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Occurrence and removal of antibiotics and antibiotic resistance genes in natural and constructed riverine wetlands in Beijing, China

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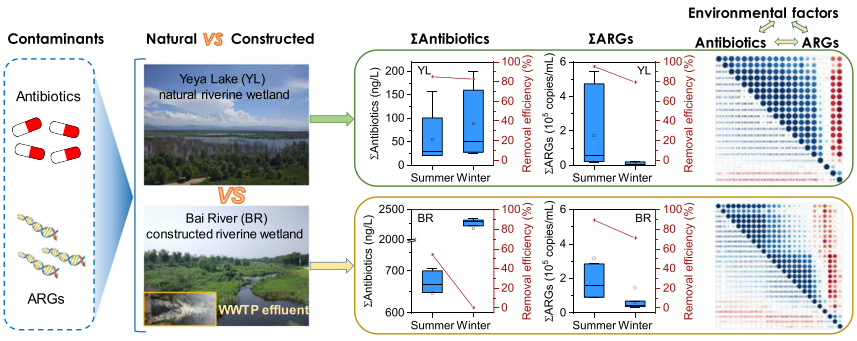
HIGHLIGHTS

- Occurrence/removal of antibiotics and ARGs were compared in two riverine wetlands.
- Wastewater effluent-receiving wetland had higher abundance of antibiotics and ARGs.
- YL wetland achieved better removal of antibiotics and ARGs than BR wetland.
- Seasonal variations in concentrations of antibiotics and ARGs were observed.
- Antibiotics, ARGs and water quality parameters strongly related in natural wetland.

GRAPHICAL ABSTRACT



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ABSTRACT

Simultaneous elimination of antibiotics and antibiotic resistance genes (ARGs) is rarely investigated in full-scale riverine wetlands. Here, we compared the occurrence, abundance, and removal of 60 antibiotics and 27 ARGs in natural (Yeya Lake (YL)) and constructed (Bai River (BR)) riverine wetlands in Beijing, China. The concentrations of antibiotics in YL wetland were ND–51.9 ng/L in water and ND–37.9 ng/g in sediments. Significantly higher concentrations were found in BR wetland (ND–546 ng/L in water and ND–118 ng/g in sediments), which locates at the downstream of a reclaimed water treatment plant. The abundances of ARGs in YL and BR wetlands were up to 5.33×10^5 and 8.41×10^5 copies/mL in water, and 1.60×10^7 and 4.67×10^8 copies/g in sediments, respectively. These results suggest that wastewater greatly contributes to the elevated abundance of antibiotics and ARGs in both water and sediments. Compared to summer, higher levels of antibiotics in water were found in winter due to the higher usage, slower attenuation and the limited dilution. But higher abundances of ARGs were found in summer than in winter, in accordance with the favored microbial growth at higher temperature as denoted by copies of 16S rRNA. Compared to BR wetland, YL wetland achieved better removal of antibiotics and ΣARGs, with average removal efficiencies of 70.0% and 87.5%. Antibiotics, ARGs and environmental factors showed strong correlations in water samples from YL wetland. However, in BR wetland that receives urban wastewater effluents, no correlation between antibiotics and ARGs was found although the distribution of antibiotics was affected by aquatic environmental factors. These results indicate that subinhibitory concentrations of antibiotics may stimulate the prevalence of ARGs in natural wetlands.

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1. Introduction

Antibiotics and antibiotic resistance genes (ARGs) are emerging contaminants (Petrie et al., 2015; Pruden et al., 2006), which have raised increasing attention worldwide. The wide application of antibiotics in human and animals led to their continuous release into environment (Kümmerer, 2009; S. Li et al., 2018b; Q.Q. Zhang et al., 2015b) and the subsequent development and dissemination of ARGs (A. Li et al., 2018; Rodriguez-Mozaz et al., 2015). As a safety barrier against their release, current wastewater treatment plants (WWTPs) cannot fully remove antibiotics (<0–100% removal) (Cai et al., 2018; Le-Minh et al., 2010; Michael et al., 2013) and ARGs (2.0–4.6 logs removal) (Gao et al., 2012; Munir et al., 2011). Consequently, WWTPs are hotspots for the release of antibiotics (Michael et al., 2013) and ARGs (Rizzo et al., 2013). The concentrations of antibiotics and ARGs in WWTP effluents ranged ND–3400 ng/L (Le-Minh et al., 2010; Michael et al., 2013) and ND–5.4 $\times 10^6$ copies/100 mL (Ben et al., 2017; Munir et al., 2011), respectively.

Wetland system offers a viable alternative for removal of antibiotics and ARGs from WWTPs effluent, because of its economic and environmental benefits (Choi et al., 2016; Fang et al., 2017; He et al., 2018a). Constructed wetlands have been successfully applied in removing pharmaceuticals and ARGs (Chen et al., 2016; Li et al., 2014; Verlicchi and Zambello, 2014). Most of the studies focused on optimization of operational parameters (physical configuration, hydraulic loading, vegetation species, etc.) to improve the removal efficiencies in mesocosm-scale constructed wetlands (Chen et al., 2016; He et al., 2018b; Li et al., 2014). Some other studies evaluated the performance of full-scale pond, ditch or lagoon wetland systems as secondary (Anderson et al., 2013; Conkle et al., 2008; Fang et al., 2017) or tertiary treatment facilities (Breitholtz et al., 2012; He et al., 2018a; Matamoros and Salvadó, 2012) for eliminating pharmaceuticals and ARGs. However, little attention is paid to the occurrence and elimination of antibiotics and ARGs in natural and constructed riverine wetland systems associated with stream or river channels.

In addition, simultaneous elimination of antibiotics and ARGs is rarely investigated in full-scale wetlands and inconsistent results were reported. For example, removal of 7 antibiotics and 18 ARGs from domestic sewage was assessed using mesocosm-scale constructed wetlands (Chen et al., 2016), with removal efficiencies of 17.9–98.5% and 50.0–85.8%, respectively. Positive or negative removal of three antibiotics and three ARGs was achieved in three wetlands with different hydraulic loading, but no correlation between antibiotics and ARGs was found (He et al., 2018a). Another recent study revealed that *sul* and *tet* genes were induced in effluent of lab-scale wetlands, although sulfamethoxazole (SMX) and tetracycline (TC) could be efficiently removed (>98%) (Song et al., 2018).

In this study, the occurrence and fate of 60 antibiotics and 27 ARGs were compared in two riverine wetlands in Beijing, China, i.e., Yeya Lake (YL) wetland and Bai River (BR) wetland. YL wetland, the largest Wetland Nature Reserve in Beijing, is a natural riverine wetland, which locates at the upstream of Guanting Reservoir. Instead, BR wetland is a constructed riverine wetland, which receives urban reclaimed water effluent. The objectives were to (1) compare the concentration levels and removal efficiencies of antibiotics in the two riverine wetlands; (2) characterize the abundance and persistence of ARGs in water and sediments; and (3) explore potential linkage between antibiotics and ARGs and evaluate the influence of water/sediment properties.

2. Materials and methods

2.1. Chemicals and reagents

Sixty antibiotic standards from five classes (sulfonamides (SAs), macrolides (MLs), quinolones (QNs), tetracyclines (TCs), and others, Table S1) were purchased from A Chemtek Inc. (Tianjin, China). Four internal standards (sulfamethoxazole-d4 (SMX-d4), roxithromycin-d7 (RTM-d7), ciprofloxacin-d8 (CIP-d8), and tetracycline-d6 (TC-d6)), were purchased from J&K Chemistry (Beijing, China). Other chemicals used in this study are shown in Text S1.

2.2. Study area and sample collection

YL wetland is the largest wetland in Beijing, with an area of 3939 hm^2. It locates in the lower reaches of the Guishui River in Yanqing District, northwest Beijing. It is characterized by mudflats, open water, and marshes, with dominant emergent vegetations of *Phragmites australis*, *Typha orientalis*, and *Zizania latifolia* (Teng et al., 2018). YL wetland mainly receives wastewater from domestic uses in rural area and agricultural non-point source pollution (Teng et al., 2018). There is no specific discharge point of wastewater into YL wetland in the investigated area.

BR wetland locates approximately 50 m downstream of the outlet of Miyun Reclaimed Water Treatment Plant in Miyun District, northeast Beijing. This plant adopts membrane bioreactor process for the treatment of combined industrial (40%) and domestic (60%) wastewater, with a daily sewage treatment capacity of 45,000 m^3. BR wetland is a constructed wetland for purification of the reclaimed water quality, with an area about 21,000 m^2. The dominant plant is *Typha orientalis* (Guo et al., 2015).

Sampling was performed in August 2017 (summer) and Jan 2018 (winter) at 7 sampling sites (YL1-YL7) around YL wetland (Fig. 1a) and 8 sampling sites (BR0-BR7) around BR wetland (Fig. 1b). YL2–YL6 (Fig. 1a) locate at the core area of YL wetland, and effluents from YL wetland flow into Guanting Reservoir (YL 7, Fig. 1a). BR1 (Fig. 1b) is the discharge point of effluents from Miyun Reclaimed Water Treatment Plant into BR, and BR2–BR6 (Fig. 1b) locate in the BR wetland. The effluent (BR7) of BR wetland is mainly used for the Chaobai River landscape and municipal use.

Water and sediment samples for antibiotic analysis were collected as previously described (A. Li et al., 2018). Water and sediment samples for ARGs detection were stored in 4.5 L plastic bottles and 50 mL sterilized polypropylene tubes, respectively. Water samples were stored at 4 °C and pretreated within 24 h. Sediment samples were stored at −80 °C.

2.3. Antibiotics extraction and analysis

Antibiotics in water samples were extracted by a solid-phase extraction (SPE) method (S. Li et al., 2018b; Li et al., 2019) (Text S2). Sediment samples were extracted by ultrasonication for three times followed by SPE (Li et al., 2019) (Text S2). The final extracts were stored at −20 °C until analysis. Target antibiotics were quantified by ultra-high performance liquid chromatography (UHPLC, Dionex UltiMate 3000 Series) in tandem with a TSQ Endura triple quadrupole mass spectrometer (MS, Thermo Scientific, USA). The mobile phase consisted of methanol and Milli-Q water with 0.1% (v/v) formic acid. Due to the differences in the properties of various classes of antibiotics, chromatographic separation of the analyses was conducted with a Syncronis C18 column (100 mm × 2.1 mm, 1.7 μm, Thermo, USA) for SAs, MLs and others, and an ACQUITY BEH C18 column (100 mm × 2.1 mm, 1.7 μm, Waters, USA) for QNs and TCs. The temperature of the columns was maintained at 40 °C. UHPLC and MS analytical conditions are presented in Tables S2 and S3.

2.4. DNA extraction and ARG quantification

2.4.1. DNA extraction and polymerase chain reaction (PCR) assays

Water samples (1.0 L) were filtered through polycarbonate membranes (0.22 μm, GTTP, Millipore, Ireland). The membranes and sediments were sealed in 50 mL sterilized polypropylene centrifuge tubes and stored at −80 °C. DNA was extracted from the frozen membranes and sediments in duplicate using the FastDNA® SPIN Kit for Soil (MP Biomedicals, USA) following the manufacture's protocol.

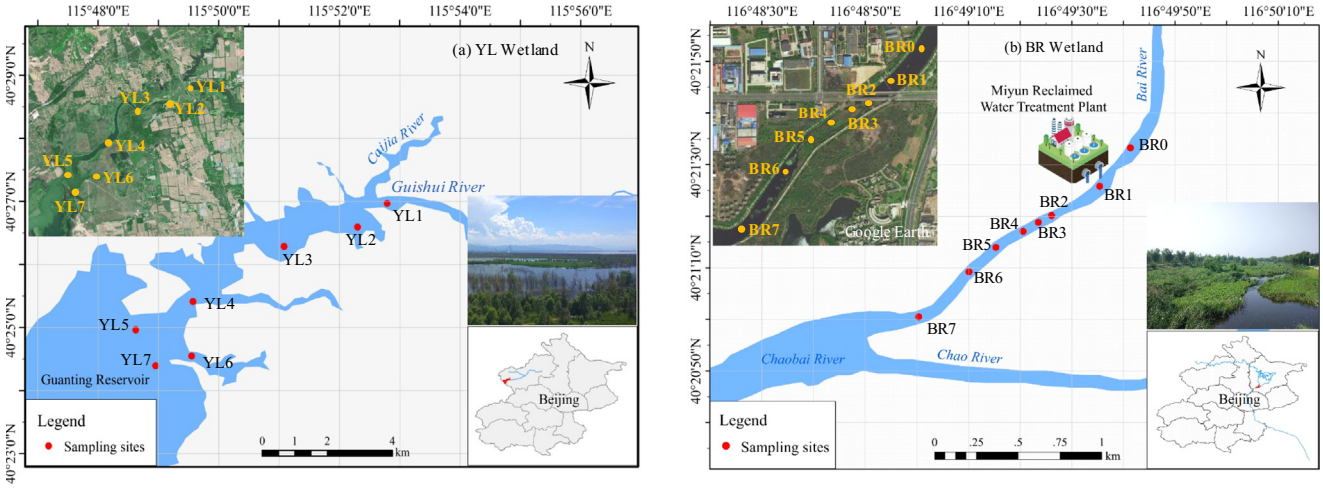

Fig. 1. Location of sampling sites in YL wetland (a) and BR wetland (b).

Concentrations of DNA were determined by spectrophotometry (NanoDrop ND-1000, Thermo Fisher, USA). The 16S rRNA gene, two integrase genes (*intI*1 and *intI*2), and 27 ARGs (Table S4) were analyzed as previously described (A. Li et al., 2018).

2.4.2. Real-time quantitative PCR (qPCR)

The abundances of 16S rRNA gene, *intI*1, and 9 frequently detected ARGs (*sul*I, *sul*II, *tet*A, *tet*C, *dfr*A1, *dfr*A12, *dfr*A13, *erm*B, and bla_{PSE-1}) were determined in triplicate using ABI 7300 qPCR (Applied Biosystems, USA). Sequence of primers and qPCR conditions are listed in Table S5. Detailed qPCR procedure is shown in Text S3.

2.5. Analysis of water/sediment properties

Water temperature (T), pH, and conductivity were measured in situ at each sampling site. Water quality parameters including total organic carbon (TOC), ammonium nitrogen (NH_4-N), nitrite nitrogen (NO_2-N), nitrate nitrogen (NO_3-N), total nitrogen (TN), and total phosphorus (TP) (Table S6) and sediment quality parameters including NH_4-N, NO_2-N, NO_3-N, and sediment organic carbon (SOC) (Table S7) were analyzed according to the national standard methods of China.

2.6. Data analysis

The venn diagrams, heatmaps, spearman correlation analysis and redundancy analysis (RDA) were performed by using R software (v3.3.1, R Foundation for Statistical Computing, Vienna, Austria). Significant differences in concentrations and removal efficiency of antibiotics were evaluated by two-sample *t*-test using OriginPro 2018 (Academic version). The removal efficiency of antibiotics/ARGs was calculated according to Eq. (1).

$$\text{Removal efficiency}\ (\%) = \frac{C_{in} - C_{eff}}{C_{in}} \times 100 \quad (1)$$

where C_{in} and C_{eff} are the concentrations of antibiotics/ARGs in influent (YL1 and BR1, respectively) and effluent (YL7 and BR7, respectively) of YL and BR wetlands. A half of LOD was used with concentrations below LOD.

3. Results and discussion

3.1. Occurrence and distribution of antibiotics in water and sediments

A total of 43 (71.7%) and 35 (58.3%) out of 60 antibiotics were detected at least once in water and sediment samples, respectively, from the two wetlands. Compared to YL wetland, more antibiotics were observed in BR wetland in water (Fig. S1a) and sediments (Fig. S1b), reflecting the influence of urban reclaimed water effluent and upstream residents' lives. The two wetlands shared 12 antibiotics in all water and sediment samples, including 3 SAs (sulfadiazine (SDZ), SMX, and trimethoprim (TMP)), 5 MLs (anhydroerythromycin (AETM), azithromycin (AZM), erythromycin (ETM), clarithromycin (CTM), and roxithromycin (RTM)), 2 QNs (flumequine (FLU) and nalidixic acid (NDA)), 1 TCs (doxycycline (DC)), and 1 others (clindamycin (CDM)). Their detection frequencies in water and sediments were 14.3–100% (Fig. S2). The prevalence of these antibiotics reflects their extensive use in bacterial infection treatment (Carvalho and Santos, 2016; Q.Q. Zhang et al., 2015b) and environmental persistence (S. Li et al., 2018b; McArdell et al., 2003).

Concentrations of antibiotics were compared between the two wetlands in water (Fig. 2) and sediments (Fig. S3). They were ND–51.9 ng/L (water) and ND–37.9 ng/g (sediment) in YL wetland, and ND–546 ng/L (water) and ND–118 ng/g (sediment) in BR wetland. Compared to YL wetland (21.0–199 ng/L), the total concentrations of antibiotics in water samples from BR wetland (323–2333 ng/L) were >1 order of magnitude higher, because BR wetland receives effluents from Miyun Reclaimed Water Treatment Plant (BR1, Fig. 1). In sediments, higher total concentrations of antibiotics were also observed in BR wetland (17.3–196 ng/g) than those in YL wetland (0.84–67.4 ng/g) (Fig. S3).

Seasonal variability of antibiotics in water was observed in both wetlands (Fig. S4a and b). In YL wetland, MLs, QNs, and others in water showed significantly higher concentrations in winter than those in summer ($p < 0.05$, Fig. S4a). In BR wetland, all classes of antibiotics in water had significant seasonal variation except TCs ($p < 0.01$, Fig. S4b). The higher levels of antibiotics in winter could be resulted from the higher human usage and slower attenuation in wetlands in cold season (water temperature -0.5–13.5 °C, Table S6), as well as limited dilution at low flow conditions (S. Li et al., 2018b; H. Zhang et al., 2015a). However, comparable concentrations of antibiotics were measured in summer and winter ($p > 0.05$) in sediments in each wetland (Fig. S4c and d).

Among different classes of antibiotics, SAs generally dominated in water in both seasons in YL wetland and BR wetland, accounting for 39.8–65.7% of the total concentrations (Figs. 2 and S5a). The persistence of SAs in water was resulted from their resistance to hydrolysis, photolysis and biodegradation (Huang et al., 2001). QNs were the second dominant group of antibiotics (10.9–30.0%), followed by MLs (7.17–20.3%). The proportions of QNs and MLs were higher in winter than those in summer in both wetlands, suggesting their increased consumption in winter. In terms of individuals, SMX presented the highest concentration in most of the sampling sites from both wetlands. The concentrations of SMX were 5.94–51.9 ng/L in YL wetland and 130–546 ng/L in

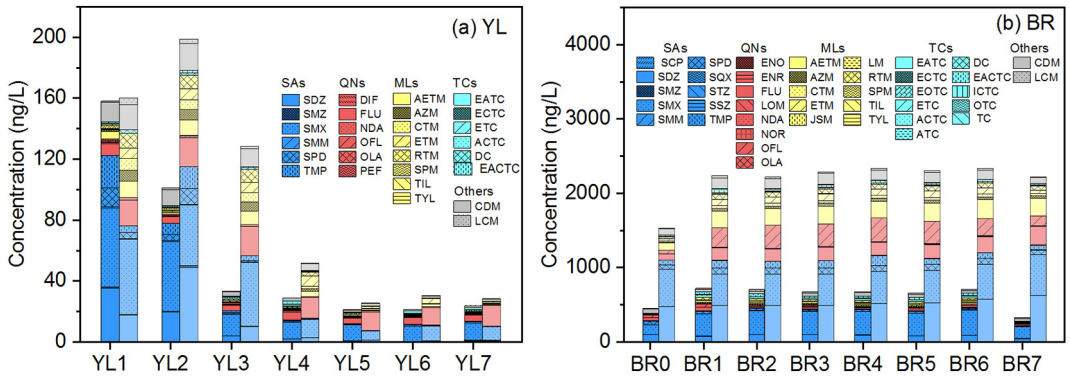

Fig. 2. Concentrations of antibiotics in water from YL wetland (a) and BR wetland (b) in summer (left column) and winter (right column).

BR wetland, accounting for 20.0–48.2% and 18.3–50.2%, respectively, of the total levels of antibiotics. A comparable level of SMX was reported in Guanting Reservoir (ND–44.4 ng/L) located at the downstream of YL wetland (Zhang et al., 2018). The levels of SMX in BR wetland were comparable to those reported in wetlands treating WWTPs effluents (49–362 ng/L, Table S8) (Breitholtz et al., 2012; He et al., 2018a).

TCs and SAs dominated in sediments of YL wetland, with average ratios of 30.1–38.2% and 28.8–36.2%, respectively (Fig. S5b). SAs were the most abundant class (55.2–69.9%) in sediments of BR wetland (Fig. S5b). The enrichment of TCs in sediments could be resulted from cation bridging and cation exchange (Luo et al., 2011). The dominance of SAs in sediments is probably resulted from their dramatically high concentrations in water, which facilitates their partition to sediments. SMX was the predominant antibiotic in sediments from YL wetland (Fig. S3), ranging from ND to 37.9 ng/g (mean 6.98 ng/g). It was consistent with those reported in Guanting Reservoir (ND–24.6 ng/g, mean 9.9 ng/g) (Zhang et al., 2018). SMX was also the dominant component in sediments (mean 34.9 ng/g) from BR wetland, with an average ratio of 40.3%.

Sediment-water distribution coefficients (K_d) of antibiotics were calculated when they were detected in >50% of both water and sediment samples (S. Li et al., 2018b; Liang et al., 2013). The K_d values were 0.73–5128 L/kg (YL wetland) and 0.09–3757 L/kg (BR wetland) (Table S9). They were comparable to those found in China's major rivers (S. Li et al., 2018b). The organic carbon normalized distribution coefficients ($\log K_{oc}$) were 2.10–5.36 and 0.60–5.12 in YL wetland and BR wetland, respectively. A positive relationship was found between $\log K_{oc}$ and $\log K_{ow}$ for 5 MLs (AETM, AZM, ETM, CTM, and RTM) and CDM in YL wetland ($R^2 = 0.86$) (Fig. S6). But no correlation between $\log K_{oc}$ and $\log K_{ow}$ was found in BR wetland, probably due to the channel lining of the Bai River, which affects the partition of antibiotics between water and sediments.

3.2. Abundance of ARGs in water and sediments

Of the 27 ARGs tested by PCR, 14 ARGs, including 2 sulfonamide resistance genes (*sul*I, *sul*II), 3 trimethoprim resistance genes (*dfr*A1, *dfr*A12, *dfr*A13), 2 tetracycline resistance genes (*tet*A, *tet*C), 1 macrolide resistance gene (*erm*B), 2 chloramphenicols resistance genes (*cat*II, *flo*R), 1 aminoglycosides resistance gene (*aac (3)*-IV), 1 glycopeptides resistance gene (*van*A), and 2 β-lactams resistance genes (bla_{SHV}, bla_{PSE-1}), were positively detected in water and sediments from the two wetlands (Table S4). Compared to YL wetland (8 ARGs in water, 5 ARGs in sediments), more ARGs were detected in BR wetland (12 ARGs in water, 12 ARGs in sediments) (Fig. S7). In different seasons, the numbers of ARGs detected in summer (8 ARGs in BR wetland, 3 ARGs in YL wetland) were comparable to those in winter (7 ARGs in BR wetland, 3 ARGs in YL wetland).

A total of 9 ARGs were successfully quantified by qPCR in water and sediments from the two wetlands (Table S10). Among them, *sul*II gene was detected in all water and sediment samples (100%), *sul*I, *dfr*A1, *dfr*A12, *tet*A, and *tet*C had the higher detection frequencies (46.7–76.7% in water and 53.3–76.7% in sediments), and *dfr*A13 gene had the lowest detection frequency (26.7% in both water and sediments). The prevalence of sulfonamide, trimethoprim, and tetracycline resistance genes were also observed in rivers (Xu et al., 2016), swine feedlot (Wang et al., 2016), and urban dust (Zhou et al., 2018) in Beijing and other wetlands (Table S11) (J. Chen et al., 2015) and rivers (Jiang et al., 2013; Luo et al., 2010; Ouyang et al., 2015) in China. The wide distribution of *sul*II gene in the environment was due to the fact that it usually exists on small non-conjugative or large transmissible multi-resistant plasmids (Enne et al., 2001; Sköld, 2000).

The absolute abundances of ARGs in water and sediments from YL wetland and BR wetland are shown in Fig. 3 and Table S12. They were ND–5.33×10^5 copies/mL and ND–8.41×10^5 copies/mL in water from YL wetland and BR wetland, respectively, which were comparable to those in full-scale wetlands (Table S11) (J. Chen et al., 2015; He et al., 2018a). In sediments, the abundances of ARGs in BR wetland (up to 4.67×10^8 copies/g) were ten times higher than in sediments of YL wetland (up to 1.60×10^7 copies/g). This indicates that WWTPs effluent greatly contributes to the enrichment of ARGs in sediments (Czekalski et al., 2014). Compared to water samples, the levels of ARGs in sediments were 2–3 orders of magnitude higher, suggesting that sediments might be more conducive to the storage of ARGs (Luo et al., 2010; Xu et al., 2016). The levels of ARGs in both wetlands were lower than those in the urban rivers in Beijing receiving hospital and domestic wastewater (7.0×10^1–5.9×10^6 copies/mL in water and 4.2×10^2–2.0×10^8 copies/g in sediment) (Xu et al., 2016) and other rivers in China such as Pearl River Estuary (10^2–10^7 copies/mL in water and 10^5–10^{10} copies/g in sediments) (B. Chen et al., 2015) and Jiulongjiang River (9.72×10^7–1.03×10^8 copies/mL) (Ouyang et al., 2015).

In contrast to the seasonal variation pattern of antibiotics (Fig. S4), higher abundances of most ARGs were found in summer than in winter in both water and sediment samples (Fig. 3 and Table S12). This is mainly ascribed to the higher contents of 16S rRNA gene in summer than in winter (Table S12). The 16S rRNA was positively related to microbial biomass, which was favored in warmer seasons (A. Li et al., 2018; Luo et al., 2010).

The *sul*II gene was predominant in YL wetland in summer (Fig. 3a and b), with the absolute abundances of 1.49×10^4–5.33×10^5 copies/mL in water and 2.07×10^6–1.60×10^7 copies/g in sediments (Table S12). In winter, *dfr*A1 and *sul*II genes dominated in water samples (Fig. 3a), while *tet*C and *sul*II genes dominated in sediment samples from YL wetland (Fig. 3b). The abundance of *sul*II gene in YL wetland was comparable to those in water of the Huangpu River (4.3×10^4–4.2×10^5 copies/mL) (Jiang et al., 2013) and lower than those in sediments of the Haihe River ($1.7 \pm 0.2 \times 10^{11}$ copies/g) (Luo et al., 2010). The findings in this study are in line with previous studies where *sul*I and *sul*II genes were the most abundant ARGs in full-scale constructed wetlands (Anderson et al., 2013; J. Chen et al., 2015; He

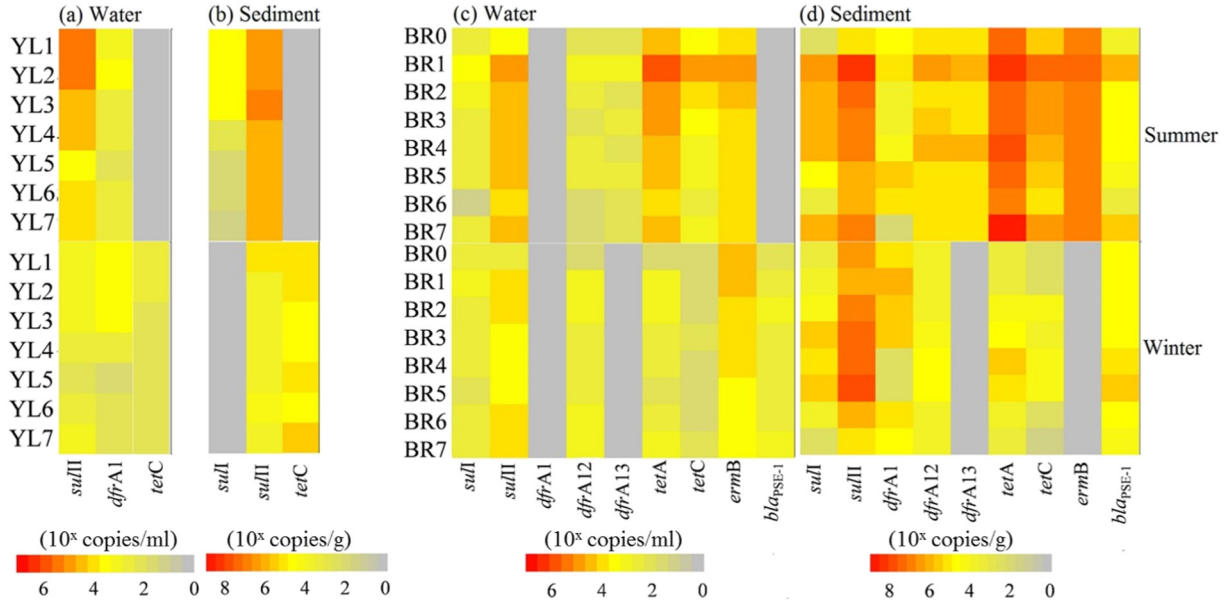

Fig. 3. The abundance of ARGs in water and sediments from YL wetland (a, b) and BR wetland (c, d).

et al., 2018a) and rivers (A. Li et al., 2018; Luo et al., 2010). The predominance of *sul* genes may be associated with the higher concentrations of SAs than the other antibiotics (Luo et al., 2010).

In summer, BR wetland exhibited high *tet*A contents in water (3.00×10^4–8.41×10^5 copies/mL) and sediments (1.93×10^7–4.67×10^8 copies/g) (Fig. 3c, d and Table S12), which were higher than those in the Taihu Lake (10^4–10^5 copies/mL in water) (Zhang et al., 2009) and Pearl River Estuary (10^6–10^7 copies/g) (A. Li et al., 2018). The *sul*II was the second abundant gene in water and sediments of BR wetland in summer. In winter, *erm*B and *sul*II dominated in water and sediment samples. The increased contents of *erm*B in winter in water samples from BR wetland was likely related to the significantly higher concentrations of MLs in winter (Fig. S4b).

Similar to the present study, seasonal differences in ARGs concentrations and compositions were also observed in constructed wetlands (Fang et al., 2017) and rivers (Chen et al., 2015; A. Li et al., 2018). This may result from the changes in antibiotic levels in the environment, ARGs from source water, microbial compositions as well as vegetation types (Fang et al., 2017; Yang et al., 2016; Yang et al., 2013).

3.3. Removal of antibiotics and ARGs in water

3.3.1. Removal of antibiotics

The average removal efficiencies of detected antibiotics were 70.0% and 45.7% in YL wetland and BR wetland, respectively (Fig. 4), indicating antibiotics could be efficiently removed in wetlands (Table S8) (J. Chen et al., 2015; He et al., 2018a; Song et al., 2018; Vymazal et al., 2017). Photodegradation, biodegradation, sediment adsorption, and plant uptake may contribute to the aqueous removal of antibiotics in wetlands (Choi et al., 2016; Song et al., 2018). Compared to BR wetland, YL wetland showed significantly higher removal efficiencies of SAs, MLs, and others ($p < 0.01$, Fig. 4), possibly due to its much lower concentration of antibiotics and favored dilution because of its larger scale. In addition, the removal efficiencies of ΣAntibiotics were 85.0% (summer) and 82.5% (winter) in YL wetland, and 54.2% (summer) and 0.30% (winter) in BR wetland. The poor performance of BR wetland in winter may be resulted from the increase in the antibiotic concentration as well as the death of plants. It was reported that plants can not only uptake antibiotics directly, but also promote microbial degradation of antibiotics through offering attachment sites on their extended roots and supplying carbon and oxygen for the growth of microbes (Choi et al., 2016; He et al., 2018b).

Environmental risks of antibiotics in effluents of YL and BR wetlands were assessed based on the risk quotient (RQ) method (Text S4). Most antibiotics posed insignificant risk (RQ < 0.01) to green algae, daphnid, and fish in both wetlands (Fig. S8). But SDZ, SMX, and AETM posed medium risk (0.1 < RQ < 1) to daphnid in effluents of BR wetland in winter, suggesting the need of upgrading and reconstruction of the Miyun Reclaimed Water Treatment Plant and BR wetland, for a better removal of these antibiotics.

3.3.2. Removal of ARGs

In YL wetland, the concentrations of ΣARGs in water decreased significantly along the stream, from 4.73×10^5 copies/mL to 2.18×10^4 copies/mL in summer (Fig. 5a) and from 2.01×10^4 copies/mL to 4.11×10^3 copies/mL in winter (Fig. 5b), with removal efficiencies of 95.4% and 79.5% in summer and winter, respectively. In BR wetland, the concentrations of ΣARGs in effluents declined by 89.2% (from 1.24×10^6 copies/mL to 1.34×10^5 copies/mL, Fig. 5c) in summer and 71.1% (from 1.45×10^5 copies/mL to 4.21×10^4 copies/mL, Fig. 5d) in winter, respectively. This suggests that the wetland systems could efficiently remove ARGs in water (Table S11) (J. Chen et al., 2015; He et al., 2018a). Attachment of the host bacteria of ARGs on sediments or plants is likely

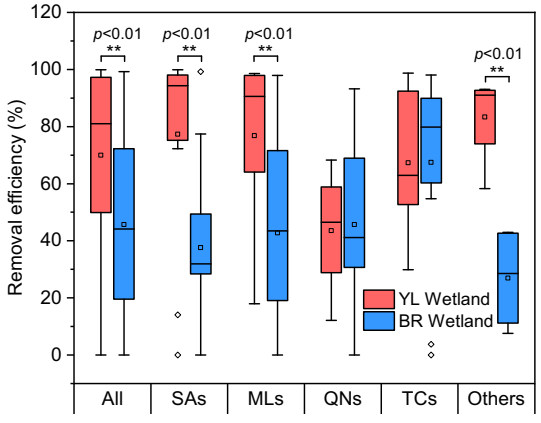

Fig. 4. Aqueous removal efficiencies of antibiotics in YL wetland and BR wetland.

responsible for ARGs elimination in the wetland systems (Chen et al., 2016; Fang et al., 2017). However, the removal mechanisms of ARGs are complicated and further studies are needed.

In different seasons, the removal efficiencies of ΣARGs in water were higher in summer than those in winter, although higher abundances of ΣARGs were observed in summer. Compared to BR wetland (80.2%), YL wetland had higher average removal efficiency (87.5%), which might mainly result from its large spatial scale and lower abundance of ΣARGs (Chen et al., 2013). In the wetland systems, the abundance of ARGs in sediments did not show obvious spatial variation trend (Fig. S9).

3.4. Relations of ARGs with antibiotics and water/sediment quality parameters

The spearman correlation and RDA of genes, antibiotics, and environmental factors are presented in Figs. 6, S10, and S11. Significant correlations ($\rho > 0.6$) among most antibiotics (QNs, MLs, SAs, and ΣAntibiotics) except TCs, genes (*sul*II, ΣARGs, *dfr*A1, and 16S rRNA), and environmental factors (TP, NH_4-N, NO_3-N, NO_2-N, and TN) were observed in water of YL wetland (Fig. 6a and b). These results were corroborated by RDA analysis (Fig. S11a and b), indicating that antibiotics and ARGs may have similar pollution sources (Chen et al., 2013; Xu et al., 2016) and antibiotics may exert selective pressure on ARGs when they are at subinhibitory levels (He et al., 2014; Luo et al., 2010).

In BR wetland, antibiotics (TCs, ΣAntibiotics, MLs, and SAs) and environmental factors (Cond, NO_3-N, TN, TOC, and TP) showed strong correlations ($\rho > 0.6$), while the coexistence of genes (*tet*C, *tet*A, ΣARGs, *dfr*A13, *dfr*A12, *int*I1, *erm*B, and *sul*II) ($\rho > 0.6$) was observed in water samples in both summer and winter (Fig. 6c and d). RDA analysis confirmed that the distribution of antibiotics was strongly affected by environmental factors (Fig. S11e and f), as reported in previous reports (S. Li et al., 2018a; Wang et al., 2015). The lack of correlation between ARGs and antibiotics may result from several reasons: (1) abundant ARGs originated from wastewater source, which were not related to antibiotic content in wastewater (He et al., 2018a); (2) the high levels of antibiotics in BR wetland (total concentrations 323–2333 ng/L) may be lethal to resistant cells (Pei et al., 2006); and (3) because of the limited scale of BR wetland, spatial variation in antibiotic concentrations was insignificant and it may lack enough time for the development of ARGs induced by antibiotics.

In sediments, seasonality correlations were observed (Fig. S10). In summer, *int*I1, 16S rRNA, *sul*II, and ΣARGs had positive correlations ($\rho > 0.6$) in YL wetland (Fig. S10a), indicating background bacteria may facilitate the spread of *sul*II in sediments (Yang et al., 2016). In addition, antibiotics (QNs, MLs, SAs, and ΣAntibiotics) and environmental factors (NH_4-N, NO_3-N, NO_2-N, and SOC) well correlated with each other (Figs. S10a and Fig. S11c) (S. Li et al., 2018a). In winter, the correlation between antibiotics, ARGs, and environmental factors in sediments was not strong in YL wetland (Figs. S10b and S11d).

In the sediments of BR wetland, antibiotics (TCs, QNs, and ΣAntibiotics) and genes (*int*I1, *dfr*A13, *dfr*A12, *erm*B, *sul*II, *sul*I, and *tet*C) exhibited significant correlations ($\rho > 0.6$) in summer (Fig. S10c), and QNs, *int*I1, 16S rRNA, and ARGs (*tet*A, bla_{PSE-1}, *sul*I, *sul*II, ΣARGs, and *tet*C) correlated with each other ($\rho > 0.6$) in winter (Fig. S10d). The RDA analysis further confirmed that most of the ARGs were grouped together and they were only affected by some antibiotics (Fig. S11g and h). The correlation between ARGs and *int*I1 suggests that *int*I1 may contribute to the propagation and spread of ARGs in the environment (Luo et al., 2010; Xu et al., 2016).

4. Conclusion

The performance of natural YL wetland and constructed BR wetland in attenuating antibiotics and ARGs was assessed in summer and winter. The concentrations of antibiotics and ARGs were up to 51.9 ng/L and 5.33×10^5 copies/mL in water, and 37.9 ng/g and 1.60×10^7 copies/g in sediments from YL wetland. Wastewater effluents greatly contributed to the enhanced concentrations of antibiotics and ARGs in BR wetland, which were up to 546 ng/L and 8.41×10^5 copies/mL in water, and 118 ng/g and 4.67×10^8 copies/g in sediments, respectively. The average removal efficiencies of antibiotics (45.7%) and ARGs (80.2%) in BR wetland were lower than those in YL wetland (antibiotics (70.0%) and ARGs (87.5%)), due to their dramatically higher concentrations in BR wetland and its smaller scale. Significantly higher concentrations of antibiotics in water were observed in winter than in summer ($p < 0.05$), resulting from differences in antibiotic usage, water flow and attenuation rates. However, more abundant ARGs were detected in summer

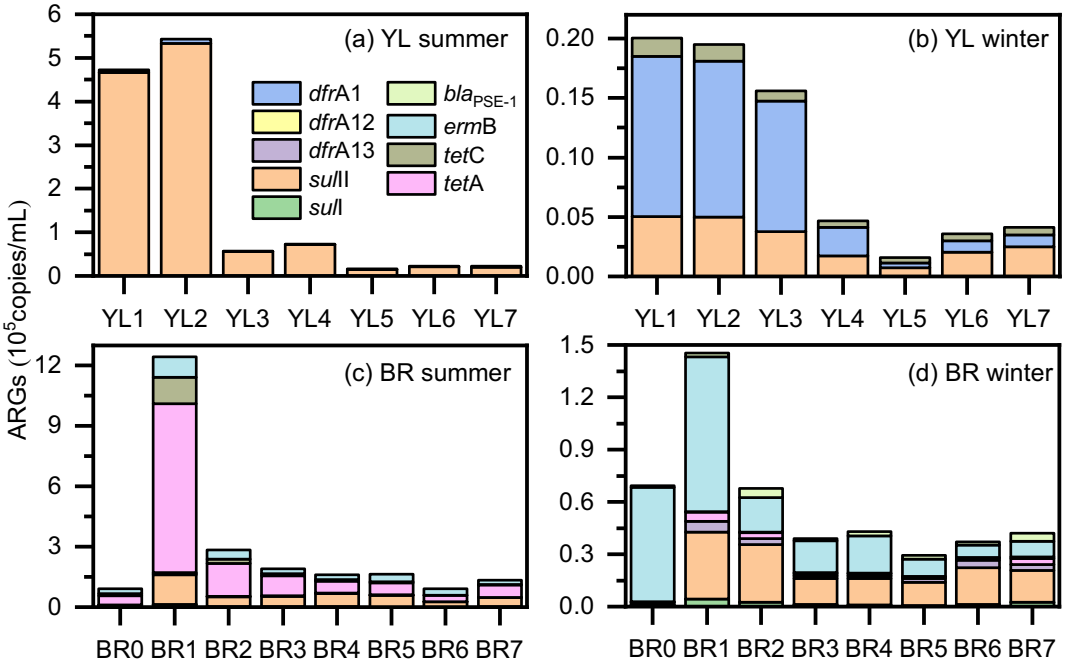

Fig. 5. The spatial variation of ARGs in water samples from YL wetland (a, b) and BR wetland (c, d).

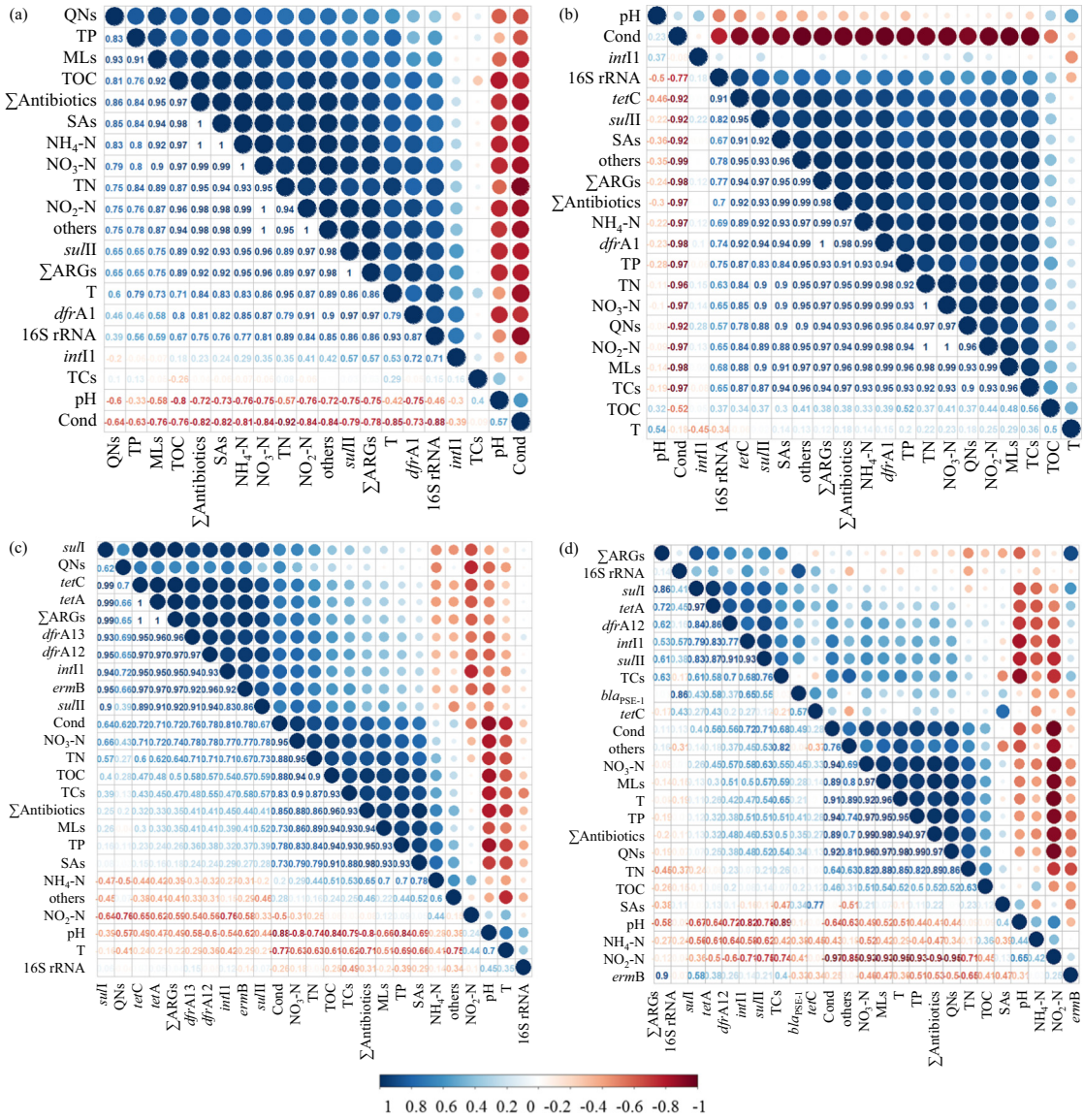

Fig. 6. The spearman correlation of genes, antibiotics, and environmental factors in water from YL (a - summer and b - winter) and BR (c - summer and d - winter) wetlands.

than in winter, possibly due to the favored growth of bacteria at higher temperature in summer. The spearman correlation and RDA analysis revealed that ARGs were correlated with antibiotics and environmental parameters in water samples from YL wetland; however, the coexistence was only observed between antibiotics and environmental parameters in BR wetland. These results suggest the role of antibiotics in exerting selective pressure for ARGs in natural wetlands when they are at subinhibitory levels.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.02.043.

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