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Effect of Manure Application on Abundance of Antibiotic Resistance Genes and Their Attenuation Rates in Soil: Field-Scale Mass Balance Approach

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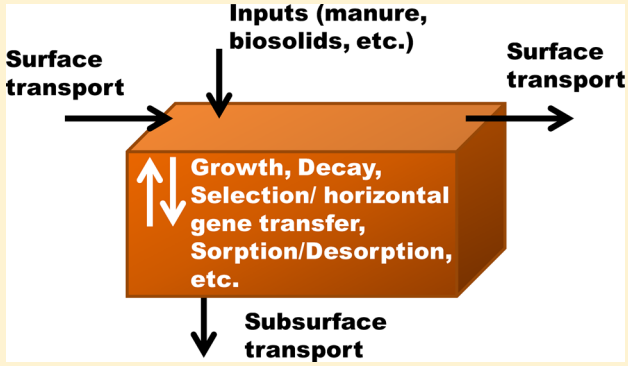
Supporting Information

ABSTRACT: The development of models for understanding antibiotic resistance gene (ARG) persistence and transport is a critical next step toward informing mitigation strategies to prevent the spread of antibiotic resistance in the environment. A field study was performed that used a mass balance approach to gain insight into the transport and dissipation of ARGs following land application of manure. Soil from a small drainage plot including a manure application site, an unmanured control site, and an adjacent stream and buffer zone were sampled for ARGs and metals before and after application of dairy manure slurry and a dry stack mixture of equine, bovine, and ovine manure. Results of mass balance suggest growth of bacterial hosts containing ARGs and/or horizontal gene transfer immediately following slurry application with respect to *ermF*, *sul1*, and *sul2* and following a lag (13 days) for dry-stack-amended soils. Generally no effects on *tet*(G), *tet*(O), or *tet*(W) soil concentrations were observed despite the presence of these genes in applied manure. Dissipation rates were fastest for *ermF* in slurry-treated soils (logarithmic decay coefficient of −3.5) and for *sul1* and *sul2* in dry-stack-amended soils (logarithmic decay coefficients of −0.54 and −0.48, respectively), and evidence for surface and subsurface transport was not observed. Results provide a mass balance approach for tracking ARG fate and insights to inform modeling and limiting the transport of manure-borne ARGs to neighboring surface water.



1. INTRODUCTION

Antibiotic resistance is a pressing human health concern and there is growing interest in potential environmental pathways by which it may originate and spread. In particular, there is a need for fundamental understanding of fate and transport mechanisms of antibiotic resistance genes (ARGs) in human-impacted environments. Several recent studies have implicated soil as a source of ARGs in major human pathogens.[1] Furthermore, community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) infections are on the rise, and recently associations have been noted with proximity to swine manure application sites.[2] Animal manure and its impact are of particular concern given that it can contain high levels of antibiotics,[3,4] antibiotic resistant microorganisms,[5] metals (which have been shown to coselect for ARGs),[6] and ARGs.[3,7−10] Elevated abundances of ARGs have been found in groundwater beneath livestock lagoons,[7,11,12] and a positive correlation between ARGs in river sediments and upstream animal counts on feedlots has been noted.[13] Therefore, manure treatment and disposal presents an important node of understanding of human effects on soil resistance levels and the potential to mitigate the spread of antibiotic resistance via environmental pathways.

Several studies have examined the effect of manure application on antibiotic resistance in soil from various perspectives. Pig manure application has been shown to increase resistance of cultivable soil microorganisms to tetracycline and sulfonamide antibiotics.[14,15] Effects of swine manure application on *sul1*,[16,17] *sul2*,[17] *ermF*, *ermB*, *tet*(Q), and *tet*(X)[18] abundance have been studied in detail in soil, while newer metagenomic approaches have revealed increases in a variety of ARGs.[19] Increases in ARG abundance in soil have been associated with manure spiked with antibiotics,[16,17] livestock fed antibiotics compared to those not fed antibiotics,[10] the presence of metals,[20,21] and general baseline increase in

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antibiotic use in archived soil samples.[22] However, manure application does not universally increase ARGs in soils.[23,24] For example, manure from three dairy farms employing therapeutic use of β-lactam antibiotics and from swine farms employing subtherapeutic use of tylosin and chlortetracycline did not increase cultivable chlortetracycline-resistant bacteria when land-applied to soil.[23]

Generally, steep increases in ARG abundance following manure application are not maintained long-term. Measurements of the persistence of ARGs in soil following pig manure slurry application ranged from <20 days for macrolide, lincosamide, and streptogramin B resistance[25] to >2 months for *sul1* in soil microcosms.[16] In other studies, <21 days were required for sulfachloropyridazine-resistant soil isolates to return to baseline levels following pig manure slurry treatment[15] and up to 6 months for tetracycline resistant isolates.[14] To date, most studies have focused on grab samples or soil microcosms, rather than monitoring the effect of manure application across manure application events under field conditions. While batch studies have suggested rapid dissipation of ARGs in soils, it is necessary to verify these rates under field conditions where real-world factors influence ARG fate, including physical transport, UV inactivation, and varying soil moisture content.

The purpose of this study was to examine the effects of application of two types of manure on abundance of a range of ARGs (tetracycline, sulfonamide, macrolide, and glycopeptide) in soil and the subsequent patterns and rates of ARG attenuation at field-scale. Sampling spanned 9 months in soils from a range of hill slopes, at two depths, and within downgradient water bodies, and where applicable dissipation rates were calculated. Loading rates of ARGs and metals (Cu, Pb) were compared and mass balance was performed on these contaminants to gain insight into mechanisms influencing ARG fate in manure-applied soils. This study provides a mass balance approach for understanding the environmental fate of ARGs and a quantitative analysis of the effects of manure application on soil ARGs at field scale.

2. METHODS

2.1. Manure Application and Sample Collection. Samples were collected from September 2012 to June 2013 to capture background levels, manure application, and time following manure application. Composite soil samples (homogenized replicates) were collected from a historically manured cornfield at two depths (five randomly selected replicates per site from 0 to 5 cm, three replicates per site from 5 to 20 cm) across a grid of 20 sites representing a range of elevations and hill slopes in the subwatershed, at the Virginia Tech StREAM Lab (http://www.bse.vt.edu/site/streamlab) (Figure 1). Sites A1−A4, B1−B3, C1−C3, and D1−D3 (n = 13) were treated with dairy manure slurry; sites A5−A7, B4, and D4 (n = 5) were treated with dry stack manure (mixture of dairy, sheep, horse, and donkey manure mixed with sawdust and straw; and sites A8 and A9 were downgradient from the manure application. Manure dry stacking is common practice on small farms where manure from different livestock is piled and liquid is allowed to evaporate or drain. This practice is different from manure composting, in which a manure pile is regularly mixed and aerated at a controlled temperature regime to favorably shift nutrient ratios and kill pathogens. Replicate grab samples of dry stack and slurry manure (three composite samples of each manure type grabbed throughout the piles/

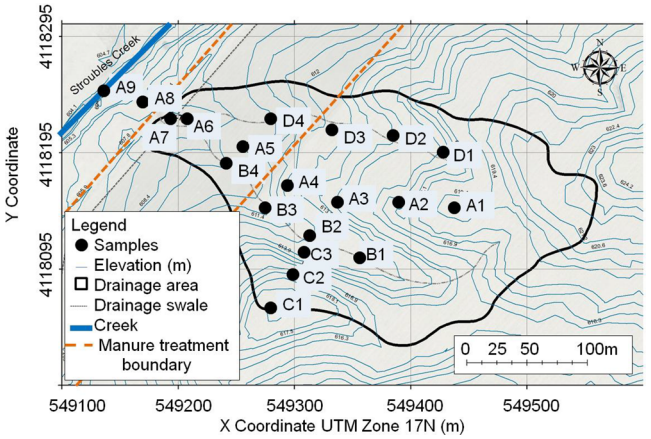

Figure 1. Map of cornfield study indicating topography, hill slopes, sample sites/codes, adjacent stream, and manure treatment zones.

slurries) were collected the day of application. The manure was surface applied at application rates of approximately 9.8 L/m^2 for slurry and 2.2 kg/m^2 for dry stack. Site E1 served as a control and was in an adjacent field that had no recent record of manure application and was used intermittently for grazing. Adjacent streamwater samples [~800 mL, concentrated onto 0.22 μm mixed cellulose ester filters (Millipore, Billerica, MA)] were collected paired with the soil sampling events.

2.2. Molecular and Chemical Analyses. DNA was extracted from homogenized soil (0.5 g wet weight), composted manure (0.5 mL of 4:1 (liquid:volume), vortexed slurry), slurried manure (0.5 mL), and filters [0.22 μm, mixed cellulose ester (Millipore, Billerica, MA)] by use of a FastDNA Spin kit (MP Biomedicals, Solon, OH) following manufacturer instructions. Quantitative polymerase chain reaction (qPCR) was performed with previously described reaction matrices and PCR protocols[26] for 16S rRNA,[27] *ermF*,[28] *sul1*,[29] *sul2*,[29] *tet*(G),[30] *tet*(O),[29] *tet*(W),[29] and *vanA*.[31] DNA extracts were diluted 1:50−1:100 to reduce inhibition. All qPCR standard curves were constructed from 10-fold serial dilutions of cloned genes ranging from 10^8 to 10^2 gene copies/μL. Based on the lowest standard on the curve and factoring in the dilutions implemented during sample processing, the limits of quantitation (LOQs) were 1.4×10^4 and 2.8×10^4 gene copies/g for ARGs and 16S rRNA, respectively. Samples were analyzed in triplicate with a standard curve, and a negative control was included in each run.

Metals were extracted via a soil acid digestion (EPA SW846 3050b), and Cu and Pb were analyzed via inductively coupled plasma mass spectrometry (ICP-MS) (Thermo Electron X-Series).

2.3. Data Analysis and Statistics. Comparisons of ARGs and metal loading rates for slurry versus dry stack manure were performed on log-normalized copy numbers with a Kruskal−Wallis rank sum test with a posthoc pairwise t test. Mass balance was performed on ARG concentrations to compare soil ARG loads expected after manure application and those observed 1 day following manure application (C_{pm}). Difference in log gene copies between predicted ($C_0 + C_m$) and measured (C_{pm}), was calculated, where C_{pm} is ARG concentration post manure application, C_0 = concentration at sampling just prior to manure application, and C_m is concentration expected to be added to soil on the basis of measurement of ARG in raw manure samples and reported application rates. Measured

(C_{pm}) and predicted (C_0 + C_m) loading rates were compared via a paired Wicoxon rank sum test. To compare the effect of manure application on soil ARG abundance, 16S rRNA gene normalized copies were Box−Cox transformed and compared via least-squares means comparison with a Satterthwaite estimation of degrees of freedom in SAS, with Tukey adjustment for multiple comparisons. Dissipation rates of 16S rRNA normalized gene copies were modeled as a logarithmic regression ($y = m \ln x + b$) from the peak average gene copies across sites for a given treatment (6−7 data points) observed following manure application versus time, by use of Microsoft Excel.

3. RESULTS

3.1. Gene Loading Rates. Gene loading rates (gene copies/m^2) were calculated on the basis of manure application rates and gene copies measured in replicate manure samples (Figure 2). The loading rate for 16S rRNA genes, indicative of total bacterial loading, was significantly lower for the dry stack manure than the slurry manure ($p < 0.001$). The *sul1*, *tet*(O), and *tet*(W) loading rates were significantly higher in the slurry manure ($p = 0.01$−0.035), while *sul2*, *tet*(G), and *ermF* levels were similar in the two manures ($p = 0.051$−0.87). All ARGs had higher copy numbers in the slurry manure when normalized to 16S rRNA gene copies (all $p < 0.02$) except *tet*(W) ($p = 0.43$). Loading rates for Cu were significantly higher for the slurry (406 ± 100 mg/m^2) than the dry stack (10.2 ± 3.4 mg/m^2) ($p = 0.0031$). There was no difference ($p = 1.0$) between the two manure types for Pb loading rates (1.83 ± 0.61 mg/m^2 slurry, 1.55 ± 0.71 mg/m^2 dry stack). Metal additions to the soil by manure application were small relative to background concentrations and natural variation (Table S1, Supporting Information).

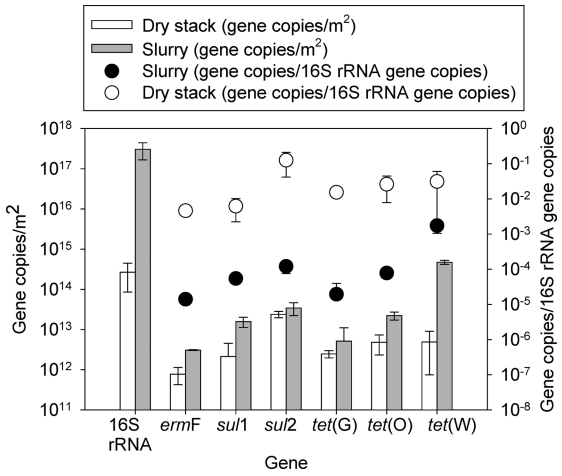

Figure 2. Gene loading rates for dry stack (white) and slurry (gray) manure per square meter (bars) and normalized to 16S rRNA gene copies (○, dry stack; ●, slurry). Error bars represent the standard deviation of three composite samples grabbed throughout the piles/slurries.

3.2. Varying Response of Soil ARGs to Manure Application. To determine the response of soil ARGs to manure application, ARGs were quantified before and after manure application in the cornfield at several points in time and space, including points downgradient from manure application. A control field was also monitored before and after it was opened for grazing of dairy cattle and sheep. No trends were observed in concentrations of any of the ARGs monitored along drainage swales compared to on hill slopes, before or after manure application.

The macrolide ARG *ermF* was rarely detected prior to application of manure in the cornfield soil (Figure 3), but spiked sharply in the first sampling (1 day) following slurry manure application and the second sampling (13 days) after dry stack application. However, *ermF* readily attenuated during the time frame of this study, returning to baseline levels by 43−55 days following dry stack and slurry manure applications, respectively. Grazing had no effect on *ermF* abundance at the control site, where *ermF* remained consistently below detection limits and *ermF* was not observed in downgradient soil samples.

In contrast, *sul2* was well-established in the soil prior to manure application (Figure S1, Supporting Information), with all 16S rRNA normalized concentrations similar in soils that were treated 6 months prior with either dry stack or slurry manure or were located downgradient from the application areas (all $p > 0.36$). A significant increase in soil *sul2* abundance was observed post- versus pre-manure application for both slurry ($p < 0.0001$) and dry stack ($p = 0.0004$). This spike in soil *sul2* gene abundance is attributed to manure application, given that there was a significant difference between post-manure application levels for soils receiving both manure types in comparison with the control site prior to grazing (both $p < 0.006$). Soil *sul2* abundances were also higher in the manured relative to the downgradient sites (both $p < 0.01$). Following grazing, *sul2* abundance at the control site also notably increased, resulting in similar gene copy levels relative to soils subject to manure application ($p = 0.75$−0.77). No difference was observed in *sul2* levels between soils treated with slurry versus dry stack manure ($p = 1.0$). Downgradient soil samples contained comparable *sul2* abundance relative to manured soils before manure application ($p = 0.76$−1.0). Results for *sul1* (Figure S2, Supporting Information) were similar to those for *sul2*.

For *tet*(O), an increase in soil gene copies was observed in post- relative to pre-slurry manure application ($p = 0.0018$) but not in dry stack manure ($p = 0.18$). No other differences were noted, including comparisons of manure treatments, manured/downgradient soils, or with controls (all $p > 0.18$) (Figure S4, Supporting Information). *tet*(W) levels were significantly higher in post- versus pre-manured soil ($p < 0.0001$) as well in slurry-manured soil relative to non-manure-treated downgradient samples ($p = 0.007$). However, no differences were observed between dry-stack-manured and downgradient soils nor between either the slurry- or dry-stack-manured soils and the control site (all $p > 0.34$) (Figure S3, Supporting Information). In several samples, *tet*(W) could be detected but could not be quantified due to qPCR interference, which effectively reduced the power of statistical comparisons. Screening indicated the presence of *tet*(G) in manure samples, but it was below detection in soil samples (data not shown).

*van*A was not detected in any of the manure or soil samples (data not shown).

3.3. Mass Balance Analysis and Dissipation Rates. Mass balance analysis was conducted on soil gene copy concentrations, based on knowledge of gene loadings in applied manure, area applied, initial soil ARG concentrations, and with the assumption of negligible loss or amplification of genes in the system (i.e., conservative contaminant under batch conditions) (Figure 4). The mass balance analysis indicated a

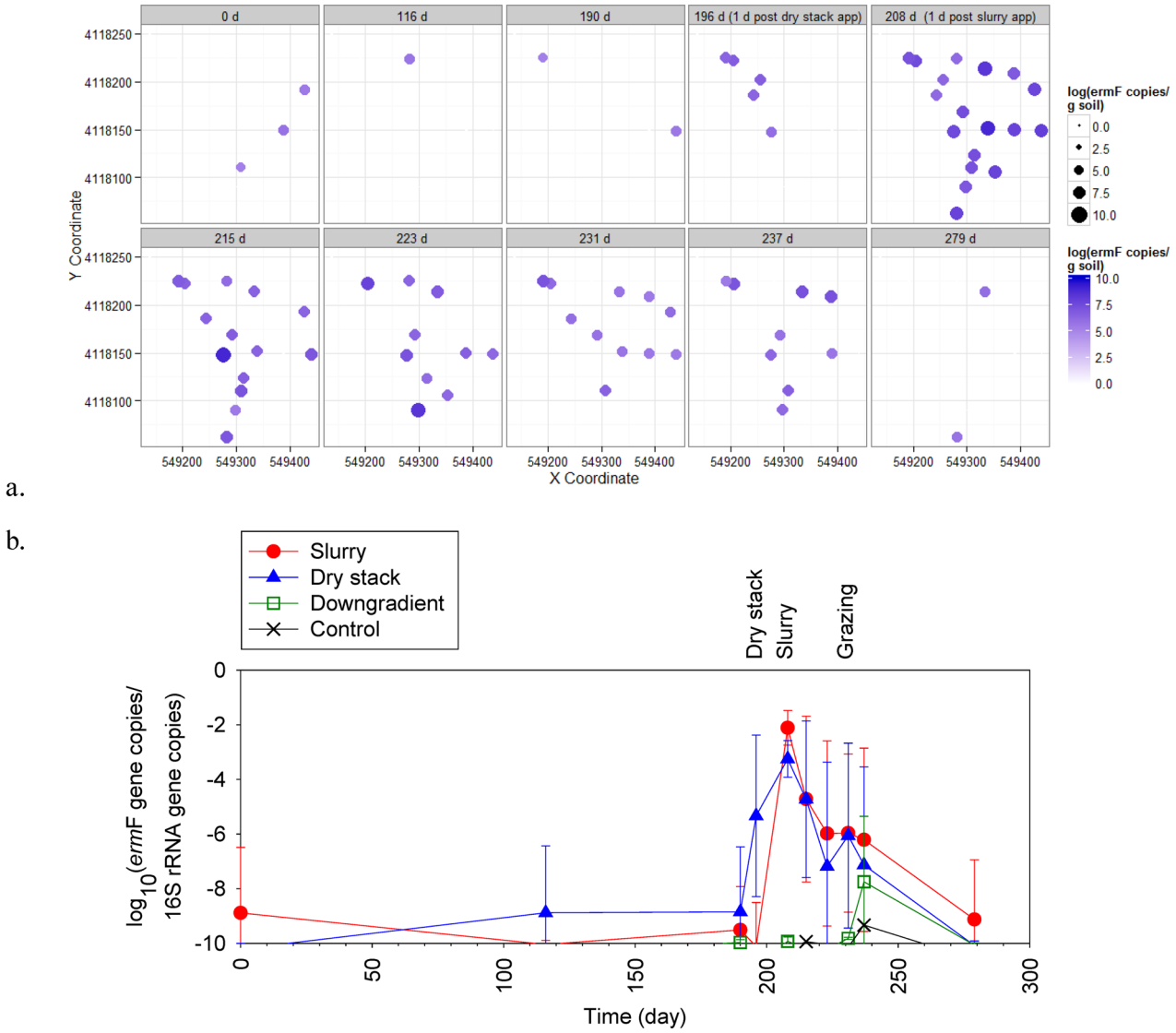

Figure 3. *ermF* copies represented (a) as a bubble plot, depicting *ermF* gene copies per gram of soil by location [Universal Transverse Mercator (UTM) zone 17N, meters] and sampling day, or (b) as 16S rRNA normalized copies by sampling date averaged by soil treatment.

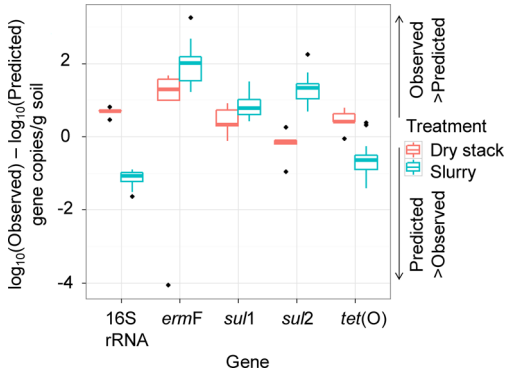

Figure 4. Comparison of observed ARG levels in soil 1 day following manure application and predicted levels based on mass balance consideration of measured ARG levels in dry stack and slurry manure and in soil in the sampling event preceding manure application. Boxes represent upper and lower quartiles, whiskers extend to high and low data points excluding outliers, and dots indicate outliers. Dry stack n = 5 sampling sites; slurry n = 13 sampling sites.

significant difference between the observed levels of 16S rRNA genes, *sul1*, *sul2*, *tet*(O), and *ermF* ($p < 0.001$, $n = 13$) in the slurry-treated soil samples and the expected levels for 1 day after manure application. There was no significant difference in the expected and observed levels in the soils treated with dry stack manure collected 1 day after manure application (p = 0.06−0.62), although the power of the dry stack analysis was lower ($n = 5$) and outliers may have affected the comparison (Figure 4). For the slurry-treated soil, observed levels were higher than expected for *ermF*, *sul1*, and *sul2* and lower than predicted for 16S rRNA and *tet*(O).

Average dissipation rates of *ermF*, *sul1*, and *sul2* in soils treated with each manure type were estimated across sites (Table 1). The dissipation rates ranged from −0.14 to −3.5 [$\log_{10}$ (gene copies/16S rRNA copies)/ln time (days)]. The greatest dissipation rate observed (following peak levels) was *ermF* in slurry-applied soils. *sul1* and *sul2* dissipation rates were noted to be higher in dry-stack-applied soils relative to slurry-applied soils.

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Table 1. Dissipation Rate Model Coefficients for Logarithmic Decay[a]

	coefficient of determination	
	dry stack	slurry
	ermF	
m	−1.5	−3.5
b	−1.8	5.6
R^2	0.92	0.93
	sul1	
m	−0.54	−0.14
b	−0.13	−1.9
R^2	0.78	0.49
	sul2	
m	−0.48	−0.17
b	−0.12	−1.5
R^2	0.86	0.8

[a] *m* = slope [$\log_{10}$ (gene copies/16S rRNA copies)/ln time (days)]. *b* = *y*-intercept [$\log_{10}$ (gene copies/16S rRNA copies)]. Coefficient of determination is listed for soils amended with dry stack or slurry manure.

4. DISCUSSION

This study provides insight into the fate of ARGs corresponding to four classes of antibiotics in soils subject to land application of two distinct manure types (dry stack and dairy slurry) and presents a mass balance approach for identifying the potential mechanisms involved in ARG fate.

4.1. Variation in Gene Responses in Soil. Application of both manure types resulted in a marked increase in soil gene copy numbers of *ermF*, *sul1*, and *sul2*. However, the abundance of all three of these ARGs did dissipate to background levels within 2 months after manure application. Spikes in *sul1* and *sul2* gene copy numbers resulting from manure application were comparable to those observed following commencement of grazing in the control field, but grazing had no effect on *ermF* abundance in soil. The only known difference in antibiotic treatment for the grazing dairy cattle relative to what was administered to the confined animals in milk production producing the manure was a second prophylactic macrolide treatment administered prior to placement of dry heifers in pasture; manure from these cows does not, therefore, enter slurry manure storage at the dairy (Table S2 in Supporting Information summarizes antibiotic regimens). However, sheep also grazed on the control field and the sheep herd was treated with Lasalocid, a carboxylic ionophore.

Interestingly, while the loading rates of *tet*(O) and *tet*(W) were comparable to or greater than those of *sul1*, *sul2*, and *ermF*, *tet*(O) and *tet*(W) did not increase in manure-amended soil relative to the control soils. Also, the observed variation of *tet*(O) in the background samplings was greater than the impact of soil amendment (Figure S4, Supporting Information), suggesting a potential seasonal variation. The behavior of *tet* ARGs was consistent with previous observations of a lack of soil *tet* gene response to cattle manure treatment[24] or following repeated irrigation with wastewater effluent.[32] Interestingly, dairy cattle (source of manure slurry) were known to be treated with tetracyclines (in addition to beta lactams, lincosadmides, cephasoporins, phenicols, and macrolides). These differences in detection underscore the finding that mechanisms involved in ARG fate and transport are complex and not necessarily driven solely by the antibiotics used. Different ARGs have different fates, likely depending not only on antibiotic selection but also on the relative growth and decay of hosts as well as the tendency for horizontal gene transfer.

4.2. Effect of Manure Type on Peak Soil ARGs. Interestingly, the two manure types resulted in comparable spikes in soil abundance of *ermF*, *sul1*, and *sul2* following land application relative to the control site. The similarities in the effects of the two manures on soil ARG abundances were despite differences in Cu concentration, moisture content, source, and initial 16S rRNA gene and ARG copy numbers.

Soils amended with the different manure types did differ in the time required to achieve peak ARG abundance: the first sampling (1 day) after slurry application and the second sampling (13 days) after application of dry stack manure. This may be due to differences in the manure matrix, especially moisture, which may facilitate more expedient integration of the manure into the soil matrix. The peak soil ARG abundances for both manure types were within the same range previously reported for soil amended with swine manure.[33,34]

4.3. Dissipation Rates. Dissipation rates were determined by use of the maximum observed soil ARG abundance as the initial concentration. The estimated dissipation rates were comparable for the two manure treatments, and the time to approach baseline was consistent with previous studies for batch tests with swine manure quantifying antibiotic resistance to macrolide, lincosamide, and streptogramin B with fluorescence in situ hybridization (FISH) probes[25] and *sul1* and *sul2*.[16] *ermF* dissipated 2.8−3 times faster than *sul1* and *sul2* in dry-stack-amended soils and 20−25 times faster than *sul1* and *sul2* in slurry-amended soils, potentially explaining why *sul1* and *sul2* were detected in background samples while *ermF* was always mainly below detection.

Several factors have been identified as potentially contributing to ARG dissipation rates, including (i) transport of bacteria hosting ARGs or transport of extracellular DNA containing ARGs,[18] (ii) binding of ARGs to soil or organic matter (which may interfere with extraction), (iii) decay of extracellular ARGs,[35] and (iv) death of bacterial host.[36] In particular, the potential importance of extracellular DNA is gaining attention. Extracellular DNA has recently been noted to be present at surprisingly high levels in environmental matrices (e.g., 5%), to persist when bound to clay particles, and to remain capable of transforming bacteria.[37] The methods incorporated in the present study include composite detection of both extracellular and intracellular DNA.

With respect to transport, runoff containing sediment particles or infiltration to the subsurface are likely two main pathways. Screening of deep soil samples (5−20 cm) indicated no obvious increase in ARG abundance in samplings following manure application (data not shown), despite several rain events presenting the opportunity for subsurface transport (Figure S5, Supporting Information). ARGs also did not notably increase in the streamwater immediately downgradient of site A9 during the study (data not shown). Likewise, patterns of ARG dissipation with time were not consistent with overland transport in that they did not increase downgradient or correspondingly decrease upgradient. Little, if any, significant overland flow or resulting sediment transport was observed at the study site during the study period. In this region, the soils have a high infiltration capacity and most runoff surfaces downgradient, due to excess saturation at the toe of slopes where hill slopes meet low-gradient floodplains (a concept known as variable source area hydrology).[38,39] Therefore, death of bacterial hosts and degradation of DNA are likely the main

mechanisms driving the observed dissipation rates. Others have observed DNA decay to be a major driver of ARG dissipation in soil column studies[40] or noted attenuation of DNA and bacteria attributed to filtration.[41] In contrast, increases in *tet*(Q) and *ermB* in runoff have been observed in controlled rainfall events immediately following pig manure slurry application,[18] indicating that differences in soil type may be driving likelihood of transport. This study was performed in a humid watershed of temperate climate, but a previous study in an arid climate found correlations between land use and ARGs in surface waters.[13]

4.4. Insight into ARG Fate from Mass Balance. The initial observed soil abundances (1 day post-manure application) of *ermF*, *sul1*, and *sul2* were higher than expected on the basis of loading rates for the slurry-amended soils, with the assumption that ARGs were conservative and there were no losses. This increase in ARG abundance above that expected from addition of slurry manure is consistent with a separate study that tracked *sul1* and *sul2* abundance in soil following pig manure addition.[17] Such differences imply either variation in loading rate, fate (+growth, +ARG selection, −decay) and transport (−transport out over land, −transport out subsurface, +transport in over land) processes at play, or a combination. For example, a generic mass balance equation for a given soil control volume (eq 1) could be elaborated upon as follows:

$$\mathrm{ARG_{acc}} = \mathrm{ARG_{load}} + \mathrm{ARG_{growth}} - \mathrm{ARG_{decay}} \pm \mathrm{ARG_{runoff}} - \mathrm{ARG_{inf}} \quad (1)$$

where $\mathrm{ARG_{acc}}$ is the rate of ARG accumulation; $\mathrm{ARG_{load}}$ is the ARG loading rate; $\mathrm{ARG_{growth}}$ includes increase in ARGs due to growth or expansion of hosts, which may be a result of selection pressure or horizontal gene transfer; $\mathrm{ARG_{decay}}$ is the ARG decay rate; $\mathrm{ARG_{runoff}}$ is the rate of surface transport of ARGs into and out of the control volume; and $\mathrm{ARG_{inf}}$ is the infiltration rate of ARGs. If the actual manure loading rate was higher or lower than estimated, the difference in expected and observed concentration should be consistent (always higher or always lower) across genes, but this was not the case. Losses due to transport are not likely, as no storm events occurred between application and the 1 day post-application sampling and no increase in ARGs was observed in deep soil samples (data not shown). Therefore, these results suggest either rapid growth/selection of bacteria carrying *ermF*, *sul1*, and *sul2* or an increase in horizontal gene transfer for these genes. The lower than expected 16S rRNA and *tet*(O) gene copies are likely due to cell death, selective sorption, or decay and inactivation (i.e., UV exposure) rather than transport, for the reasons described above. The decrease in 16S rRNA is consistent with observations that while community shifts occur after the addition of nutrients associated with manure application, these shifts do not generally result in manure microbes outcompeting soil microbes.[3,33]

The first day following dry stack manure application, the mass balance analysis indicated that the observed soil ARG abundances were consistent with what was expected on the basis of estimated loading rates of ARGs in the manure, assuming no loss of ARGs. Interestingly, a significantly higher concentration of *ermF*, *sul1*, and *sul2* than expected was observed by the second sampling after dry stack manure application (13 days). This suggests growth of bacterial hosts containing ARGs and/or horizontal gene transfer between the sampling periods may have similarly occurred in the soil receiving dry stack manure but was delayed relative to the manure slurry application. Rainfall may be a critical factor governing impact of dry stack manure to soil, as suggested by two precipitation events subsequent to dry stack application (Figure S5, Supporting Information).

Overall, results indicate that land application of dairy and dry stack manures resulted in significant increases of *sul1*, *sul2*, and *ermF* ARGs following manure application and provide insight into dissipation rates and mechanisms. Given the transient nature of ARGs in soil following manure application observed in this and other studies with different manure types,[10,16,19] there is evidence that efforts to prevent exposure to and transport to neighboring surface water of ARGs in land-applied manure may be best targeted in the 1−2 months immediately following manure application. However, this target range may vary with local hydrologic and soil conditions.

■ ASSOCIATED CONTENT

Supporting Information

Five figures showing distributions of *sul2*, *sul1*, *tet*(W), and *tet*(O) in soil plot with time as well as precipitation data; and two tables listing comparison of Cu and Pb in soil and manure loading rate and a summary of antibiotic regimens. 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 interest.

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