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Article

Impact of NH_4^+-N on Organic Micropollutant Removal and Antibiotic Resistance Gene Occurrence during Simulated Riverbank Filtration

Jian Zhao, Qiaojuan Wang, Yangrui Huang, Shangbiao Fang, Gang Liu, Weixiao Qi, Yaohui Bai, Walter van der Meer, Jiuhui Qu, and Huijuan Liu*

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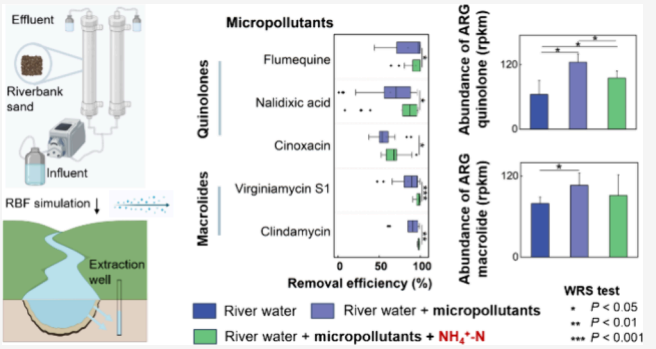
ABSTRACT: Organic micropollutants (OMPs) facilitate the spread of antibiotic resistance genes (ARGs). Ammonia-oxidizing microorganisms (AOMs) are crucial for OMP degradation during riverbank filtration (RBF) and significantly influenced by NH_4^+-N concentrations. However, the effect of NH_4^+-N on OMP removal and ARG occurrence in RBF remains unclear. This study aimed to examine the effects of low (~0.1 mg/L) and high (~2.2 mg/L) NH_4^+-N concentrations on OMP removal, ARG occurrence, and microbial communities. NH_4^+-N addition had no significant effect on the removal of 108 out of 128 OMPs, suggesting that other factors primarily govern the removal process. Notably, NH_4^+-N addition enhanced the removal of 20 OMPs by 3−70%, including three quinolones (e.g., flumequine), indicating its promotion of specific OMP removals. This effect may primarily result from NH_4^+-N enhancing OMP biotransformation through the stimulation of AOMs (particularly AOA and comammox) and heterotrophs (e.g., *Bradyrhizobium*). Furthermore, NH_4^+-N addition significantly reduced the abundance of eight ARGs, including quinolone ARGs, likely due to its inhibition of antibiotic-resistant bacteria. Additionally, we hypothesize that NH_4^+-N alleviates OMP selective pressure on microorganisms by promoting OMP conversion through AOMs. This study enhances the understanding of microbe-mediated OMP removal in the presence of NH_4^+-N and its impact on ARG occurrence during RBF.



KEYWORDS: *riverbank filtration, organic micropollutants, NH_4^+-N, antibiotic resistance genes, ammonia-oxidizing microorganisms*

INTRODUCTION

The contamination of the global surface waters by a wide range of organic micropollutants (OMPs), including antibiotic and nonantibiotic pharmaceuticals, remains a major challenge, critically compromising drinking water safety worldwide.[1−4] Riverbank filtration (RBF), a century-old natural pretreatment technology with eco-friendly and sustainable advantages, has been widely implemented in Europe and North America.[2,5,6] RBF also shows great promise for countries like China and South Korea and is a potential solution for global water supply challenges.[7,8] Initially, research primarily focused on the ability of RBF to remove turbidity,[9] NH_4^+-N,[7] and pathogenic microorganisms.[10] More recently, studies have highlighted RBF's capability to reduce OMPs,[2,3,6] enhancing its appeal owing to its environmentally benign and chemical-free nature.[1,11] Antibiotic resistance is an escalating global health crisis.[12] The increasing prevalence of antibiotic resistance genes (ARGs) and pathogenic bacteria is intensifying socio-economic burdens and threatening human health, with projections estimating costs of $100 trillion and 10 million deaths by 2050.[13]

OMPs drive ARG proliferation through coselection pressures, as antibiotic and nonantibiotic pharmaceuticals (e.g., carbamazepine and gemfibrozil) have demonstrated ARG-enhancing potential.[14,15] Although OMP concentrations in RBF systems are extremely low (ranging from a few ng/L to several hundred ng/L),[2−4] the potential risks associated with ARGs in OMP-contaminated surface water should not be underestimated. However, the mechanisms underlying ARG changes during the removal of various OMPs at ng/L concentrations in RBF remain poorly understood.

Factors influencing OMP removal in RBF may indirectly affect ARG occurrence. Previous studies have suggested that OMP removal efficiency in RBF is influenced by environmental factors (e.g., temperature and redox conditions), soil characteristics (e.g., grain size and organic matter content), and

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water quality parameters (e.g., dissolved organic carbon composition and concentration).[3,16,17] Biotransformation is the most significant OMP removal mechanism in RBF.[18−20] Notably, microbial community composition—and, consequently, OMP removal in RBF—is primarily determined by water quality parameters.[21] As a crucial water quality parameter, NH_4^+-N fluctuates in river water (typically between 0.1 and 1.7 mg/L) and is a key regulator of microbial activity.[22] Ammonia-oxidizing microorganisms (AOMs) can degrade OMPs.[23] Specifically, ammonia-oxidizing bacteria (AOB), archaea (AOA), and complete ammonia oxidizers (comammox) have demonstrated OMP biotransformation capabilities via ammonia monooxygenase-catalyzed nonspecific reactions (e.g., hydroxylation and amide hydrolysis), direct chemical reactions with hydroxylamine, and indirect oxidative actions mediated by reactive nitrogen species, such as nitrite and nitric oxide.[24,25] We have previously reported that AOA and comammox are predominant in RBF systems and are vital in the transformation of 32 types of OMPs.[19] NH_4^+-N directly serves as a substrate for AOMs and influences their abundance and community structure.[26] However, the impact of NH_4^+-N on OMP removal and ARG occurrence in RBF systems remains poorly understood.

This study aims to investigate the patterns and mechanisms through which NH_4^+-N influences OMP removal and ARG occurrence in RBF. Six sand columns simulating RBF were continuously operated for 20 months: two control columns (C_{Ctrl}); two with only OMP addition (C_{OMPs}); two with both NH_4^+-N and OMP addition ($C_{AMM+OMPs}$). Over 8 months, we compared OMP removal between $C_{AMM+OMPs}$ and C_{OMPs} to evaluate the effects of NH_4^+-N. Additionally, ARG abundance was analyzed across all setups to assess the effects of OMP and NH_4^+-N co-occurrence on ARG dynamics. We also evaluated the relative contributions of adsorption and biotransformation to OMP removal and analyzed AOM community shifts via metagenomic sequencing to elucidate the mechanisms by which NH_4^+-N affects OMP removal and ARG occurrence. The findings of this study expand our understanding of how RBF addresses the simultaneous contamination of NH_4^+-N and OMPs and the associated variations in ARG occurrence.

MATERIALS AND METHODS

Chemicals. A total of 128 OMPs, comprising 110 pharmaceuticals and personal care products, including 41 antibiotics and 16 psychiatric drugs, were chosen according to prescription rates, sales metrics, and environmental priorities.[19] Table S1 presents detailed information on OMPs, including their CAS number, molecular weight, Log P, and pharmaceutical classification. Two distinct OMP preparations (powder: ≥95% purity; solution: 100 μg/mL, ≥99% purity) were acquired through Sigma-Aldrich (MO, USA) and First Standard (Tianjin, China), followed by cryopreservation at −20 °C. Additionally, 28 isotopic substances (e.g., sulfamethoxazole-D4 and caffeine-D9) used as internal standards were obtained from First Standard in 100 μg/mL solutions and similarly stored at −20 °C (Table S2). LC-MS grade solvents from Thermo Fisher Scientific (USA) and Macklin (China) constituted the mobile phase system. Methanol and acetonitrile (≥99.9% purity) served as eluents, with formic acid (≥99%) added as a mobile phase modifier for ultrahigh-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) analysis. NH_4Cl powder (99.99% purity) was procured from Shanghai Aladdin Biochemical Technology Co., Ltd. (China). Solid phase extraction (SPE) was conducted for sample preparation, using Oasis HLB cartridges (500 mg, 6 mL capacity) purchased from Waters Corporation (Milford, MA, USA).

RBF Simulation Experiments. The experimental apparatus (Figure 1) comprised six black polyvinyl chloride columns

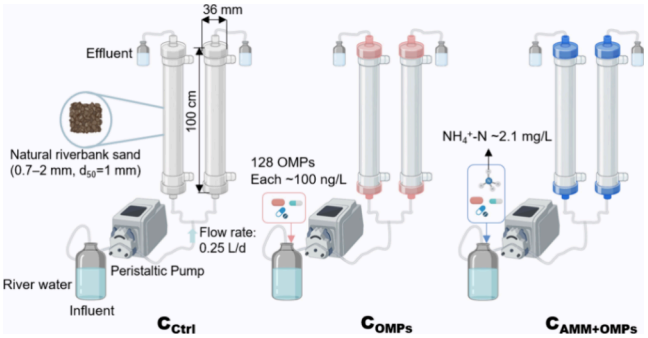

Figure 1. Experimental setups for riverbank filtration simulation. OMPs, organic micropollutants.

(1 m in length, 36 mm in diameter) filled with natural riverbank sand (0.7−2 mm, median grain size 1 mm) to simulate the aerobic section of an RBF. The setup included duplicate control columns (C_{Ctrl}), OMP treatment columns (C_{OMPs}), and combined NH_4^+-N and OMP treatment columns ($C_{AMM+OMPs}$). The physicochemical parameters of the sand, including pH, cation exchange capacity, granulometric distribution, and organic matter content, are detailed in Table S3. Feed water, which was renewed weekly, was sourced from the Yongding River, Beijing, China. The water parameters, including NH_4^+-N, Ca^{2+}, K^+, Mg^{2+}, dissolved oxygen (DO), F^-, Cl^-, NO_3^--N, SO_4^{2-}, and dissolved organic carbon (DOC), are listed in Table S4. The sand columns, with a bottom-to-top flow, maintained a flow rate of 0.25 L/day and a residence time of 36 h.

The 20-month sand column experiment, conducted at 20−25 °C, consisted of three stages based on treatment types and objectives. Initially, a 6-month microbial cultivation period used surface water (SW) as the influent for C_{Ctrl}, C_{OMPs}, and $C_{AMM+OMPs}$. This was followed by a 6-month NH_4^+-N adaptation phase using SW for C_{Ctrl} and C_{OMPs} and SW plus NH_4^+-N (~2.1 mg/L) for $C_{AMM+OMPs}$. The final 8-month stage focused on OMP exposure, where C_{Ctrl} received SW, C_{OMPs} received SW plus 128 OMPs (100 ng/L each), and $C_{AMM+OMPs}$ was treated with SW, NH_4^+-N (~2.1 mg/L), and 128 OMPs. From months 7−20, SW was filtered through a 0.45-μm glass fiber filter before being used as feedwater to prevent NH_4^+-N and OMP degradation in the inlet tank. OMP concentrations were measured 8 times in the influent and 22 times in the effluent during the OMP exposure phase. After completing the experiment, 5 g of sand from each column's base was collected for DNA analysis and stored at −80 °C.

Batch adsorption experiments were performed in triplicate using 1 L amber flasks incubated at 25 °C (120 rpm, 36 h) to evaluate sand's OMP adsorption capacity and NH_4^+-N effects. The experimental groups were as follows: (i) Control: 800 mL ultrapure water with 128 OMPs (100 ng/L each) and 400 mg/L NaN_3 (microbial inhibitor); (ii) Sand treatment: 800 mL Yongding River water amended with 200 g natural sand, 128 OMPs, and 400 mg/L NaN_3; (iii) NH_4^+-N treatment: Sand

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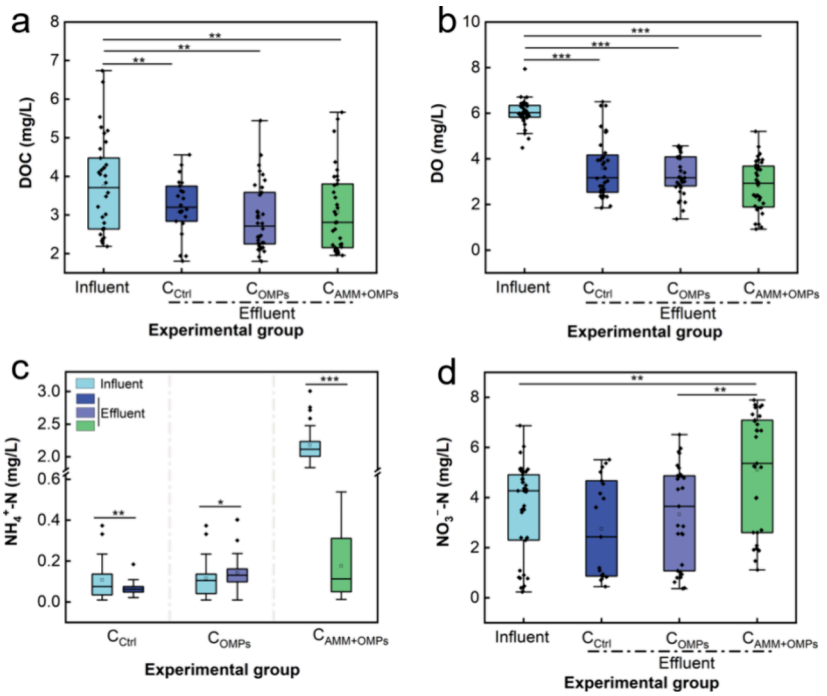

Figure 2. Concentration of (a) dissolved organic carbon (DOC), (b) dissolved oxygen (DO), (c) NH_4^+-N, and (d) NO_3^--N in influent and effluent of the columns. Statistical analysis was performed using the Wilcoxon rank-sum test, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

treatment components supplemented with approximately 2.1 mg/L NH_4^+-N.

Analytical Methods for OMPs. The quantitative analysis of the 128 OMPs was conducted using SPE coupled with UHPLC-MS/MS (AB SCIEX QTRAP 5500, USA). The SPE procedures are provided in Text S1. MS/MS parameters for the OMPs and internal standards, including precursor ions, product ions, and collision energies, are summarized in Tables S1 and S2. Detailed UHPLC-MS/MS analytical methods, such as the mobile phases and injection volumes, are provided in Text S2.

Metagenomic Analysis of Microbial Communities. DNA was isolated from all sand samples collected at the 20-month time point using the soil FastDNA SPIN Kit (MP Biomedicals, Irvine, CA, USA). The metagenomic library construction and sequencing procedures were carried out by BGI Genomics (Shenzhen, China).

All raw sequencing data were processed using SOAPnuke v.1.5.2.[27] To identify and remove reads of host origin, the trimmed sequences were aligned to the host genome using SOAP2 software (applicable only for host-originated samples). Following MEGAHIT-driven *de novo* reconstruction,[28] stringent size selection (≥200 bp) was applied, eliminating substandard genomic fragments prior to functional profiling. Genes were predicted from the contigs using MetaGeneMark software.[29] The CD-HIT algorithm was employed to filter out redundant genetic sequences, with parameters set at 95% identity and 90% coverage.[30] Salmon software was used for quantification to construct the gene abundance matrix.[31] To generate functional annotations, the protein sequences of the genes were blasted against functional databases, such as CARD, EggNOG, and KEGG via DIAMOND, with an E-value threshold of 10^{-5}.[32]

The methods for genomic binning, recovery of metagenome-assembled genomes (MAGs), and quantification of the relative abundance of MAGs in individual samples are described in Text S3. The identification of potential nitrogen cycle open reading frames (ORFs), as well as the methods for quantifying the abundance of antibiotic-resistant bacteria (ARB), mobile genetic elements (MGEs), and pathogenic ARB (PARB) are detailed in Text S4.

Statistical Analysis. Intergroup disparities in OMP removal efficacy and microbial abundance (C_{OMPs}- vs $C_{AMM+OMPs}$-exposed groups) were statistically compared using the nonparametric Wilcoxon rank-sum test in SPSS 26.0 (IBM SPSS Statistics, Chicago, IL, USA). The significance threshold for comparisons was set at $P < 0.05$. Genus-level discriminative taxa in $C_{AMM+OMPs}$ were identified through LEfSe analysis (LDA effect size >3; see Text S5 for methodological details).

RESULTS

Removal of DOC, DO, and NH_4^+-N in Simulated RBF Systems. DOC removal efficiencies ranged from 14 to 22% and did not significantly ($P > 0.05$) differ across the groups (Figure 2a), suggesting that the addition of OMPs and NH_4^+-N did not notably influence DOC removal. For DO, the concentrations decreased from 6.0 ± 0.6 mg/L to 3.5 ± 1.3 mg/L, 3.3 ± 0.9 mg/L, and 2.8 ± 1.1 mg/L in C_{Ctrl}, C_{OMPs}, and $C_{AMM+OMPs}$, respectively (Figure 2b), indicating that the columns simulated the aerobic section of an RBF. $C_{AMM+OMPs}$ demonstrated enhanced DO consumption, likely due to higher NH_4^+-N levels.

We further examined the effectiveness and mechanisms underlying NH_4^+-N removal in simulated RBF. In C_{Ctrl}, NH_4^+-N levels significantly decreased from 0.11 ± 0.09 to 0.07 ± 0.04 mg/L (Figure 2c), achieving a 36% removal efficiency ($P < 0.01$). Conversely, C_{OMPs} showed increased NH_4^+-N to 0.14 ± 0.08 mg/L (Figure 2c), suggesting impaired NH_4^+-N removal when exposed to OMPs. Meanwhile, $C_{AMM+OMPs}$ initially began with a higher NH_4^+-N level of 2.18 ± 0.26 mg/L and was significantly reduced to 0.18 ± 0.15 mg/L,

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demonstrating a 92% removal efficiency ($P < 0.001$). This setup also showed a ~1.9-mg/L increase in NO_3^--N levels (Figure 2d), indicating that NH_4^+-N was primarily removed through nitrification.

Concentration of OMPs in the River. Thirty OMPs were detected in the river, with total concentrations ranging from 57 to 123 ng/L (Figure S1a). Caffeine had the highest concentration of approximately 20 ng/L, followed by sulfamethoxazole and phenazone, which reached peak concentrations of 19 and 17 ng/L, respectively. The detected OMPs encompassed nine antibiotics (four macrolides, one sulfonamide, two quinolones, and two broad-spectrum), six psychiatric drugs, five cardiovascular medications, three doping agents, two insecticides, and five other types of medications (Figure S1b). Notably, sulfonamide and quinolone antibiotics showed median concentrations of 15 and 10 ng/L, respectively (Figure S1c). In river water, various target OMPs were detected at low concentrations, constituting <1% of the total concentration of introduced OMPs.

Removal Efficiency of OMPs in Simulated RBF Systems. To assess the OMP removal efficiency of the simulated RBF system, 128 OMPs were analyzed in the C_{OMPs} influent and effluent. The results showed that 32 OMPs were nearly completely removed (>99%), while 86 were partially removed, including 61 OMPs removed by more than 70% and 25 OMPs removed by 30−70% (Figure S2). However, 10 OMPs, including fluconazole, were removed by less than 30%, with fluconazole exhibiting no removal. This suggests that while the simulated RBF system effectively removed most OMPs, its efficiency was limited for certain substances.

Further investigation into the removal mechanisms focused on sand adsorption capabilities. Triple-batch tests evaluated the adsorption capacity of the sand and revealed that 41 OMPs were not adsorbed at all, 40 showed less than 30% adsorption, 30 were adsorbed between 30 and 70%, and only 17 had strong adsorption rates of over 70% (Figure S2). Of the OMPs that were not adsorbed, all except acetaminophen were effectively removed from the sand columns, indicating that biotransformation was crucial for their elimination.

Impact of NH_4^+-N Addition on OMP Removal. The impact of varying NH_4^+-N concentrations on the removal efficiency of OMPs was examined by comparing the performances of C_{OMPs} and $C_{AMM+OMPs}$. NH_4^+-N addition significantly ($P < 0.05$) increased the removal efficiencies of 20 out of 128 OMPs by 3−70% (Figure 3a), including 3 quinolones (flumequine, nalidixic acid, and cinoxacin), 2 macrolides (virginiamycin S1 and clindamycin), sulfaguanidine, thiabendazole, cardiovascular medications, such as losartan and digoxin, and other pharmaceuticals. Specifically, 5 OMPs, including virginiamycin S1, exhibited consistently higher removal rates in the presence of elevated NH_4^+-N concentrations (Figure 3b), while the remaining 15 exhibited varying removal patterns, with removal efficiencies improving either during the initial phase and specific time intervals (e.g., sulfaguanidine, Figure 3c) or within particular intervals (e.g., glipizide, Figure 3d) following NH_4^+-N addition.

The addition of NH_4^+-N enhanced the removal efficiencies of 20 OMPs. For 10 OMPs, which were scarcely adsorbed by sand (adsorption efficiencies ≈ 0%, Figure S2), NH_4^+-N addition likely improved their removal through enhanced biotransformation. For the remaining 10 OMPs (e.g., thiabendazole), sand adsorption efficiencies ranged from 2% to 58%. NH_4^+-N addition enhanced the adsorption of

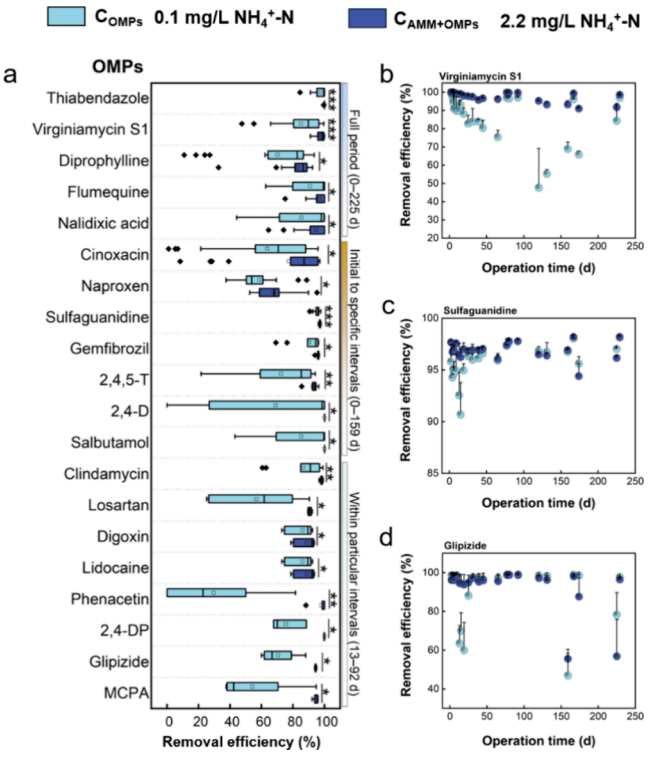

Figure 3. Removal efficiency of (a) 20 organic micropollutants (OMPs), (b) virginiamycin S1, (c) sulfaguanidine, and (d) glipizide. Statistical analysis was performed using the Wilcoxon rank-sum test, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

virginiamycin S1 and salbutamol by ~8% and 9%, respectively, suggesting a potential role in promoting OMP removal via increased adsorption. The improved removal of the other 8 OMPs likely stems from enhanced biotransformation.

Impact of OMP and NH_4^+-N Addition on AOMs. Genus-level LEfSe analysis identified 22 biomarkers in $C_{AMM+OMPs}$, as compared to C_{Ctrl} and C_{OMPs} (Figure S3), including AOMs (e.g., *Nitrospira*) and heterotrophic bacteria (e.g., *Bradyrhizobium*), suggesting that NH_4^+-N addition enriched specific microbial taxa.

The abundance of *amo*A was similar in C_{Ctrl} and C_{OMPs} (Figure 4a), indicating that multiple OMPs at the investigated concentrations did not significantly ($P > 0.05$) affect *amo*A abundance. In contrast, $C_{AMM+OMPs}$ showed significantly ($P < 0.05$) higher *amo*A abundance (Figure 4a), suggesting that NH_4^+-N addition could increase *amo*A abundance. To further examine the impact of OMPs and NH_4^+-N on the AOM community, seven AOA MAGs, five comammox MAGs, and one AOB MAG were obtained via metagenomic binning (Table S5). The abundance of AOMs was similar between C_{Ctrl} (0.021%) and C_{OMPs} (0.020%), indicating that OMPs did not significantly ($P > 0.05$) affect AOM abundance. However, in $C_{AMM+OMPs}$, AOM abundance increased to 0.055%, indicating that NH_4^+-N significantly ($P < 0.05$) enhanced AOM growth. The abundance of AOB MAG was significantly ($P < 0.05$) higher in C_{OMPs} than in C_{Ctrl} (Figure 4b), suggesting that OMPs may increase AOB MAG. Conversely, the abundance of comammox MAGs was significantly ($P < 0.05$) lower in C_{OMPs} (Figure 4b), indicating that OMPs may inhibit comammox MAGs. Moreover, AOA, AOB, and comammox were more abundant in $C_{AMM+OMPs}$ than those in

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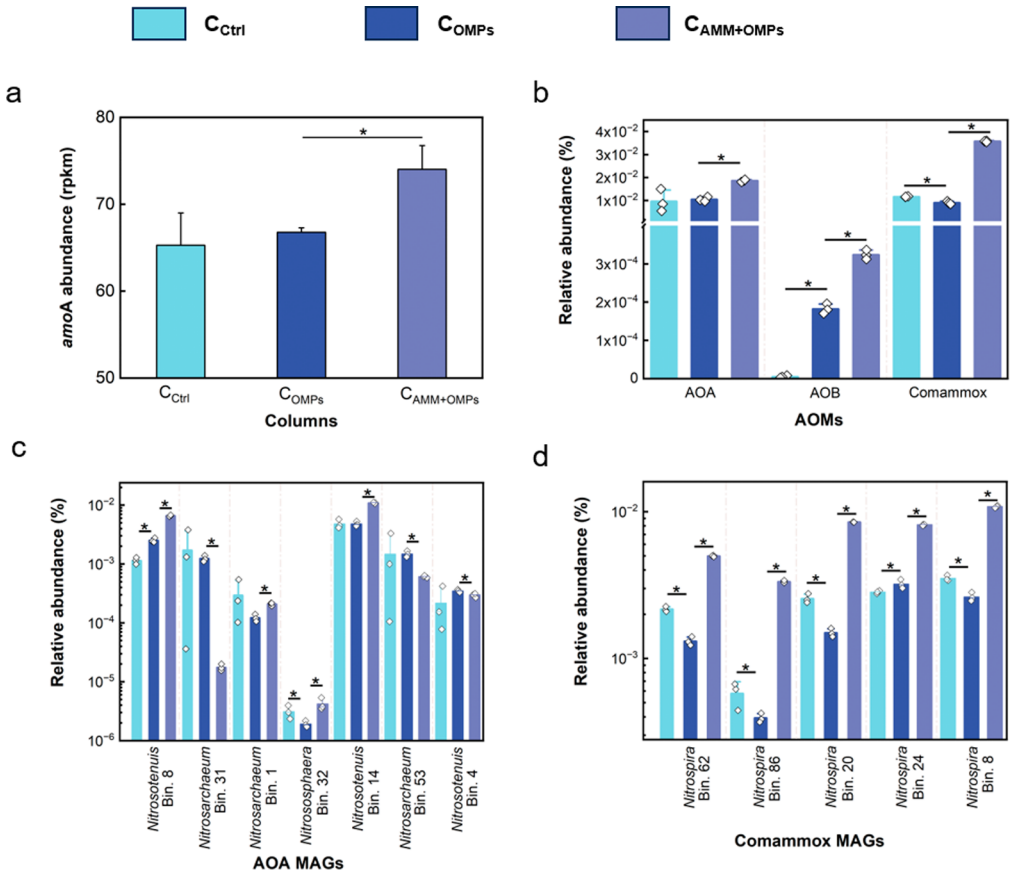

Figure 4. (a) Abundance of *amo*A. Relative abundance of (b) ammonia-oxidizing archaea (AOA), ammonia-oxidizing bacteria (AOB), and complete ammonia oxidizers (comammox); (c) AOA metagenome-assembled genomes (MAGs); and (d) comammox MAGs. Statistical analysis was performed using the Wilcoxon rank-sum test, $*P < 0.05$.

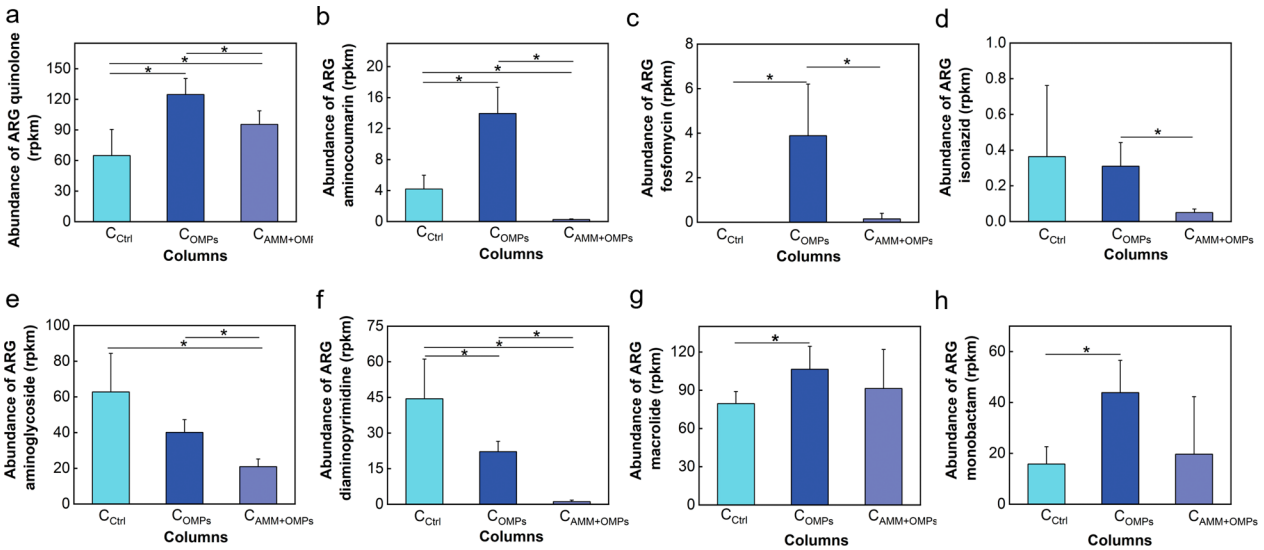

Figure 5. (a–h) Abundance of antibiotic resistance genes (ARGs) in inlet sand samples of C_{Ctrl}, C_{OMPs}, and $C_{AMM+OMPs}$. Statistical analysis was performed using the Wilcoxon rank-sum test, $*P< 0.05$.

C_{OMPs} (Figure 4b), showing that NH_4^+-N addition benefits these AOMs.

In the simulated RBF system, comammox and AOA were the predominant ammonia oxidizers, accounting for >99% of the AOM MAGs (Figure 4b). We examined their responses to OMPs and NH_4^+-N. Among the seven AOA MAGs compared between C_{Ctrl} and C_{OMPs}, only *Nitrososphaera* (Bin. 32) significantly ($P < 0.05$) decreased in abundance (Figure 4c), whereas five remained stable and one increased. Conversely, four out of five comammox MAGs in C_{OMPs} showed significantly ($P < 0.05$) reduced abundance (Figure 4d), suggesting a negative effect of OMPs on most comammox, except for *Nitrospira* (Bin. 24), implying their resilience to OMPs. Overall, OMPs inhibited specific comammox and AOA

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MAGs. However, AOA MAGs generally exhibited greater tolerance to OMPs than comammox MAGs.

Compared with C_{OMPs}, four AOA MAGs in $C_{AMM+OMP}$ increased significantly ($P < 0.05$), e.g., *Nitrosotenuis* (Bin. 8) (Figure 4c). Conversely, three AOA MAGs, including *Nitrosarchaeum* (Bin. 31), were significantly ($P < 0.05$) decreased. Hence, with the concentration of NH_4^+-N rising from 0.1 to 2.2 mg/L, a selective impact was exerted on AOA MAGs, including enhancement and inhibition. Additionally, all five comammox draft genomes in $C_{AMM+OMPs}$ were significantly ($P < 0.05$) higher than those in C_{OMPs} (Figure 4d). Therefore, unlike AOA, NH_4^+-N positively influenced all the comammox MAGs examined.

Effects of OMP and NH_4^+-N Addition on the Abundance of ARGs. To assess the impact of OMPs on ARG occurrence, we compared the abundance of 25 ARGs between C_{Ctrl} and C_{OMPs}. The detection of 22 out of 25 ARGs in C_{Ctrl} (Table S6), including genes resistant to quinolones and macrolides, indicated their potential natural presence in RBF systems. Notably, the overall abundance of ARGs in C_{OMPs} was significantly ($P < 0.05$) higher than that in C_{Ctrl} (Table S6), suggesting that the presence of multiple OMPs at ng/L levels could promote ARG dissemination. Specifically, seven ARGs in C_{OMPs} exhibited significantly higher abundance than in C_{Ctrl} ($P < 0.05$), with resistance genes for cephamycins increasing 2.33-fold, quinolones 1.93-fold, macrolides 1.34-fold, monobactams 2.78-fold, aminocoumarins 3.32-fold, and tetracyclines 1.38-fold, along with the new detection of fosfomycin resistance (Figure S4). Conversely, the abundance of four ARGs (diaminopyrimidine, glycopeptide, glycylcyclines, and pleuromutilin resistance genes) significantly decreased ($P < 0.05$) in C_{OMPs} compared with that in C_{Ctrl}. These findings suggest that multiple OMPs at ng/L levels can selectively influence ARG abundance.

To investigate the impact of NH_4^+-N on ARG occurrence, a comparative analysis was conducted among C_{OMPs} and $C_{AMM+OMPs}$, alongside an evaluation of their respective increases compared with C_{Ctrl}. The abundances of six ARGs were significantly lower in $C_{AMM+OMPs}$ compared with C_{OMPs} ($P < 0.05$), specifically those conferring resistance to quinolones (Figure 5a), aminocoumarins (Figure 5b), fosfomycin (Figure 5c), isoniazid (Figure 5d), aminoglycosides (Figure 5e), and diaminopyrimidines (Figure 5f). Conversely, 19 ARGs showed similar abundances between $C_{AMM+OMPs}$ and C_{OMPs}. These results suggest that a higher concentration of NH_4^+-N, as simulated in this study, could potentially reduce the spread of specific ARGs. Furthermore, the abundance of ARGs conferring resistance to macrolides (Figure 5g) and monobactams (Figure 5h) was significantly higher in C_{OMPs} than in C_{Ctrl} ($P < 0.05$). Contrastingly, the abundance of these ARGs between $C_{AMM+OMPs}$ and C_{Ctrl} did not significantly differ ($P < 0.05$), suggesting that NH_4^+-N addition mitigates the effect of OMPs on ARG dissemination.

Further analysis revealed that NH_4^+-N addition significantly decreased ARB and PARB populations by 60% and 68%, respectively ($P < 0.05$; Figure S5a,b). Concurrently, MGE abundance declined by 13% ($P < 0.05$; Figure S5c). These results demonstrate NH_4^+-N's dual mitigation mechanism: directly suppressing resistant microbial communities while limiting horizontal gene transfer via MGE suppression, ultimately reducing environmental antimicrobial resistance risks.

DISCUSSION

Impact of NH_4^+-N on OMP Removal Efficiency. In the aerobic zone of the RBF, particularly within the first few meters, OMP removal is the most efficient.[2] Our simulation results support this observation, demonstrating that most OMPs are effectively removed within the first meter of the simulated RBF, with 93 out of 128 OMPs removed by >70%. Previous studies have also indicated that RBF does not achieve uniform removal across all OMPs.[5,6,33] Consistent with this, our results showed that 10 OMPs were removed by <30%, with fluconazole exhibiting almost no removal (≈0%). To enhance OMP removal efficiency, further water purification in the anoxic and anaerobic zones of the RBF,[16,34] or combining the RBF process with technologies such as reverse osmosis,[1] could be effective strategies. Key mechanisms of OMP removal during RBF include adsorption and biotransformation.[18,19] In our simulations, 40 OMPs (e.g., clindamycin) were primarily removed through biotransformation. Adsorption was confirmed for the remaining 87 OMPs, including azithromycin; however, the specific role of biotransformation requires further investigation.

In enriched nitrifier cultures, an increase in NH_4^+-N concentrations (50−500 mg/L) positively impacted the removal of some OMPs, such as naproxen, carbamazepine, sulfamethoxazole, and atenolol (>10 μg/L).[35−38] Consistent with these findings, our long-term RBF simulation results demonstrated that adding NH_4^+-N (0.1−2.2 mg/L) enhanced the removal of 20 out of 128 OMPs (e.g., naproxen, approximately 100 ng/L) by 3−70%. This suggests that low-level increases in NH_4^+-N concentration are beneficial for removing certain OMPs at ng/L concentrations, whereas most are likely influenced by other factors. Generally, the positive effect of NH_4^+-N on OMP removal is primarily linked to nitrification activity[38−40] and can be largely attributed to AOMs, including AOA, AOB, and comammox, which can transform OMPs.[23,25,41,42] In the present study, the higher concentrations of NH_4^+-N were primarily removed through nitrification. Consistently, NH_4^+-N addition significantly increased the abundance of *amo*A and the relative abundance of recovered AOA, AOB, and comammox MAGs. Our previous research highlighted the important role of AOA and comammox in RBF systems for the removal of 32 OMPs,[19] with 10 of these, namely phenacetin, lidocaine, glipizide, MCPA, 2,4-D, losartan, clindamycin, salbutamol, gemfibrozil, and digoxin, showing enhanced removal due to NH_4^+-N. Therefore, it is reasonable to consider the increase in AOA, AOB, and comammox MAGs due to NH_4^+-N as a potential mechanism underlying the enhanced removal of OMPs.

In most ecosystems, AOA, AOB, and comammox often coexist.[43] This pattern was also observed in the simulated RBF system. Since AOA and comammox typically thrive under low NH_4^+-N and oligotrophic conditions,[26,44] they emerged as the dominant ammonia oxidizers in the simulated RBF system, accounting for >99%, as anticipated. Even when NH_4^+-N concentration was increased to 2.2 mg/L, AOA and comammox retained their dominance. However, NH_4^+-N inhibited the three AOA MAGs in the simulated RBF systems, likely due to their higher ammonia affinity, which could result in substrate inhibition at elevated NH_4^+-N concentrations.[43] Notably, NH_4^+-N significantly enhanced the relative abundance of the four AOA MAGs and five comammox MAGs. Thus, we hypothesized that the four AOA MAGs and five

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comammox MAGs with significantly increased relative abundance actively contributed to the NH_4^+-N-enhanced removal of OMPs.

Beyond the role of AOMs, heterotrophic microorganisms mediate OMP biodegradation.[19,45,46] *Bradyrhizobium*, recognized as 2,4-D degraders,[47] showed significant increases in relative abundance with NH_4^+-N addition in the current study. This suggests that NH_4^+-N enhances specific OMP removal (e.g., 2,4-D) by stimulating heterotrophic bacteria. Given the extensive diversity and functional versatility of heterotrophic communities, their contributions to NH_4^+-N-enhanced removal of specific OMPs merit systematic investigation.

Adding NH_4^+-N may enhance the adsorption of specific OMPs, such as salbutamol, indicating a potential mechanism by which NH_4^+-N enhances OMP removal. However, the detailed patterns and mechanisms underlying NH_4^+-N's promotion of OMP adsorption warrant further investigation. While biosorption is considered minor for OMP removal during RBF,[18] elevated NH_4^+-N may stimulate extracellular polymeric substance production, enhancing OMP capture through biofilm adsorption. This mechanism warrants further systematic investigation.

The positive effect of NH_4^+-N addition on the removal of some specific OMPs may reflect the collective action of the microbial community throughout the sand column. However, microbiological data were only collected from the influent region, where microbial abundance is typically highest, potentially up to 100 times greater than at the effluent end of the 1-m sand column.[10] Despite this, microbial activity in other regions of the column may offer alternative explanations for the observed enhanced effect of NH_4^+-N addition on OMP removal.

Impact of OMPs and NH_4^+-N on ARG Abundance. Antibiotic resistance evolved long before naturally occurring antibiotics and their derivatives were used to treat human diseases.[48] ARGs have been identified in various environments associated with RBF, including river water, sediments, aquifers, and groundwater.[49,50] As expected, 22 of the 25 ARGs were identified in the control group (C_{Ctrl}), which simulated a natural RBF system using river water. The presence of ARGs in the RBF medium may be attributed to two key processes: (i) The RBF process may enhance the retention of ARGs within the filtration media, since rivers are major reservoirs for these genes.[51−53] (ii) The presence of the 30 OMPs in river water, including macrolides and quinolones, at concentrations ranging from 57 to 123 ng/L may further facilitate ARG spread, as antibiotic and nonantibiotic OMPs can accelerate this process.[12,14,54]

The total abundance of ARGs in C_{OMPs} was significantly higher than that in C_{Ctrl} ($P < 0.05$), suggesting that multiple OMPs at ng/L levels promote ARG dissemination. Although these results do not directly confirm an increase in ARG abundance in practical RBF applications, they highlight the potential risk of enhanced ARG transmission through RBF usage. Future research should monitor ARG levels in media during RBF trials to balance water purification with preventing and controlling ARG risks. Antibiotics exert a selective pressure that favors the spread of ARGs.[55,56] The increased abundance of quinolone and macrolide ARGs was likely attributed to their addition to the influent (approximately 1,400 ng/L for quinolones and 900 ng/L for macrolides). Additionally, common nonantibiotic pharmaceuticals, such as naproxen and gemfibrozil, contribute to antibiotic resistance by enhancing the uptake of exogenous ARGs at clinically and environmentally relevant concentrations.[14,54] OMPs decreased the abundance of four ARGs (diaminopyrimidine, glycopeptide, glycocycline, and thymine resistance genes), indicating that selective pressure from OMPs can enhance and suppress specific ARGs. This finding underscores the need for a comprehensive analysis of ARGs when evaluating the impact of OMPs or other factors on them.

In the simulated RBF system, NH_4^+-N decreased the abundance of eight ARGs. NH_4^+-N concentration is a crucial water quality parameter that shapes microbial community structure.[57,58] In this study, NH_4^+-N significantly increased AOM abundance while reducing ARB abundance. The decline in ARBs likely resulted from increased competition for essential resources like DO, driven by higher AOM levels. Notably, OMPs showed no enrichment effect on three specific ARGs associated with isoniazid, aminoglycosides, and diaminopyrimidines. Conversely, the abundance of these ARGs exhibited a significant reduction following NH_4^+-N addition (Figure 5). This trend suggests that NH_4^+-N selectively inhibits ARBs, reducing these specific ARGs (Figure S6).

Previous studies have shown that antibiotic and nonantibiotic pharmaceuticals, such as gemfibrozil and naproxen, accelerate ARG dissemination.[54] The selective pressure induced by antibiotics is generally more intense in environments with higher concentrations of these compounds.[59] Herein, we observed that the addition of NH_4^+-N enhanced the removal efficiencies of three quinolone antibiotics (flumequine, nalidixic acid, and cinoxacin) and two macrolide antibiotics (virginiamycin S1 and clindamycin), while simultaneously reducing the abundance of the corresponding quinolone and macrolide ARGs. The increase in the relative abundance of AOA and comammox MAGs following NH_4^+-N addition may contribute to the enhanced removal of certain OMPs. Moreover, microbial-mediated reactions, such as hydroxylation and acetylation, can inactivate antibiotics like macrolides.[60,61] AOA and comammox may modify antibiotics through similar biochemical processes, facilitating the transformation and detoxification of these pollutants.[24,36] Based on our analysis, we hypothesize that NH_4^+-N stimulates AOA and comammox activity to enhance OMP removal, thereby reducing both OMP concentrations and antimicrobial efficacy (particularly for quinolone- and macrolide-associated ARGs), ultimately leading to decreased ARG abundance (Figure S6). Evidence from recent findings supports our hypothesis, suggesting that NH_4^+-N can stimulate AOMs to degrade oxacillin while also inhibiting the production and spread of ARGs during managed aquifer recharge.[62] However, the evidence supporting this hypothesis remains indirect, necessitating further studies to identify specific degradation products and elucidate the underlying mechanisms linking AOM activity to the inactivation of antimicrobial agents.

■ ENVIRONMENTAL IMPLICATIONS

The findings of this study suggest that NH_4^+-N coexistence enhances the removal of specific OMPs (e.g., flumequine) in RBF, possibly due to the increased biotransformation facilitated by NH_4^+-N's impact on AOMs (e.g., AOA and comammox) and heterotrophic bacteria, such as *Bradyrhizobium*. These results collectively highlight that optimizing microbial communities is an effective strategy for enhancing the removal of specific OMPs. Water quality parameters (e.g.,

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DOC concentration and composition) exert a stronger influence on OMP removal efficiency in RBF systems compared to the soil type.[17,21] This work advances the theoretical framework connecting water quality parameters to OMP attenuation, while providing new empirical evidence and mechanistic insights. Integration of NH_4^+-N monitoring into multivariate modeling frameworks is therefore recommended to improve site-specific evaluation and predictive capacity of OMP removal performance in operational RBF systems.

The results further reveal that trace-level OMP mixtures significantly increase the abundance of ARGs, whereas the coexistence of NH_4^+-N reduces the abundance of specific ARGs (e.g., quinolone ARGs). This reduction may be due to the inhibitory effect of NH_4^+-N on ARBs. Additionally, we hypothesize that the coexistence of NH_4^+-N stimulates AOMs to enhance OMP removal, thereby reducing both OMP concentrations and antimicrobial efficacy, ultimately leading to decreased ARG abundance. Compared to conventional physical separation and chemical oxidation methods (e.g., chlorination), microbe-mediated biotransformation technologies demonstrate superior advantages in gently degrading OMPs while also mitigating the dissemination of ARGs. When NH_4^+-N co-occurs with OMPs at relatively high concentrations, it enhances the control of specific OMPs and ARGs by influencing the microbial community, potentially serving as a natural mechanism in environmental settings. However, in scenarios where NH_4^+-N concentrations are relatively low, it is also important to consider improving OMP removal efficiency while simultaneously strengthening risk management for ARGs. Since NH_4^+-N and its transformation product (NO_3^--N) are typically priority targets in aquatic environmental pollution control, artificially supplementing NH_4^+-N to enhance OMP and ARG control could be seen as a "drinking poison to quench thirst" approach, which has limited practicality. Consequently, future research should focus on developing alternative, NH_4^+-N-independent microbial enhancement strategies to simultaneously achieve efficient OMP removal and effective ARG risk mitigation.

■ ASSOCIATED CONTENT

Data Availability Statement

The metagenomic sequencing data are archived at the National Center for Biotechnology Information under accession number PRJNA970413.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.4c13440.

Additional experimental details, including the SPE procedure, analysis of OMPs using UHPLC−MS/MS, the genomic binning method, and the LEfSe analysis method; figures presenting the concentrations of OMPs in the river, OMP removal efficiency by simulated RBF, genus-level biomarkers, the abundance of ARGs (with significant changes), ARBs, PARBs, and MGEs, and the proposed mechanism of NH_4^+-N in reducing ARGs; tables showing analyte information and MS/MS properties, internal standard information and MS/MS properties, sand parameters, feedwater parameters, information on AOM MAGs, and the abundances of 25 ARGs (PDF)

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Notes

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■ REFERENCES

(1) Zhai, Y.; Liu, G.; van der Meer, W. G. J. One-Step Reverse Osmosis Based on Riverbank Filtration for Future Drinking Water Purification. *Engineering.* **2022**, *9*, 27−34.

(2) Hollender, J.; Rothardt, J.; Radny, D.; Loos, M.; Epting, J.; Huggenberger, P.; Borer, P.; Singer, H. Comprehensive micropollutant screening using LC-HRMS/MS at three riverbank filtration sites to assess natural attenuation and potential implications for human health. *Water Res. X.* **2018**, *1*, No. 100007.
(3) Oberleitner, D.; Schulz, W.; Bergmann, A.; Achten, C. Impact of seasonality, redox conditions, travel distances and initial concentrations on micropollutant removal during riverbank filtration at four sites. *Chemosphere.* **2020**, *250*, No. 126255.
(4) Zhao, J.; Kang, J.; Cao, X.; Bian, R.; Liu, G.; Hu, S.; Wu, X.; Li, C.; Wang, D.; Qi, W.; Huang, C.; Liu, H.; Qu, J. Impacts of Comorbidity and Mental Shock on Organic Micropollutants in Surface Water During and After the First Wave of COVID-19 Pandemic in Wuhan (2019−2021). *China. Engineering.* **2024**, *37*, 40−48.
(5) Hoppe-Jones, C.; Oldham, G.; Drewes, J. E. Attenuation of total organic carbon and unregulated trace organic chemicals in U.S. riverbank filtration systems. *Water Res.* **2010**, *44* (15), 4643−4659.
(6) Hamann, E.; Stuyfzand, P. J.; Greskowiak, J.; Timmer, H.; Massmann, G. The fate of organic micropollutants during long-term/long-distance river bank filtration. *Sci. Total Environ.* **2016**, *545*, 629−640.
(7) Hu, B.; Teng, Y.; Zhai, Y.; Zuo, R.; Li, J.; Chen, H. Riverbank filtration in China: A review and perspective. *J. Hydrol.* **2016**, *541*, 914−927.
(8) Ray, C. Worldwide potential of riverbank filtration. *Clean Technol. Environ. Policy.* **2008**, *10* (3), 223−225.
(9) Ray, C.; Grischek, T.; Schubert, J.; Wang, J. Z.; Speth, T. F. A perspective of riverbank filtration. *Journal-American Water Works Association.* **2002**, *94* (4), 149−160.
(10) Weiss, W. J.; Bouwer, E. J.; Aboytes, R.; LeChevallier, M. W.; O'Melia, C. R.; Le, B. T.; Schwab, K. J. Riverbank filtration for control of microorganisms: Results from field monitoring. *Water Res.* **2005**, *39* (10), 1990−2001.
(11) Reid, E.; Igou, T.; Zhao, Y.; Crittenden, J.; Huang, C.; Westerhoff, P.; Rittmann, B.; Drewes, J. E.; Chen, Y. The minus approach can redefine the standard of practice of drinking water treatment. *Environ. Sci. Technol.* **2023**, *57* (18), 7150−7161.
(12) Larsson, D. G. J.; Flach, C. Antibiotic resistance in the environment. *Nat. Rev. Microbiol.* **2022**, *20* (5), 257−269.
(13) MacLean, R. C.; San Millan, A. The evolution of antibiotic resistance. *Science* **2019**, *365* (6458), 1082−1083.
(14) Wang, Y.; Lu, J.; Mao, L.; Li, J.; Yuan, Z.; Bond, P. L.; Guo, J. Antiepileptic drug carbamazepine promotes horizontal transfer of plasmid-borne multi-antibiotic resistance genes within and across bacterial genera. *Isme Journal.* **2019**, *13* (2), 509−522.
(15) Wang, Y.; Qiao, M.; Zhu, D.; Zhu, Y. Antibiotic Resistance in the Collembolan Gut Microbiome Accelerated by the Nonantibiotic Drug Carbamazepine. *Environ. Sci. Technol.* **2020**, *54* (17), 10754−10762.
(16) Munz, M.; Oswald, S. E.; Schäfferling, R.; Lensing, H. Temperature-dependent redox zonation, nitrate removal and attenuation of organic micropollutants during bank filtration. *Water Res.* **2019**, *162*, 225−235.
(17) Alidina, M.; Li, D.; Ouf, M.; Drewes, J. E. Role of primary substrate composition and concentration on attenuation of trace organic chemicals in managed aquifer recharge systems. *J. Environ. Manage.* **2014**, *144*, 58−66.
(18) Bertelkamp, C.; Reungoat, J.; Cornelissen, E. R.; Singhal, N.; Reynisson, J.; Cabo, A. J.; van der Hoek, J. P.; Verliefde, A. R. D. Sorption and biodegradation of organic micropollutants during river bank filtration: A laboratory column study. *Water Res.* **2014**, *52*, 231−241.
(19) Zhao, J.; Fang, S.; Liu, G.; Qi, W.; Bai, Y.; Liu, H.; Qu, J. Role of ammonia-oxidizing microorganisms in the removal of organic micropollutants during simulated riverbank filtration. *Water Res.* **2022**, *226*, No. 119250.
(20) Huntscha, S.; Rodriguez Velosa, D. M.; Schroth, M. H.; Hollender, J. Degradation of Polar Organic Micropollutants during Riverbank Filtration: Complementary Results from Spatiotemporal Sampling and Push-Pull Tests. *Environ. Sci. Technol.* **2013**, *47* (20), 11512−11521.
(21) Bertelkamp, C.; Schoutteten, K.; Vanhaecke, L.; Vanden Bussche, J.; Callewaert, C.; Boon, N.; Singhal, N.; van der Hoek, J. P.; Verliefde, A. R. D. A laboratory-scale column study comparing organic micropollutant removal and microbial diversity for two soil types. *Sci. Total Environ.* **2015**, *536*, 632−638.
(22) Covatti, G.; Grischek, T. Sources and behavior of ammonium during riverbank filtration. *Water Res.* **2021**, *191*, No. 116788.
(23) Rattier, M.; Reungoat, J.; Keller, J.; Gernjak, W. Removal of micropollutants during tertiary wastewater treatment by biofiltration: Role of nitrifiers and removal mechanisms. *Water Res.* **2014**, *54*, 89−99.
(24) Zhou, L.; Han, P.; Zhao, M.; Yu, Y.; Sun, D.; Hou, L.; Liu, M.; Zhao, Q.; Tang, X.; Klümper, U.; Gu, J.; Men, Y.; Wu, Q. L. Biotransformation of lincomycin and fluoroquinolone antibiotics by the ammonia oxidizers AOA, AOB and comammox: A comparison of removal, pathways, and mechanisms. *Water Res.* **2021**, *196*, No. 117003.
(25) Han, P.; Yu, Y.; Zhou, L.; Tian, Z.; Li, Z.; Hou, L.; Liu, M.; Wu, Q.; Wagner, M.; Men, Y. Specific Micropollutant Biotransformation Pattern by the comammox Bacterium Nitrospira inopinata. *Environ. Sci. Technol.* **2019**, *53* (15), 8695−8705.
(26) Verhamme, D. T.; Prosser, J. I.; Nicol, G. W. Ammonia concentration determines differential growth of ammonia-oxidising archaea and bacteria in soil microcosms. *Isme Journal.* **2011**, *5* (6), 1067−1071.
(27) Chen, Y.; Chen, Y.; Shi, C.; Huang, Z.; Zhang, Y.; Li, S.; Li, Y.; Ye, J.; Yu, C.; Li, Z.; Zhang, X.; Wang, J.; Yang, H.; Fang, L.; Chen, Q. SOAPnuke: a MapReduce acceleration-supported software for integrated quality control and preprocessing of high-throughput sequencing data. *Gigascience.* **2018**, *7* (1), gix120.
(28) Li, D.; Liu, C.; Luo, R.; Sadakane, K.; Lam, T. MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. *Bioinformatics.* **2015**, *31* (10), 1674−1676.
(29) Zhu, W.; Lomsadze, A.; Borodovsky, M. Ab initio gene identification in metagenomic sequences. *Nucleic Acids Res.* **2010**, *38* (12), No. e132.
(30) Fu, L.; Niu, B.; Zhu, Z.; Wu, S.; Li, W. CD-HIT: accelerated for clustering the next-generation sequencing data. *Bioinformatics.* **2012**, *28* (23), 3150−3152.
(31) Patro, R.; Duggal, G.; Love, M. I.; Irizarry, R. A.; Kingsford, C. Salmon provides fast and bias-aware quantification of transcript expression. *Nat. Methods.* **2017**, *14* (4), 417−419.
(32) Buchfink, B.; Xie, C.; Huson, D. H. Fast and sensitive protein alignment using DIAMOND. *Nat. Methods.* **2015**, *12* (1), 59−60.
(33) Kondor, A. C.; Jakab, G.; Vancsik, A.; Filep, T.; Szeberényi, J.; Szabó, L.; Maász, G.; Ferincz, Á.; Dobosy, P.; Szalai, Z. Occurrence of pharmaceuticals in the Danube and drinking water wells: Efficiency of riverbank filtration. *Environ. Pollut.* **2020**, *265*, No. 114893.
(34) Bai, Y.; Ruan, X.; Wang, F.; Antoine, G.; van der Hoek, J. P. Sulfonamides removal under different redox conditions and microbial response to sulfonamides stress during riverbank filtration: A laboratory column study. *Chemosphere.* **2019**, *220*, 668−677.
(35) Tran, N. H.; Urase, T.; Kusakabe, O. The characteristics of enriched nitrifier culture in the degradation of selected pharmaceutically active compounds. *J. Hazard. Mater.* **2009**, *171* (1), 1051−1057.
(36) Kassotaki, E.; Buttiglieri, G.; Ferrando-Climent, L.; Rodriguez-Roda, I.; Pijuan, M. Enhanced sulfamethoxazole degradation through ammonia oxidizing bacteria co-metabolism and fate of transformation products. *Water Res.* **2016**, *94*, 111−119.
(37) Xu, Y.; Radjenovic, J.; Yuan, Z.; Ni, B. Biodegradation of atenolol by an enriched nitrifying sludge: Products and pathways. *Chem. Eng. J.* **2017**, *312*, 351−359.
(38) Fernandez-Fontaina, E.; Omil, F.; Lema, J. M.; Carballa, M. Influence of nitrifying conditions on the biodegradation and sorption of emerging micropollutants. *Water Res.* **2012**, *46* (16), 5434−5444.

https://doi.org/10.1021/acs.est.4c13440
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

(39) Helbling, D. E.; Johnson, D. R.; Honti, M.; Fenner, K. Micropollutant Biotransformation Kinetics Associate with WWTP Process Parameters and Microbial Community Characteristics. *Environ. Sci. Technol.* **2012**, *46* (19), 10579−10588.
(40) Su, Q.; Schittich, A.; Jensen, M. M.; Ng, H.; Smets, B. F. Role of Ammonia Oxidation in Organic Micropollutant Transformation during Wastewater Treatment: Insights from Molecular, Cellular, and Community Level Observations. *Environ. Sci. Technol.* **2021**, *55* (4), 2173−2188.
(41) Zhou, L.; Han, P.; Yu, Y.; Wang, B.; Men, Y.; Wagner, M.; Wu, Q. L. Cometabolic biotransformation and microbial-mediated abiotic transformation of sulfonamides by three ammonia oxidizers. *Water Res.* **2019**, *159*, 444−453.
(42) Men, Y.; Han, P.; Helbling, D. E.; Jehmlich, N.; Herbold, C.; Gulde, R.; Onnis-Hayden, A.; Gu, A. Z.; Johnson, D. R.; Wagner, M.; Fenner, K. Biotransformation of Two Pharmaceuticals by the Ammonia-Oxidizing Archaeon Nitrososphaera gargensis. *Environ. Sci. Technol.* **2016**, *50* (9), 4682−4692.
(43) Jung, M.; Sedlacek, C. J.; Kits, K. D.; Mueller, A. J.; Rhee, S.; Hink, L.; Nicol, G. W.; Bayer, B.; Lehtovirta-Morley, L.; Wright, C.; de la Torre, J. R.; Herbold, C. W.; Pjevac, P.; Daims, H.; Wagner, M. Ammonia-oxidizing archaea possess a wide range of cellular ammonia affinities. *Isme Journal.* **2022**, *16* (1), 272−283.
(44) Kits, K. D.; Sedlacek, C. J.; Lebedeva, E. V.; Han, P.; Bulaev, A.; Pjevac, P.; Daebeler, A.; Romano, S.; Albertsen, M.; Stein, L. Y.; Daims, H.; Wagner, M. Kinetic analysis of a complete nitrifier reveals an oligotrophic lifestyle. *Nature.* **2017**, *549* (7671), 269−272.
(45) Men, Y.; Achermann, S.; Helbling, D. E.; Johnson, D. R.; Fenner, K. Relative contribution of ammonia oxidizing bacteria and other members of nitrifying activated sludge communities to micropollutant biotransformation. *Water Res.* **2017**, *109*, 217−226.
(46) Fernandez-Fontaina, E.; Gomes, I. B.; Aga, D. S.; Omil, F.; Lema, J. M.; Carballa, M. Biotransformation of pharmaceuticals under nitrification, nitratation and heterotrophic conditions. *Sci. Total Environ.* **2016**, *541*, 1439−1447.
(47) Kitagawa, W.; Takami, S.; Miyauchi, K.; Masai, E.; Kamagata, Y.; Tiedje, J. M.; Fukuda, M. Novel 2,4-Dichlorophenoxyacetic Acid Degradation Genes from Oligotrophic Bradyrhizobium sp. Strain HW13 Isolated from a Pristine Environment. *J. Bacteriol.* **2002**, *184* (2), 509−518.
(48) D'Costa, V. M.; King, C. E.; Kalan, L.; Morar, M.; Sung, W. W. L.; Schwarz, C.; Froese, D.; Zazula, G.; Calmels, F.; Debruyne, R.; Golding, G. B.; Poinar, H. N.; Wright, G. D. Antibiotic resistance is ancient. *Nature* **2011**, *477* (7365), 457−461.
(49) Ma, L.; Li, B.; Jiang, X. T.; Wang, Y. L.; Xia, Y.; Li, A. D.; Zhang, T. Catalogue of antibiotic resistome and host-tracking in drinking water deciphered by a large scale survey. *Microbiome* **2017**, *5*, 1−12.
(50) Zainab, S. M.; Junaid, M.; Xu, N.; Malik, R. N. Antibiotics and antibiotic resistant genes (ARGs) in groundwater: A global review on dissemination, sources, interactions, environmental and human health risks. *Water Res.* **2020**, *187*, No. 116455.
(51) Pruden, A.; Arabi, M.; Storteboom, H. N. Correlation Between Upstream Human Activities and Riverine Antibiotic Resistance Genes. *Environ. Sci. Technol.* **2012**, *46* (21), 11541−11549.
(52) Muurinen, J.; Muziasari, W. I.; Hultman, J.; Pärnänen, K.; Narita, V.; Lyra, C.; Fadlillah, L. N.; Rizki, L. P.; Nurmi, W.; Tiedje, J. M.; Dwiprahasto, I.; Hadi, P.; Virta, M. P. J. Antibiotic Resistomes and Microbiomes in the Surface Water along the Code River in Indonesia Reflect Drainage Basin Anthropogenic Activities. *Environ. Sci. Technol.* **2022**, *56* (21), 14994−15006.
(53) Allen, H. K.; Donato, J.; Wang, H. H.; Cloud-Hansen, K. A.; Davies, J.; Handelsman, J. Call of the wild: antibiotic resistance genes in natural environments. *Nat. Rev. Microbiol.* **2010**, *8* (4), 251−259.
(54) Wang, Y.; Lu, J.; Engelstädter, J.; Zhang, S.; Ding, P.; Mao, L.; Yuan, Z.; Bond, P. L.; Guo, J. Non-antibiotic pharmaceuticals enhance the transmission of exogenous antibiotic resistance genes through bacterial transformation. *Isme Journal.* **2020**, *14* (8), 2179−2196.
(55) Neu, H. C. The Crisis in Antibiotic Resistance. *Science.* **1992**, *257* (5073), 1064−1073.
(56) Kümmerer, K. Drugs in the environment: emission of drugs, diagnostic aids and disinfectants into wastewater by hospitals in relation to other sources − a review. *Chemosphere.* **2001**, *45* (6), 957−969.
(57) Yang, L.; Yao, H.; Jia, F.; Han, B.; Chen, Y.; Jiang, J.; Liu, T.; Guo, J. Effects of ammonia nitrogen and organic carbon availability on microbial community structure and ecological interactions in a full-scale partial nitritation and anammox (PN/A) system. *Water Res.* **2023**, *244*, No. 120524.
(58) Sui, Q.; Liu, C.; Zhang, J.; Dong, H.; Zhu, Z.; Wang, Y. Response of nitrite accumulation and microbial community to free ammonia and dissolved oxygen treatment of high ammonium wastewater. *Appl. Microbiol. Biotechnol.* **2016**, *100* (9), 4177−4187.
(59) Martínez, J. L. Antibiotics and Antibiotic Resistance Genes in Natural Environments. *Science.* **2008**, *321* (5887), 365−367.
(60) Davies, J. Inactivation of Antibiotics and the Dissemination of Resistance Genes. *Science.* **1994**, *264* (5157), 375−382.
(61) Das, B.; Verma, J.; Kumar, P.; Ghosh, A.; Ramamurthy, T. Antibiotic resistance in Vibrio cholerae: Understanding the ecology of resistance genes and mechanisms. *Vaccine.* **2020**, *38*, A83−A92.
(62) Fang, Y.; Chen, C.; Cui, B.; Li, H.; Zhou, D. Key role of NH_4^+-N in the removal of oxacillin during managed aquifer recharge: Reconsidering the recharge limitation. *Water Res.* **2024**, *266*, No. 122375.

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