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Influencing factors of antibiotic resistance genes removal in constructed wetlands: A meta-analysis assisted by multivariate statistical methods

Ling Zhang [a,b], Changzhou Yan [a,*], Ce Wen [a,b], Ziyue Yu [a,b]

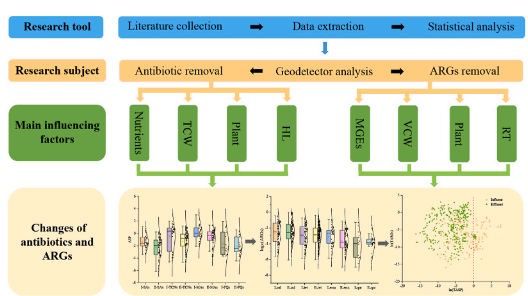
[a] *Key Laboratory of Urban Environment and Health, Fujian Key Laboratory of Watershed Ecology, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen, 361021, China*

[b] *University of Chinese Academy of Sciences, Beijing, 100049, China*

HIGHLIGHTS

- A framework is proposed to analyze the influencing factors of ARGs removal.
- Nutrients, plant and hydraulic load dominate majority antibiotics removal.
- MGEs, plant and running time dominate majority ARGs removal.
- The relative abundance of ARGs did not decrease significantly in effluent.

GRAPHICAL ABSTRACT



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ABSTRACT

In order to control antibiotic resistance genes (ARGs) diffusion in constructed wetlands, it is critical to explore the main factors influencing ARGs removal and understand its mechanism. Despite the fact that numerous studies have been conducted to determine the factors influencing ARGs removal by constructed wetlands in recent years, attempts to use published data and incorporate them into a comprehensive comparison and analysis are still limited. A framework for literature collection, data extraction and statistical analysis (LDS) was constructed in this study. The main factors influencing antibiotics and ARGs removal by constructed wetlands were identified using this framework. The results showed that nutrients, types of constructed wetlands and hydraulic loading were the principal factors influencing the removal of most antibiotics. The principal factors influencing the most ARGs removal were mobile genetic elements, plants, volume of constructed wetlands and running time. After purification by constructed wetlands, the risk coefficient of antibiotics decreased significantly, while the relative abundance of most ARGs did not change significantly. The analysis results of linear mixed model showed that the relationship between antibiotics and ARGs in effluent was closer than that in influent. LDS framework provides a new platform for the study of influencing factors of pollutant removal based on data mining.

* Corresponding author.
E-mail address: czyan@iue.ac.cn (C. Yan).

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1. Introduction

The overuse of antibiotics will exert selective pressure on bacteria carrying antibiotic resistance genes (ARGs), resulting in ARGs diffusion (Liang et al., 2018a). ARGs have the characteristics of persistence and rapid diffusion in the environment as an emergent pollutant (D. Zhu et al., 2019). With the continuous advancement of economic globalization, the globalization of ARGs pollution has also initially appeared. Its rapid spread poses a serious threat to public health around the world (Hu et al., 2020). Many academics have focused on developing efficient ARGs removal processes in the past years.

In recent years, with the wide application of constructed wetlands in the treatment of industrial wastewater, domestic wastewater and aquaculture wastewater (Chen et al., 2016b; Liu et al., 2019; Vymazal, 2011), the ARGs removal by constructed wetlands has attracted the attention of many researchers. Some researchers were committed to optimizing the operating conditions of constructed wetlands in order to improve the removal efficiency of ARGs (Chen et al., 2016a; Huang et al., 2017; Song et al., 2018a). Other researchers attempted to reveal the mechanism of specific influencing factors on ARGs removal by constructed wetland (Li et al., 2019; Song et al., 2018b; Zhang et al., 2022b). However, these studies have no uniform conclusion due to the differences in climate, type of constructed wetland, and scale. Researchers are puzzled over these results, which seemed contradictory to each other. Therefore, up to now, it was difficulty to illustrate the influence of antibiotics on ARGs removal and effectively improve the removal efficiency of ARGs in constructed wetlands. If the relevant data of previous researches can be incorporated into a unified model for analysis, the problems caused by the differences in operating conditions may be eliminated.

In recent years, relevant studies based on transcontinental scale have generally been conducted as meta-analysis, such as the study on the influencing factors of pollutant distribution in reservoirs and soil nitrogen cycle (Guo et al., 2021; Liu et al., 2018, 2022). These studies provide good reference works for solving the current dilemma. How to mine and statistically analyze the data in the current literature is the core of studying the influencing factors of antibiotic and ARGs removal by constructed wetlands. The removal of antibiotic and ARGs by constructed wetland involves many influencing factors, the selection of suitable analysis methods is very important to this study. Because the factors influencing antibiotics and ARGs removal include continuous variables (nutrients, operation time, hydraulic load and temperature, etc.) and classification variables (plant species and constructed wetland types, etc.), the coexistence of different types of variables limits the selection of analysis methods. Geodetector is a statistical method to reveal the driving force of influencing factors, which can analyze both classification variables and continuous variables (appropriate discretization is required). Therefore, Geodetector is an effective method to quantify the contribution of various influencing factors (Wang et al., 2010, 2016).

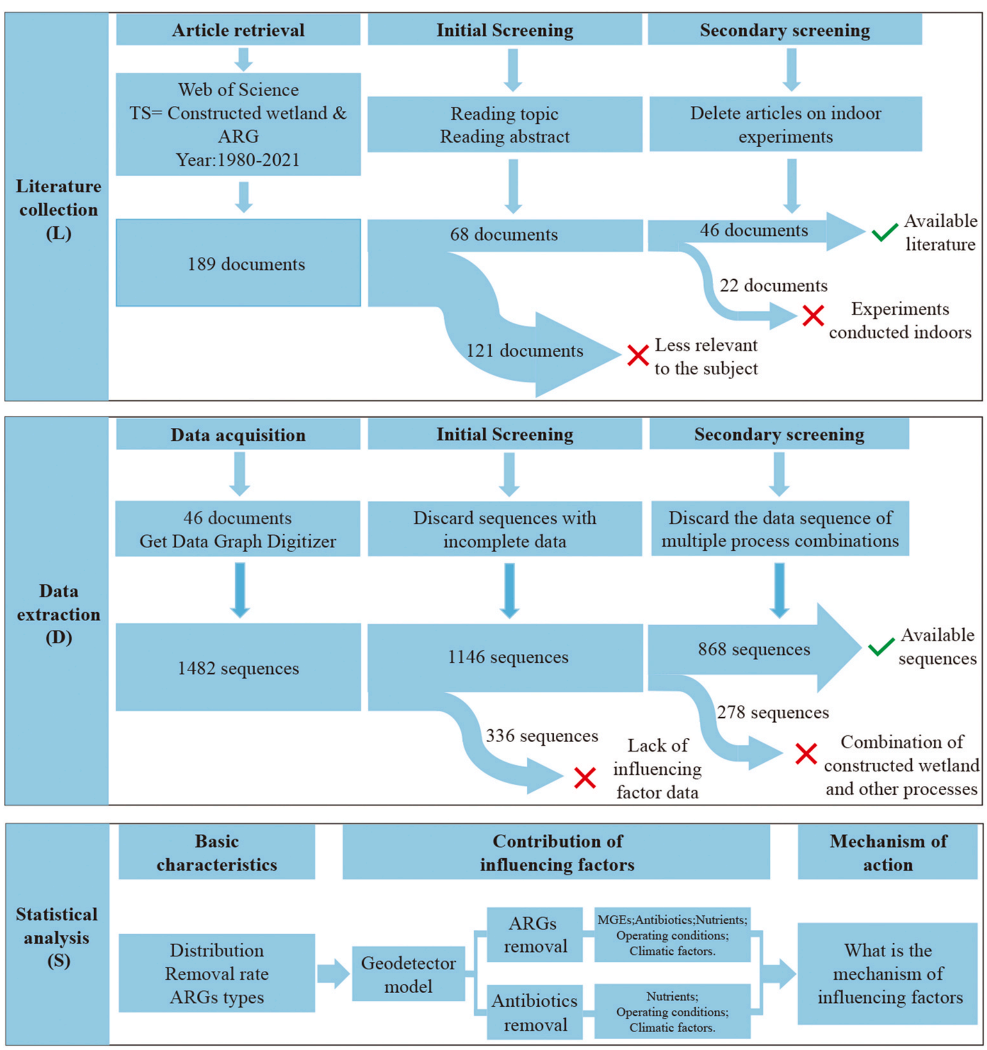

Fig. 1. Literature collection, Data screening and Statistical analysis framework (LDS framework).

In order to effectively integrate and analyze the relevant data of ARGs removal by constructed wetlands from the current literature, this study constructs a systematic procedure for literature collection, data screening and statistical analysis (LDS). The purpose of formulating and implementing this procedure were: 1) to explore the main factors that affect the removal of ARGs in constructed wetlands; 2) to reveal the differences of influencing factors between antibiotics and ARG removal in constructed wetlands; 3) to analyze the relationship between antibiotics and ARG in sewage after purification by constructed wetland.

2. Methods

Fig. 1 showed the whole operation process of LDS framework with literature collection, data extraction and statistical analysis as the core, taking the influencing factors of ARGs removal by constructed wetland as an example. Firstly, the screening conditions were set for literature retrieval and screening in the specified database. Then, extracted data sequence from the literature were preprocessed. Finally, the data meeting the requirements were statistically analyzed using Geodetector to obtain the main factors influencing the removal of ARGs.

2.1. Literature collection

In this study, the relevant studies involving ARGs removal by constructed wetlands were searched in the core collection of Web of Science. The time span of the retrieved papers is from 2000 to 2021. The subject words used were “constructed wetland”, “artificial wetland”, “human made wetland”, “antibiotic resistance gene” and “ARG”. The scope of topic retrieval includes title, abstract and keywords. The search formula is as follows: subject = (“Constructed wetland” OR “Artificial wetland” OR “Human made wetland”) AND (“Antibiotic resistance gene” OR “ARG”). The language and type of papers were set as English and research papers, respectively. A total of 189 papers were retrieved. Considering that this study focuses on the removal of ARGs by constructed wetlands, some of the retrieved papers may only have subject words in the abstract and title, but the research emphasis is not related to this. In addition, some experimental devices were placed indoors, which obviously did not meet the requirements of this study. Repeating screen was performed by reading the materials and methods section of the papers, screen out papers that meet our needs (Fig. 1).

2.2. Data extraction

Based on the selected papers, the relevant data from those papers was extracted. In this study, the factors influencing ARGs removal in constructed wetland were divided into five categories, namely mobile genetic elements (MGEs), antibiotics, nutrients, operating conditions of constructed wetlands and geographical-climatic factors. MGEs, antibiotics and nutrient data were expressed as removal rate. Nutrients included chemical oxygen demand (COD), total nitrogen (TN) and total phosphorus (TP). The operating conditions of constructed wetlands included the type of constructed wetlands (TCW), plant species (PS), specific surface area of substrate (SSA), hydraulic load (HL), volume of constructed wetlands (VCW) and running time of constructed wetlands (RT). Geographical-climatic factors include geographical location (GL), average temperature (AT) and relative humidity (RH). There were 14 influencing factors in five categories. Compared with ARGs removal, MGEs and antibiotic removal rate were not included in the influencing factors of antibiotic removal. The required data were obtained by reading the text, pictures, tables and supplementary materials of the papers. Among them, the data in the pictures were extracted by Get data graph digitizer software (Version 2.25.0.32). If the required data is not available from the papers, it can be obtained by contacting the corresponding authors of the papers. If the required data is still unavailable, the corresponding data sequences were deleted. Based on the above data extraction rules, 868 effective data sequences were extracted in this study (Fig. 1).

2.3. Statistical analysis

In order to quantify the contribution of each influencing factor and reveal the interaction between influencing factors, the Geodetector analysis model was used in the study (http://www.geodetector.cn/). Compared with the common correlation analysis and regression analysis, the Geodetector analysis model can quantify the driving force and interaction of each independent variable on the dependent variable, and does not need to make linear assumptions (Z. Zhu et al., 2019). Based on the above advantages, the Geodetector analysis model has been widely used in the fields of land use, public health, ecology and environmental protection (Luo et al., 2016; Hu et al., 2021; Yin et al., 2019; Zhang et al., 2019). In the Geodetector analysis model, the q value is used to evaluate the driving force or explanatory force of the independent variable on the dependent variable. The range of q value is 0–1. The larger the q value, the greater driving force of the independent variable on the dependent variable.

In this study, the linear mixed models of the total relative abundance of ARGs (TARG) and antibiotics in influent and effluent were established with TARG as response variable, the total selective pressure of antibiotics (TASP) as explanatory variable, while ARGs type, COD, TN and TP as covariables. There are 15 model combinations of random effects in this model (Fig. S1, Supplementary materials). The significance of each random effect model and the fixed effect model was calculated, and then the models with the strongest significance were selected.

The relative abundance of ARGs is calculated as follows:

$$rARG_{ij} = \frac{ARG_{ij}}{16S\ rRNA_j}$$

Among them, $rARG_{ij}$ represents the relative abundance of a certain ARG subspecies i at sample j, ARG_{ij} represents the absolute abundance of a certain ARG subspecies i at sample j, and $16S\ rRNA_j$ represents the absolute abundance of 16 S rRNA at sample j.

The formula for calculating the total relative abundance of specific types of ARGs is as follows (Duarte et al., 2019):

$$TARG_{yj} = \sum_{i \in y} rARG_{ij}$$

Where $TARG_{yj}$ represents the total relative abundance of class y ARG at sample j.

The formula for calculating the selection pressure potential of antibiotics (risk coefficient) is as follows:

$$ASP_{ij} = \frac{MEC_{ij}}{PNEC_{ij}}$$

Among them, ASP_{ij} represents the selective pressure of certain antibiotic subspecies i at sample j, MEC_{ij} represents the concentration of antibiotic i at sample j, and $PNEC_{ij}$ represents the predicted no-effect concentration of antibiotic i.

The formula for calculating the total selective pressure of certain types of antibiotics is as follows (Duarte et al., 2019):

$$TASP_{yj} = \sum_{i \in j} ASP_{ij}$$

Where $TASP_{yj}$ represents the total selective pressure of class y antibiotics at sample j.

The indexes such as ASP and rARG were logarithmically processed in this study.

In this study, K-means cluster analysis was used to discretize continuous variables (Wang and Xu, 2017). Nonlinear curve fitting was used to analyze the driving mode of each influencing factor, and nonlinear curve fitting was completed by Origin software (ver. 2017, Origin Lab Corporation, USA). The data collation involved in this study was completed with Excel 2021 (Microsoft Corp. Redmond, WA, USA). K-means cluster analysis was completed by SPSS (Version 23; SPSS Inc, Chicago, IL, USA). The analysis based on linear mixed model was completed by R software (Duarte et al., 2019).

3. Results and discussion

3.1. The distribution of constructed wetlands for ARGs removal

In this study, these constructed wetlands were distributed across Asia, Europe and North America (Fig. S2). In the screened literatures, a total of 30 kinds of ARGs were studied, mainly including sulfonamide, tetracycline, erythromycin, quinolone and chloramphenicol resistance genes. Among them, 220 data sequences of sulfonamides ARGs (*sul1*:78; *sul2*:89; *sul3*:49) were screened, accounting for 25.3% (Fig. S3, Supplementary materials); 382 data sequences of tetracycline ARGs (*tetA*: 53; *tetB*: 9; *tetC*: 12; *tetG*: 31; *tetH*: 3; *tetM*: 78; *tetO*: 85; *tetQ*: 12; *tetW*: 49; *tetX*: 46) were screened, accounting for 44.0% (Fig. S3, Supplementary materials); There were 106 data sequences of erythromycin ARGs, accounting for 12.2% (Fig. S3, Supplementary materials); 70 data sequences of quinolone ARGs were screened, accounting for 8.1% (Fig. S3, Supplementary materials); 80 data sequences of chloramphenicol ARGs were screened, accounting for 9.2% (Fig. S3, Supplementary materials).

The visualization of ARGs removal in constructed wetlands showed that there is no significant difference in the median removal rates of the common types of ARGs. The median removal rate of sulfonamide ARGs subspecies (*sul1*, *sul2* and *sul3*) were between 73% and 84% (Fig. 2a). Among the common subspecies of tetracycline ARGs, the median removal rates of *tetM*, *tetO* and *tetX* were all about 80% (Fig. 2b). Among the common subspecies of macrolide ARGs, the median removal rate of *ermB* was significantly lower than that of *ermC*, 68% and 82%, respectively (Fig. 2c). The median removal rates of common subspecies of quinolone ARGs and chloramphenicol ARGs were all about 80% (Fig. 2d).

3.2. The influencing factors of antibiotics removal by constructed wetlands

The effect of influencing factor on antibiotics removal in constructed wetland was investigated using the Geodetector model. Fig. 3 showed the main factors influencing the removal of four types antibiotics in constructed wetlands. For example, the q value (the larger q value, the greater effect) of COD, TP, constructed wetland type, plant species and hydraulic load were 0.547, 0.397, 0.369, 0.522 and 0.345 respectively in the sulfonamide antibiotics removal (Fig. 3a). In general, nutrients, types of constructed wetlands, plant and hydraulic load were principal influence factors on the majority antibiotics removal (Table S1, Supplementary materials). It is worth noting that the average temperature, relative humidity and geographical location were not the principal factors influencing antibiotics removal in constructed wetlands, which may be related to the strong buffer capacity of constructed wetlands to climate change (Vymazal, 2011).

By comparing the antibiotics removal efficiency of different types

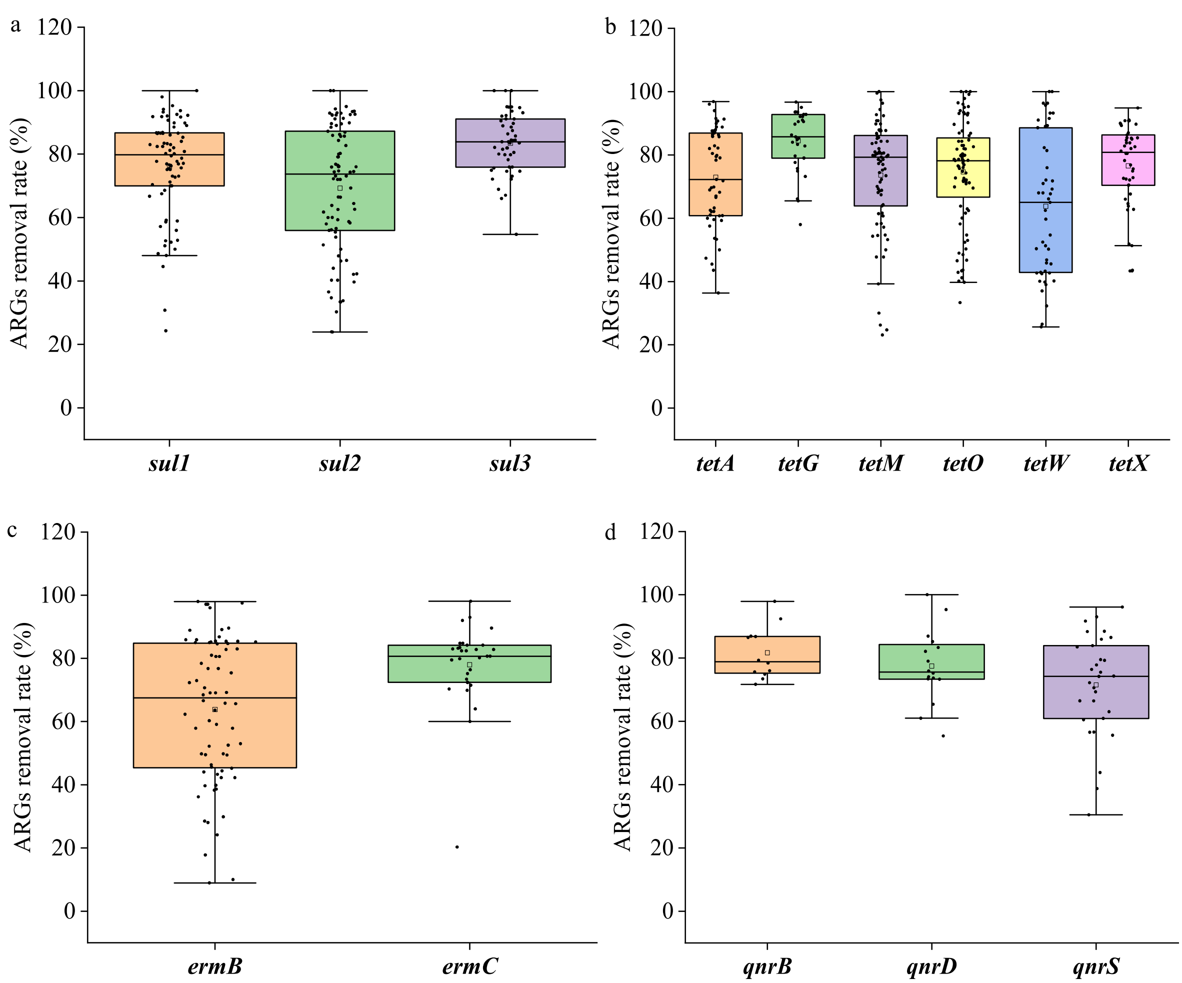

Fig. 2. Removal rate of common ARGs. a: Sulfonamide ARGs; b: Tetracycline ARGs; c: Macrolide ARGs; d: Quinolone ARGs. Sul: Sulfonamide ARGs; tet: Tetracycline ARGs; erm: Macrolide ARGs; qnr: Quinolone ARGs.

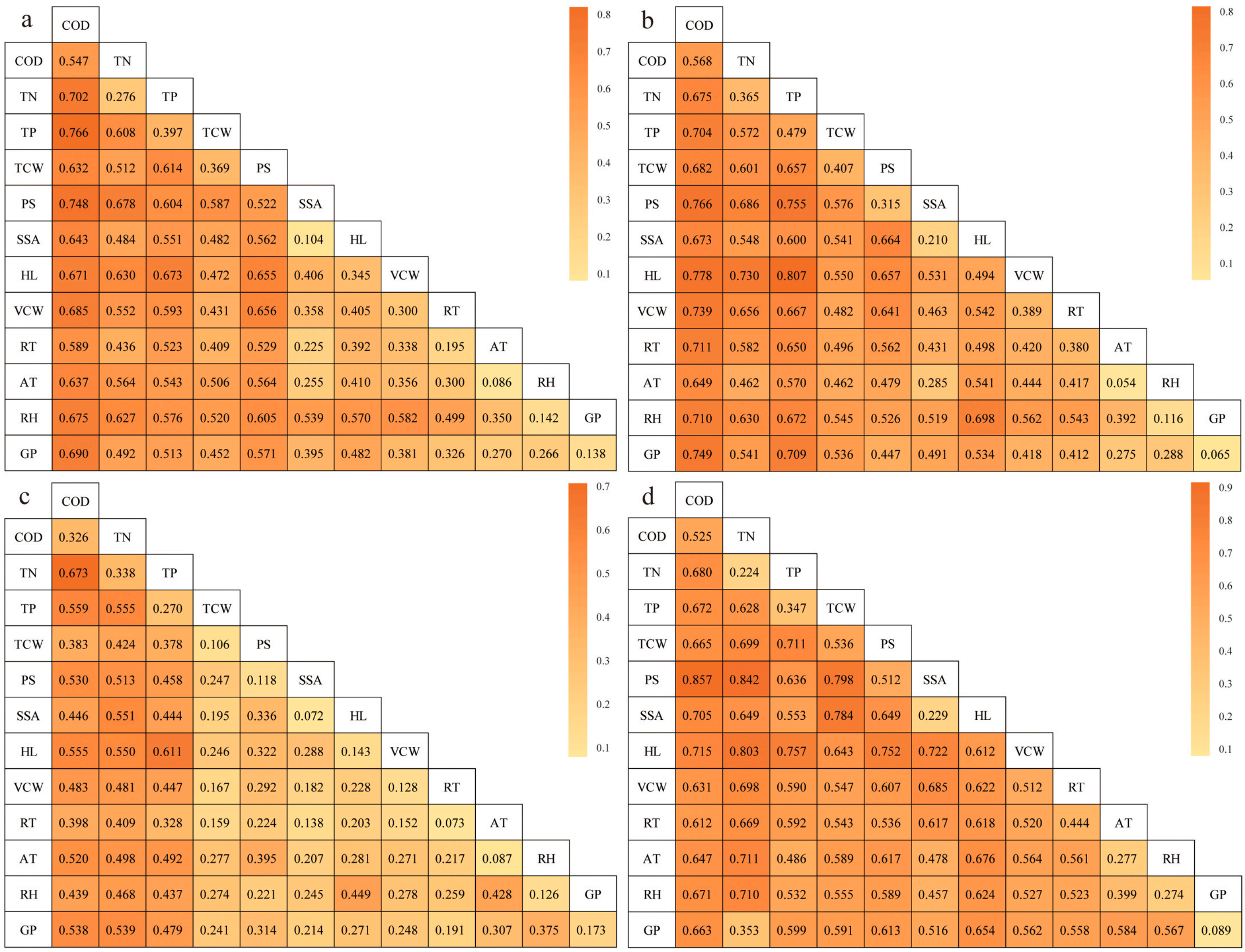

Fig. 3. The influencing factors of antibiotic removal in constructed wetlands. a: Sulfonamide antibiotics; b: Tetracycline antibiotics; c: Macrolide antibiotics; d: Quinolone antibiotics. COD: chemical oxygen demand; TN: total nitrogen; TP: total phosphorus; TCW: types of constructed wetlands; PS: plant species; SSA: specific surface area of substrate; HL: hydraulic load; VCW: volume of constructed wetlands; RT: running time of constructed wetlands; GP: geographical position; AT: average temperature; RH: relative humidity.

constructed wetlands, it was found that the median removal rate of four common antibiotics in subsurface flow constructed wetlands was significantly higher than that in surface flow constructed wetlands, but there was no significant difference in antibiotic removal rates between vertical and horizontal subsurface flow constructed wetlands (Fig. S4, Supplementary materials). In addition, compared with sulfonamides, tetracyclines and macrolides, the median removal rate of quinolone antibiotics in the three types of constructed wetlands was relatively low (Fig. S4, Supplementary materials). The removal rate of quinolone antibiotics decreased gradually with the increase of hydraulic load, while the trend of sulfonamides, tetracyclines and macrolides was not obvious (Fig. S5, Supplementary materials), which meant that most antibiotics have a high removal rate within a certain hydraulic load range.

The above results showed that nutrients, types of constructed wetlands and hydraulic load have important effects on antibiotics removal. Antibiotic removal mainly relies on microbial degradation, substrate adsorption, plant absorption and hydrolysis in constructed wetlands, among which microbial degradation and substrate adsorption contribute greatly (Zhang et al., 2022a). Microorganisms biodegrade antibiotics through direct degradation and co-metabolism. Nutrients provide enough carbon and nitrogen sources for the microorganisms in antibiotics removal. Dissolved oxygen changes caused by constructed wetland types and plants form different types of microbial communities. Therefore, nutrients and type of constructed wetland had a greater effect on antibiotic removal in this study. The change of hydraulic load will also influence the removal of antibiotics in constructed wetland. The inappropriate hydraulic load are not conducive to the removal of antibiotics. Too small hydraulic load makes the microorganisms in the constructed wetland unable to obtain enough nutrients and impact the antibiotic removal efficiency. The excessive hydraulic load will impact the antibiotic removal efficiency because the pollutants cannot be fully utilized by microorganisms (Dan A et al., 2013). Risk factor detection also confirmed that both oversize and undersize hydraulic loads are not high-frequency areas of high antibiotic removal rates (Table S2, Supplementary materials). Therefore, hydraulic load has a great effect on the removal of antibiotics.

Many studies have shown that substrate adsorption is one of the main way to remove antibiotics in constructed wetlands (Hijosa-Valsero et al., 2011; Liu et al., 2013). Antibiotics are adsorbed on the adsorption sites of the substrate, and the specific surface area of the substrate is the principal factor (Zhang et al., 2022a). Surprisingly, the specific surface area of substrate had little effect on the removal of antibiotics by constructed wetlands in this study. The possible reason is that the substrate surface is covered by microbial membrane in constructed wetlands, which prevents the direct contact of antibiotics and substrate (Ding et al., 2018). In addition, due to the large running time span of the constructed wetlands involved in this study, the substrate state at the initial stage is different from that at other periods. Therefore, the specific surface area of the substrate has little effect on antibiotics removal.

Antibiotics can also be removed from constructed wetlands by plant absorption (Huang et al., 2015; Song et al., 2018b). Some antibiotics remain in the form of parent molecule (Bartrons and Peñuelas, 2017; Madikizela et al., 2018), while others may be metabolized in the plant. Previous studies have confirmed that the amount of antibiotics accumulated in plants is low (Huang et al., 2019; Liang et al., 2018b). It is worth noting that the effect of plants on the removal of antibiotics is not limited to direct absorption. Many previous studies have confirmed that plants rhizosphere exudates play an indirect role in the removal of antibiotics (Hongbin et al., 2020). On the one hand, it can ameliorate the root microbial community and improve the removal rate of antibiotics (Reichel et al., 2013). On the other hand, it has the ability to adsorb antibiotics. Furthermore, different plants have varied root scales, and plants with larger roots have inherent advantages in releasing oxygen and exudates from roots, which makes plants with developed roots become the first choice for plants in constructed wetlands. Therefore, plants have a great influence on antibiotic removal in constructed wetlands. Hydrolysis and photolysis are also a way for constructed wetlands to remove antibiotics (Abramović et al., 2021). However, previous studies have confirmed that they contribute little to the removal of antibiotics (Huang et al., 2019; Liu et al., 2014).

3.3. The influencing factors of ARGs removal by constructed wetlands

Based on the Geodetector analysis model, the contribution of various influencing factors in ARGs removal by constructed wetlands was quantified. Fig. 4 showed the main factors influencing the removal of four types ARGs in constructed wetlands. A larger q value represents a greater effect of that factor on ARGs removal. By summarizing the main influencing factors of four types ARGs removal, it was found that MGEs, plant, volume of constructed wetland, running time and types of constructed wetland were principal influence factors on most ARG removal, while antibiotics only had greater influence on part of ARGs removal (Table S3, Supplementary materials).

By comparing the ARGs removal efficiency of different types constructed wetlands, it is found that the median removal rates of four common types of ARGs in surface flow constructed wetlands were lower than those in subsurface flow constructed wetlands, and the median removal rates of four types of ARGs (sulfonamide ARGs, tetracycline

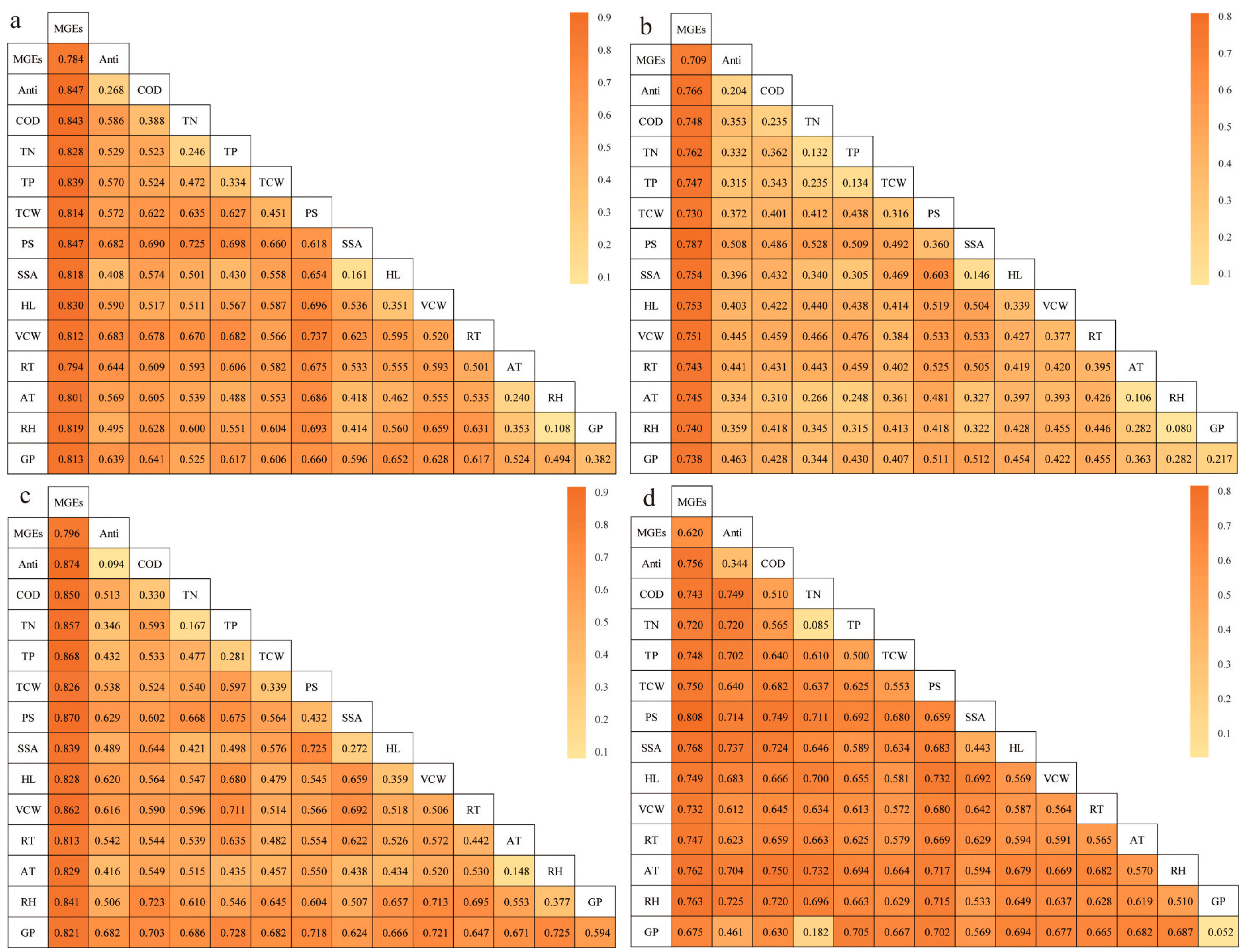

Fig. 4. The influencing factors of ARGs removal in constructed wetlands. a: Sulfonamide ARGs; b: Tetracycline ARGs; c: Macrolide ARGs; d: Quinolone ARGs. MGEs: mobile genetic elements; Anti: antibiotics; COD: chemical oxygen demand; TN: total nitrogen; TP: total phosphorus; TCW: types of constructed wetlands; PS: plant species; SSA: specific surface area of substrate; HL: hydraulic load; VCW: volume of constructed wetlands; RT: running time of constructed wetland; GP: geographical position; AT: average temperature; RH: relative humidity.

ARGs, macrolide ARGs and quinolone ARGs) in surface flow constructed wetlands were all between 40% and 50% (Fig. S6, Supplementary materials). There was no significant difference in the removal of four kinds of common ARGs between vertical subsurface flow constructed wetland and horizontal subsurface flow constructed wetland (Fig. S6, Supplementary materials).

The relationship between several main influencing factors and ARGs removal was investigated by curve fitting. The results showed that there were significant positive linear correlation between MGEs and ARGs among the four types of common ARGs (Fig. 5). This is mainly because many ARGs are part of MGEs, which results MGEs have a great influence on ARGs removal. Other studies have also confirmed the strong correlation between MGEs and ARGs (Li et al., 2019; Wang et al., 2021). The removal rates of the four common ARGs gradually decreased with the increase of the constructed wetland volume (Fig. S7, Supplementary materials). The fitting results of four common ARGs removal rate and running time of constructed wetland accorded with the two-phase exponential attenuation model, which showed that the ARGs removal efficiency decreases gradually with the increase of running time. As shown in Fig. S8, when the running time of constructed wetlands exceed ten years, the removal rate of ARGs was generally less than 50%. Therefore, when the running time of constructed wetland exceeds ten years, it should be considered to renew the constructed wetland.

The above results showed that MGEs, plants, the volume and running time of constructed wetlands play important role in ARGs removal. Different from antibiotic removal, ARGs are mainly carried by microorganisms (Hu et al., 2020). ARGs removal mainly depends on filtration, interception, secretion oxidation and other paths in constructed wetlands (Wu et al., 2016). Considering the rapid replication of ARGs, controlling ARGs diffusion is also part of ARGs removal.

Filtration and adsorption are main pathways to remove ARGs in constructed wetlands. The substrate and plant roots indiscriminately filter and absorb pollutants in water, ARGs and its carrier are trapped around the substrate and plant roots. The interception effect is mainly determined by the substrate porosity and the size of the plant root system. The microbial membrane covering the surface of the substrate causes pore blockage as a result of the constructed wetland's long-term operation (Ding et al., 2018), which weakens the effect of filtration and adsorption. Based on this fact, the substrate specific surface area did not have a significant effect on ARGs removal in this study. Further analysis of the research results also confirmed that the removal rate of ARGs gradually decreased with the increase of running time (Fig. S8, Supplementary materials). Plant root filtration mainly depends on the full contact between plant roots and pollutants. The larger plant root system, the higher contact probability. Through risk factor detection of the plants involved, it was found that *Cyperus rotundus*, *Iris tectorum Maxim*, *Arundo donax* and *Thalia dealbata Fraser* are high-frequency plants with high removal rate (Table S4, Supplementary materials). Most of these plants are fibrous root type plants with developed root systems, which creates suitable conditions for filtering and intercepting pollutants. In addition to root filtration, the removal of ARGs by plants through the release of secretions is also a path that can not be ignored. The secretions (lysozyme, etc.) can oxidize bacteria, resulting in the death of microorganisms and the loss of ARGs carrier (Wu et al., 2016). Root exudates can also adsorb ARGs carriers around the roots to achieve the purpose of removing ARGs (Yan et al., 2016). Therefore, the results showed that plants have great influence on ARGs removal in constructed wetlands.

In terms of controlling the spread of ARGs, both plants and the running time of constructed wetlands exhibited great influence. The long-running constructed wetlands will lead to the decline of filtration

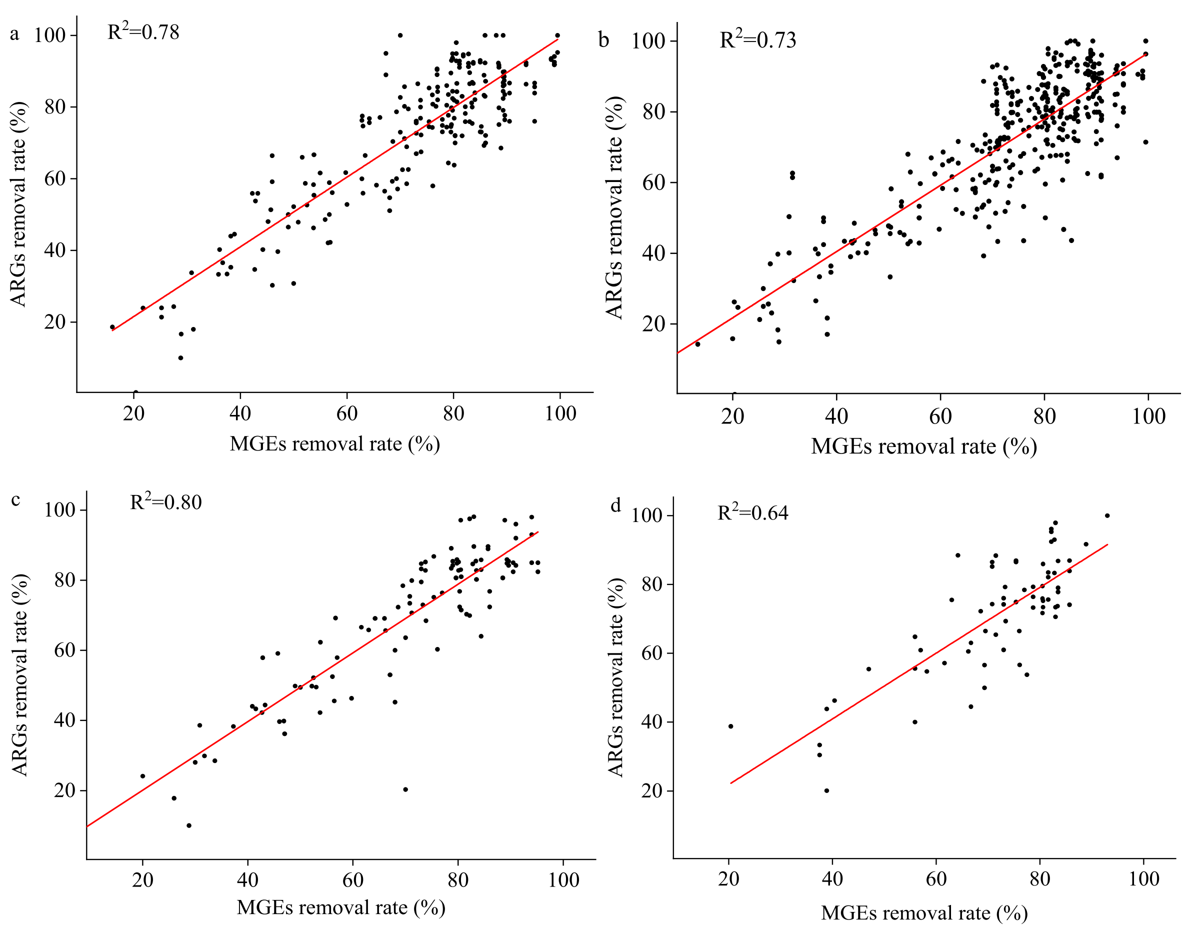

Fig. 5. Relationship between ARGs removal rate and MGEs removal rate. a: Sulfonamide ARGs; b: Tetracycline ARGs; c: Macrolide ARGs; d: Quinolone ARGs.

performance due to substrate blockage, which makes the constructed wetlands become the focus of horizontal gene transfer and lead to the diffusion of ARGs (Abou-Kandil et al., 2021). Through the risk factor detection of running time, we found that the running time less than six years is the high-frequency stage of high removal rate, and the longer the running time, the less the frequency of high removal rate (Table S5, Supplementary materials). In addition, oxygen released from the plant rhizosphere increased the survival rate of pathogens, thus increasing the abundance of ARG (Vivant et al., 2016; Wu et al., 2015).

3.4. The changes of ARGs and antibiotics in influent and effluent

After the purification of constructed wetland, the relative abundance of majority kinds of ARGs in the effluent did not change obviously. As shown in Fig. 6a, it can be found that the relative abundance of most types ARGs in the effluent did not decrease significantly compared with the influent, and there was no obvious variation in the normal distribution of each group of data. Compared with the influent, the relative abundance of macrolides ARGs decreased (Fig. 6a). These results are related to the following process: the exchange behavior between ARGs and microbial membrane in constructed wetland. Due to the long-term contact between microbial membrane and sewage containing ARGs in constructed wetlands, it is inevitable to infect ARGs through horizontal gene transfer. Biofilms carrying ARGs may transmit ARGs to residual microorganisms in wastewater. In addition, due to the limited microbial life, the microbial membrane in the constructed wetland will fall off and enter the effluent in a certain period of time. These factors keep the relative abundance of ARGs in effluent stable to some extent. Based on the calculation of ARGs relative abundance reduction, the median reduction of ARGs relative abundance was the largest when the running time was 3–5 years, while the reduction of ARGs relative abundance was relatively small when the running time was more than 10 years (Fig. S9, Supplementary materials). This is because constructed wetland systems are not stable at the initial stage of operation, and the removal of ARGs relative abundance only reaches the maximum reduction after stabilization. However, with the longer running time, the function of constructed wetland gradually declines and the reduction of ARGs relative abundance decreases gradually. At the same time, Fig. 6b showed that the risk coefficients of four common antibiotics decreased significantly after purification by constructed wetlands. Specifically, the average risk coefficient of sulfonamides, tetracyclines, macrolides, quinolone decreased from −1.4 to −2.2, −0.47 to −1.28, 0.16 to −0.78 and −1.70 to −2.03, respectively. This showed that constructed wetlands can significantly reduce the ecological risk of antibiotics.

Due to the different removal pathways of ARGs and antibiotics in constructed wetlands, the coexistence of antibiotics and ARGs does not necessarily indicate a causal relationship between them. Other factors in influent and effluent may also have effect on ARGs. Therefore, it is necessary to incorporate antibiotics, ARGs types and nutrients into the system, and reveal the rules of antibiotic inducing ARGs diffusion and evaluate the strength of the association between antibiotics and ARGs in influent and effluent based on linear mixed model. The results of the linear mixed model analysis showed that the all 15 influent model combinations showed no significance. Among the 15 effluent model combinations, the TARG ~ TASP+(1|COD) +(1|TN) model showed strong significance. This indicated that the association between ARGs and antibiotics was enhanced after purification. Visualizing total antibiotic selection pressure and total ARGs showed that the risk threshold of antibiotics after the constructed wetland treatment was mostly less than 0. However, the total ARGs levels of tetracycline ARGs, macrolide ARGs and quinolone ARGs were not significantly reduced in the effluent (Fig. S10, Supplementary materials). These results are closely related to the induction behavior of antibiotics on ARGs. The studies on the removal of antibiotics in constructed wetlands are mostly based on control experiments, and there are great differences in the amount of antibiotics added to the influent according to individual needs (Duarte et al., 2019). As a result, most of the microorganisms in the influent containing high concentrations of antibiotics may be killed and the abundance of ARGs is relatively low. However, the concentration of antibiotics in some influent is relatively low. Due to the induction of ARGs by sub-inhibitory concentration of antibiotics, the abundance of ARGs may be at a high level (Song et al., 2018a). The existence of these conditions makes the relationship between antibiotics and ARGs in influent seems not close. In the effluent, the concentration of antibiotics decreased significantly, and the low concentration of antibiotics reduced the pressure of microbial survival, which provided the induction conditions for the lateral gene transfer and diffusion of ARGs (Komijani et al., 2021; Lin et al., 2021; Wang et al., 2021), which made it possible for ARGs to spread among the residual microorganisms. As a result, the connection between antibiotics and ARGs in effluent was improved. This connection depends on the concentration of antibiotics. In other words, the effect of antibiotics on ARGs is more obvious in low concentration antibiotic environment. However, the antibiotic concentration threshold which can have a significant effect on ARGs needs to be proved by follow-up studies.

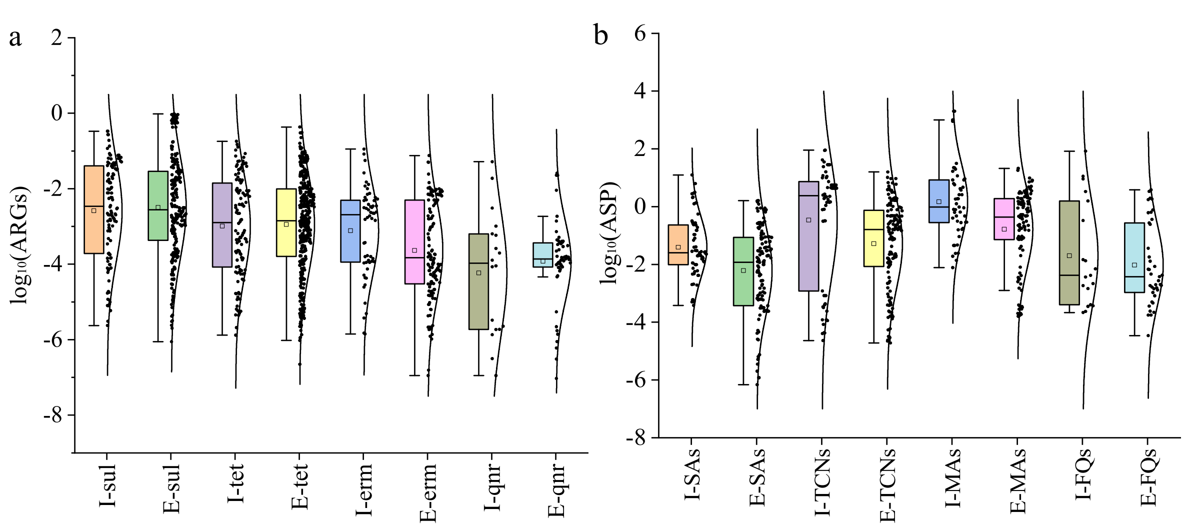

Fig. 6. a: The relative abundance of ARGs in influent and effluent; b: The risk coefficient of antibiotics in influent and effluent. “I” stands for influent, “E” stands for effluent. Sul: Sulfonamide ARGs; tet: Tetracycline ARGs; erm: Macrolide ARGs; qnr: Quinolone ARGs. SAs: Sulfonamide antibiotics; TCNs: Tetracycline antibiotics; MAs: Macrolide antibiotics; FQs: Quinolone antibiotics.

4. Conclusions and future suggestions

Based on the LDS framework, this study explored the factors affecting ARGs removal in constructed wetlands from a global perspective. The factors influencing the removal of antibiotics and ARGs were different. Nutrients, the type of constructed wetland and hydraulic load have significant influence on the removal of majority antibiotics, while the factors that have great influence on majority ARGs removal were MGEs, plants, the volume and running time of constructed wetland. The essence of this difference is that the main pathways of ARGs removal and antibiotic removal are different. After purification in constructed wetlands, the ARGs relative abundance did not decrease significantly, and the relationship between ARGs and antibiotics is enhanced. Therefore, it is still necessary to pay attention to the potential risk of ARGs diffusion in the effluent of constructed wetlands, especially for running time more than ten years older constructed wetlands.

Application of this LDS framework can incorporate the relevant data of previous researches into a unified model for analysis. However, there are still some deficiencies that are expected to be improved in the follow-up study. First, microorganisms play a major role in the removal of antibiotics and ARGs. However, the existing work is unable to obtain complete microbial data and quantify the influence of microorganisms. Then, the constructed wetlands involved in this study are mainly concentrated in Asia, Europe and North America, while there is a lack of data in Africa, Oceania and South America. Therefore, follow-up research still needs to pay attention to data sharing, which needs to take into account principles of accessibility and reusability. We hope that the proposed LDS framework can be extended to other environmental processes to provide a new tool for exploring their influencing factors.

Author contribution statement

Ling Zhang: Software, Methodology, Writing–original draft, Investigation, Visualization, Writing–original draft preparation. Changzhou Yan: Conceptualization, Writing–review & editing, Project administration, Funding acquisition. Ce Wen: Resources, Validation. Ziyue Yu: Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2023.137755.

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