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Effects of Sulfamethazine on Denitrification and the Associated N_2O Release in Estuarine and Coastal Sediments

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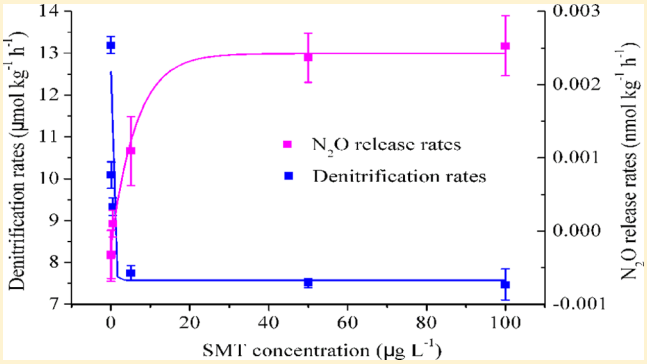
Supporting Information

ABSTRACT: Denitrification is an important pathway of nitrogen removal and nitrous oxide (N_2O) production in estuarine and coastal ecosystems, and plays a significant role in counteracting aquatic eutrophication induced by excessive nitrogen loads. Estuarine and coastal environments also suffer from increasing antibiotic contamination because of the growing production and usage of antibiotics. In this study, sediment slurry incubation experiments were conducted to determine the influence of sulfamethazine (SMT, a sulphonamide antibiotic) on denitrification and the associated N_2O production. Genes important for denitrification and antibiotic resistance were quantified to investigate the microbial physiological mechanisms underlying SMT's effects on denitrification. SMT was observed to significantly inhibit denitrification rates, but increasing concentrations of SMT enhanced N_2O release rates. The negative exponential relationships between denitrifying gene abundances and SMT concentrations showed that SMT reduced denitrification rates by restricting the growth of denitrifying bacteria, although the presence of the antibiotic resistance gene was detected during the incubation period. These results imply that the wide occurrence of residual antibiotics in estuarine and coastal ecosystems may influence eutrophication control, greenhouse effects, and atmospheric ozone depletion by inhibiting denitrification and stimulating the release of N_2O.



INTRODUCTION

Global reactive nitrogen production has increased dramatically over the past few decades.[1−4] A substantial amount of this reactive nitrogen (primarily in the form of nitrate) is transported into estuarine and coastal ecosystems via river flow, atmospheric deposition, and groundwater discharge.[2,4,5] An increasing reactive nitrogen load is recognized as an important driver of water pollution (e.g., eutrophication, harmful algae blooms, seasonal hypoxia, and acidification) in most estuarine and coastal regions around the world.[6−10] Therefore, pathways for reactive nitrogen removal from aquatic environments have attracted attention.[1,2,4,5] Denitrification has been identified as a dominant dissimilatory pathway to permanently remove reactive nitrogen from estuarine and coastal ecosystems.[11−14] However, this process is susceptible to interference from environmental changes caused by human activities.[2,3,14−16] An improved understanding of the denitrification response to anthropogenic influence is required to protect these aquatic ecosystems.[7,9,10]

Although antibiotics are generally easily degraded compared with persistent organic pollutants (POPs),[17] they are considered to be pseudopersistent contaminants in aquatic ecosystems due to their high usage in human medicine, veterinary medicine, farming, and aquaculture for the prevention and/or treatment of diseases.[18−23] Currently, the global usage of antibiotics is estimated at 100 000 to 200 000 tons annually.[22] However, due to the partial metabolism of antibiotics in organisms, as much as 80−90% of the compounds are excreted directly as parent compounds via urine and feces into aquatic environments.[20,22,24,25] Numerous reports have shown a wide occurrence and distribution of antibiotics in most estuarine and coastal environments.[22,23,26−28] Antibiotic residues, even at very low levels, can cause various environmental problems and may affect microbial population dynamics.[22,25,29,30] The antibacterial properties of antibiotics may influence denitrifying bacteria and their denitrification rates.[30−32] Despite the potential significance of antibiotics in controlling reactive nitrogen pollution and eutrophication, the importance of this issue remains unclear because studies are limited in aquatic ecosystems.[33−35]

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The misuse of antibiotics in China is common.[21,22] Currently, the annual antibiotic usage in China accounts for more than 20% of the global usage.[22,29,36] Sulfonamides, chloramphenicols, macrolides, and tetracyclines are the most prevalent antibiotics used in China.[19,22,37] Of these antibiotics, sulfonamides are widely detected in the estuarine and coastal regions of China at relatively high residual concentrations (approximately 50−1550 ng L^{-1}).[22,24,29,37,38] In this study, sulfamethazine (SMT), a common sulfonamide, was selected as a representative antibiotic to investigate the fate of antibiotics in estuarine and coastal environments and to reveal the potential influence of residual antibiotics on denitrification and the physiological response of denitrifying bacteria to the presence of antibiotics at the gene level. This study can also provide primary data for the control of reactive nitrogen and antibiotic pollution along with an evaluation of associated eco-environmental risks in these aquatic ecosystems.

MATERIALS AND METHODS

Sample Collection and Pretreatment. The Yangtze Estuary was selected as the sampling site to decipher the effects of antibiotics on the denitrification process. The Yangtze Estuary is one of the most hypereutrophic areas along the estuarine and coastal zone of China due to the reactive nitrogen overload in this region.[39,40] Surface (0−5 cm) sediments were collected from the study site using box corers in July 2013 (Figure S1, SI). After returning the samples to the laboratory, the ambient antibiotics in the sediment samples were removed by a preincubation experiment to eliminate their potential interference with the antibiotic addition experiments. Briefly, the sediment cores were placed into a recirculating glass container filled with artificial seawater[41] having near in situ salinity and nutrient levels,[42,43] and preincubated at room temperature (25 °C) to remove the ambient antibiotics in the sediments. The preincubation lasted approximately 2 months until the concentrations of all of the detected antibiotics in the sediments were below the detection limits.[22] After preincubation, the core sediments were homogenized under a N_2 atmosphere for subsequent antibiotic-added slurry incubation experiments.

Antibiotic Degradation Assays. Degradation experiments were conducted to determine the antibiotic dynamics during the slurry incubation experiments.[44] A 100 ng L^{-1} antibiotic solution was prepared using SMT (99.0%, Dr. Ehrenstorfer GmbH, Germany) and artificial seawater[41] with in situ salinity. The solution was purged with helium for 15 min. Subsequently, 120 mL gastight borosilicate vials were filled with the SMT solution, sealed with butyl-rubber stoppers, and crimped with aluminum caps to prevent leakage of the solution and gas. Four replicates were prepared, and these vials were designated as the control group. The collected sediments (150 g) were mixed with the SMT solution at a ratio of 1:7, and the mixture was stirred vigorously to make homogenized slurries. After poisoning with 1 g of NaN_3,[45] the vials were filled with the slurries and sealed. Four replicates were prepared and designated as the poisoned group to reveal the antibiotic adsorption ability of the sediments. An unpoisoned group was also prepared to determine the biotic degradation of the antibiotic. In this group, the sediments were mixed directly with the SMT solution to make slurries, and the vials were filled with the slurries and sealed. All of the slurry sample groups were incubated in the dark at room temperature (25 °C). At incubation times of 0, 1, 4, 8, 12, 24, 36, and 48 h, 1 mL of slurry was extracted from each sample using a syringe and used to analyze the SMT concentration. After filtration through 0.22 μm Millipore filters, SMT concentrations were determined using Waters Acquity ultra performance liquid chromatograph-tandem mass spectrometry (UPLC-MS/MS), with a detection limit of 1.2 ng L^{-1} for SMT.[22,29]

Measurement of the Denitrification Rates. The collected sediment samples were mixed with in situ salinity artificial seawater[41] at a ratio of 1:7, and the mixture was purged with helium for 15 min and stirred vigorously to make homogenized slurries.[40] Gas-tight borosilicate vials (12 mL, Labco Exetainer) were filled with the slurries, sealed with butyl-rubber septa, and screw caps to prevent leakage of the solution and gas. The samples were preincubated for 24 h to eliminate background nitrate before performing the following incubation experiments.[12,40]

In the SMT concentration gradient experiments, the slurry vials were spiked with $^{15}NO_3^-$ (final concentration of ∼100 μmol L^{-1}, final % ^{15}N: ∼ 90−99%, depending on the concentrations of the residual nitrate).[12] The SMT antibiotic solution was then injected into the vials at final concentrations of 0, 0.05, 0.5, 5, 50, and 100 μg L^{-1}. Subsequently, one-half of the replicates were designated as the initial samples and were preserved with 0.1 mL of saturated $HgCl_2$ solution.[40] The remaining slurries were shaken (200 rpm) and incubated for approximately 8 h at room temperature (25 °C).[12] At the end of the incubation, the remaining sample replicates were also preserved with $HgCl_2$, as described for the initial samples. The nitrogen gases ($^{29}N_2$ and $^{30}N_2$) produced during the incubations were analyzed with a membrane inlet mass spectrometer (MIMS),[46] with a detection limit of 0.05 μmol L^{-1} for N_2.[5] Four replicates were prepared for each concentration. The rates of denitrification were determined by quantifying the differences in the process-specific ^{15}N-labeled products between the final and initial samples of the experiments.[12,40]

In the time series experiments, the slurry vials were spiked with $^{15}NO_3^-$ as described above, and half of the replicates were injected with SMT at a final concentration of 5 μg L^{-1}. The antibiotic was not added to the other half of the vials, which were designated as the control group. Within the incubations, the slurry samples were harvested at 0, 2, 4, 6, 8, 12, 24, 36, and 48 h for both the control and SMT treatment groups. Four replicates for each time interval were analyzed using the MIMS. The rates of denitrification were estimated by quantifying the differences in the process-specific ^{15}N-labeled products in the samples between each time interval.[12,40]

The rates of denitrification in the concentration gradient and time series experiments were estimated according to the following equation:[12]

$$D = \frac{T_{30} \times 2 \times (1 - F_n)}{F_n} + 2 \times T_{30}$$

where D (μmol ^{15}N kg^{-1} h^{-1}) denotes the total $^{15}NO_3^-$-based denitrification rates, T_{30} (μmol $^{30}N_2$ kg^{-1} h^{-1}) indicates the total, measured production rates of $^{15}NO_3^-$-derived $^{30}N_2$ during the incubation, and F_n (%) denotes the fraction of ^{15}N in NO_3^-, which is obtained according to the added $^{15}NO_3^-$ and the measured residual NO_3^- concentrations in the incubation slurries.

Measurement of N_2O Production Rates. The concentration gradient and time series experiments were conducted as

described above to investigate the effects of the antibiotics on N_2O release during denitrification. After incubation, a headspace equilibrium technique was used for the dissolved N_2O measurement.[47] Briefly, 2 mL of ultrahigh-purity nitrogen gas was injected into each 12 mL vial to replace the water phase and create a headspace. The vials were shaken vigorously for 1 h to equilibrate the gas and liquid phases. Subsequently, the headspace gas was extracted using a syringe and quantified using a gas chromatography (Shimadzu GC-14B) with a detection limit of 0.1 ppb for N_2O.[47] The Bunsen solubility coefficient for the determined salinity and temperature after phase equilibration was used with the determined N_2O in the headspace to estimate the total N_2O concentration in the sediment slurries.[48] The production rates of N_2O over the incubations were calculated using the following equation:

$$R = \frac{(N_f - N_i) \times V}{T}$$

where R (nmol kg^{-1} h^{-1}) denotes the N_2O production rate, N_i and N_f (nmol mL^{-1}) indicate the total contents of N_2O dissolved in the initial and final slurry samples, respectively, V (mL) is the volume of the incubation vials, and T (h) represents the incubation time.

Molecular Microbial Analysis. Denitrification genes encoding nitrite reductase (*nirS*) and nitrous oxide reductase (*nosZ*), and the sulfonamide resistance gene (*sul*1, one of the most common marker genes for sulfonamide resistance) were quantified in the sediment slurries from the denitrification incubation experiments to explore the microbial physiological mechanisms underlying the interaction between antibiotics and denitrification.[32,49,50] Briefly, Powersoil DNA Isolation Kits (MO BIO) were used to extract total DNA from the incubated slurries according to the manufacturer's protocols. The extracted DNA was used for the real-time quantitative PCR determination of the abundances of the *nirS*, *nosZ*, and *sul*1 genes in the concentration gradient and time series incubation experiments. The nitrite reductase gene (*nirS*) in the extracted DNA was amplified with primers cd3aF (5′-GTS AAC GTS AAG GAR ACS GG-3′) and R3 cd (5′-GAS TTC GGR TGS GTC TTG A-3′).[31] The nitrous oxide reductase gene (*nosZ*) was amplified with primers nosZ2F (5′-CGC RAC GGC AAS AAG GTS MSS GT-3′) and nosZ2R (5′-CAK RTG CAK SGC RTG GCA GAA-3′).[49] The sulfonamide resistance gene (*sul*1) was amplified using primers sul1-F (5′-CGC ACC GGA AAC ATC GCT GCA C-3′) and sul1-R (5′-TGA AGT TCC GCC GCA AGG CTC G-3′).[50] The gene copy numbers of *nirS*, *nosZ*, and *sul*1 were determined in quadruplicate with an ABI 7500 Sequence Detection System (Applied Biosystems, Canada) using the SYBR green qPCR method. The 25 μL qPCR reaction consisted of 12.5 μL of Maxima SYBR Green/RoxqPCR Master Mix (Fermentas, Lithuania), 1 μL of each primer (10 μmol L^{-1}) and 1 μL of template DNA. All reactions were performed in 8-strip thin-well PCR tubes with ultraclean cap strips (ABgene, UK). The thermal cycling conditions were as follows: 2 min at 50 °C, 10 min at 95 °C, followed by 40 cycles of 30 s at 95 °C, 1 min at 57 °C for *nirS*, 60 °C for *nosZ* and 65 °C for *sul*1, and 1 min at 72 °C. The cycle thresholds were determined by comparison with standard curves constructed using a serial dilution of a known amount of plasmid DNA containing the target fragment. In this study, the standard curves ranged from 1.3×10^4 to 1.3×10^9 copies μL^{-1} for *nirS*, 1.2×10^2 to 1.2×10^7 copies μL^{-1} for *nosZ*, and 1.5×10^4 to 1.5×10^9 copies μL^{-1} for *sul*1. The amplification efficiencies were 97.4%, 99.2%, and 95.6% for the *nirS*, *nosZ*, and *sul*1 genes, respectively. The real-time qPCR consistency was confirmed by the strong linear relationships among the threshold cycle (C_T) and the log10 values of the gene copy numbers of the standard curves (R^2 = 0.9978 for *nirS*, R^2 = 0.9959 for *nosZ*, and R^2 = 0.9947 for *sul*1). Three no-template controls were run for all of the experiments to detect and exclude any possible contamination. In addition, the melting curve analyses showed no detectable peaks associated with primer-dimer artifacts or other nonspecific PCR amplification products.

Statistical Analysis. In this study, an analysis of variance (ANOVA) was performed to examine whether changes in the obtained data were statistically significant at the 95% confidence level. Correlation and regression analyses were also performed in the present study. The Statistical Package of Social Sciences (SPSS, version-19.0) was used for all statistical analyses.

RESULTS AND DISCUSSION

Antibiotic Degradation. In the degradation experiments, the control group data showed that SMT was degraded slightly (but significantly) at 48 h without sediments (repeated measures ANOVA, $p < 0.0001$) (Figure 1). The SMT concentrations in the control group decreased by 4.8 ± 0.04% during the incubation period. Because SMT is relatively resistant to hydrolysis,[51] it was not degraded extensively during incubation without photocatalytic enhancement. A significant decrease (7.7 ± 0.08%) in the poisoned group was detected during the incubation experiments (repeated measures ANOVA, $p < 0.0001$). Compared with the control group, 2.9 ± 0.04% of the antibiotic was apparently adsorbed by the sediments. The relatively small amount of adsorbed SMT indicates that the tendency for the sediment adsorption of SMT is low, likely due to the low distribution coefficient (K_d, 0.23–43.1 L kg^{-1}) of the compound.[52−54] In the unpoisoned group, the concentrations of SMT were significantly lower than those in the poisoned group (repeated measures ANOVA, $p < 0.0001$), with a 13.4 ± 0.01% decrease during the incubation period. Because the sediments used in the unpoisoned group

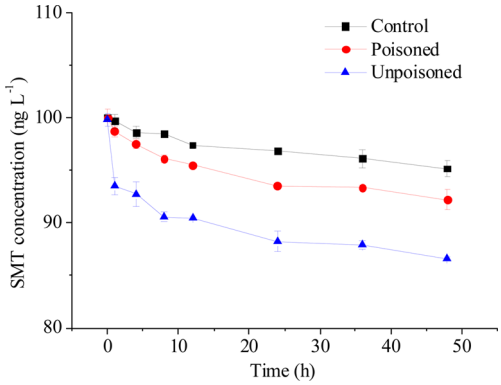

Figure 1. Temporal changes in antibiotic (SMT) concentrations in the degradation experiments. Control denotes the group with no sediments; poisoned denotes the sediment slurry groups with NaN_3 poisoned sediments; and unpoisoned denotes the sediment slurry groups with untreated sediments. Bars denote the standard error of quadruplicate samples.

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had the same geochemical properties as the sediments in the poisoned group, the difference in the SMT concentrations between the two groups (5.7 ± 0.02%) was primarily caused by microbial degradation and/or transformation.[51,55] These comparisons show that, in addition to hydrolysis and sediment adsorption,[17,54] microorganisms also played a significant role in controlling the fate of SMT.[51,55] The present study also indicates that although microorganisms combined with hydrolysis could accelerate antibiotic removal,[17,22,55] most of the antibiotic was retained in the anaerobic and aphotic estuarine and coastal sediments. Therefore, the high antibiotic residue may affect nitrogen cycling processes.[30]

Antibiotic Influence on Denitrification Rates. Antibiotics may inhibit the metabolism of denitrifying bacteria due to their antibacterial properties,[22,30] although they may also lead to the development of resistance genes.[25,37,56,57] Therefore, we tested the response speed and pattern of denitrification in the presence of SMT. In this study, the concentration gradient experiments indicated that the rates of denitrification were related negatively and exponentially to the antibiotic concentrations (R^2 = 0.994, Figure 2). As the concentrations of the added SMT increased from 0 to 5 μg L^{-1}, the denitrification rates significantly decrease in the incubation experiments (one-way ANOVA, $p < 0.0001$). At the SMT concentration of 50 ng L^{-1}, which is near the in situ average concentration in the Yangtze Estuary,[22,24,29] the denitrification rates were reduced by 20−30% based on the incubation experiments (Figure 2). Our previous study showed that approximately 30% of the riverine inorganic nitrogen (mainly nitrate) transported into the Yangtze Estuary is removed by denitrification.[40] We estimated that the nitrogen removal efficiency caused by denitrification may increase up to 50−60% in the estuarine ecosystem, assuming no inhibition of denitrification by antibiotics. Therefore, the antibiotic inhibition of denitrification may be a factor causing the retention of excessive inorganic nitrogen in the estuarine and coastal ecosystem, thereby further contributing to eutrophication in the study area. However, the decrease in the denitrification rate was less remarkable as the concentration of SMT continued to increase (one-way ANOVA, $p > 0.05$), and the denitrification rates remained nearly constant at the concentrations exceeding 5 μg L^{-1} (Figure 2). This response pattern most likely demonstrated the existence of antibiotic resistant genes in the denitrifying bacteria. Although antibiotic resistant genes are considered a potential threat to human health, they may help the function of denitrifying bacteria.[25,56,58]

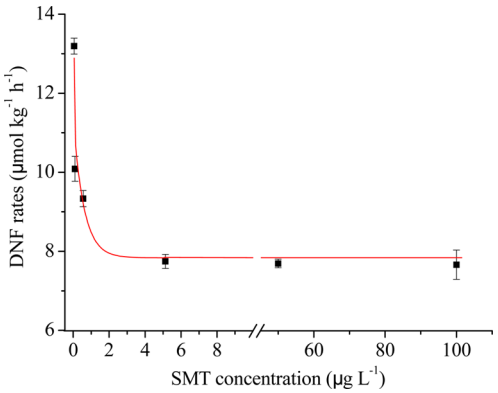

Figure 2. Relationship between denitrification (DNF) rate and the antibiotic (SMT) concentration during 8-h incubation experiments. Bars denote the standard error of quadruplicate samples.

In the time series experiments, the instantaneous denitrification rates peaked at 8 h and then dropped quickly (Figure S2, SI). The denitrification rates of the SMT treatment group were significantly lower than those of the control group during the first 8 h (repeated measures ANOVA, $p < 0.0001$), mainly due to antibiotic inhibition. After 8 h, the rates of the SMT treatment group resembled those of the control group (repeated measures ANOVA, $p > 0.05$). The consumption of nitrate during the incubation period could be ignored (Figure S3, SI); therefore, we concluded that the changes in the denitrification rates might be caused primarily by antibiotic resistant genes and the depletion of readily available electron donors. However, with the sustained input of antibiotics and nutrients, decreases in denitrification rates may occur in estuarine and coastal regions, consequently leading to a reduction in the nitrogen removal ability and an increase in the overloaded nitrogen residue in these aquatic ecosystems.

Antibiotic Influence on N_2O Production. Nitrous oxide (N_2O) is an intermediate product in the denitrification process. Because N_2O has a higher radiative forcing potential than CO_2 and CH_4, it is considered a major greenhouse gas that may affect climate changes and ozone depletion in the atmosphere.[59,60] Although the antibiotic was shown to inhibit nitrogen gas production via denitrification, we noted that the production of N_2O might not be suppressed by the added antibiotic (Figure 3). With an increasing concentration of SMT, the production of N_2O followed Michaelis−Menten kinetics in the N_2O release experiments (R^2 = 0.992). The N_2O could be largely involved with N_2O reduction to N_2 when the SMT concentration was less than 50 ng L^{-1}, leading to N_2O uptake during the incubations (Figure 3). However, as the SMT concentration increased (higher than 50 ng L^{-1}), the produced N_2O significantly accumulated over the incubation period (one-way ANOVA, $p < 0.0001$). The N_2O production has also been reported to increase in soil incubated with chloramphenicol.[61] The N_2O accumulation induced by SMT and chloramphenicol suggested that antibiotics may more strongly inhibit N_2O reduction to N_2 than N_2O production. The time series experiments of N_2O release also indicated that SMT could significantly accelerate the production of N_2O in sediments (repeated measures ANOVA, $p < 0.0001$) (Figure S4, SI). Therefore, the presence of antibiotics at relatively high levels

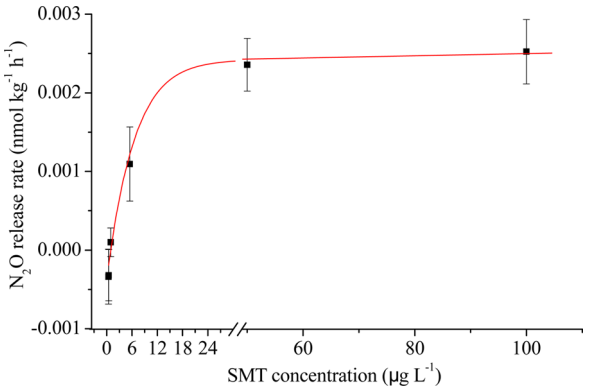

Figure 3. Relationship between N_2O release rate and antibiotic (SMT) concentration during 8 h incubation experiments. Bars denote the standard error of quadruplicate samples.

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may lead to increased N_2O production in hypereutrophic estuarine and coastal regions, and the resulting release of N_2O would contribute to greenhouse effects and ozone depletion in the atmosphere.[59,60,62]

Microbial Physiological Response to the Antibiotics. In addition to the availability of the substrates and the microenvironmental conditions, the denitrification rates may be related to the abundance of the denitrifying bacteria.[32,50,63] The *nirS* gene encoding nitrite reductase and the *nosZ* gene encoding nitrous oxide reductase are directly involved in the key steps of the denitrification process,[32,64] whereas most of the bacterial resistance to sulfonamide can be explained by the presence of the *sul*1 gene.[34,65] In the present study, the *nirS*, *nosZ*, and *sul*1 genes were quantified to reveal the microbial physiological mechanisms underlying the interaction between antibiotics and denitrification.

In the concentration gradient experiments, the concentrations of SMT were negatively and exponentially related to the abundances of the *nirS* (R^2 = 0.998) and *nosZ* (R^2 = 0.999) genes (Figure 4), as observed for the changes in the denitrification rates with SMT. These negative exponential relationships likely provided the genetic evidence for the microbial antibiotic inhibition of denitrification. Pearson's correlations between the denitrification rates and the abundances of both denitrifying genes also support this conclusion ($p < 0.05$). As the concentration of SMT increased, the abundances of both denitrifying genes decreased remarkably over the incubation period (one-way ANOVA, $p < 0.0001$), which is likely attributed to the SMT-mediated inhibition of folic acid synthesis, which is required for bacterial growth.[66] However, the inhibition of SMT appeared to reach an upper limit when the concentration of SMT exceeded 5 $\mu g\ L^{-1}$; thus, the abundances of the *nirS* and *nosZ* genes remained nearly constant (one-way ANOVA, $p > 0.05$). Despite an identical response of both denitrifying genes to SMT, the differences in the SMT-mediated inhibition of the *nirS* and *nosZ* genes were significant (one-way ANOVA, $p = 0.0003$). SMT inhibition was greater for the *nosZ* gene (50−53% decrease in number), which controls N_2O reduction to N_2, than for the *nirS* gene (39−42% decrease in number). This inhibition difference may explain N_2O accumulation in response to the SMT concentration during the incubation experiments. In contrast, the abundance of the *sul*1 gene was positively and exponentially related to the SMT concentration (R^2 = 0.995, Figure 4). This relationship indicates that the presence of the antibiotic stimulated the increase in the *sul*1 gene number during incubation, likely via the transfer and autonomous replication of plasmids carrying the *sul*1 gene throughout the entire bacterial community.[34,67,68] Because antibiotic resistance could be spread among bacteria,[34] it might also appear in certain denitrifiers, although not directly evidenced by the data in the present study. However, the incubation experiments suggested that the antibiotic resistance developed in denitrifying bacteria had an upper limit, as reflected by the changes in the denitrification rates (Figure 2).

In the time series experiments, the abundances of the *nirS* and *nosZ* genes were significantly higher in the control group than in the SMT treatment group (repeated measures ANOVA, $p < 0.0001$) (Figure S5, SI). The highest copy numbers of the denitrifying genes in the control group were observed to be more than twice the values of the SMT treatment group. The lower abundances of the *nirS* and *nosZ* genes in the SMT treatment group demonstrated that the growth of denitrifying bacteria was inhibited by the antibiotic, as confirmed by the changes in the denitrification rates. The *sul*1 gene number in the control group did not change significantly during the incubations (repeated measures ANOVA, $p > 0.05$), but it distinctly increased in the SMT treatment group (repeated measures ANOVA, $p < 0.0001$) (Figure S5, SI). This result also showed that the antibiotic addition likely stimulated the transfer and autonomous replication of plasmids carrying the *sul*1 gene,[67,68] thus enhancing the *sul*1 gene number. The abundance of the *sul*1 gene began to increase when SMT was added and continued to increase with time, indicating that the increase in the *sul*1 gene number is rapid. Although the total number of denitrifiers harboring the *nirS* and/or *nosZ* genes decreased in the time series experiments, a portion of denitrifying bacteria might develop resistance to the antibiotic,

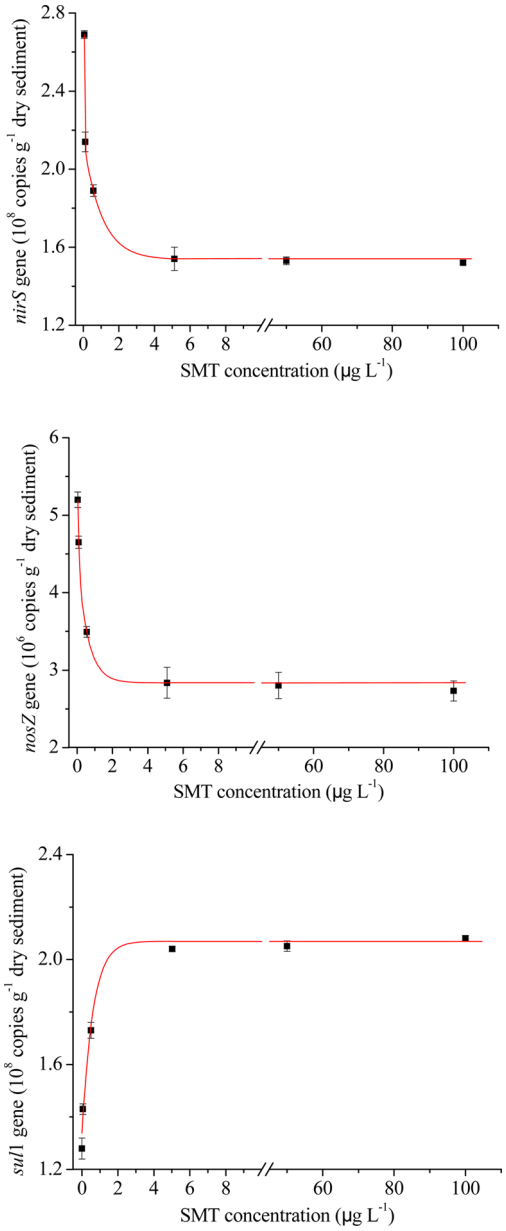

Figure 4. Relationship between the *nirS*, *nosZ*, and *sul*1 gene numbers and antibiotic (SMT) concentration during 8 h incubation experiments. Gene copy numbers were obtained from quadruplicate samples in real-time quantitative PCR experiments. Bars denote the standard error of quadruplicate samples.

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partly causing the denitrification rates in the SMT treatment group to resemble those in the control group after 8 h of incubation.

In summary, the present study shows that the antibiotic residues may decrease the denitrification rates in estuarine and coastal ecosystems via inhibiting the growth of denitrifying bacteria, thus leading to the retention of more reactive nitrogen in these aquatic environments. However, the antibiotic residues can enhance the production and release of N_2O from the sediment−water systems, primarily due to the greater inhibition of N_2O reduction to N_2 than N_2O production during denitrification. This response of N_2O emission to the antibiotic residues may contribute to greenhouse effects and atmospheric ozone depletion. The results observed in this study could guide future efforts to control reactive nitrogen and antibiotics pollution in the hypereutrophic estuarine and coastal ecosystems, which suffer from an increasing input of antibiotics and reactive nitrogen.

■ ASSOCIATED CONTENT

Supporting Information

Figure S1 shows the study area and sampling site; Figures S2 shows the time series change of the denitrification rates under the antibiotic effect; Figure S3 gives the nitrate concentrations in the sediment slurries of the incubation experiments; Figures S4 and S5 show the time series changes of the N_2O production and denitrifying gene abundance under the antibiotic effect, respectively. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interests.

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