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The impact of manure and soil texture on antimicrobial resistance gene levels in farmlands and adjacent ditches

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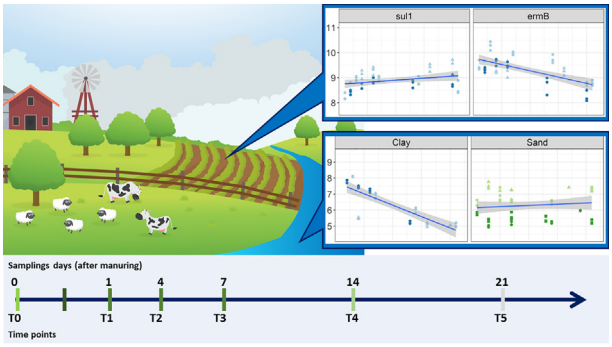
HIGHLIGHTS

- Manure application resulted in the increase of *erm(B)* and *tet(W)*, but not of *sul1*.
- Soil gene decay in soils was dependent on the type of ARG (*erm(B)* < *tet(W)* < *sul1*).
- Soil texture affected gene decay in adjacent surface water (clay < peat < sand).
- Linear models predicted restoration of ARG levels shortly after sampled timeframe.

GRAPHICAL ABSTRACT



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ABSTRACT

Manure application can spread antimicrobial resistance (AMR) from manure to soil and surface water. This study evaluated the role of the soil texture on the dynamics of antimicrobial resistance genes (ARGs) in soils and surrounding surface waters. Six dairy farms with distinct soil textures (clay, sand, and peat) were sampled at different time points after the application of manure, and three representative ARGs *sul1*, *erm(B)*, and *tet(W)* were quantified with qPCR. Manuring initially increased levels of *erm(B)* by 1.5 ± 0.5 log copies/kg of soil and *tet(W)* by 0.8 ± 0.4 log copies/kg across soil textures, after which levels gradually declined. In surface waters from clay environments, regardless of the ARG, the gene levels initially increased by 2.6 ± 1.6 log copies/L, after which levels gradually declined. The gene decay in soils was strongly dependent on the type of ARG (*erm(B)* < *tet(W)* < *sul1*; half-lives of 7, 11, and 75 days, respectively), while in water, the decay was primarily dependent on the soil texture adjacent to the sampled surface water (clay < peat < sand; half-lives of 2, 6, and 10 days, respectively). Finally, recovery of ARG levels was predicted after 29–42 days. The results thus showed that there was not a complete restoration of ARGs in soils between rounds of manure application. In conclusion, this study demonstrates that rather than showing similar dynamics of decay, factors such as the type of ARG and soil texture drive the ARG persistence in the environment.

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1. Introduction

Antimicrobial resistance (AMR) is considered as one of the most significant challenges to global public health (WHO, 2016). AMR is currently approached from a "One Health" perspective, which includes exploring the occurrence of AMR in animals, humans, the environment, and its transmission between reservoirs (One Health, 2019). The presence of antimicrobial resistance genes (ARGs) in manure, related to the usage of antibiotics in veterinary practices (Hoelzer et al., 2017; Topp et al., 2018), results in environmental contamination of manured soils and surface water.

In the Netherlands, per year, approximately 1.6 million cows are present in the dairy industry, and 57 million chickens in layer poultry farming (de Greeff and Mouton, 2017), annually producing over 40.8 million tons of manure. While it is known that manure application introduces antibiotics and other pharmaceuticals to soils (Heuer et al., 2011; Jechalke et al., 2014) and can result in antimicrobial-resistant bacteria and ARGs entering the environment in soil and water systems (Agga et al., 2015; Chee-Sanford et al., 2009), the contribution of the soil texture to the spread of AMR to the environment has not yet been thoroughly addressed. The soil texture (relative content of particles from different size classes) comprises a set of physicochemical parameters, which may interact in a non-independent manner. Whether by protection against predation, limited organic carbon availability, or other, the soil texture has proven effects on the survivability of fecal bacteria in soils (Franz et al., 2014; van Veen et al., 1997) and on the structure of the soil bacterial community (Blau et al., 2018; Girvan et al., 2003). Thus, one can also assume that the soil texture will also play a significant role on the fate of ARGs. Changes in soil bacterial communities and resistomes have been studied after the application of different types of manure (Han et al., 2018; Zhang et al., 2017), of different manure loads (Gou et al., 2018), or after multiple manure applications (Chessa et al., 2016; Wang et al., 2018). However, these studies were performed under microcosm settings. Only a few recent studies investigated the fate and transport of ARGs after manure application in soils and water runoffs simultaneously in field experiments (Fahrenfeld et al., 2014; He et al., 2016; Joy et al., 2013; Luby et al., 2016; Muurinen et al., 2017; Soni et al., 2015), and even fewer studies aimed to model the spread of resistance (Baker et al., 2016; Volkova et al., 2013), while no study so far addressed the role of soil texture in a field setting.

The soil resistome has been correlated with the microbial phylogenetic and taxonomic structure across soil textures, indicating that the soil native bacterial composition is the primary determinant of the ARG content in agricultural and grassland soils (Forsberg et al., 2014). Additionally, recent field studies focus on changes in resistome diversity after manure application measured with high-throughput qPCR (Chen et al., 2019; Cheng et al., 2019; Pu et al., 2018; Xie et al., 2018a, 2018b), but this approach does not provide the quantitative data needed to determine the fate of the different ARGs in the environment in terms of decay rates.

This study analyzed the role of the soil texture on the dynamics of AMR in soils and adjacent surface waters (runoff). To achieve this goal, we (i) evaluated the impact of manure application on selected ARG levels, over time, in manured soil and watercourses adjacent to the soil; and (ii) tested the role of soil texture on the dynamics of ARG decay in soils and surrounding surface water over time. Manure, soil, and water samples were examined by qPCR to quantify β-lactam (bla_{TEM}), sulfonamide (*sul1*), macrolide (*erm(B)*), and tetracycline (*tet(W)*) resistance genes levels. These ARGs were selected because they represent resistance to the most consumed antimicrobial families in animal health (de Greeff and Mouton, 2018; ECDC et al., 2017), and are measured in manured soils worldwide (Blau et al., 2018; McKinney et al., 2018; Tien et al., 2017). In this work, we focus on qPCR on a large number of samples, as qPCR – in contrast to metagenomics or resistance gene arrays which can generate data on a wide scale of resistance genes – can provide information on the absolute gene concentrations per g of soil which are needed to determine resistance gene kinetics. We hypothesized that the soil texture would have a significant impact on the decay rates of ARGs introduced by manure application in soils and water streams.

2. Materials and methods

2.1. Sampling locations and soil characteristics

In the Netherlands, manure application to soils is only permitted between February and August (spring and summer; RVO 2017). During the rest of the year, cattle manure is collected and stored below the stables until the compartment's holding capacity is full, after which it is transferred to a storage silo until the manuring season starts. The manure is then applied by injection, and no-tillage is performed. For this study, six dairy farms were selected, each of which had distinct soil textures (clay, sand, or peat; Table 1). For the last five years before the sampling campaign, the sampled fields had similar usage (grassland) and fertilization rates (farmers' personal communication). In these farms, the animals pasture during the day, but not in the parcels to which manure was applied. Farmers regularly perform soil analysis on their fields every four to five years; however, the results were not available to us. Therefore, the physicochemical properties of the soils were determined, following standardized procedures (Eurofins Agro; Netherlands), and detailed information about the soil characteristics can be found in Table S1.

2.2. Sample collection

Sampling occurred from February to August 2017, during the manuring season. The manure samples were collected shortly before being applied to the field (after mixing). Soil and water samples were collected within one week before manuring (time point T0) and at defined time intervals after manuring (1, 4, 7, 14, and 21 days, time points T1, T2, T3, T4, and T5, respectively). In addition, never manured soil samples from each soil texture (NM) were used as controls and were collected from gardens nearby the sampled farms due to the inability to find buffer or forest areas of the same soil types. Each sampling cycle was repeated after each round of manure application, and occurred 34–80 days after the previous round, except for the first round. Composite soil samples were collected and prepared according to ISO guidelines (ISO 10381-6:2009). Briefly, after walking the fields in a "W" pattern, in which >25 grab samples of each field (0–10 cm soil depth, 4–8 samples taken on the manure bands) were collected every 40 steps with a soil probe. To avoid an excess of plant biomass and rhizosphere, the grass turfs were pushed aside by foot before collection with the probe. The manure bands were visible until two weeks after manure application. After collection, the soil was homogenized with a regular 3-prong gardening tool and enclosed in plastic zip-lock bags. Water samples were collected with sterile 2-L bottles, from the adjacent discharge ditch (closed and independent systems). Because the drainage pipes ended below the ditch water surface, the water samples were collected as close as possible to the drainage pipe exit, except the first round of farm F01, which was collected directly from the drainage pipe. All samples were kept on ice during transport and were processed for *E. coli* enumeration within 24 h and stored at −20 °C before DNA extraction and further chemical analysis.

2.3. E. coli enumeration

Manure samples (10 g) were homogenized in a blender with 10 mL of sterile saline solution (0.85% NaCl, m/v) and were diluted before being plated on Tryptone Bile X-glucuronide (TBX) agar media (Oxoid, UK). Soil samples (100 g) were homogenized for 1 min with 100 mL sterile saline solution and then diluted 10-fold. The homogenate and dilution were plated on TBX, in duplicate, and incubated. Volumes of 1, 3,

Table 1
Characteristics of the farms included in the study.

Farm code	Soil texture	Clay (%)	Silt (%)	Sand (%)	Org. matter (%)	Nr. animals	Manure applied (tons/ha)			Area (ha)[a]
							Round 1	Round 2	Round 3	
F01	Clay	18	32	45	4,8	700	32	25	–	5,0
F02	Clay	35	41	9	14,6	200	30	–	–	8,7
F03	Sand	5	15	70	10,3	340	30	15	–	1,7
F04	Sand	2	10	79	8,6	100	25	15	15	1,0
F05	Peat	36	28	17	19,3	120	40	10	–	7,0
F06	Peat	31	34	9	25,1	400	30	20	15	10,0

Footnote Note: Even though the chemical analysis of the farm F05 classified it as clay, the previous analysis performed by the farmers indicated that its soil texture was peat. Similarly, the analysis classified the farm F01 as loam, but the previous analysis indicated that its texture is clay.
[a] Estimated via https://boerenbunder.nl.

10, 30- and 100-mL of ditch water were filtered through 0.45 μm pore cellulose nitrate membranes (Merck-Millipore, USA), and filters were placed on TBX. All plates were prepared in duplicate and were incubated between 16 and 24 h at 37 °C before counting. Bacterial enumeration was calculated according to ISO guidelines (ISO 8199:2018). Briefly, the final bacterial concentration (CFUs/kg or L) resulted from the sum of the total CFUs obtained in a sample divided by the total amount of the same sample tested. The limit of quantification (LOQ) was calculated by assuming a count of 10 CFUs in the highest volume of the original sample.

2.4. DNA extraction and qPCR

Total DNA extracts were obtained from 200 mg of manure with the QIAamp DNA Stool Mini Kit (QIAGEN, Germany), 250 mg of soil with the FastDNA™ Spin Kit for Soil (MP Biomedicals, USA), and 100 mL of water samples with the DNeasy® PowerWater® Kit (QIAGEN), in triplicate. The water samples were filtered through 0.22 μm pore PVDF filters (Merck-Millipore, USA) prior to DNA extraction. At each sampling cycle, an internal standard was spiked in at least one sample type from each farm to assess the DNA extraction efficiency (Fig. S1). The spike consisted of 50 μL containing 6.34×10^6 gene copies of a synthetic blue fluorescence protein as a 720 bp DNA fragment (BFP; gBlocks; IDT technologies, Belgium) added at the first step of the extraction procedure, before cell lysis. As the after-lysis recoveries were comparable within soil and water samples (Fig. S1), the concentrations were not corrected for after-lysis recovery, and no lysis efficiency was measured. DNA extraction proceeded according to the manufacturer's instructions with one adaptation, which consisted of adding or adjusting the bead-beating step with Precellys Evolution (Bertin Instruments, France) for enhanced cell lysis. DNA quantification was performed using Quantus Fluorometer (Promega, USA) according to the manufacturer's protocol, and checked for purity with NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific, USA).

The 16S rRNA gene and the selected ARGs were quantified by qPCR in a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, USA). Each qPCR reaction contained 1× iQ™ SYBR Green Supermix (Bio-Rad), 300 mM of both forward and reverse primers (except for *ermB* and bla_{TEM} where 400 mM was used), 2 μg of bovine serum albumin (BSA; Thermo Scientific, USA), and 2 μL of 10-fold diluted DNA (1–10 ng), in a final volume of 20 μL (Table M2). The following thermal cycling conditions were applied: 95 °C for 10 min (1 cycle); 95 °C for 15 s, 60 °C for 1 min (except for *sul1*, which required 65 °C), in 40 cycles. Samples were tested with a standard curve ranging between 10^{-3}–10^{-7}, or 10^{-5}–10^{-9} target copies of standardized gene fragments (gBlocks) for the 16S rRNA gene, or ARGs, respectively, and non-template controls for each run. Cut-off values were calculated based on the lowest amount of genes included in the calibration curve of all plates. All calibration curves had a signal intensity of >2 ct difference to the non-template controls (only for 16S rRNA gene, other genes did not show amplification in non-template controls). The average of ct obtained from this point across all plates was taken, and the standard deviation added to it. Melting curves were performed to confirm the specificity of each reaction, starting at 65 °C with successive increments of 0.5 °C, up to 95 °C. Quantifications for each extract was performed in duplicates, following the Standard Curve method described elsewhere (Brankatschk et al., 2012). Possible qPCR inhibition was verified by quantifying the 16S rRNA gene using 10-, 100-, and 1000-fold diluted extracts. The primer sets and concentrations used in this study can be found in the supplemental information (Table S2). Predicted values were estimated based on the average ARG concentration in manure and soil previous to amendment, on the amount of manure applied and soil sampled (top 10 cm) per hectare, and on assumed densities of 1.0 and 1.5 kg/dm^3 for manure and soil, respectively.

2.5. Data analysis

For the comparison of gene levels (log copies/kg soil) before and after manuring, outcome variables were tested for normality, and when verified, an analysis of variance (ANOVA) was applied, using Tukey post-hoc analysis. When normality was not achieved, group comparison was performed using the equivalent non-parametric test (Kruskal-Wallis). For analysis of decay rates of gene levels (log copies/kg soil), linear mixed models were used treating the farms and the rounds of manuring as random effects. Because higher gene levels were observed at T2 than at T1, the first three days (i.e., T0 and T1) after manure application were excluded from the models for soil and water models. Relevant factors were identified through model reduction. The full model included the following variables as fixed effects: days after manure application (4–21 days), gene type, the soil texture, the amount of rain, the interaction between days after manuring and soil texture, and interaction between days after manuring and gene type. Resulting models were inspected for normality of residues, and significance scores of $p < 0.05$ were considered for all performed tests. The half-lives were calculated based on the slope of the models. The statistical analyses were performed with R version 3.5.1 (R Core Team, 2020) and RStudio (Version 1.1.456; https://www.rstudio.com/) using the software packages *dplyr* (Wickham et al., 2015), *tidyr* (Wickham and Henry, 2019) and *reshape2* (Wickham, 2007) to trim, clean, and transform data; *lme4* (Bates et al., 2015), *lmerTest* (Kuznetsova et al., 2017), and *MuMIn* (Barton, 2018) to create the models; *Rcmdr* (Fox, 2005), and *sjstats* (Lüdecke, 2019) to perform the group comparisons; and *ggplot2* (Wickham, 2016) to produce the graphs.

3. Results and discussion

3.1. Gene identity determines ARG persistence in soil fertilized with dairy manure

In this study, ARG levels were measured to understand the role of manure and soil type on the persistence of AMR in grasslands and adjacent watercourses after manure application. Across soil textures, a

significant increase of *erm(B)* and *tet(W)* was observed directly after manuring (Fig. 1), followed by a gradual decrease, while *sul1* levels remained roughly constant throughout time (Fig. 2). Overall, the decay rates differed between genes, but the soil textures did not affect resistance gene decay rates. bla_{TEM} was only incidentally detected in the manure samples used in the first round of manuring from farms F01 and F05 (8.75 and 8.83 log copies/kg, respectively). Thus, it was excluded from further analysis.

The *erm(B)* levels increased significantly after manuring ($p < 0.01$). After being transported from manure (manure concentrations 10.25–11.30 log copies/kg; Table S3) and reaching its highest concentrations in soils, *erm(B)* decreased by roughly −0.05 log copies/kg per day across soil textures ($t_{1/2} = 7$ days; Table 2; $p < 0.01$), as predicted from linear mixed models that were used to relate the gene decay to soil texture and gene identity (Table 2). These findings are corroborated by Tien et al. (2017), who found an identical reduction of this ARG in soil 30 days after manure application. In the present study, *erm(B)* levels in soils immediately after manuring differed between the rounds of manure application, but ARG decay was identical.

Moreover, *erm(B)* was not detected in never-manured soils (NM), nor before the first spring manure amendment (T0; Fig. 1). Recently, it has been found that *erm(B)* was only detected in soils amended with either pig or cattle manure, and not in soils that either received chemical fertilizer or were not fertilized (Peng et al., 2017). Others were able to detect *erm(B)* in composite and band samples of manure-injected soils, but not between the manure bands of the same soils (Luby et al., 2016). This suggests that *erm(B)* is mainly introduced to soils via manure application, and our findings support this hypothesis. According to the Comprehensive Antibiotic Resistance Database (CARD) (Jia et al., 2017), *erm(B)* is often found in Gram-positive bacteria, namely in *Enterococcus* spp. and the obligate anaerobic *Clostridium* spp., which are common manure microbiota (Hodgson et al., 2016; Leclercq et al., 2016). The relatively fast decay of *erm(B)* could be related to the decline of *Clostridia*, as they are less fit to thrive in aerobic soil habitats (Pourcher et al., 2007).

Despite being found in manure (10.20–10.83 log copies/kg), and in contrast to *erm(B)* findings, *tet(W)* was present in NM samples and at T0 (Fig. 1) and showed a slower decay rate than *erm(B)* ($t_{1/2} =$ 11 days; Table 2). This finding is in accordance with recent publications (Fahrenfeld et al., 2014; McKinney et al., 2018), where *tet(W)* could be quantified in soils before manure application, but at higher levels than the ones described here. However, in two studies focusing on the prevalence of ARG after long-term manure application (Li et al., 2017; Peng et al., 2017), *tet(W)* was not found in the non-manured soils used as controls. A similar decay of *tet(W)* was found 20 days after the application of poultry litter in untilled soils (Cook et al., 2014), and after the application of cattle manure in soils with different pastures (Kyselková et al., 2015), although in these studies fewer time points were measured. In one of those long-term studies, where soils were continuously applied with different manures for 30 years (Peng et al., 2017), *Firmicutes*, *Gammaproteobacteria,* and *Bacteroidetes* were found positively correlated with most of the ARGs, including *tet(W)*. These phyla represent bacteria commonly found in the gut, whose adaptability to agricultural environments (e.g., manure, soils) differs – *Clostridia* and *Bacteroidetes* represent obligate anaerobes that will quickly die-off in mostly aerobic soils, in contrast to *Bacilli*. Given this condition, the relatively moderate decay rate of *tet(W)* could be explained by the survival potential of its host bacteria. Recently, different bacterial hosts have

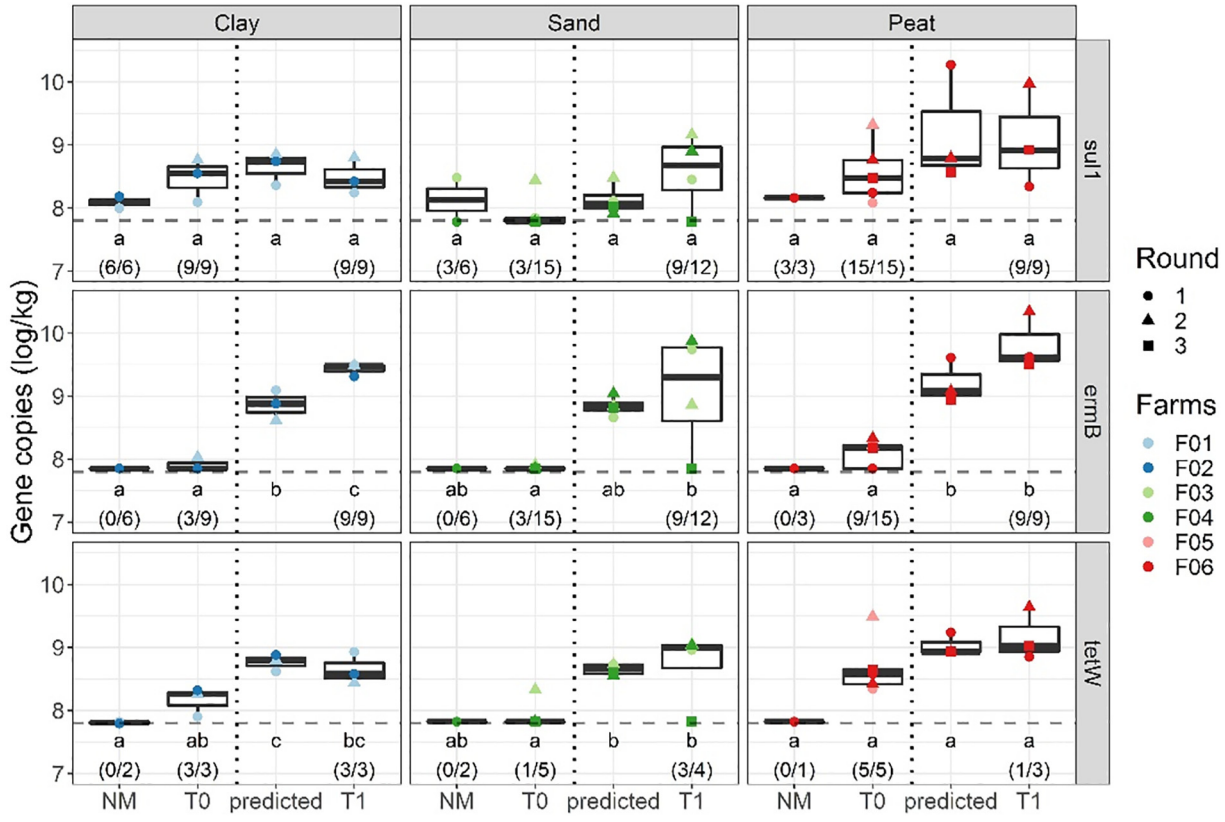

Fig. 1. ARG concentrations in soil samples that were never manured (NM), and before (T0) and after (T1) manure application on farms using different manure types and manure application systems. Predicted values were estimated based on the average ARG concentration in manure and soil previous to amendment, on the amount of manure applied and soil sampled, and on assumed densities of 1.0 and 1.5 kg/dm^3 for manure and soil, respectively. The horizontal dashed bar represents the limits of quantification (LOQ), which were determined based on the experimentally defined cut-off values for qPCR (7.78, 7.85, and 7.82 log copies/kg of soil, for *sul1*, *erm(B)*, and *tet(W)*, respectively). Values between brackets represent the number of DNA replicates above the LOQ used to calculate the represented averages. [a, b, c] Indicate significantly different groups ($p < 0.05$) of gene concentrations between each time point.

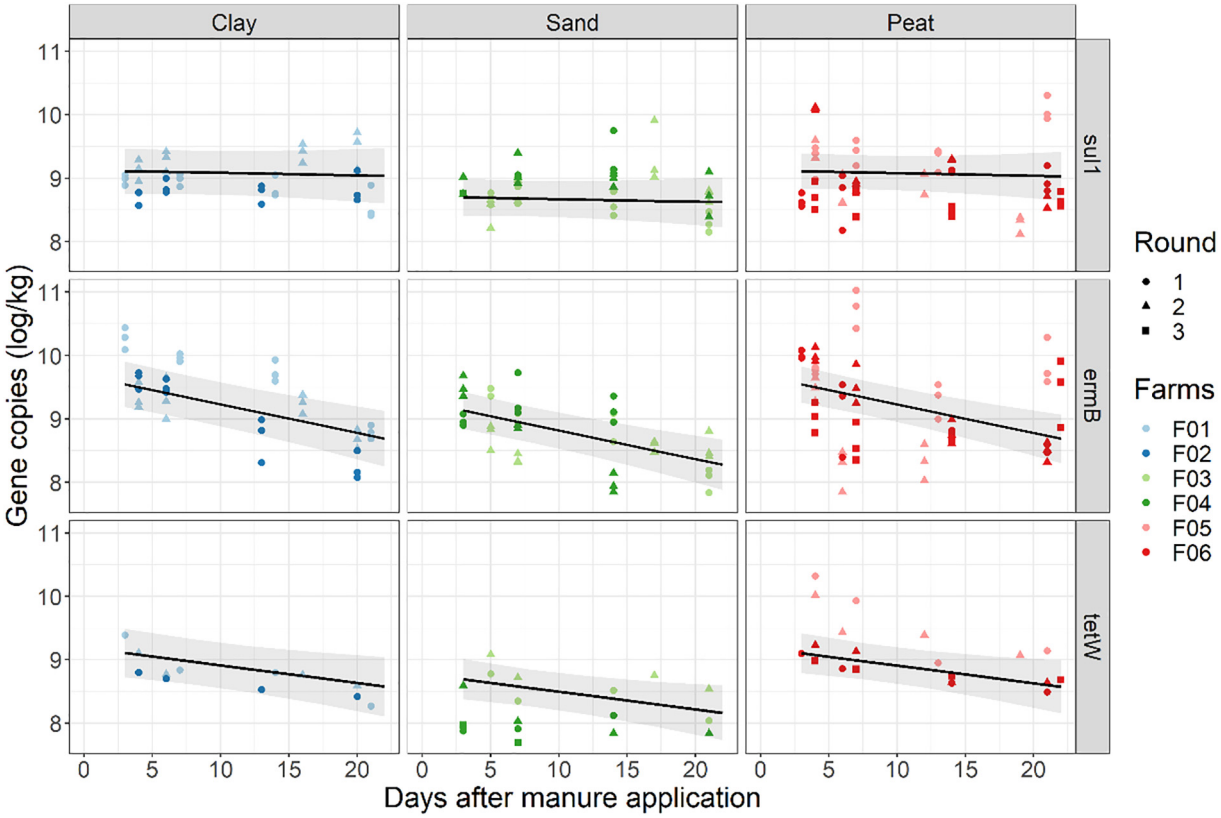

Fig. 2. ARG level in soil samples after manure application in farms from different soil textures.

been associated with single types of ARGs (Stalder et al., 2018), reinforcing the argument that the bacterial hosts can vary greatly, and that fate of genes will depend to a large extent on the fate of host bacteria. An alternative hypothesis would be that the application of manure, and its organic compounds and other components, could stimulate the soil bacterial communities that carry the targeted ARGs (Udikovic-Kolic et al., 2014; Xie et al., 2018a), thus contributing to the maintenance (slow decay) of the ARGs. This might be particularly relevant for the case of *tet(W)* since it was detected before manure application (T0) and in NM samples.

The *sul1* gene was present in manure (9.33–11.96 log copies/kg), but did not increase after manure application, and consequently, the decay rate was the lowest of the targeted ARGs in this study (−0,01 log units per day; $t_{1/2} = 75$ days; Table 2; $p < 0.01$). The constant levels of *sul1* (Fig. 2; $p = 0.15$) can be explained by the high prevalence of this ARG in soils previous to manure application (NM and T0; Fig. 1), which confirms the ubiquity of *sul1* in the environment (Gillings et al., 2008). Recently, Wang et al. (2017) also observed that *sul1* had the lowest decay rates in manure-amended soil microcosms, over 96 days. Nevertheless, that decay rate was calculated based on the ARG relative abundance (ARG copies/16S rRNA copies), which limits the comparison with the results of this study. However, in contrast to our findings, during a microcosm study where manure, with and without antibiotics, was applied to different soil textures (Heuer and Smalla, 2007), an increase of *sul1* in both soils was observed after manure application, and after 32 days, the *sul1* levels had decreased close to 1 log, which corresponded to a much higher decay rate than in the current study (roughly −0.03 log units per day). In other field studies, the *sul1* levels after application of dairy manure were similar to the ones in the current study, even though it was increased after manuring (Munir and Xagoraraki, 2011; Nõlvak et al., 2016). Increases of *sul1* after manuring were also observed by (McKinney et al., 2018).

Furthermore, the results showed that in most cases, there was not a complete recovery of soil resistome from one round to the next. According to the model-predicted decay rates, it would take on average 42 days (47 and 33 days in round 1 and 2, respectively) for the levels of *erm(B)* to decrease to the LOQ levels before manure application in clayey soils. In sandy soils, the model predicted that it would take an average of 29 days for *erm(B)* levels to decrease back to original levels, while in peaty soils, *erm(B)* levels were predicted to decrease to LOQ

Table 2
Best models for soil and water samples in farms with different soil textures (excl. 0–3 days after manuring).

Sample type	Explanatory factor	Estimate (log/kg or log/L)	Std. error	p-Score
Soil	(Intercept)	9.6	0.19	<0.01
	Gene decay per day	−0.05	0.01	<0.01
	tet(W)	−0.5	0.1	<0.01
	sul1	−0.5	0.1	<0.01
	Soil texture: sand	−0.4	0.2	0.09
	Soil texture: peat	−0.0	0.2	0.99
	Rainfall	0.04	0.01	<0.01
	Interaction: days and *tet (W)*	0.02	0.01	0.08
	Interaction: days and *sul1*	0.04	0.01	<0.01
Water	(Intercept)	7.8	0.5	<0.01
	Gene decay	−0.15	0.02	<0.01
	tet(W)	−0.26	0.25	0.29
	sul1	−0.4	0.2	0.01
	Soil texture: sand	−1.7	0.7	0.04
	Soil texture: peat	−1.7	0.7	0.03
	Rainfall	0.04	0.02	0.01
	Interaction: days and *tet (W)*	0.04	0.02	0.07
	Interaction: days and *sul1*	0.05	0.01	<0.01
	Interaction: days and sand	0.12	0.03	<0.01
	Interaction: days and peat	0.10	0.03	<0.01

in roughly 40 days. Similar trends were also found for *tet(W)*, as it would take on average 38, 36, and 37 days for the *tet(W)* levels to decrease to the levels found before the first application of manure in clayey, sandy, and peaty soils, respectively. As the pasture soils are often repeatedly manure-fertilized after 34–80 days, soil resistance levels do not recover entirely between manuring rounds. Decay at later timepoints might be related to the input of fresh manure from grazing cows.

In contrast to gene identity, the soil texture did not affect gene decay rates in soil samples. This was shown in the linear mixed models by non-significant interactions between soil texture and time after manuring, and from the fact that this interaction was not included in the final, best models after model reduction (Table 2). It was initially hypothesized that the soil texture would play a significant role in the fate of ARGs in manure-amended soils. The fate of added microorganisms can vary with soil texture, as shown for *E. coli*, for which survival was lower in organically managed sandy soils (Franz et al., 2005). Recently, it has been found that physicochemical properties, such as heavy metals, moisture content, and organic matter, can affect the decay kinetics of some ARGs in soils (Sui et al., 2019). However, according to the results observed in this study, the type of ARG rather than the texture of soil was the determining variable affecting the decay of the measured ARGs (Table 2). This discrepancy might indicate that the fate of the resistance gene host and the background of resistance genes has stronger effects than the soil textures. Additionally, the rhizosphere might also contribute to the maintenance of the gene in soils as it is a known hotspot for horizontal gene transfer because they promote the occurrence of high densities of active cells (Jechalke et al., 2013; Kopmann et al., 2013; Van Elsas et al., 2003).

E. coli is commonly used as an indicator organism of fecal contamination, and it was tested to evaluate whether fecal bacteria would be able to remain viable in manured soils throughout the sampling time frame, complementing resistance gene measurements, which also detect genes from dead cells posing smaller public health risks. *E. coli* was only detected after manure application (except in round 3 of farms with peaty soil), and although no clear survival trends were observed, *E. coli* was more abundant at round 2, and it was still quantifiable three weeks after manuring in all soil types, except in farms with sandy soils (Fig. S2).

Rainfall had an overall increasing effect on the levels of ARG found in soils according to the linear mixed models (Table 2). Rainfall has been linked to the transport of *erm* and *tet* genes in agricultural runoffs (Joy et al., 2013; Soni et al., 2015), supposedly through mobilization from the upper soil fraction through infiltration and surface transportation. Also, the shorter survival of *E. coli* has been found in soils with higher moisture content (Oliver et al., 2006; Rothrock et al., 2012). Given this, one would assume that the ARG levels in topsoil would decrease after rainfall; however, that was not the case. On the other hand, the water content of a soil microcosm set-up had a negligible effect on the decay of ARGs (Sandberg and LaPara, 2016). Also, the findings of this study are consistent with the findings of Joy et al. (2013), where the levels of *erm* and *tet* genes increased in the top manure-broadcasted soils even after three rainfall events. This increase is likely to be due to the dissemination of the manure bands in the field caused by the rainfall, making it more homogeneous, but it can also be due to ARG-carrying bacterial growth.

3.2. Soil texture determines ARG persistence in water

In the ditch water samples, the rates of the ARG decrease (i.e., slope) were similar within the same soil texture, regardless of the ARG (Fig. 3). The ARG levels decreased quicker in ditches than in soil: by roughly -0.15 ($t_{1/2} = 2$ days; $p < 0.01$), -0.03 ($t_{1/2} = 10$ days; $p < 0.01$), and -0.05 ($t_{1/2} = 6$ days; $p < 0.01$) log copies / L per day in clay, sand, and peat, respectively (Table 2).

ARG transport from the fields to the ditches can depend on rainfall as well as on the strength of bacterial and gene sorption to soil particles, which in turn can depend on the soil type. Here, water samples from two clayey soils showed to have the highest ARG decline. This might be related to rainfall-induced transport of ARG to the ditches shortly

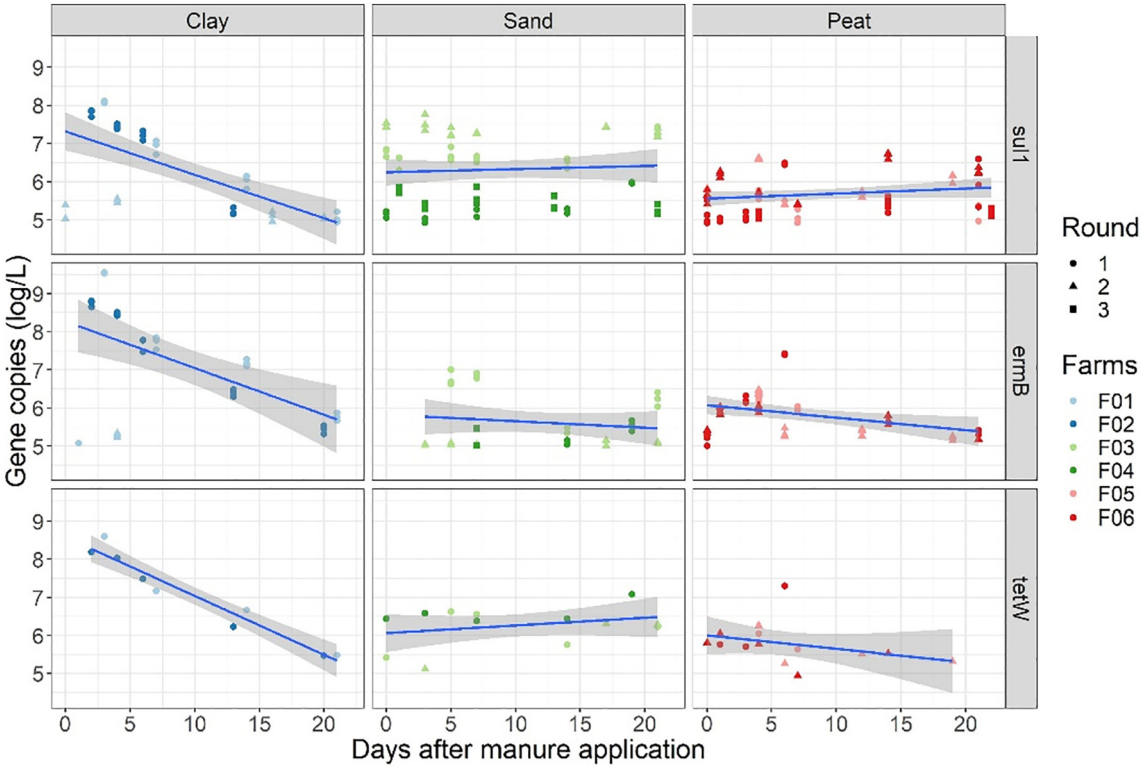

Fig. 3. ARG level in water samples after manure application in farms from different soil textures.

after manure fertilization: on two of the three sampling occasions in clayey soils, >8 mm precipitation per day occurred shortly after manuring. As the permeability of clay particles is low (Schramm et al., 1986), surface run-off from clay might, therefore, have resulted in peaks of resistance genes in the receiving water early on. On the other hand, high levels of ARGs have also been found in farm F01 in round 1 in water, although this represented the only water sample taken at the outlet of a drainage pipe and, therefore, representing soil infiltrate. Thus, in clay soils, infiltration is another mechanism of transfer to adjacent ditches next to surface run-off, in contrast to high sorption of bacteria (Cho et al., 2016; Pachepsky et al., 2006) and nucleic acids (Ogram et al., 1988) to clayey soils. Arguably, even though the water ditches were surrounded solely by fields owned by one farmer, adjacent soil parcels owned by the same farmer can follow a different manure application cycle, possibly leading to additional leaching into the sampled ditches. It should be noted that the ARG persistence within ditches depends on hydrological parameters that determine the dilution of soil run-off, such as flow and volume of ditches, which were not recorded in this study.

4. Conclusions

In this field study, the role of the soil texture on the dynamics of AMR in soils and adjacent surface waters was addressed. Overall, this study demonstrated that the persistence of the measured ARGs in soils differed, and largely depended on the type of gene. The descending decay rates (*sul1* > *tet(W)* > *erm(B)*) were related to the level of the ARG prior to manure application and possibly influenced by the different survival capabilities of the bacteria hosting these ARGs in soils. Nonetheless, in water samples, the texture of soil to which manure was applied determined the persistence of the targeted ARGs (clay < peat < sand), thus affecting the fate of AMR in the environment. Finally, ARG levels were predicted to recover to levels before manure fertilization after 29–42 days of manure application, i.e., do not recover between rounds of manure application. To conclude, this study demonstrates that rather than showing similar dynamics of decay, factors such as the type of ARG and soil texture drive the ARG persistence in the environment.

CRediT authorship contribution statement

Gonçalo Macedo: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration. **Lucia Hernandez-Leal:** Conceptualization, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Peter van der Maas:** Writing - review & editing, Funding acquisition. **Dick Heederik:** Conceptualization, Writing - review & editing. **Dik Mevius:** Conceptualization, Writing - review & editing. **Heike Schmitt:** Conceptualization, Methodology, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.139563.

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