. Hence, cross section may be an important reason for the role of TETs on *sul* genes in this study.

Honghu Lake is a closed and Yangtze-isolated lake had less water exchange with Yangtze River, so the retention time of antibiotics may be longer than those in East Dongting Lake (Yangtze-connected lake), so the antibiotic concentration in Honghu Lake was higher than in East Dongting Lake. But, the ARG diversity and abundance in East Dongting Lake were higher than Honghu Lake, due to high input source of ARGs (wastewater treatment plant) and existence of other selective pressures on ARGs. Heavy metals had also important influence on the abundance of ARGs [41, 43]. The ecological risk of heavy metals in sediment of Dongting Lake was higher than that of Honghu Lake [44]. So, the difference in heavy metals may also have impact on the abundance of ARGs in both lakes. Although we give a profile of antibiotics and ARGs in East Dongting Lake and Honghu Lake, still, more work should be done to investigate the biodiversity of antibiotic-resistant bacteria and the spread mechanism of ARGs in lakes.

Conclusion

Honghu Lake and East Dongting Lake suffered high intensity of human disturbance. The most sites in East Dongting Lake

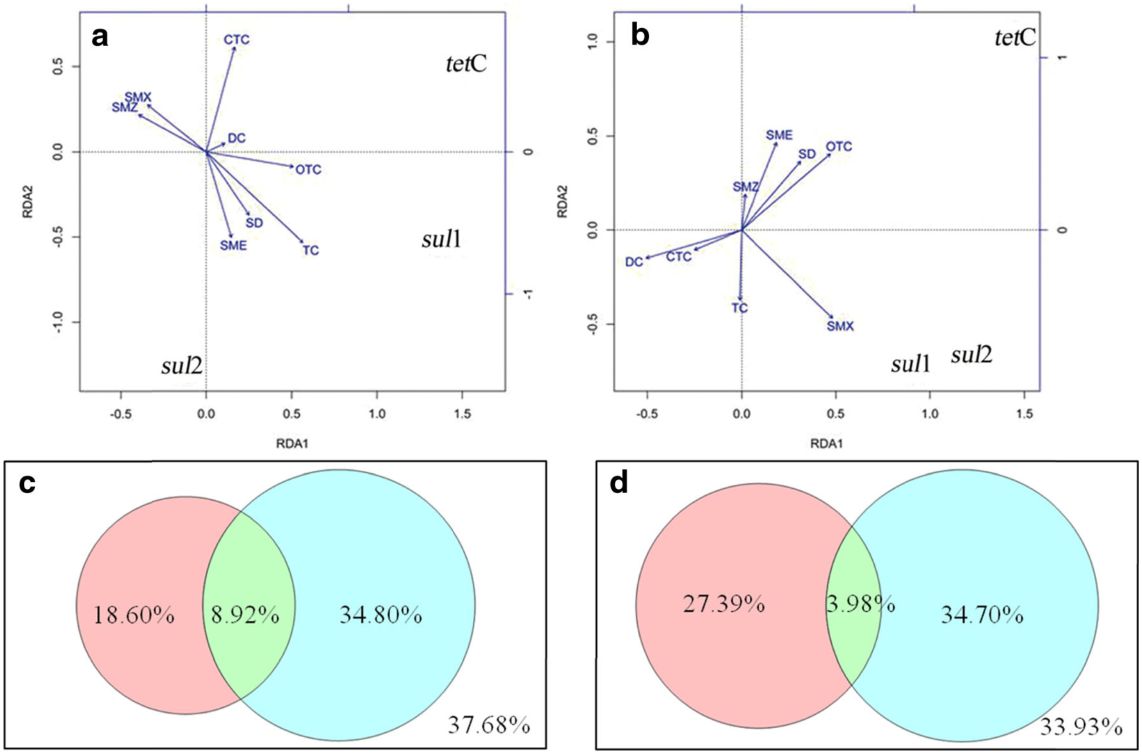


Fig. 7 The redundancy analysis (RDA) between the antibiotics and *sul*1, *sul*2, and *tet*C genes in East Dongting Lake (**a**) and Honghu Lake (**b**) and Venn diagram of four SAs (*brick red*) and four TETs (*light blue*) and their interaction (*light green*) accounted for the total variability of *sul*1, *sul*2, and *tet*C in East Dongting Lake (**c**) and Honghu Lake (**d**) (Color figure online)

had higher total concentrations of SAs compared to total concentrations of TETs, whereas the opposite trend was found in Honghu Lake. TOC may be an important factor influencing the distribution of TC and TETs in both lakes. *sul* genes are the main ARGs compared to *tet* genes in Honghu Lake. SMX and OTC may play a role in the abundance of *sul*1 and *tet*C in sediments of Honghu Lake. The detected gene species and relative abundance in East Dongting Lake were higher than those in Honghu Lake.

Acknowledgments This project was supported in part by the National Natural Science Foundation of China (Nos. 31400113, 41401542), Funding Project of Sino-Africa Joint Research Center, Chinese Academy of Sciences (Y623321K01), Knowledge Innovation Program of the Chinese Academy of Sciences (No. Y455436K02), Youth Innovation Promotion Association of Chinese Academy of Sciences (No. 2015282), and the Hundred Talents Program of the Chinese Academy of Sciences (Y329671K01).

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