Environmental Science & Technology

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Article

Persistence of Antibiotic Resistance Genes Varies with Particle Size and Substrate Conditions in Recirculating Streams

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Cite This: *Environ. Sci. Technol.* 2023, 57, 8902−8910

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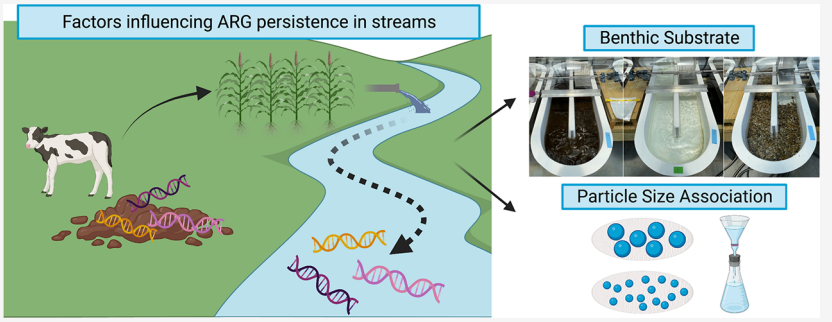

ABSTRACT: Antibiotic resistance (AR) determinants are enriched in animal manures, a significant portion of which is land-applied as a soil amendment or as fertilizer, leading to potential AR runoff and microbial pollution in adjacent surface waters. To effectively inform AR monitoring and mitigation efforts, a thorough understanding and description of the persistence and transport of manure-derived AR in flowing waters are needed. We used experimental recirculating mesocosms to assess water-column removal rates of antibiotic resistance genes (ARGs) originating from a cow manure slurry collected from a dairy farm. We quantified the effect of three benthic (i.e., bottom) substrate variations and particle sizes of manure slurry on water column removal rates. Overall, we observed variation in ARG behavior across substrate treatments and particle sizes. For ARGs associated with small particles, removal rates were higher in mesocosms with a substrate. *tetW* was typically removed at the highest rates across particle size and treatment, followed by *ermB* and bla_{TEM}. Our data suggests that both substrate character and particle size exert control on the fate and transport of ARGs in surface waters, laying the foundation for future research in this area to establish a predictive framework for AR persistence and fate in flowing waters.

KEYWORDS: *Antibiotic resistance, fate and transport, agricultural impacts, human health, environment*

INTRODUCTION

The global crisis of antimicrobial resistance (AMR), dubbed the "silent pandemic",[1,2] is one of the top issues facing the world according to the Group of Seven (G7) highly industrialized countries,[3] the World Health Organization,[4] and other government institutions. A recent meta-analysis revealed that in 2019, AMR was the confirmed cause for ∼1.27 million deaths and ranks only behind tuberculosis and COVID-19 for global deaths caused by infection.[5] The UK Government's Review on Antimicrobial Resistance estimated that if steps are not taken to control the growing issue, AMR could kill 10 million people by 2050.[6]

One of the major drivers of the AMR crisis is the widespread use of antibiotics which leads to selective pressure on both clinical and environmental bacteria to acquire antibiotic resistance (AR). In 2018, global antibiotic use was estimated to total 40 billion defined daily doses, which is an increase of 46% since 2000.[7] Globally, the majority of antibiotic use is accounted for by animal husbandry[8] which can largely be explained by the rise in demand for animal protein.[9] In the United States (US), >60% of antibiotic consumption is attributed to animal husbandry,[10] which has been generally linked to increased antibiotic resistance in animals and in some cases humans.[11−13] Antibiotics consumed by livestock exert selective pressure on the microbiome of animals, enriching AR within their systems.[10] Further, because they can be largely excreted untransformed,[10] the intact antibiotics and their residues will enrich AR in the resulting manure and exposed environments. The US produces >1.1 billion tons of animal manure per year which is most commonly land-applied as a soil

Received: March 30, 2023
Revised: May 12, 2023
Accepted: May 15, 2023
Published: June 8, 2023

https://doi.org/10.1021/acs.est.3c02374
Environ. Sci. Technol. 2023, 57, 8902−8910

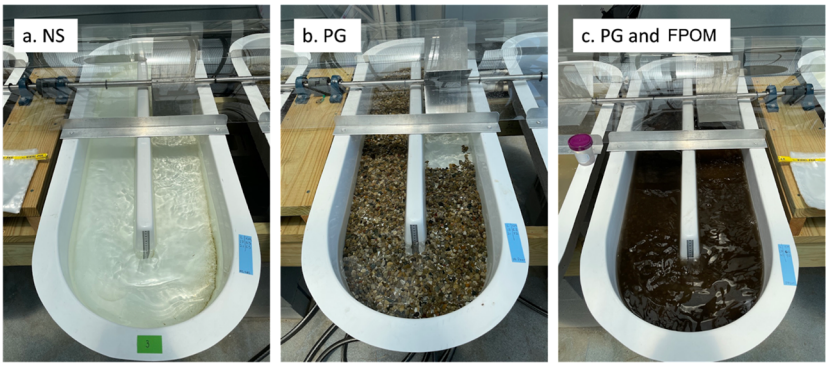

Figure 1. Three treatments for assessing antimicrobial resistance removal from controlled recirculating streams: a) No substrate (NS), b) PG only (PG), c) PG and Fine Particulate Organic Matter (PG+FPOM).

amendment or fertilizer.[14] Genes encoding resistance to antibiotics or antibiotic resistance genes (ARGs) persist in the manure along with residual antibiotics, increasing and selecting for AR in the soils where manure is applied.[15−18] Researchers have found perfect DNA matches between the resistomes of common soil bacteria and a diverse array of human pathogens,[19] suggesting that the exchange of resistance between environmental bacteria and clinical pathogens exists with as yet uncharacterized consequences.

In 1999, the United States Environmental Protection Agency (EPA) and the United States Department of Agriculture (USDA) outlined a national strategy for animal feeding operations to responsibly and sustainably handle animal manure.[20] However, many of these strategies are focused on nutrient pollution, and the suggestions are outdated given the evolution of livestock production over the past several decades. Federal regulations have further attempted to define and encourage environmentally conscious practices for land application of manure, but even these remain primarily qualitative; and enforcement is challenging and typically ineffective.[21] Further, no regulations exist regarding the dissemination of antibiotic resistance through the land application of manure because the mechanisms behind ARG transport from fields to the surrounding environment are largely unexplored, necessitating comprehensive characterization of the fate and transport of AR after land application. In particular, while mechanisms driving the removal and persistence of AR through soils have been assessed,[22−24] we lack characterization of AR in streams and ditches receiving runoff from agricultural fields and their potential to transport ARGs even further from their source. Given that AR levels are highest at the surface of the soil[17,18] and that ARG concentrations in soil remain stable for up to four weeks post land-application,[23] precipitation-driven runoff poses notable concerns especially under a changing climate, where extreme precipitation events are forecasted for the future.[25] Understanding the underlying mechanisms driving off-site transport of AR could inform regulations and encourage targeted mitigation and monitoring efforts in order to minimize human exposure to environmental AR.

The conditions of benthic substrate in streams, as well as the particle size distribution of manure inputs, may influence AR transport in flowing waters. In general, microbial biofilms in aquatic ecosystems are strongly influenced by stream geomorphology and benthic (i.e., bottom) sediment composition,[26] and underlying substrate has been shown to impact the fate and transport of environmental DNA originating from eukaryotes.[27,28] We therefore hypothesized that substrate/sediment might similarly alter ARG dynamics in streams. Additionally, particle size distribution impacts transport distances and rates of deposition of particulate runoff, with the removal of larger particles occurring faster than smaller particles.[29] We therefore hypothesized that ARGs associated with smaller particles would be transported in the water column longer than ARGs associated with larger particles. Across both hypotheses, we expected removal to vary between ARG targets. To test for the effect of substrate and particle size on ARG persistence, we quantified water-column removal rates of ARGs originating from a cow manure slurry using replicated recirculating mesocosms with three different benthic substrate treatments.

METHODS

Experimental Design and Sample Collection. To isolate the mechanisms driving ARG removal in streams, we conducted a controlled experiment utilizing the Experimental Mesocosm Facilities (EMF) at the University of Notre Dame. This design allowed for a unique assessment under controlled conditions that is not possible in a natural environment. Our experimental design included 12 identical mesocosms filled with 25 L of groundwater. Water was recirculated continuously at an average velocity of 0.2 m s^{-1}, with n = 4 replicates of three substrate variations including no substrate (NS), pea gravel only (PG), and pea gravel with fine particulate organic matter (PG+FPOM). Pea gravel was added so that it completely covered the bottom of the mesocosms in one layer. We purchased PG (D_{50} = 0.5 cm) from a local gravel supplier and rinsed and soaked it in water before adding it to the mesocosms. FPOM was collected from the bottom of a local stream in Northwestern Indiana (41.7277, −86.2633) on the day prior to the start of the experiment and filtered through a 1 mm sieve to remove large particulate organic matter and other debris. We concentrated FPOM by precipitation and added 2.5 L of the concentrated FPOM mixture to each stream (dry weight: 0.02 g/mL), a quantity deemed enough to cover the bottom of mesocosms evenly with particles. After setup, to stabilize the systems, we ran the mesocosms for 72 h before starting the experiment (Figure 1).

We collected fresh fecal samples from a domestic cow (*Bos taurus*) from a herd at a local dairy farm that was receiving treatment with Spectramast (a third generation cephalosporin) and Polyflex (ampicillin). This animal was being individually treated for illness and was isolated from healthy cows in the herd. Upon collection (i.e., via rectal retrieval), the sample was

https://doi.org/10.1021/acs.est.3c02374
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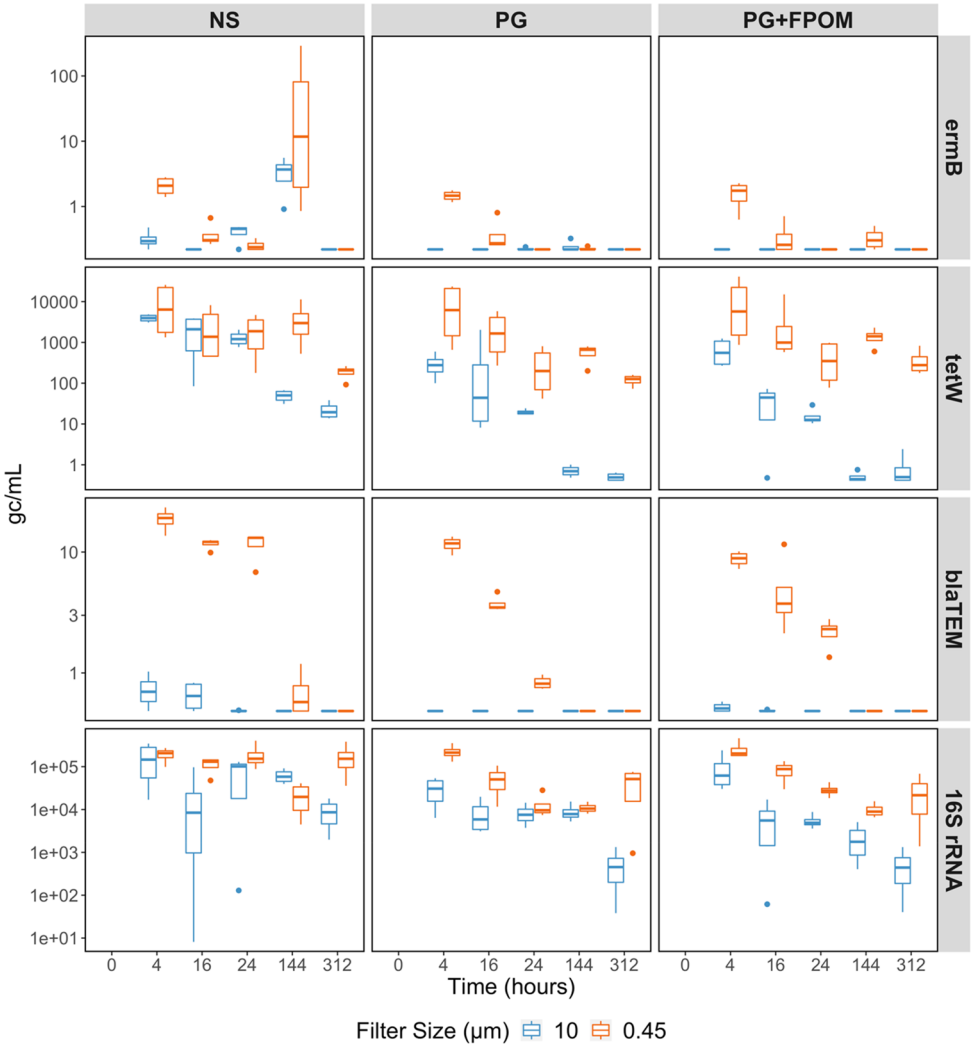

Figure 2. Box plots of ARG concentrations at each time point of the experiment. The *x*-axis is discrete, and *y*-axis scales are variable for visualization purposes.

placed in a sterilized 1 L plastic bottle (Thermo Fisher Scientific Inc., Massachusetts, USA) and transported 45 min back to the laboratory in a cooler on ice. We immediately diluted the sample to create a manure slurry of 10 g wet wt per 1 L of DI water and stored the slurry at 4 °C for approximately 12−15 h until we began the experiment the following day. Biosafety requirements necessitated that the concentration of *E. coli* was less than 100 CFUs/mL (i.e., the US EPA recreational water limit) at the experiment's conclusion. The dose was calculated based upon prior *E. coli* concentration measurements in manure samples.

Prior to spiking the mesocosms with 200 mL of manure slurry, we collected a 300 mL grab sample of water from each mesocosm directly upstream of the mesocosm rotors (n = 12 total) to assess background ARG levels using Whirl-Pak Standard Bags (Whirl-Pak, Madison, WI). After addition of manure slurry, we allowed the mesocosms to run for 20 min to achieve complete mixing and then collected one 300 mL grab sample of water from each mesocosm at the same place directly upstream of the mesocosm rotors at 20 min, 4 h, 16 h, 24 h, 1 week, and 2 weeks (n = 72 total samples). On each sampling day (n = 4), we collected a groundwater sample as a control from the hose used to fill the mesocosms (hose blank, n = 4). Throughout the experiment, we accounted for evaporation from the mesocosms by refilling them to their original volume (25 L) using groundwater before each sampling.

Sample Processing. We transported samples approximately 400 m between buildings to the laboratory on ice and then immediately processed them using vacuum membrane filtration. We sequentially filtered up to 250 mL of sample volume using two sizes (10 and 0.45 μm) of Mixed Cellulose Ester (MCE) filters (Advantech, Taipei, Taiwan). We chose two size classes to compare the behavior of large (>10 μm) and small (>0.45 μm and <10 μm) particle-associated ARGs. For background samples, we used just the 0.45 μm filters. For each sampling event, we also filtered the hose blank. We placed all filters in sterile 1.5 mL tubes which we kept frozen at −20 °C until extraction. Extractions took place in 24 sample batches during the few months following the experiment. Negative extraction controls were included with each batch (n = 9).

We transferred filters from the 1.5 mL tubes to *PowerBead tubes* (Qiagen, Hilden, Germany) and subsequently extracted DNA/RNA using the *DNEasy PowerSoil Pro Kit* according to the manufacturer's instructions. Briefly, we lysed filters via chemical and mechanical homogenization, cleaned the lysate by mixing with a DNA binding solution, and finally passed the

https://doi.org/10.1021/acs.est.3c02374
Environ. Sci. Technol. 2023, 57, 8902−8910

Table 1. Average Removal Rates (k = h^{-1}) across No Substrate (NS), Pea Gravel Only (PG), and Pea Gravel and Fine Particulate Organic Matter (PG+FPOM) for 0.45 and 10 μm Filters

	0.45 μm Filter Removal (k = h^{-1} ± standard error)			10 μm Filter Removal (k = h^{-1} ± standard error)		
	PG+FPOM	PG	NS	PG+FPOM	PG	NS
ermB	0.11 ± 0.0062	0.11 ± 0.0040	0.096 ± 0.0033	0.024 ± 0.0083	0.0037 ± 0.0030	0.0034 ± 0.010
tetW	0.15 ± 0.012	0.16 ± 0.0094	0.087 ± 0.022	0.27 ± 0.012	0.16 ± 0.013	0.065 ± 0.016
bla_{TEM}	0.079 ± 0.0088	0.12 ± 0.0049	0.018 ± 0.0053	0.026 ± 0.0093	0.00064 ± 0.00040	0.013 ± 0.0068

lysate through a silica spin filter membrane. We washed the membrane and eluted the silica membrane using 50 μL of Tris buffer. We assessed nucleic acid quantities using Qubit dsDNA HS assays according to the manufacturer's instructions (Thermo Fisher Scientific Inc., Massachusetts, USA). We stored the extracted nucleic acids at −20 °C until further analysis. All analyses were conducted within six months of storage.

Molecular Analysis. We conducted absolute quantification of select ARG targets as markers for AR using droplet digital PCR (ddPCR; QX200 Droplet Digital PCR System, Bio-Rad, Hercules, CA, USA). Here, ARGs are used to broadly refer to both intercellular and free-floating ARGs. During ddPCR, the PCR reaction is partitioned into thousands of individual reactions before amplification, and all droplets are analyzed at end-point to enable absolute quantification of target DNA.[30] We included targets for ARGs encoding resistance to tetracycline (*tetW*),[31] erythromycin (*ermB*),[32] and beta-lactams (bla_{TEM}).[33] Resistance rates tend to be highest against tetracyclines[34] as it is among the most commonly used classes of antimicrobials in animal production, accounting for 66% of total antibiotics sold for the industry.[35] Erythromycin, a critically important antimicrobial in human medicine,[36] is also used in animal husbandry and also has high rates of resistance.[34] Recent studies have found an increase in extended-spectrum beta-lactamases producing *Enterobacteriaceae* in both humans and dairy cattle, likely associated with the use of beta lactam antibiotics.[37] The bla_{TEM} assay used in our analysis incorporates 135 variants within the TEM family of beta-lactam resistance including resistance to cephalosporins, which was an antibiotic being used to treat the cow that provided the manure sample for the study.[33] We also include concentrations of 16S rRNA as a marker for total bacterial population.[38] We detail the complete information for our digital PCR experiments in the Supporting Information as outlined by the digital MIQE Guidelines. We experimentally determined 95% limits of detection (LODs) for each assay using a ten-replicate serial dilution series of positive control material and a probit analysis outlined by Stokdyk (2016).[39] The 95% LOD represents the concentration for which the probability of a single ddPCR reaction being positive is 95%. Detections at or below the LOD were replaced with the LOD for the analysis.

Data Analysis. We processed all data in RStudio version 2022.2.3.492.[40] To control for manure slurry concentration differences between mesocosms, we assessed removal in terms of the measured concentration at each sampling time $C(t_i)$ normalized by the measured concentration at the first sampling time after manure slurry addition ($C(t_0)$). To estimate water column removal rates for ARGs, we applied the best fitting model, a single-phase exponential degradation model, on a log−linear scale. When we measured a significant decrease in concentration over time, we expressed removal rate constants as the metric k ($hour^{-1}$) reflecting the statistically significant slope of the regression for each target and substrate treatment combination. Regressions where significant removal was not observed were removed from the calculation of k. We used ANCOVA to determine whether treatment replicates were statistically similar. When statistically similar, we calculated the average slope across treatment replicates for each assay to determine an average removal rate ($\bar{k}$ = h^{-1}) over time. To analyze the effect of the three different substrate treatments on removal rates and any differences among ARG targets, we compared the mean removal rates for each treatment using ANOVA and a post hoc Tukey HSD test. A complete reporting of statistical results is included in the SI.

RESULTS

In general, we observed rapid, exponential removal within the first 24 h (Figure 2) and a spike in concentration at the one-week time point for some of the targets, most dramatically for *tetW* and *ermB* (Figure 2, *x*-axis discrete).

This spike occurred across multiple targets and in multiple mesocosms and could be due to a number of factors including resuspension, microbial community growth, biofilm detachment, and horizontal gene transfer in the water column or biofilms. Our experiment was not designed to address these possible outcomes, but we acknowledge that the one- and two-week data are important; we require further experimentation to interpret the ambiguities that do not follow the same trend as the data for the first 24 h. We are interested in long-term transport of ARGs in surface waters, so we will design future experiments to capture these dynamics using more frequent sampling up to the two week mark. But we emphasize that within 24 h, ARG concentrations decrease exponentially by multiple logs of magnitude. By focusing on removal during this time period, our subsequent assessments relate to control measures that can be implemented closer to the source of contamination with important implications for downstream fate and transport. We collected 48 samples within the first 24 h. Background concentrations of the three ARG targets were negligible in the mesocosms compared to after manure addition (n = 12 samples) though we include these data in the SI for reference (Figure S1). Furthermore, all hose blanks and extraction blanks were negative for all targets. After the addition of the manure slurry, we found that the percentage of detection on 10 μm filters was only higher at the first two time points for 16S rRNA, and the majority of the ARG targets was captured on 0.45 μm filters across all treatments and time points, with the percentage of detection on 10 μm filters decreasing over time (Figure 3).

Rates of Water Column Removal Differed by Size Class. We quantified water column removal rates (k) of ARGs over the sampling sequence (Table 1). For ARGs captured on 0.45 μm filters (small particle-associated ARGs), water column concentrations of *ermB*, *tetW*, and bla_{TEM} decreased across all treatments over time. We calculated statistically similar removal between treatment replicates for all targets except in

https://doi.org/10.1021/acs.est.3c02374
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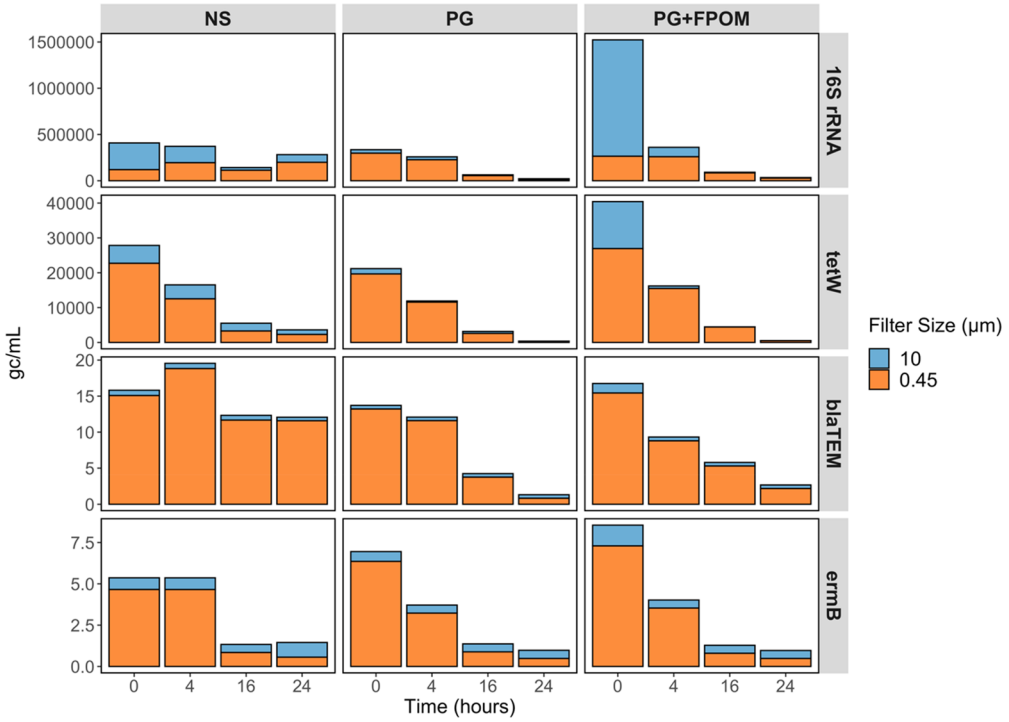

Figure 3. Raw concentrations of ARGs over time with size distribution of detections indicated by color. There were $n = 4$ mesocosm replicates for each treatment, and bar height represents the average between those. The y-axis differs for each target, with concentration increasing per target from bottom to top.

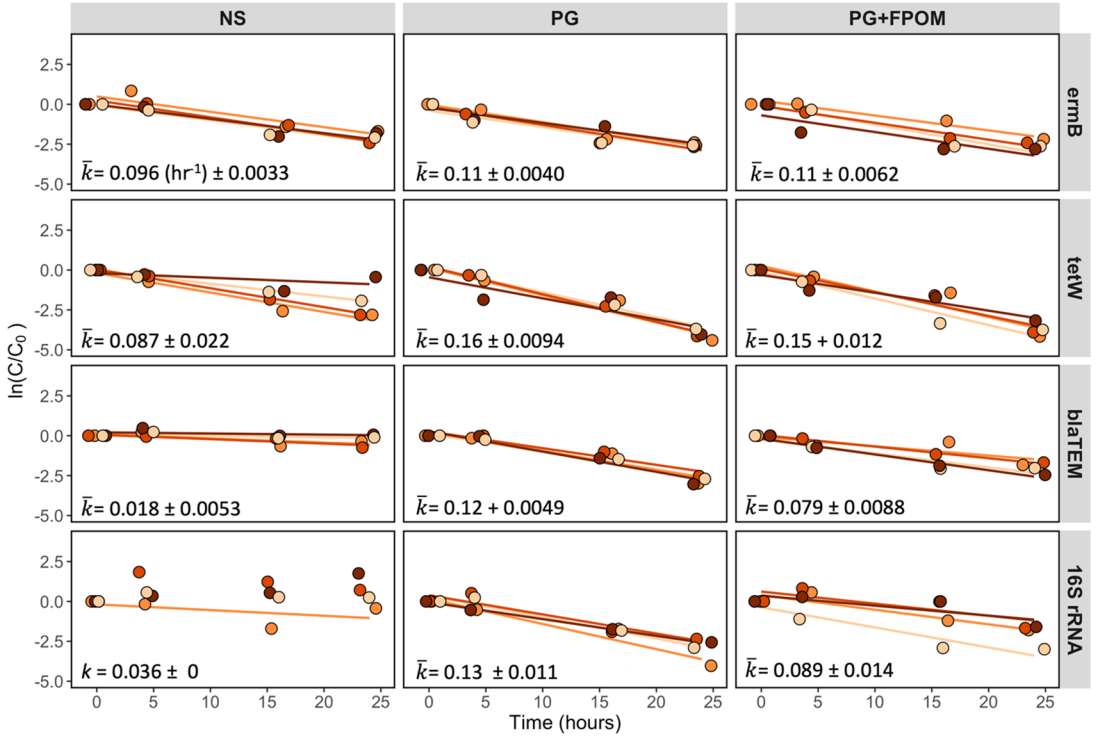

Figure 4. ARG average removal rates across mesocosm replicates ± standard error for 0.45 μm filters. Mesocosm replicates for each treatment are visualized separately by color. Points are jittered with respect to the x-axis. Only regression lines whose slope was negative and significant are included.

one case, where removal of *tetW* varied significantly across no substrate treatment replicates ($p = 0.03$).

We compared k values between treatments using ANOVA and posthoc Tukey HSD. For most small particle-associated ARGs, there was an effect of the presence of substrate versus no substrate on water column removal rates (Figure 4). For *tetW*, the presence of substrate significantly increased water column removal rates compared to mesocosms with no

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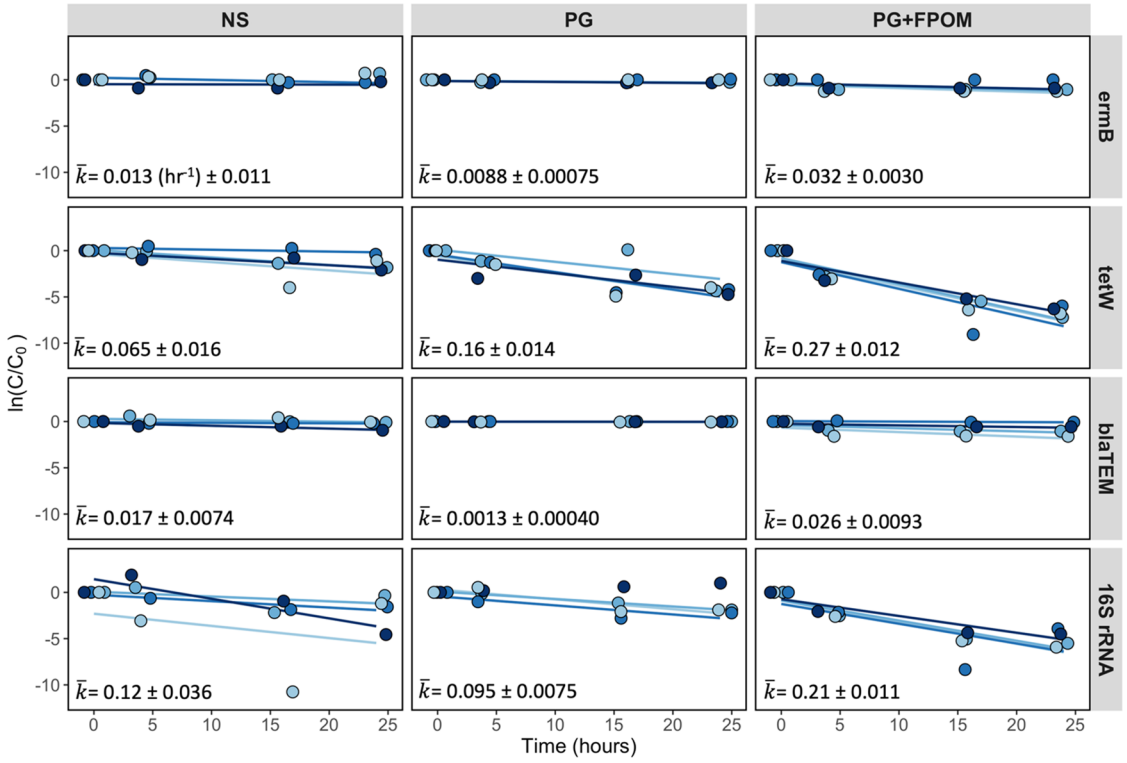

Figure 5. ARG average removal rates across mesocosm replicates ± standard error for 10 μm filters. Mesocosm replicates for each treatment are visualized separately by color. Points are jittered with respect to the x-axis. Only regression lines whose slope was negative and significant are included.

substrate (p = 0.05 and p = 0.02 for PG+FPOM and PG, respectively). For bla_{TEM}, substrate also significantly increased removal (p < 0.001 for PG+FPOM and PG). Additionally, removal rates for PG and PG+FPOM treatments differed for bla_{TEM} with a higher removal rate when only PG was present (p = 0.01).

Within each treatment, we assessed variation between small particle-associated ARGs to assess whether water column removal is target specific. In mesocosms without substrate (Figure 4, Column 1), the water column removal rates for *ermB* and *tetW* were higher than for bla_{TEM} (p = 0.02 and p = 0.03, respectively). With PG (Figure 4, Column 2), *tetW* removal rates were higher than bla_{TEM} (p = 0.01) and *ermB* (p = 0.002). With PG+FPOM (Figure 4, Column 3), the removal rate of *tetW* was significantly higher than the removal rate of bla_{TEM} (p = 0.004). Overall, on 0.45 μm filters, removal varied between ARG targets within each treatment.

The effect of substrate on ARG removal rates was distinctly different for ARGs captured on 10 μm filters (large particle-associated ARGs). In general, ARG removal rates were lower than for small particle-associated ARGs (Figure 5). Of the large particle associated ARGs, *tetW* removal rates were significantly higher with PG+FPOM and PG compared to NS (p < 0.001 and p = 0.002, respectively). Additionally, *tetW* removal rates were higher with PG+FPOM than with PG (p = 0.001).

Similar to the smaller size class, the effect of substrate on behavior for large particle-associated ARGs varied among targets. For the PG treatment (Figure 5, Column 2), *tetW* removal rates were higher than all other targets (p < 0.001 for *ermB* and bla_{TEM}), and for the PG+FPOM treatment (Figure 5, Column 3), *tetW* removal rates were higher than for all other targets (p < 0.001 for *ermB* and bla_{TEM}). Overall, removal rates with each treatment are highest for *tetW* associated with the larger size class.

Difference in Removal between Size Classes. We also considered the differences in removal rates between the two particle size classes for all ARG targets with each treatment. With PG+FPOM, removal rates for small particle-associated ARGs (>0.45 μm but <10 μm) were significantly higher for *ermB* (p < 0.001) and bla_{TEM} (p = 0.01) but significantly lower for *tetW* (p < 0.001) when compared to removal rates for large particle-associated ARGs (>10 μm). With PG, removal rates for small particle-associated ARGs were significantly higher than large particle-associated ARGs for *ermB* (p < 0.001) and bla_{TEM} (p < 0.001). For NS, small particle-associated *ermB* removal rates were significantly higher for than for large particle-associated *ermB* (p < 0.001). Across all treatments, *ermB* was removed at consistently higher rates when associated with small particles compared to large particles. Overall, we further show that size class did have an effect on *ARG* removal, and this varies between targets and within treatments.

DISCUSSION

Substrate Impacts ARG Removal. Using the replicated experimental mesocosm platform, we show that benthic substrate affects ARG water column removal rates and that these rates vary among ARG targets and with particle size. For ARGs associated with small particles (>0.45 μm but <10 μm), removal rates were higher in mesocosms with substrate. The presence of interstitial spaces created by the addition of substrate allows for particle retention, as demonstrated by one study's findings that more interstitial space increases eukaryotic associated DNA retention.[41] In our case, the presence of

https://doi.org/10.1021/acs.est.3c02374
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substrate is an important variable controlling ARG removal rates from the water column. In the single case where we observed a significant difference in removal rates between PG and PG+FPOM for 0.45 μm filters, bla_{TEM} was removed faster with PG. In contrast, for 10 μm filters, when removal differed significantly between the two substrate treatments, *tetW* was removed faster with PG+FPOM. These cases of substrate effect varying between size classes are interesting, particularly in light of the findings of one study that show turbidity is a strong predictor of environmental DNA association with 10 μm filters.[42] The addition of FPOM to the water column provides more opportunity for ARGs to adsorb to particles and potentially aggregate, causing them to settle out of the water column more quickly. It is important to note that a major difference between the PG and PG+FPOM treatments is the extent of biofilm growth. While we did not directly assess biofilm effects on ARG removal, we know that FPOM serves as a rich nutrient source that stimulates the growth of biofilm on substrate and that biofilm can impact DNA removal from the water column.[43] However, since this trend is not consistent across all targets in our study, we can hypothesize that other potentially target-specific characteristics are at play. The difference in removal rates between the treatments indicates that ARG removal in aquatic ecosystems may depend on additional factors like additional substrate types, organic matter content, and biofilm growth.

Particle Size Association Impacts ARG Removal. In general, we see higher removal rates of ARGs associated with small particles compared to large particles, which could simply be a surface area phenomena, with more "sticky" surface area, or biofilm, associated with smaller particles. Aggregation of smaller particles transitioning to larger ones is also a possibility, such that what we are measuring as slower removal of large particles could actually be the replenishment of large particles by the aggregation of small particles.[44]

Water Column Removal Rates Varied by Target. For larger particles (>10 μm), *tetW* had higher water column removal rates than all targets across all treatments, while for smaller particles (>0.45 μm but <10 μm), removal rates for *tetW* and *ermB* were similar and typically higher than bla_{TEM}. Variation in water column removal rates among ARG targets, between different particle sizes, and across substrate treatments suggests that we cannot assume that all ARGs will be transported and/or retained similarly in the water column, making the modeling and prediction of ARG occurrence, persistence, and fate even more complex.

Though few studies have characterized the underlying mechanisms driving water column removal of ARGs in agriculturally impacted streams, some studies have assessed removal in land-applied soils.[22,23,45,46] One study assessing impacts of soil type on ARG dynamics found that removal was highly dependent on the type of ARG, regardless of soil, with decay rates higher for *tetW* than *ermB*,[22] and our removal rates documented here are consistent with these findings; water column removal rates of *tetW* were frequently higher than *ermB* across all substrate treatments in both size classes. Another previous study assessing swine manure in pond mesocosms (i.e., nonflowing water) documented similar heterogeneity in the persistence and fate of different ARGs.[47] While the source materials for the ARGs may vary, it is possible ARGs follow a similar trend

It is likely that host- and gene-specific factors such as genomic location or association with other functional genes impact ARG behavior in the environment.[48] Previous research has shown that *tetW* incurs resistance by ribosomal protection mechanisms and has been reported to be chromosomally colocated with a conjugative transposon in some human and rumen abundant gut microbes.[49,50] In contrast, bla_{TEM} incurs resistance through antibiotic inactivation[51] and is frequently found on plasmids,[52] while *ermB* occurs on a wide range of mobile genetic elements.[53] Though not all genes are associated with mobility in bacterial communities, plasmids are non-chromosomal DNA that can replicate independently within a bacterium, which could be a potential reason for the differences in removal between targets in our study and others. Additionally, morphological variations and host survival are likely to impact retention, settling times, and resuspension. Future work establishing the ARG state (i.e., intact cells or as free DNA) and characterizing their hosts will better provide mechanisms of removal between varying ARGs. We also acknowledge that the genes we assessed are not an exhaustive list, and given the variability of removal rates and persistence in the water column among targets, a broader suite of ARGs should be analyzed in the future.

Overall, we showed that substrate and particle size class influence water column removal rates for ARGs and that removal rates are also target-specific. As the risks of environmental exposure, via precipitation-driven runoff events, increase under a changing climate, it will be important to consider particle size association and gene-specific characteristics when assessing the factors driving ARG transport and removal in flowing waters, especially in agricultural landscapes. Water column removal rates are an integrative response metric that can be used in future research, as they capture differences in environmental persistence among ARG targets and reflect variation in environmental drivers responsible for ARG retention and resuspension. Ongoing work on ARG transport and retention will be critical to inform potential regulation, mitigation, and monitoring of ARG targets in order to reduce human exposure to environmental AR.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.3c02374.

A complete accounting of statistical results and ARG concentrations before the experiment and after the first 24 h of the experiment (PDF)

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https://doi.org/10.1021/acs.est.3c02374
Environ. Sci. Technol. 2023, 57, 8902−8910

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This study was funded by USDA Grant #1024735.

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https://doi.org/10.1021/acs.est.3c02374
Environ. Sci. Technol. 2023, 57, 8902−8910