The insignificant effect of increased sulfate concentration on nitrogen dynamics in eutrophic lakes: the neglected role of iron ions

Baogui Liu[A,B,#], Yu Peng[A,#], Miaotong Yu[A], Muchun Zhou[A,C], Lanqing Zhang[A], Li Chen[A], Ruoyu Jia[A], Chuanqiao Zhou[A,D,*] (iD), Yiting Wu[A], Xiaoguang Xu[A] and Guoxiang Wang[A]

For full list of author affiliations and declarations see end of paper

*Correspondence to:
Chuanqiao Zhou
School of Environment, Jiangsu Center for Collaborative Innovation in Geographical Information Resource Development and Application, Jiangsu Key Laboratory of Environmental Change and Ecological Construction, Nanjing Normal University, Nanjing, 210023, PR China.
Present address: 1 Wenyuan Road, Xianlin University District, Nanjing, 210023, PR China.
Email: chuanqiaozhou@163.com

#These authors contributed equally to this work

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ABSTRACT

Context. Sulfate (SO_4^{2-}) concentrations in eutrophic lakes are continuously increasing. **Aims.** This study aimed to explore the effects on nitrogen and their limiting factors in eutrophic lakes. **Methods.** We constructed a series of microcosms with different initial SO_4^{2-} concentrations (0–180 mg L^{-1}) to simulate the SO_4^{2-} concentration in eutrophic lakes. **Key results.** The NO_3^--N concentration rapidly decreased in all treatments. The respective 2.21 and 2.77 mg L^{-1} concentrations of NO_3^--N recorded on Day 5 in the initial 150 and 180 mg L^{-1} SO_4^{2-} concentration treatments were higher than in other treatments. The highest N_2O concentrations in all treatments showed insignificant differences. The existence of free Fe^{2+} that was released from iron reduction resulted in an insignificant effect of increased SO_4^{2-} concentration on nitrogen dynamics. With a decrease in Fe^{2+}, the acid volatile sulfate (AVS) increased, and the highest AVS concentrations were positively correlated with the initial SO_4^{2-} concentration (7.20–12.88 mg kg^{-1}). **Conclusion.** This effect could be ascribed to the fact that the rapid combination of sulfide (ΣS^{2-}) and Fe^{2+} prevents ΣS^{2-} from producing a toxic effect on the denitrification. **Implications.** Our study highlighted the role of iron in eutrophic lakes, especially in the coupling of sulfate reduction and nitrogen dynamics.

Keywords: cyanobacteria, denitrification, eutrophic lake, iron concentration, nitrogen dynamics, sulfate reduction, sulfate-reducing bacteria, sulfide.

Introduction

Lakes play a crucial role in the interactions among each layer of the Earth's surface system (Davidson *et al.* 2018; Wang *et al.* 2018). Exogenous nutrients, such as nitrogen and phosphorus, have been introduced into freshwater lakes in recent years, resulting in cyanobacterial blooms that negatively influence lake ecosystems (Huisman *et al.* 2004). Studies have shown that lakes are important natural sources of greenhouse gas emissions (Bastviken *et al.* 2011). Despite contributing to global warming by only 5%, N_2O has a very high global warming potential that is 289 times higher than that of CO_2 and 12 times higher than that of CH_4 (Karlén 2001; Sturm *et al.* 2014). Therefore, the emission and production processes of N_2O in eutrophic lakes have become a hot research topic (Yan *et al.* 2017; Zhou *et al.* 2021).

Researchers generally agree that cyanobacterial blooms contribute to more N_2O emissions (Yan *et al.* 2017; Liu *et al.* 2022). The majority of the N_2O emissions in lakes are triggered by microbial activity in sediments, including nitrification, denitrification and dissimilatory nitrate reduction to ammonium (DNRA), with nitrification and denitrification accounting for 90% (Gödde and Conrad 2000). Cyanobacteria decomposition changes the overlying water from an aerobic state to an anaerobic reduction state (Li *et al.* 2011; Xing *et al.* 2011). Therefore, denitrification plays a dominant role in N_2O production because nitrification requires the participation of oxygen (Well *et al.* 2006;

Morse and Bernhardt 2013). The participation of denitrifying bacteria is essential to denitrification (Prosser and Nicol 2012). At the same time, complete denitrification requires the participation of nitrate reductase (NAR), nitrite reductase (NIR), nitric oxide reductase (NOR) and nitrous oxide reductase (NOS) (Kool *et al.* 2011). Denitrification is affected by many factors, including dissolved oxygen (DO), temperature, microbial community structure and activity (Yang and Silver 2016). Studies have shown that sulfide (ΣS^{2-}), which is produced by sulfate reduction, has toxic effects on microorganisms and microbial enzymes in denitrification and, subsequently, affects nitrogen dynamics by inhibiting denitrification (Senga *et al.* 2006; Jones *et al.* 2017). However, in eutrophic biological systems, the inhibiting effect of ΣS^{2-} on denitrification has not always been observed for several reasons, including the presence of high metal ions and the low concentration of ΣS^{2-} (Baldwin and Mitchell 2012).

As one of its important products, ΣS^{2-} concentration is greatly affected by sulfate reduction (Saxton *et al.* 2021; Zhou *et al.* 2022). In recent years, an input of a large amount of exogenous sulfur has led to a significant increase in SO_4^{2-} in freshwater bodies across the world (Lamers *et al.* 2001; Yu *et al.* 2013). For instance, the SO_4^{2-} concentration in Lake Taihu, one of the typical eutrophic lakes worldwide, has increased from 30 to 100 mg L^{-1} in the past 70 years, and it will continue to rise in the future (Yu *et al.* 2013; Zhou *et al.* 2022). An increase in SO_4^{2-} (as the charged electron in sulfate reduction) concentration also increases the sulfate reduction strength and produces additional ΣS^{2-} (Holmer and Storkholm 2001; Zhou *et al.* 2022). Previous studies have shown that ΣS^{2-} exists only in the system for a very short time (Friedrich and Finster 2014; Zhao *et al.* 2019) because of two factors, namely, the hydrogen sulfide overflowing from the incubator and the downward migration of sulfide, which then combines with other substances in the sediment and becomes immobilised (Poulton 2003; Fedorov *et al.* 2019). In eutrophic lakes, the formation of stable acid volatile sulfide (AVS) by the combination of ΣS^{2-} with iron ions is a common phenomenon that releases endogenous phosphorus and results in the rapid disappearance of ΣS^{2-} (Zhang *et al.* 2021). At the same time, the anaerobic reducing environment formed by cyanobacteria decomposition promotes iron reduction and significantly increases the Fe^{2+} concentration in the overlying water (Chen *et al.* 2016). Therefore, the high concentration of iron ions in eutrophic lakes is an important factor that restricts the influence of ΣS^{2-} (Baldwin and Mitchell 2012).

In this study, a series of microcosmic systems with initial SO_4^{2-} concentrations of 0, 30, 60, 90, 120, 150 and 180 mg L^{-1} was constructed to explore the effect of increasing SO_4^{2-} concentrations and cyanobacteria decomposition on nitrogen dynamics in eutrophic lakes. The dynamic variations of S, Fe and N in water columns and the N_2O concentration in the head space were intensively investigated. Results highlighted the effect of increased SO_4^{2-} concentration in eutrophic lakes on N cycling. Following previous research, the change in Fe^{2+} in this study limits the change in the N cycle caused by SO_4^{2-} in lakes, thereby providing novel ideas for follow-up research.

Materials and methods

Sample collection and preparation

Samples of sediments and cyanobacteria were collected from the eutrophic Lake Taihu (31°24′45″N, 120°0′42″E) in July 2020. Cyanobacteria were collected by using a 64-μm mesh plankton net. Sediments from this site were collected by using a gravity core sampler. The sediment samples were blended thoroughly, homogenised, sieved with a 100 mesh and placed in a polyethylene bag. Cyanobacteria were collected and concentrated by sieving water through a fine plankton mesh (250 meshes) and stored in an incubator with ice packs before being immediately taken to a laboratory. The cyanobacteria samples were flushed and centrifuged at 1500 revolutions min^{-1} for 5 min at an experimental temperature of 25°C with a CT15RT versatile refrigerated centrifuge (SC-3610, Zonkia, PR China) and freeze-dried using Biosafer-10A. Different gradient sulfate concentrations were prepared from high-purity water and Na_2SO_4.

Incubation experiment setup

Microcosm systems were constructed to simulate the dramatic increase in SO_4^{2-} concentration and cyanobacteria bloom in Lake Taihu. These systems comprised 51 anaerobic bottles for each SO_4^{2-} concentration treatment (diameter 75 mm, length 180 mm and volume 500 mL), and three replicates were used for each system. According to the ratio of surface sediments, the average water depth and the cyanobacteria accumulation density in Lake Taihu during the cyanobacteria bloom (2500 g m^{-2}), 100 g of sediment, 200 mL of water and 0.11 g of cyanobacteria powder were added into each bottle to simulate cyanobacteria decomposition process (Zhang *et al.* 2021). On the basis of the changes in the SO_4^{2-} concentration of Lake Taihu over the years (Yu *et al.* 2013), the SO_4^{2-} concentrations in the microcosm systems were set to 30, 60, 90, 120, 150 and 180 mg L^{-1}. A control without SO_4^{2-} was also used. All systems were then placed in a biochemical incubator at a temperature of 25°C. Each treatment was sampled on Days 1, 2, 3, 4, 5, 6, 7, 9, 11, 14, 18, 23, 28, 33, 38, 43 and 48. The water, gas and soil samples were collected by destructive sampling, and three anaerobic bottles were collected for each group, at each sampling point, 18 anaerobic bottles were opened for testing, which ensured the anaerobic environment and air pressure for other bottles. Gas was extracted from the microcosm systems by using a syringe, and one part of the sediment was used for determining microorganisms and was stored in a refrigerator at −80°C. The rest of the

sediments and other samples were kept at 0–4°C for less than 24 h before the analysis.

Chemical analytical methods

Water dissolved oxygen (DO) and oxidation and reduction potential (ORP) were measured using calibrated probes (MP525, China). All water-column and pore-water samples were filtered through 0.45-μm nylon filters before measuring total nitrogen (TN), ammonium nitrogen (NH_3-N), nitrate nitrogen (NO_3^--N), NO_2^--N, SO_4^{2-} and ΣS^{2-}. SO_4^{2-} and ΣS^{2-} were detected by turbidimetry (Tabatabai 1974) and methylene blue colourimetry (Cline 1969) respectively, whereas the Fe^{2+} and Fe^{3+} in the overlying water were determined using the calorimetrical method (Lovley and Phillips 1988). TN was determined photometrically using a UV-vis spectrophotometer (UV-6100, Mapada, PR China) (Raveh and Avnimelech 1979). NH_3-N, NO_3^--N and NO_2^--N were determined using an auto-analyser (Auto-analyzer 3, SEAI, Germany). The AVS in the sediment was determined using the zinc cold diffusion method (Hsieh and Shieh 1997), and the N_2O cocentration was determined using a gas chromatograph (GC) (7890B, Aglient).

Quantification of sulfate-reducing bacteria (SRB) in sediments

Sequencing and real-time reverse-transcriptase quantitative polymerase chain reaction (RT-qPCR) technologies were used to confirm the changes in sediment SRB in the microcosm systems. The sediments from microcosm systems on Days 0 and 38 were collected to characterise the abundance of SRB.

The sediment samples were collected and frozen at −80°C in an ultra-low-temperature freezer. The E.Z.N.A. Soil DNA Kit (Omega Bio-Tek, Norcross, GA, USA) was used to extract the total genomic DNA from each soil sample, following the instructions of the manufacturer. Nucleic acid quality and concentration were determined by 1% agarose gel electrophoresis and a NanoDrop 2000 UV spectrophotometer (Thermo Scientific, USA) respectively.

The SRB in sediments was quantified using the qPCR technique. The primer pair for *dsrB* was DSR1F+/DSR-R. The qPCR experiments were performed on an ABI7300 qPCR instrument (Applied Biosystems, USA) using ChamQ SYBR Color qPCR Master Mix as the signal dye. Each 20 μL reaction mixture contained 2 μL of the template DNA and 16.5 μL of the ChamQ SYBR Color qPCR Master Mix. Standard curves for each gene were obtained by a 10-fold serial dilution of standard plasmids containing the target functional gene. All procedures followed the MIQE guidelines (Bustin *et al.* 2009).

Statistical analysis

SPSS (ver. 18.0, IBM, see https://www.ibm.com/au-en/products/spss-statistics) was used for the statistical analysis,

and the one-way ANOVA and correlation analysis were performed by bivariate correlations analysis.

Results

DO and ORP dynamics in overlying water

Cyanobacteria decomposition influenced the DO and ORP in the overlying water (Supplementary Fig. S1). No significant differences in DO and ORP were detected among the treatments with different initial SO_4^{2-} concentrations. All treatments shifted to an anaerobic state on Day 2, and the DO concentrations were 0.48, 0.44, 0.43, 0.48, 0.46 and 0.49 mg L^{-1} in the treatments. The overlying water of all treatments remained anoxic until the end of incubation. The ORP in all treatments showed a consistent change trend with DO. The ORP in all treatments was below 100 mV on Day 2 (−111.63, −115.37, −123.05, −122.43, −120.43, −122.63 and −106.53 mV).

### SO_4^{2-} and ΣS^{2-} dynamics in overlying water

Sulfate reduction was observed in all treatments with cyanobacteria decomposition. The SO_4^{2-} concentrations decreased substantially and eventually reached close to 0 (Fig. 1). The lowest-reported SO_4^{2-} concentrations were 1.18 and 0.24 mg L^{-1} in the treatments with initial SO_4^{2-} concentrations of 150 and 180 mg L^{-1} on Day 21 respectively. However, the treatment with an initial SO_4^{2-} concentration of 30 mg L^{-1} reported the lowest SO_4^{2-} concentration of 2.92 mg L^{-1} on Day 5. The lowest SO_4^{2-} concentrations in the remaining treatments with initial SO_4^{2-} concentrations of 60 and 90 mg L^{-1} were respectively 2.56 and 7.2 mg L^{-1} on Day 9. The lowest SO_4^{2-} concentration in the treatment with an initial SO_4^{2-} concentration of 12.0 was 5.33 mg L^{-1} 1 day later than that in the treatment with initial SO_4^{2-} concentration of 90 mg L^{-1}.

The maximum ΣS^{2-} concentration was positively correlated with the initial SO_4^{2-} concentration in the overlying water (Fig. 1). Higher initial SO_4^{2-} concentrations were associated with higher maximum ΣS^{2-} concentrations. The maximum ΣS^{2-} concentration in the treatment with an initial SO_4^{2-} concentration of 180 mg L^{-1} was approximately six times larger than that in the treatment with an initial SO_4^{2-} concentration of 30 mg L^{-1} (i.e. 3.59 and 0.61 mg L^{-1}).

TN, NH_3-N, NO_3^--N and NO_2^--N dynamics in overlying water

As a result of the cyanobacteria decomposition, the morphological nitrogen in the overlying water showed different trends (Fig. 2). Specifically, TN initially increased and then decreased before stabilising at the end of the incubation. The maximum TN concentration was observed on Day 5, and no significant differences were observed among the
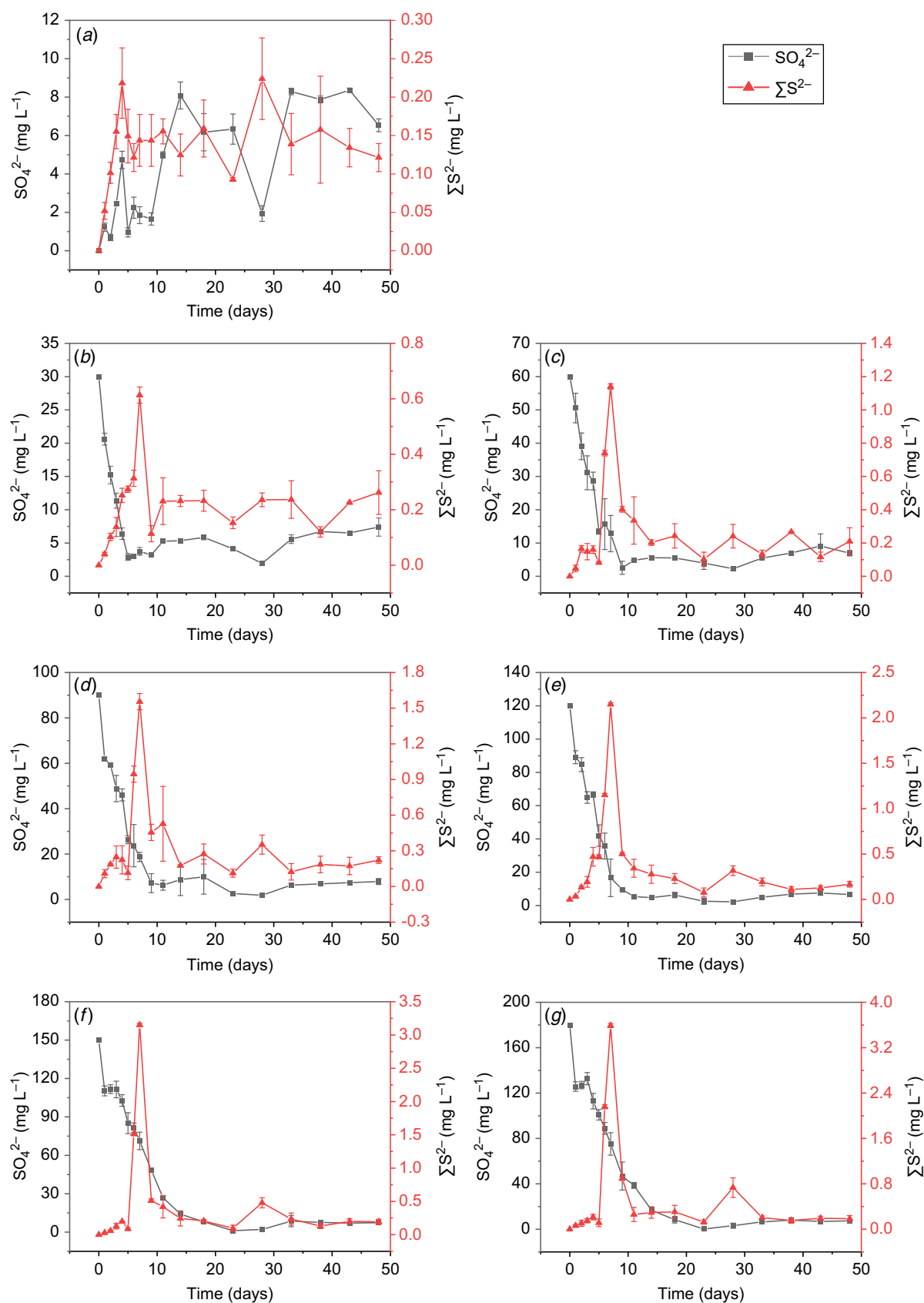
Fig. 1. Dynamic change of SO_4^{2-} and ΣS^{2-} in the water column from different initial SO_4^{2-} concentration microcosms during the incubation (*a*) 0 mg L^{-1}; (*b*) 30 mg L^{-1}; (*c*) 60 mg L^{-1}; (*d*) 90 mg L^{-1}; (*e*) 1200 mg L^{-1}; (*f*) 0 mg L^{-1}; and (*g*) 180 mg L^{-1}.

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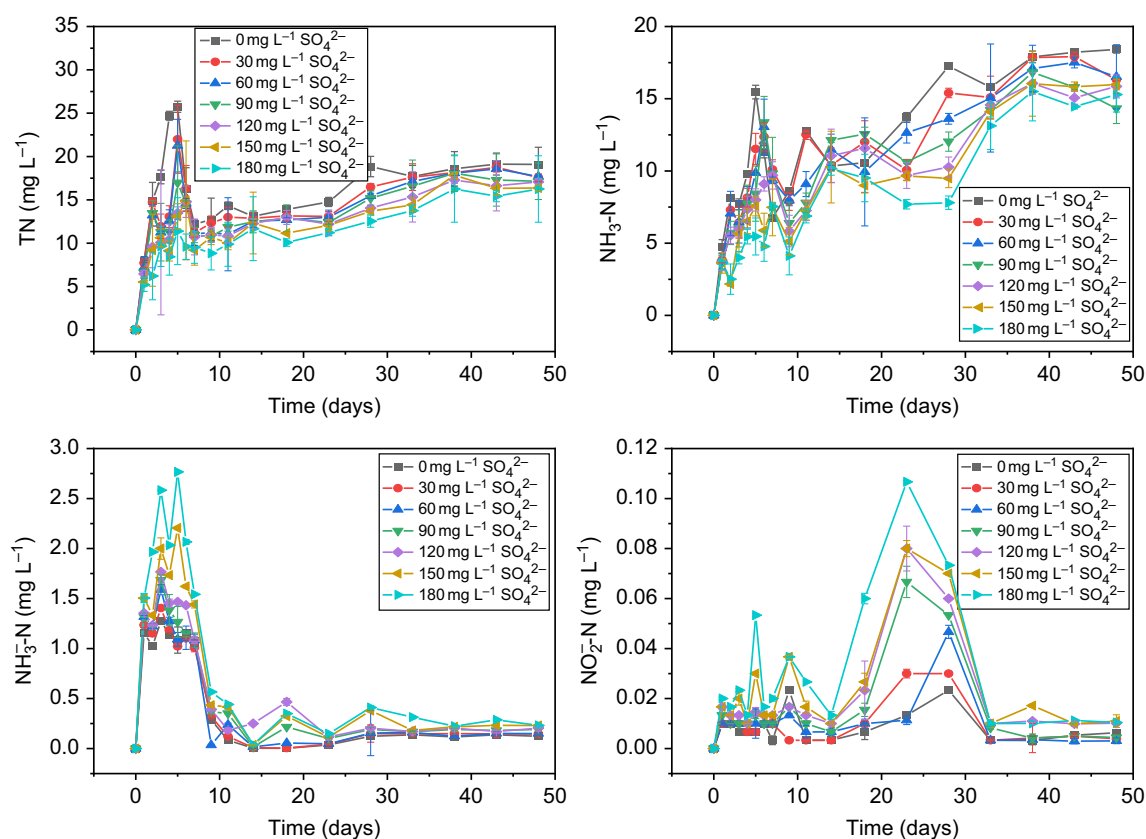
Fig. 2. Dynamic change of TN, NH$_3$-N, NO$_3^-$-N and NO$_2^-$-N in the water column during the incubation.

treatments. NH$_3$-N continuously increased before becoming stable on Day 28. The NH$_3$-N concentrations in the initial SO$_4^{2-}$ treatments of 0, 30, 60, 90, 120, 150 and 180 mg L^{-1} were 17.25, 15.4, 13.61, 12.03, 10.27, 9.49 and 7.80 mg L^{-1} respectively. The concentration of NO$_3^-$-N increased significantly at the beginning of the incubation and reached its peak on Day 3. The treatments with initial SO$_4^{2-}$ of 150 and 180 mg L^{-1} had the highest NO$_3^-$-N of 2.00 and 2.58 mg L^{-1} respectively. From Days 7 to 11, NO$_3^-$-N decreased significantly and eventually became stable. The NO$_2^-$-N concentration was low at the initial stage, gradually increased after the reduction of NO$_3^-$-N concentration, reached its peak on Day 23 and decreased to a stable stage.

Dynamics change in Fe^{2+}, Fe^{3+}, total Fe and AVS

The variations in the concentrations of Fe^{2+}, Fe^{3+} and total Fe in overlying water during the incubation are shown in Fig. 3. The concentrations of Fe^{2+}, Fe^{3+} and total Fe in the treatment without SO$_4^{2-}$ were always the highest compared with the other treatments. The Fe^{3+} and total Fe continuously increased during the incubation, and the Fe^{3+} and total Fe concentrations in the treatment without SO$_4^{2-}$ remained the highest on Days 38 and 23 (9.9 and 19.7 mg L^{-1} respectively). The higher the concentration of SO$_4^{2-}$ was, the lower the concentration of Fe^{2+}, showing a negative

correlation. The Fe^{2+} concentration in all treatments began to decrease on Day 9, which corresponded to an AVS increase in the sediments. However, in the treatment without SO$_4^{2-}$, the Fe^{2+} concentration increased after 14 days, which contradicted the trends observed in the other treatments, and the highest Fe^{2+} concentration reached 16.7 mg L^{-1} on Day 33. The AVS concentrations in the sediment were positively correlated with the initial SO$_4^{2-}$ concentration. The AVS reached the highest concentrations of 7.2, 7.9, 8.5, 8.9, 9.3, 11.1 and 12.9 mg kg^{-1} in all treatments on Day 11.

## Dynamics change in N$_2$O

Cyanobacteria decomposition promoted N$_2$O emissions during the incubation (Fig. 4). The N$_2$O concentration in all treatments initially increased and then decreased. The N$_2$O concentration in the treatment without SO$_4^{2-}$ reached 0.013 μmol L^{-1}, which was higher than that in the other treatments on Day 11. However, throughout the whole incubation period, no significant differences in N$_2$O concentrations were observed across all treatments (Supplementary Fig. S2).
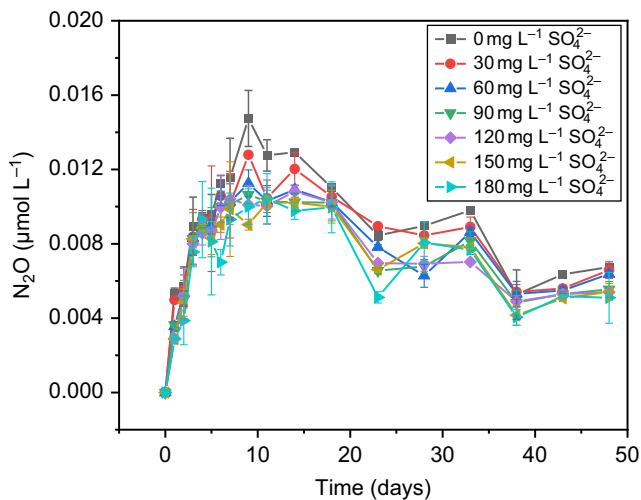
SRB in the sediments

The final abundance of SRB on Day 38 increased, along with the initial SO$_4^{2-}$ concentrations. Among all treatments, the

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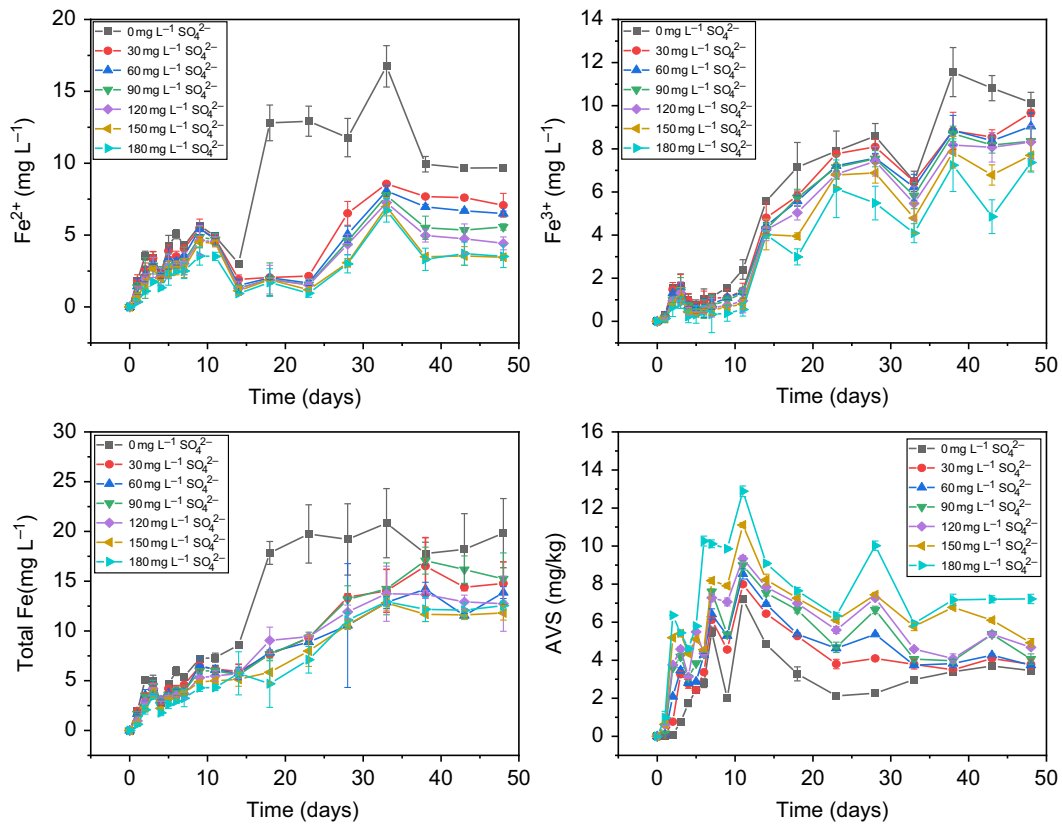
Fig. 3. Dynamic change of Fe^{2+}, Fe^{3+} and total Fe in the water column, and AVS in the sediment during the incubation.

Fig. 4. Dynamic change of N_2O concentration during the incubation.

final abundance of SRB in the treatments with initial SO_4^{2-} concentrations of 0, 30 and 60 mg L^{-1} was less than the initial abundance. The highest abundance of SRB $(2.98 \times 10^8$ copies $g^{-1})$ was observed in the treatment with an initial SO_4^{2-} concentration of 180 mg L^{-1} and it was

3.86 times higher than that in the treatment with an initial SO_4^{2-} concentration of 30 mg L^{-1}.

Discussion

Cyanobacteria bloom has a serious impact on the formation of anaerobic environments in the overlying water of shallow lakes (Ye *et al.* 2010; Xing *et al.* 2011). In the microcosmic systems constructed for this study, the cyanobacteria decomposition consumed DO and formed a strong anaerobic reduction environment (Fig. S1) that also released large amounts of nutrients (TN, NH_3-N, NO_3^--N and NO_2^--N; Fig. 2). Similar results have been observed *in situ* in eutrophic lakes (Zhou *et al.* 2021). Denitrification is the principal nitrogen removal reaction in anaerobic settings. Given that the enzyme activity required to complete the reaction is greatly affected by DO concentration (Gong *et al.* 2012; Morse and Bernhardt 2013), the anaerobic environment created by cyanobacteria decomposition promotes denitrification (Zhu *et al.* 2020). Denitrification is one of the important processes of N_2O emission, and cyanobacteria bloom promotes N_2O emission in eutrophic lakes (Yan *et al.* 2017). The *in situ* data showed that the N_2O emission from the cyanobacteria accumulation area is higher than that

from the open lake area (27.85 and 10.77 µmol m^{-2} day^{-1} respectively; Supplementary Fig. S3). In addition, the N$_2$O concentration increased significantly along with cyanobacteria decomposition in the microcosm system (Fig. 4). In the denitrification process, organic compounds were used as electron donors to NO$_3^-$-N and N$_2$O (Adouani *et al.* 2010). Therefore, the NO$_3^-$-N concentrations decreased significantly from Days 7 to 11 (Fig. 2). NO$_2^-$-N is an important intermediate product of the denitrification process. NO$_3^-$-N was reduced to NO$_2^-$-N by NAR and then to N$_2$O (Gödde and Conrad 2000; Kool *et al.* 2011). Fig. 2 shows that NO$_2^-$-N begins to rise after NO$_3^-$-N reaches its lowest concentration. Denitrification is influenced by many factors, including DO, temperature and microbial enzyme activity (Schreiber *et al.* 2012). Under different conditions, the factors that affect nitrogen dynamics are also restricted by other conditions. Previous studies have shown that increasing SO$_4^{2-}$ concentration has no obvious effect on the nitrogen cycle in eutrophic lakes, probably owing to the high iron concentration in the system (Baldwin and Mitchell 2012).

The sulfate reduction rate increases along with the SO$_4^{2-}$ concentration in the overlying water (Chen *et al.* 2016; Zhou *et al.* 2022). The SO$_4^{2-}$ concentration in Lake Taihu demonstrated significant seasonal variations but remained high (Supplementary Fig. S4). Sulfate reduction is an important mode of organic metabolism in natural ecosystems, especially in water layers with a low DO (Hausmann *et al.* 2016; Jørgensen *et al.* 2019). Previous studies have shown that sulfate reduction significantly affects the matter cycling in Lake Taihu, including the dynamic changes in phosphorus and other matters (Zhao *et al.* 2021). The increase in the initial SO$_4^{2-}$ concentration promoted the abundance of SRB (Fig. 5; Zhang *et al.* 2021). As the charged electron in the sulfate reduction reaction, SO$_4^{2-}$ was reduced to ΣS^{2-} after the reaction (Holmer and Storkholm 2001). Therefore, the SO$_4^{2-}$ concentration decreased after the sulfate reduction, which

resulted in the formation of a large amount of ΣS^{2-} through its participation in the sulfate reduction reaction, and its concentration increased along with the initial concentration of SO$_4^{2-}$ (Fig. 1). After its formation, part of ΣS^{2-} was released in the form of H$_2$S, whereas the other part was combined with metal elements in the sediments. The concentration of ΣS^{2-} in the overlying water rapidly decreased (Zhang *et al.* 2021). The bond of ΣS^{2-} with iron ions resulted in the formation of AVS, which was fixed in sediments and is considered one of the important destinations of ΣS^{2-} (Wu *et al.* 2019; Zhang *et al.* 2021). The AVS in sediments significantly increased (Fig. 3). Fe-bound P is abundant in lake sediments and is consumed by microorganisms when the concentration of organic matter is high (Mort *et al.* 2010; Zhao *et al.* 2019). This process releases large amounts of iron ions in the overlying water (Fig. 3). The formation of an anaerobic reduction environment is conducive to the gradual increase of Fe^{2+} concentrations in the overlying water (Fig. 3). Therefore, in this study, the presence of high iron ions was an important reason behind the short-lived presence of ΣS^{2-}.

Although increased SO$_4^{2-}$ concentration stimulates sulfate reduction and releases more ΣS^{2-} (Chen *et al.* 2016; Zhou *et al.* 2022), its effects on N dynamics are not obvious, because majority of ΣS^{2-} combined with Fe^{2+} to form AVS was fixed in the sediment (Fig. 2, 5). In this study, the higher the concentration of SO$_4^{2-}$ was, the lower concentration of Fe^{2+}, which proved that Fe^{2+} combined with the ΣS^{2-} to form the AVS (Fig. 3). ΣS^{2-} is toxic to microbial enzymes, hence inhibiting the activity and abundance of microorganisms (Kosolapov *et al.* 2004). Denitrification process, including NAR, NIR, NOR and NOS, requires the participation of a variety of microbial enzymes that are susceptible to the toxic effect of ΣS^{2-} (Yang and Silver 2016). Sulfide has been shown to affect nitrogen dynamics in aquatic sediments, principally through the inhibition of denitrification and stimulation of dissimilatory DNRA (Baldwin and Mitchell 2012). In addition, denitrification is superior to sulfate reduction in its competition for organic matter (Liu *et al.* 2007). Therefore, the competition for organic matter is unlikely to be one of the factors affecting the denitrification process of sulfate reduction, especially in the case where cyanobacteria decomposition releases sufficient organic matter (Ye *et al.* 2015; Yan *et al.* 2017). Moreover, an increased SO$_4^{2-}$ concentration has no significant effect on N dynamics given the lack of any significant correlation between N$_2$O concentration and the initial SO$_4^{2-}$ concentration ($R^2 = 0.21$; Fig. S2).

A conceptual diagram was built to confirm that an increased SO$_4^{2-}$ concentration has no significant effect on N dynamics in the system of high iron concentration (Fig. 6). Using exogenous sulfur as an input increased the SO$_4^{2-}$ concentration (Yu *et al.* 2013) and promoted the intensity of sulfate reduction, thereby triggering the release of additional ΣS^{2-} (Chen *et al.* 2016). Moreover, cyanobacteria
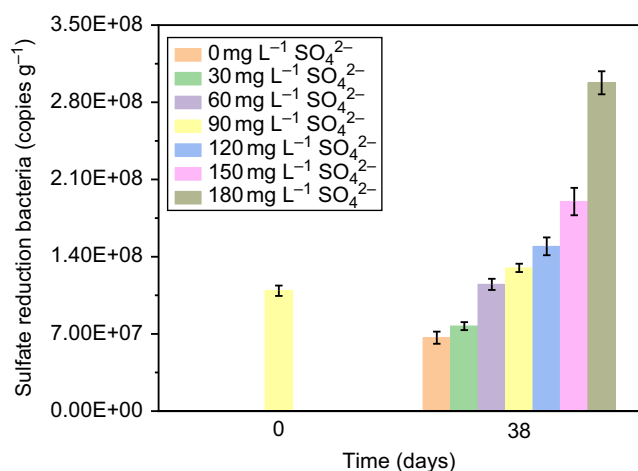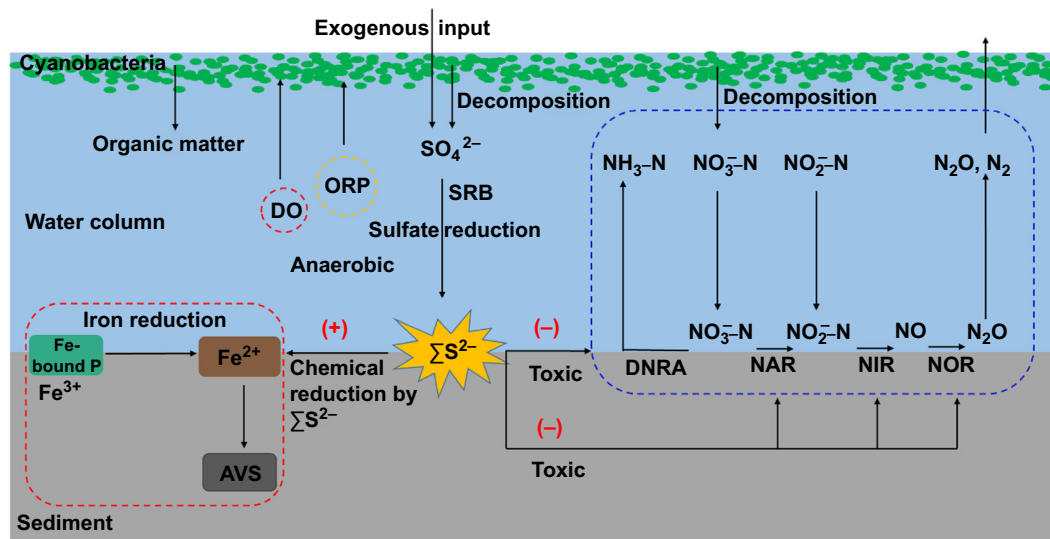

Fig. 5. Dynamic changes of SRB in sediments of microcosms.

Fig. 6. A simplified scheme of the effect of sulfur reduction on the N dynamics inhibited by the presence of high Fe ions in eutrophic lakes.

decomposition released organic matter, created an anaerobic reduction environment, drove iron reduction and released Fe^{2+}. Free Fe^{2+} was readily combined with ΣS^{2-} to form AVS that was fixed in sediments (Zhang *et al.* 2021), thereby rapidly reducing the ΣS^{2-} in the overlying water (Zhao *et al.* 2019). ΣS^{2-} affects the denitrification process driven by microorganisms and is toxic to all microorganisms involved in such process (Kosolapov *et al.* 2004). However, because of the existence of free Fe^{2+}, ΣS^{2-} cannot remain for a long time, hence explaining its insignificant influence on N dynamics (Fig. 2). This study has provided new insights into the effect of increasing SO_4^{2-} concentration on N cycling and its possible limiting factors in eutrophic lakes. When treating N concentration in eutrophic lakes, the effect of the coupling between sulfate reduction and iron reduction needs to be considered.

Conclusions

The increased SO_4^{2-} concentration had no significant effect on N dynamics in the system of high iron concentrations. In eutrophic lakes, cyanobacteria decomposition creates an anaerobic environment in the overlying water, which leads to sulfate reduction and iron reduction. With an increasing initial SO_4^{2-} concentration, the sulfate reduction gradually increases, along with the production of ΣS^{2-}. The produced Fe^{2+} in the iron reduction process rapidly combined with ΣS^{2-} to form a stable Fe-bound S in sediments. The cyanobacteria decomposition process releases a large amount of nitrogen, including NH_3-N, NO_3^--N and NO_2^--N, which act as substrates that participate in the nitrogen cycle and promote N_2O production. Microorganisms play an important role in

the nitrogen cycle. Given its toxicity to microorganisms, ΣS^{2-} can inhibit their activity and abundance and, subsequently, affect the nitrogen dynamics. However, in a system with high iron ion concentration, ΣS^{2-} is rapidly bound, and its toxic effect on microorganisms is significantly inhibited, thereby eliminating its significant influence on the nitrogen dynamics and N_2O production. Therefore, when managing the effects of increased SO_4^{2-} concentration on nitrogen dynamics in eutrophic lakes, the possible effects of Fe^{2+} concentration should be considered.

Supplementary material

Supplementary material is available online.

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Author affiliations

[A]School of Environment, Jiangsu Center for Collaborative Innovation in Geographical Information Resource Development and Application, Jiangsu Key Laboratory of Environmental Change and Ecological Construction, Nanjing Normal University, Nanjing, 210023, PR China.

[B]School of Geography, Nanjing Normal University, Nanjing, 210023, PR China.

[C]China Aerospace Science and Industry Nanjing Chenguang Group, Nanjing, 210022, PR China.

[D]Present address: 1 Wenyuan Road, Xianlin University District, Nanjing, 210023, PR China.