



Influence of sulfate reduction on fraction and regeneration of phosphorus at sediment–water interface of urban malodorous river

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Abstract

The effective control of the release of endogenous phosphorus is an urgent problem in the management of urban malodorous rivers. This research explored the fraction and regeneration of phosphorus of urban malodorous river in the context of sulfate reduction. It was found that sulfate reduction could promote sediment phosphorus release. The contents of total phosphorus (TP) and soluble reactive phosphorus (SRP) in the overlying water presented a decreasing trend after the initial increase during the operation of 120 days. The phosphorus release was positively related to the input of sulfate, and the maximum values of TP and SRP (14.01 mg/L and 12.27 mg/L, respectively) in the overlying water were observed when 8 mM Na₂SO₄ was added. Moreover, the addition of sulfate could significantly affect the distribution of phosphorus fraction in the sediment and promote the transformation of moderately active phosphorus (NaOH-P, D, HCl-P) to more active phosphorus Resin-P), which resulted in more release of phosphorus to the overlying water. In addition, it was observed that sulfate input could increase the relative abundance of phosphate solubilizing bacteria (PSB) and sulfate-reducing bacteria (SRB) from 0.69 to 1.1% and 4.92 to 9.03%, respectively.

Keywords Endogenous phosphorus · Sulfate reduction · P fractions · Malodorous river

Introduction

Phosphorus (P) is the key limiting factor for water eutrophication (Loganathan et al. 2014; Pant and Reddy 2001; Tang et al. 2014). Furthermore, the concentration of sulfur (S), an important biogenic element of the hydrosphere geochemical cycle, is often higher

by 2–3 orders of magnitude than that of phosphorus in the aquatic system (Baldwin and Mitchell 2012), and its role in the geochemical cycle is receiving increasing attention. Sulfate reduction is the main route of organic matter mineralization in sediments, and it is the beginning of the sulfur cycle. How it affects the endogenous P release is a scientific question worthy of discussion.

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Nowadays, only a part of the research on phosphorus regeneration in water have focused mainly on the oceans (Yang et al. 2016), estuaries (Myrbo et al. 2017; Pollman et al. 2017; Stagg et al. 2017), wetlands (Coleman Wasik et al. 2015; Johnson et al. 2016), and lake sediments (Zhang et al. 2017; Wang and Benoit 2017; Ren et al. 2018). Some studies have investigated the spatiotemporal distribution of sulfur and phosphorus in water environments (Wang et al. 2018) and the effect of exogenous sulfate on phosphate migration and transformation (Yuan et al. 2012; Fan et al. 2014). These previous studies confirmed the effects of sulfate on P release in sediments, since sulfate reduction significantly changed the physical and chemical properties of the sediments and thus affected the environmental behavior of phosphate. Different P fractions in the sediment have different effects on water eutrophication (Huang et al. 2003; Liu et al. 2019; Zhu et al. 2017). Nevertheless, none of these studies involved the effects of sulfate on phosphorus fractions. And few studies have focused on the effect of sulfate on phosphorus regeneration at the sediment–water interface.

Moreover, malodorous urban rivers have been common in many cities especially in developing countries (Lu et al. 2010; He et al. 2013). Due to human activities, the sulfate content in the urban black malodorous river water changes significantly. (Coleman Wasik et al. 2015). Therefore, it is of scientific importance to ascertain how sulfate reduction affects the endogenous P release in urban malodorous rivers. However, little information is available regarding the effect of sulfate reduction on the fraction and regeneration of phosphorus in an urban malodorous river.

The objectives of this study are to (1) investigate the distribution characteristics of phosphorus and sulfur fractions in the sediments of an urban malodorous river, (2) analyze the effects of sulfate reduction on the distribution and regeneration of phosphorus, and (3) identify the possible coupling mechanism of sulfate reduction and phosphorus release.

Materials and methods

Sample characteristics

All of the water and sediments in the study are sampled from the river *Taopu* located in the town of *Taopu* in Shanghai. Sediments were collected using a bottom sampler and stored at 4 °C. The basic physicochemical properties of overlying water and sediment are measured and shown in Table 1.

Experimental setup

For all the experiments, each reactor was filled with 1 L of sediments and 3 L of the overlying water siphoned over the sediments. The outer flank of each column reactor was covered in order to prevent light exposure. The whole experiment included six runs, i.e., run 0 (control), run 1 (10 mM Na₂MoO₄ as a

sulfate-reduction inhibitor), run 2 (2 mM Na₂SO₄), run 3 (4 mM Na₂SO₄), run 4 (6 mM Na₂SO₄), and run 5 (8 mM Na₂SO₄).

Different P fractions

Different P fractions were analyzed by the modified Hedley fractionation method by Tiessen (Tiessen and Moir 1993; He et al. 2008). The sediment-derived phosphorus was classified into six fractions: Resin-P, NaHCO₃-P, NaHCO₃-P, NaOH-P, dilute-HCl-extractable P (D. HCl-P), concentrated HCl-extractable P (C. HCl-P), and Residual-P. Resin-P denotes freely exchangeable inorganic phosphorus, while NaHCO₃-P stands for organic and inorganic phosphorus loosely adsorbed on the surface of the sediment. Further, NaOH-P denotes inorganic phosphorus that was tightly bound to Fe–Al compounds and organic phosphorus bound to humic acid (Wang et al. 2006). In addition, D. HCl-P and C. HCl-P refer to inorganic phosphorus synthesized with calcium and occluded phosphorus, respectively, whereas Residual-P is unusable phosphorus or other occluded phosphorus in the residue after all of the extraction steps.

Microbial community analyses

After genomic DNA extraction according to the instructions of the DNA extraction kit corresponding to each type of sample, the integrity and purity of the DNA were detected by 1% agarose gel electrophoresis, and the concentration and purity of the DNA were detected by using NanoDrop One. For the polymerase chain reaction (PCR) amplification and product electrophoresis detection, genomic DNA was used as the template. Furthermore, primers 338F (5'-ACTCCTACGGGAGG CAGCAG-3') and 806R (5'-GGACTACHVGGGTW TCTAA T-3') with barcodes and Premix Taq (TaKaRa) were used for PCR amplification according to the specifically selected sequencing segment.

Subsequently, the NEBNext® Ultra™ DNA Library Prep Kit for Illumina® was employed for database construction. After its completion, the high-throughput sequencing platform HiSeq and MiSeq were used to perform on-line sequencing. The sequences analyses were done directly on I-Sanger Cloud platform.

Analytical and statistical methods

All of the chemical analyses were performed in accordance with standard methods (APHA 1998). The TP, soluble reactive phosphorus (SRP), sulfate, and acidic volatile sulfide (AVS) concentrations were measured colorimetrically. Furthermore, the pH, DO, and temperature (T) were measured using a relevant portable electrode.

Table 1 Basic physicochemical properties of overlying water and sediment

Overlying water						Sediment	
pH	DO (mg/L)	TP (mg/L)	PO ₄ ³⁻ (mg/L)	S ²⁻ (mg/L)	SO ₄ ²⁻ (mg/L)	AVS (mg/kg)	TP (mg/kg)
6.9 ~ 7.1	9.8	0.488	0.172	0.10 ± 0.0	97.24 ± 2.8	4185 ± 10.8	7855 ± 20.4

Accession numbers

The sequence data reported in this study was deposited in the NCBI Sequence Read Archive database under accession number SRR11516698-SRR11516699.

Results and discussions

Variations in sulfate and acid volatile sulfide contents in different treatment groups

Figure 1 shows the profiles of sulfate concentration in each group. The sulfate content decreased gradually for all the experimental groups, which was mainly due to the sulfate reduction in the anoxic environment at the sediment–water interface. When sulfate enters the water body, it is used by soluble reactive phosphorus (SRB) as an electron acceptor (Berner and Westrich 1985; Dornblaser et al. 1994). Sulfates react in the system as follows (Herlihy and Mills 1985; van Houten et al. 1994):

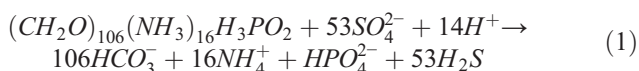
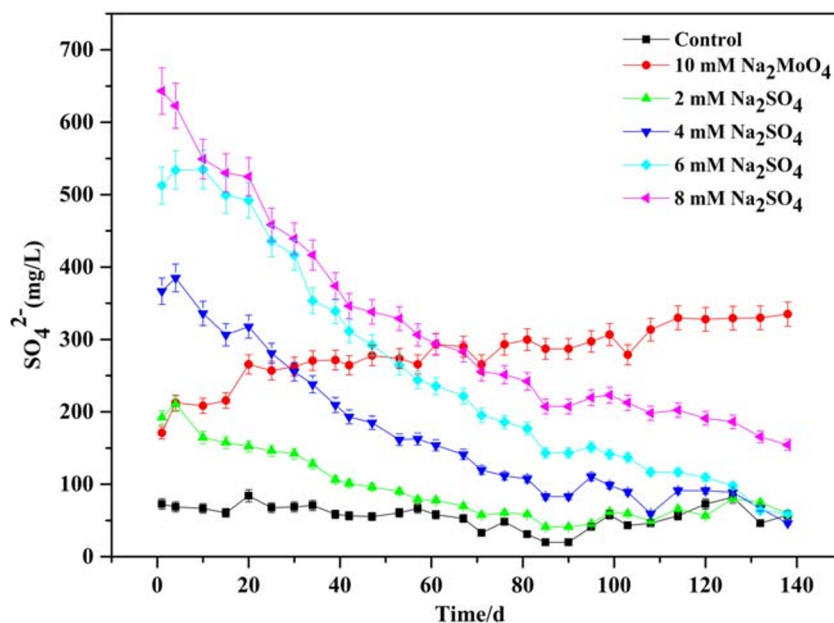


Fig. 1 Comparison of sulfate content in overlying water between different treatment groups (0 (control), 2, 4, 6, 8 mM Na₂SO₄, and 10 mM Na₂MoO₄)



During sulfate reduction, some organic phosphorus is converted to HPO₄⁻ and released. The sulfate content in the presence of 10 mM Na₂MoO₄ remained basically unchanged, indicating that the presence of Na₂MoO₄ had an inhibitory effect on sulfate reduction. As a sulfate-reduction inhibitor, excess Na₂MoO₄ can greatly inhibit the growth and anaerobic respiration of sulfate-reducing bacteria (Nair et al. 2015), thereby decreasing the consumption of sulfates and resulting in the sulfate concentration being higher than that in the control group.

In an anaerobic reduction environment, the organic matter in the sediments can be utilized by sulfate-reducing bacteria with SO₄²⁻ receiving electrons to be reduced. Both the oxidation of sulfur-containing organic matter and the reduction of sulfate can produce S²⁻ (Thode-Andersen and Jorgensen 1989). Fe(II) and Fe(III) and S²⁻ in sediments react to form various iron sulfide minerals (Oehm et al. 1997).

The results showed that the AVS content in the sulfate-addition groups increased significantly in the following order: 8 mM > 6 mM > 4 mM > 2 mM (Fig. 2). This was because of the generated S²⁻ from sulfate reduction to combine with Fe²⁺ from active iron reduction to form FeS₂, which is the main form of AVS. The increase in sulfate content will promote the formation of reduction sulfur include the production of AVS in sediments.

Fig. 2 Comparison of the sediment acid volatile sulfide (AVS) content among different treatment groups (control, 10 mM Na_2MoO_4 , 2 mM-, 4 mM-, 6 mM- and 8 mM Na_2SO_4)

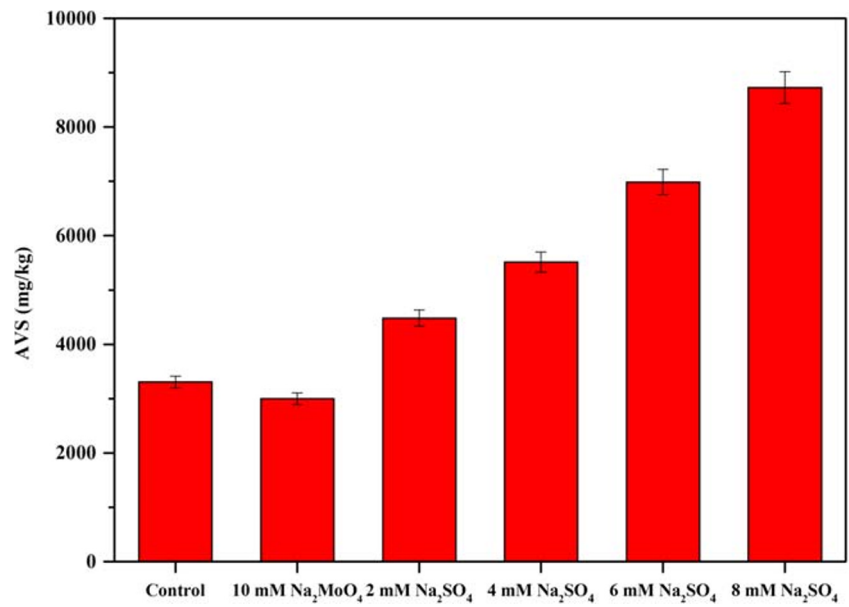
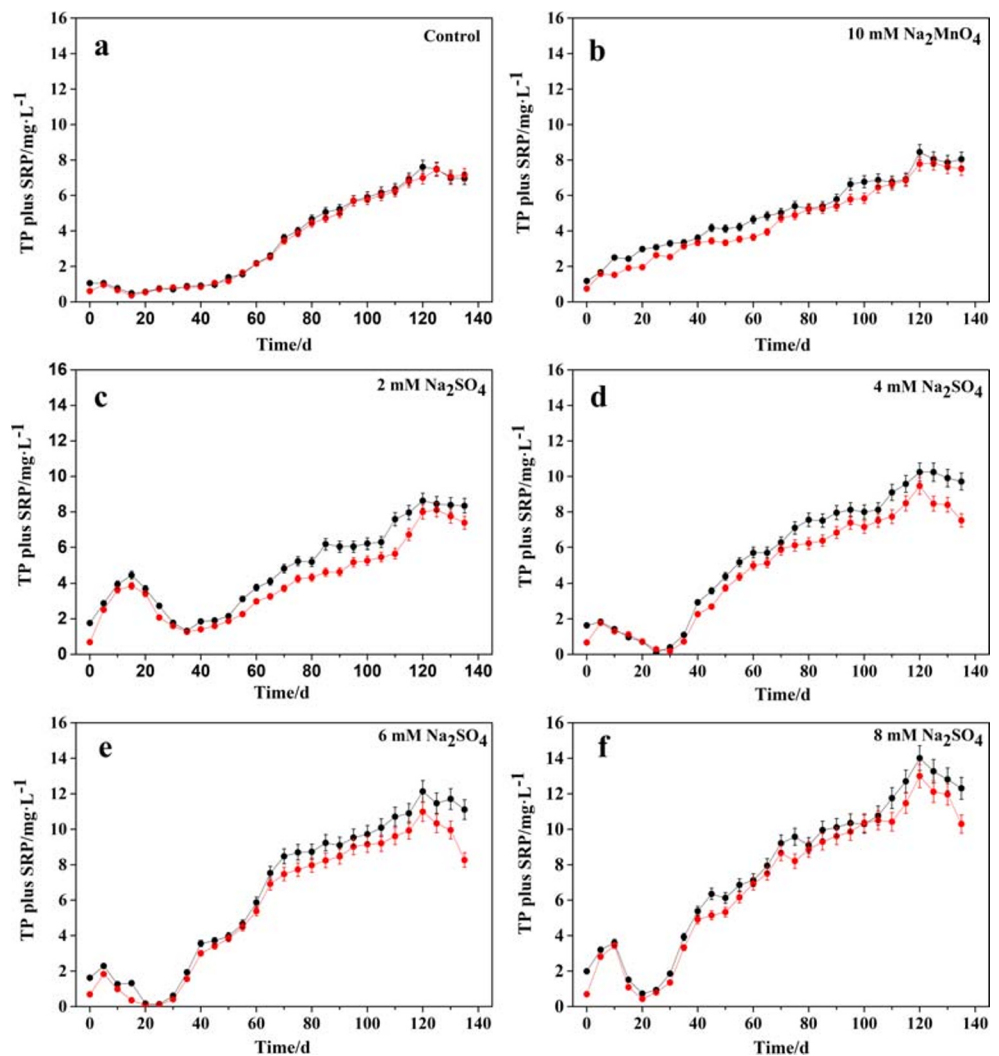


Fig. 3 Comparison of total phosphorus (TP) and soluble reactive phosphorus (SRP) contents in the overlying water between different treatment groups (a (control), b (10 mM Na_2MoO_4), c (2 mM Na_2SO_4), d (4 mM Na_2SO_4), e (6 mM Na_2SO_4), and f (8 mM Na_2SO_4)) during 140 days. Red dots denote SRP; black squares denote TP



Variations in TP and SRP contents in overlying water

Figure 3 demonstrates that the concentrations of SRP and TP in the overlying water increased first and then decreased in the first 30 days. After 30 days, the concentrations of SRP and TP showed an obvious increasing trend, and the increase was positively related to the sulfate level. The SRP and TP contents in the overlying water in the 8 mM sulfate group were significantly higher than the initial value and reached the maximum values of 14.01 mg/L and 12.27 mg/L, respectively, in 120 days (Fig. 3f). The P release followed the order: 2 mM < 4 mM < 6 mM < 8 mM. The addition of sulfate affects the P release in the sediment, which causes the migration of P from the sediment to the water body and gradually increases the P concentration in the overlying water.

In the later stage of the experiment, SRP and TP concentrations in the overlying water showed a decreasing trend (Fig. 3). With the lower sulfate concentration in the later stage, the sulfate reduction rate decreased, and the released phosphate was absorbed into the sediment. Before being permanently deposited, phosphorus generally undergoes multiple release-deposition-re-release-redeposition processes, and only a small part of phosphorus is finally deposited in sediments (Hupher et al. 1995).

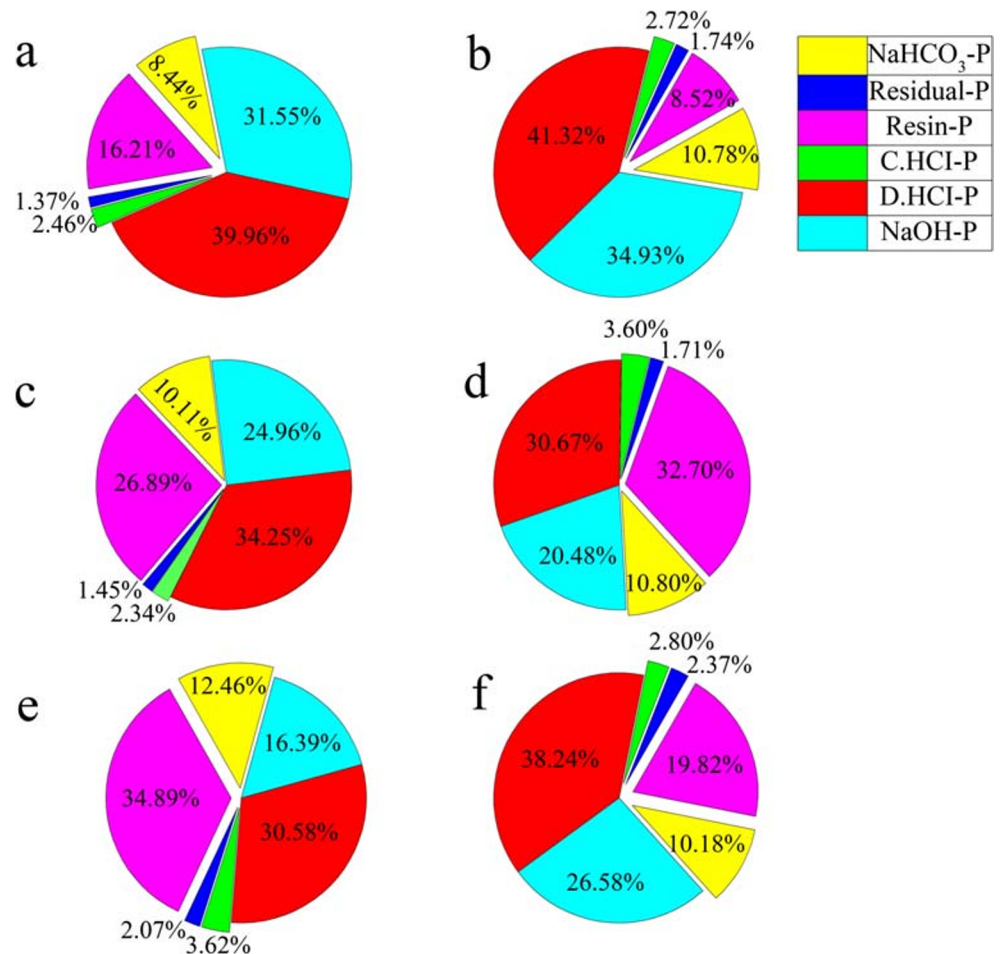
SRP and TP concentrations in the overlying water were highest in the 10 mM Na_2MoO_4 group (Fig. 3b). Its concentration of SO_4^{2-} was higher than that in the other groups from 70 to 140 days. However, the high sulfate concentration did not produce a high concentration of released P, indicating sulfate reduction was important for inducing the endogenous phosphorus.

Transformation of P fractions in sediments after sulfate input

The P fractions are critical for the bioavailability of phosphorus. As such, the changes of P fractions in sediments after different levels of sulfate input were investigated (Fig. 4). Generally, sediment phosphorus is present in six different fractions and the moderate active phosphorus (NaOH-P and D. HCl-P) were dominant forms (46.97–76.25%) for all groups. An interesting observation was that the percentage of these two dominant P fractions decreased with the addition of sulfate. Additionally, the degree of decrease was positively related to the content of sulfate.

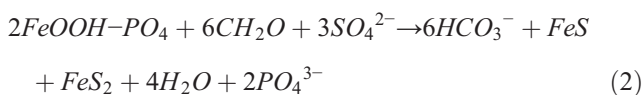
NaOH-P consists of inorganic phosphorus closely bound to iron and aluminum compounds as well as organic phosphorus bound to humic acid (Norton et al. 2008; Jan et al. 2013).

Fig. 4 Comparison of the average percentage of different P fractions (Resin-P, NaHCO_3 -P, NaOH-P, D. HCl-P, C. HCl-P, Residual-P) of the sediments among different treatment groups (a (control), b (10 mM Na_2MoO_4), c (2 mM Na_2SO_4), d (4 mM Na_2SO_4), e (6 mM Na_2SO_4), and f (8 mM Na_2SO_4))



Since aluminum and aluminum hydroxide are relatively stable under aerobic and anaerobic conditions (Jansen et al. 2003; Steinman et al. 2004), the change of Al-bound phosphorus content in the sediment is very small (Zhang and Huang 2007). Hence, the active inorganic phosphorus of sediments can be basically represented by the iron-bound phosphorus and sulfate reduction promoted the transformation and release of iron-bound phosphorus. The reason for the sulfate-driven transformation and release of iron-bound phosphorus (FeOOH-PO₄ compound) is mainly attributed to the competitive advantage of reductant sulfur for iron ions with phosphate. Sulfate reduction can produce the reductant sulfur (S²⁻) to react with FePO₄ to replace PO₄³⁻.

(Eq. 2) (Rutterberg and Berner 1993).



D. HCl-P is an inorganic phosphorus bound to Ca. Compared with the control group, the percentage of D. HCl in the sediment in runs 2–5 also decreased (Fig. 4). Phosphate-solubilizing bacteria (PSB) are bacteria associated with P release. Through the series of desorption, decomposition, and mineralization, phosphorus from insoluble inorganic phosphorus and phosphorous organic compounds is converted into soluble orthophosphates and released into water, producing organic acids—such as lactic acid, citric acid, succinic acid, acetic acid, and formic acid—and inorganic acids—such as nitric acid and sulfuric acid—and releasing positrons from Ca₃(PO₄)₂ (Adnan et al. 2019).

Different from the decrease in NaOH-P and D. HCl-P, the increase in the percentage of active phosphorus (Resin-P) was observed with the input of sulfate. These observations showed

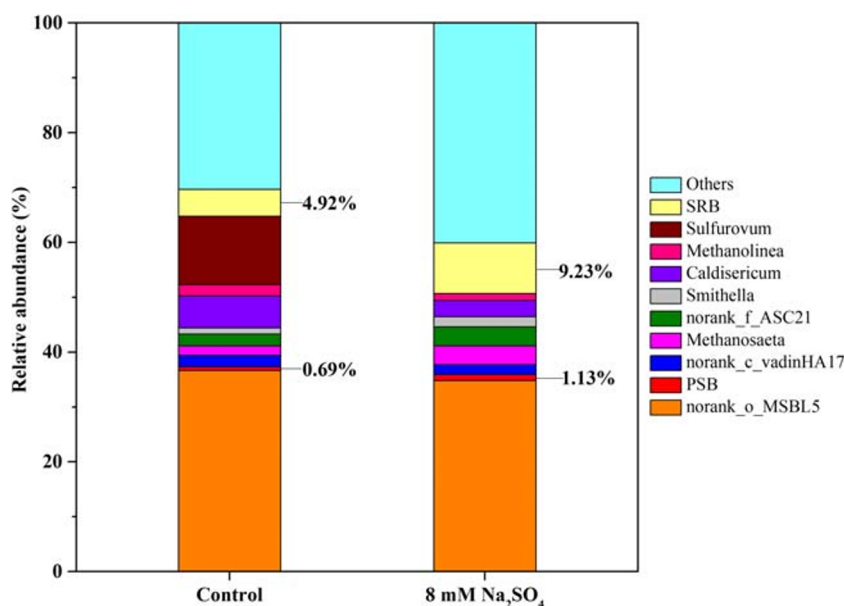
the transformation of NaOH-P and D. HCl-P to Resin-P. Resin-P is free exchangeable inorganic phosphate that can enter the overlying water following the concentrate gradient and is the direct source of the phosphorus release. Compared with moderately active phosphorus, Resin-P is relatively more active. The transformation from NaOH-P and D. HCl-P to Resin-P, from less active to more active, and from the potential source to the direct source of phosphorus release increased the contents of TP and SPR in the overlying water. Moreover, our previous work showed that Resin-P is more favorable for algal growth, compared with NaOH-P and D. HCl-P (Zhu et al. 2017). Additionally, the contents of total phosphorus in the sediments decreased with the addition of sulfate. The decreases for 2 mM-, 4 mM-, 6 mM-, and 8 mM sulfate-addition group were separately 495 ± 16.4, 609 ± 10.8, 717 ± 15.3, and 846 ± 15.0 mg/kg, which further confirms the sulfate-driven release of endogenous P. Therefore, it is concluded that the sulfate input increases the risk of the eutrophication of water body.

Analysis of diversity and structure of bacterial flora

The alpha diversity reflects the species richness and diversity of microbial flora in samples. It was found that no obvious differences between the control and 8 mM sulfate group, as evidenced by the values of the richness of operational taxonomic units (OTUs) and Chao1 index. However, according to the Simpson index, the evenness of bacterial flora for 8 mM sulfate group (0.125) was higher than that of the control group (0.0937).

Figure 5 illustrates the bacterial flora structure and relative abundance at the genus level in the control and 8 mM Na₂SO₄ groups. The relative abundance of norank_o_MSBL5 (OTU₂) in the control and 8 mM sulfate-addition group

Fig. 5 The flora distribution of bacterial communities in the genus level in the control and 8 mM Na₂SO₄ groups. Bacteria with a relative abundance of < 0.5% in either group are included under “others”



was 36.61% and 34.8%, respectively. It belongs to the category of *Dehalococcoidia*, which includes microorganisms that exhibit strictly anaerobic organohalide respiration. Furthermore, the organic acids produced after dehalogenation are a carbon source or energy source for other types of microorganisms (Yang et al. 2019). Some studies have reported that an unnamed genus similar to *Dehalococcoidia* exists in the phylum *Chloroflexi*, and it has the potential to decompose carbohydrates, plant lignin, and cellulose in the sediment. Hence, diverse bacteria from the phylum *Chloroflexi* are widespread in the sediment (Hug et al. 2013).

Additionally, significantly different relative abundances of *Sulfurovum* were observed between the control (12.39%) and 8 mM sulfate-addition group (1%) (Fig. 5). *Sulfurovum* is a Gram-negative bacterium, chemoautotroph, and facultative anaerobe, and it uses elemental sulfur or thiosulfate as the electron donor (Inagaki et al. 2004; Mino et al. 2014). The 8 mM sulfate group is apt to convert the sulfate to negative-valence sulfur, which causes the relative abundance of *Sulfurovum* to be low due to the lack of electron donors (Mori et al. 2018).

Analysis of characteristics of target flora

In this study, target bacteria mainly included PSB (e.g., *Bacillus*, *Flavobacterium*, *Streptomyces*, and *Micrococcus*) and SRB (e.g., *Desulfobacterium*, *Desulfurococcus*, and *Desulfuromonas* of class Deltaproteobacteria). PSB are a group of bacteria related to P release. They produce organic acids, such as lactic acid, citric acid, succinic acid, acetic acid, and formic acid, as well as inorganic acids, such as nitric acid and sulfuric acid, to promote the dissolution of insoluble phosphorus fractions. For example, PSB are mainly responsible for the decomposition and release of D. HCl-P (Li et al. 2019). However, their ability to release positrons from Fe–Al compounds is much lower (Bautista-Cruz et al. 2019). The relative abundance of PSB was 1.13%, which is higher than that of control (0.69%). The dominant PSB genus in both groups was *Thiobacillus*, which is common sulfur-oxidizing denitrifying bacteria (Fig. 5). In the 8 mM sulfate group, there were more sulfide ions produced by sulfate reduction (Thode-Andersen and Jorgensen 1989). They were available for the process of denitrification of *Thiobacillus* who contributed to more D. HCl-P release.

SRB are chemotrophs and heterotrophs that can use sulfate as the electron acceptor and H₂, lactate, fatty acid, ethanol, dicarboxylic acid, and aromatic compounds as the electron donor. They can reduce SO₄²⁻ to H₂S, and they are strictly anaerobic or facultative anaerobic (Cai et al. 2009; Feng and Ma 2005). SRB accounted for 9.23% of the total bacteria for the sulfate-addition group, which is higher than 4.92% in the control group (Fig. 5). This finding indicated that a high sulfate concentration was favorable for the growth of SRB, which could take more part in sulfate-driven phosphorus release.

Conclusions

These results presented in this study showed that sulfate reduction was closely related to the phosphorus release at sediment–water interface of the urban malodorous river. It was found that the phosphorus release was positively related to the level of sulfate, and the 8 mM Na₂SO₄-addition group demonstrated the maximum P release with the highest contents of TP and SRP (14.01 mg/L and 12.27 mg/L, respectively) in the overlying water. A clear transformation of NaOH-P and D. HCl-P to highly bioavailable Resin-P was observed for all sulfate-addition groups, which resulted in more release of phosphorus to the overlying water. It was also observed the relative abundance of PSB and SRB increased separately from 0.69 to 1.1% and 4.92 to 9.03% with the addition of sulfate. Taken together, the sulfate-driven release of endogenous P was mainly due to the competitive advantage of reductant sulfur for iron ions with phosphate. Sulfate reduction can produce the reductant sulfur (S²⁻) to react with FePO₄ to replace PO₄³⁻. Further study is needed to clarify the above deduction by exploring the effects of different forms of sulfur on phosphorus release and fractions.

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Authors' contributions Guan Linchang and Xia Zhenyu: writing—original draft preparation, investigation, and data analysis. Jin Lili: investigation, data analysis, and visualization. Xu Yiwen: investigation and data analysis. He Yan: conceptualization, writing—review and editing, project administration, and funding acquisition.

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Data availability The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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