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Abstract

The objective of this study was to determine the fate of commonly used veterinary antibiotics in their naturally excreted form when manure-based amendments are applied to soil. Beef cattle were administered sulfamethazine, tylosin, and chlortetracycline and dairy cows were treated with pirlimycin according to standard animal production practice. The resulting manure was composted for 42 days under static or turned conditions and applied at agronomic N rates to sandy, silt, and silty clay loam soils and compared with amendment with corresponding raw manures in sacrificial microcosms over a 120-day period. Antibiotic dissipation in the raw manure-amended soils followed bi-phasic first order kinetics. The first phase half-lives for sulfamethazine, tylosin, chlortetracycline, and pirlimycin ranged from 6.0 to 18 days, 2.7 to 3.7 days, 23 to 25 days, and 5.5 to 8.2 days, respectively. During the second phase, dissipation of sulfamethazine was negligible, while the half-lives for tylosin, chlortetracycline, and pirlimycin ranged from 41 to 44 days, 75 to 144 days, and 87 to 142 days, respectively. By contrast, antibiotic dissipation in the compost-amended soils followed single-phase first order kinetics with negligible dissipation of sulfamethazine and half-lives of tylosin and chlortetracycline ranging from 15 to 16 days and 49 to 104 days, respectively. Pirlimycin was below the detection limit in the compost-amended soils. After incubating 120-days, antibiotics in compost-amended soils (up to 3.1 $\mu g\ kg^{-1}$) were significantly lower than in the manure-amended soils (up to 19 $\mu g\ kg^{-1}$; $p<0.0001$), with no major effect of soil type on the dissipation. Risk assessment suggested that manure composting can reduce antibiotic resistance selection potential in manure-amended soils.

Keywords: environmental fate, antibiotics, dairy, beef, soil, risk assessment

Highlights

- Antibiotic dissipation follows bi-phasic 1st-order kinetics in manure-amended soils
- Antibiotic dissipation obeys single-phase 1st-order kinetics in compost-amended soils
- Manure-borne antibiotics persist in soils at low concentrations
- Soil type had negligible effect on dissipation kinetics of manure-borne antibiotics
- Manure composting can reduce antibiotic resistance selection potential in manure-amended soils

1. Introduction

Antibiotics are widely used in livestock for therapeutic and sub-therapeutic uses (Chee-Sanford et al., 2009). About 80% of the 13.5 million kilograms of antibiotics sold yearly in the USA is used in animal production (Done et al., 2015), among which macrolides are categorized as “critically important” while sulfonamides, tetracyclines and cephalosporins are categorized as “highly important” in human medicine by World Health Organization (Collignon et al., 2016). Administered antibiotics are not fully metabolized, leading to their excretion in animal manure (Chiesa et al., 2015). In the environment, soil is the primary receiver of antibiotics used in animal production, mainly through land application of manure (Jechalke et al., 2014). A wide range of antibiotics has been detected worldwide in various manure and manure-amended soils, at concentrations up to several thousand μg kg^{-1} (Martinez-Carballo et al., 2007; Ho et al., 2014; Li et al., 2015; Yang et al., 2016).

Although composting has been proposed as a mean to reduce antibiotic levels in manure before land application, recent studies have indicated that it cannot completely remove antibiotics (Dolliver et al., 2008; Cessna et al., 2011; Ray et al., 2017). There is concern that antibiotics released into soils could create a selective pressure on the native microbial community (Cleary et al., 2016; Nordenholt et al., 2016) and enrich resistant bacteria and resistance genes (Jechalke et al., 2014).

Previous studies demonstrated that antibiotic dissipation in soils is influenced by environmental factors, such as temperature, pH, and soil physicochemical properties, as well as the initial concentrations of antibiotics and their associated matrixes (Otker and Akmehmet-Balcioglu, 2005; Cengiz et al., 2010; Srinivasan and Sarmah, 2014). Chemical properties of the antibiotics can also influence their interactions with soil minerals and organic matter (Otker and Akmehmet-Balcioglu, 2005; Wegst-Uhrich et al., 2014). To date, investigations on antibiotic fate in soils have largely been conducted on antibiotic-spiked soils or soils mixed with antibiotic-spiked manure (Carlson and Mabury, 2006; Fang et al., 2014; Pan and Chu, 2016). Although such approaches are in accordance with the US EPA guidelines for pesticides (USEPA., 2008a, b), they ignore effects of passing through the animal gut and subsequent manure management on the interactions of antibiotics with manure matrix before land application. Such influences are important because: ***1)*** antibiotics typically enter the soil via a manure matrix, whereas pesticides are normally directly applied to soils; ***2)*** passage through the animal gut will influence their sorption within the manure matrix, partially biodegrade antibiotics, and alter microbial communities involved; and ***3)*** manure amendment alters soil physical, chemical, and biological properties, all of which will influence the fate of antibiotics.

Although there is limited information comparing the environmental fate of manure-borne versus spiked antibiotics, studies of biosolids have demonstrated significantly faster dissipation rates of the antimicrobial triclosan when spiked into soils compared to when it is biosolids-borne (Kwon and Xia, 2012). Decreased diffusion, increased sorption, and reduced bioavailability when biosolids-borne could contribute to prolonged persistence, compared to predicted values and those determined using spiked soils.

The objective of this study was to determine the effect of composting and soil type on the dissipation of manure-borne antibiotics following land-application, using manure derived from antibiotic-administrated dairy cows and beef cattle. The results help to evaluate whether benefits of composting using FDA FSMA guidelines (FDA, 2014) extend towards reducing antibiotics and their potential impacts in soils, particularly within the USDA National Organic Program's recommended 120-day waiting period between raw manure application and harvest of crops that are in contact with soil (USDA, 2012).

2. Materials and methods

2.1 Raw manure and compost

Raw manure was collected from sulfamethazine, tylosin and chlortetracycline-treated beef cattle or pirlimycin-treated dairy cows (Table S1 and Text S1 of Supplemental Information (SI)). After laboratory-scale composting for 42-days, using static and turned techniques as recommended by the FDA (FDA, 2014) and described in a previous study (Ray et al., 2017), compost was collected from composting tumblers (Text S1, SI). The physical properties and antibiotic concentrations in raw manure and the compost are listed in Table S2.

2.2 Soil microcosms

The sandy loam, silt loam, and silty clay loam soils were top soils (0-5 cm) collected from three locations in Virginia (Text S2, SI and Table S3).

For each microcosm unit, 15 g air-dried soil sieved through 2-mm was added to a 150 mL pre-washed (70% ethanol) and air-dried glass jar. Calculated based on the typical agronomic nitrogen application rate in Virginia (Evanylo, 2009), 2.44 g raw manure or 1.60 g compost was then added to a glass jar and mixed by hand shaking and stirring. Ultrapure water was added to the manure-soil mixture to bring the soil moisture to 50% of its field moisture capacity and maintained by recording the total weight of each microcosm jar. Each jar was covered loosely with an aluminum foil sheet to reduce moisture loss while maintaining aerobic conditions. All microcosms were kept in the dark at room temperature (20°C) with soil moisture adjusted weekly by adding ultrapure water to bring the total weight of each jar to its recorded weight. Soils were collected in triplicate on days 0, 1, 3, 7, 29, 57, 90 and 120 via destructive sampling, freeze dried, and stored at -20°C for antibiotics analysis.

Antibiotics were analyzed using SPE-UPLC-MS/MS method with small modifications (Text S3, SI) (Ray et al., 2014; Ray et al., 2017). The samples were extracted using sonication with methanol: McIlvaine buffer (50:50, v/v) or methanol: phosphate buffer (70:30, v/v). The extracts were cleaned up using OASIS HLB Cartridges (Waters, Milford, MA). After the sample preparation, the antibiotics were analyzed using Agilent 1290 UPLC coupled with Agilent 6490 Triple Quad tandem mass spectrometry (Agilent Technologies Inc., Santa Clara, CA). To estimate the method detection limits (MDLs), series dilutions of antibiotic standards were spiked into manure amended soils. The spiked samples were processed through the entire analytical method. The MDLs were then determined using seven samples spiked near the lowest concentration that was detected. The MDLs for sulfamethazine, tylosin, chlortetracycline, and pirlimycin were 0.13, 0.25, 0.59, and 1.54 $\mu g\ kg^{-1}$, respectively. The method quantification limits (3.3 time MDLs) for sulfamethazine, tylosin, chlortetracycline, and pirlimycin were 0.43, 0.83, 1.95, and 1.78 $\mu g\ kg^{-1}$, respectively. The recoveries for sulfamethazine, tylosin, chlortetracycline, and pirlimycin ranged from 90% to 118%, 81% to 121%, 58% to 76%, and 89% to 91% in manure amended soils, respectively.

2.3 Data graphing and statistical analysis

Bi-phasic or single phasic first order kinetic models were used to fit the C_t/C_o vs t curves. The rate constants were acquired based on the slope of the curve fit of $\ln(C_t/C_o)$ vs t to the respective model and the half-lives were derived from the calculated rate constants. If all triplicate samples were non-detect, then the C_t/C_o point was noted as below the detection limit (BDL).

All statistical analyses were carried out using JMP (JMP®, Version 12.0. SAS Institute Inc., Cary, NC, 1989-2007). For non-detect samples, half of the method detection limit was used for statistical purposes. Two-way analysis of variance (ANOVA) was carried out at the 95% confidence level to test the effects of amendment type (raw manure, static compost, or turned compost), soil type, and their interactions on antibiotic dissipation. One-way ANOVA was used to test the effect of amendment type on the final antibiotic concentrations in soils at the end of microcosm study. Multiple paired comparisons were conducted using the Tukey-Kramer method (Tukey, 1949).

2.4 Assessment of antibiotic resistance selection potential

Risk quotient (RQ) values were applied to assess the potential impact of antibiotic residue on antibiotic resistance selection potential in soils at day 0 and 120 days after manure application. The RQ value was calculated as the ratio of the measured concentrations in the soils before and after incubation (Table 1) to the predicted no-effect concentrations for antibiotic resistance selection in soils (PNECs_{soil}). The predicted no-effect concentration in soil for an antibiotic (PNEC_{soil}) can be calculated using the following equations (Thomaidi et al., 2016):

$$\mathrm{PNEC}_{soil} = \mathrm{PNEC}_{water} \times k_d = \mathrm{PNEC}_{water} \times k_{oc} \times f_{oc}$$

Where PNEC_{water} is the predicted no-effect concentration for resistance selection in water (μg L^{-1}), k_d is the soil-water partition coefficient of antibiotics (L kg^{-1}), k_{oc} is the soil organic carbon-water partitioning coefficient of an antibiotic. The PNEC_{soil} for sulfamethazine, tylosin, chlortetracycline, and pirlimycin were estimated (Text S4, SI) and ranged from 58-100, 7.0-12, 0.68-1.2, and 10-18 μg kg^{-1}, respectively (Table S8). Similar to the classification used for ecological risk evaluation (Ho et al., 2015), RQ values <0.1, 0.1-1, and >1 are categorized to three levels, as "low", "medium", and "high" antibiotic resistance selection potentials, respectively.

3. Results and discussion

3.1 Antibiotic dissipation patterns in the soil microcosms

The term "dissipation" is used here to refer to the collective effects of biodegradation, transformation, sorption, loss of extractability, and other processes that contribute to a net decrease in measured antibiotic. Limited field studies have reported the dissipation of manure-borne antibiotics (Halling-Sorensen et al., 2005; Heuer et al., 2008). While such field studies are of value to gain a general sense of antibiotic behavior in the real world, it is not possible to isolate the effects of various environmental processes in governing their fate. For example, transport, plant uptake, and photodegradation will all contribute to some extent to the persistence of antibiotics in soils at field-scale and cannot be distinguished from other processes, such as biodegradation. In contrast, microcosms provide the advantage of a closed system with a limited number of variables.

3.1.1 Raw manure-amended soils

The initial concentrations (dry weight basis) of sulfamethazine, tylosin, and chlortetracycline in the three cattle manure-amended soils ranged from 29-47, 8.4-10, and 46-80 μg kg^{-1}, respectively (Table 1). These concentrations were more relevant to real-world conditions compared to levels typically spiked to soils (100 to 1,000,000 μg kg^{-1}) (Accinelli et al., 2007; Pan and Chu, 2016).

Dissipation of sulfamethazine was rapid in all three soils within the first 7 days, with 43 to 77% remaining at day 7 (Fig. 1), but slowed significantly thereafter. Sulfamethazine remained constant at levels of 32 to 45% in the three soils until day 120 (Fig. 1). Our observations are consistent with a prior field study examining the dissipation of five sulfonamides, including sulfamethazine, in manure-amended soils (Stoob et al., 2007), where the dissipation was initially fast, but slowed down considerably after 14 days. Similar results were also reported when examining the sulfamethazine dissipation in a swine manure-amended sandy loam soil with an initial spiked concentration of 100,000 μg kg^{-1} (Lertpaitoonpan et al., 2015). Other studies of structurally-related sulfonamides also yielded similar results (Wang et al., 2006; Liu et al., 2010; Fang et al., 2014). For example, Fang et al. (2014) observed rapid dissipation of sulfadiazine within 7 days, followed by dramatically slower dissipation in manure-amended soils with an initial spiked concentration of 20,000 μg kg^{-1} (Fang et al., 2014). This study supports the overall conclusion that sulfamethazine becomes more persistent and less bioavailable with time.

Similar to the case with sulfamethazine, initial dissipation of tylosin was rapid in raw manure-amended soils (Fig. 1). By day 7, 44% to 58% of tylosin remained in the three soils. However, in contrast to sulfamethazine, the dissipation of tylosin continued through 120 days, although at a slower rate. By 120 days, only 5.9 to 8.4% of tylosin remained in the three soils, with final concentrations ranging 0.51 to 0.87 μg kg^{-1} (Fig. 1, Table 1). Continuous dissipation of tylosin in soils were observed in previous studies (Halling-Sorensen et al., 2005; Carlson and Mabury, 2006; Schlusener and Bester, 2006; Hu and Coats, 2007; Sassman and Lee, 2007; Liu et al., 2010). A field study using manure-borne antibiotics showed that the concentrations of tylosin declined from 50 μg kg^{-1} to 10 μg kg^{-1} in a sandy loam soil and from 25 to 3 μg kg^{-1} in a sand soil within 155 days (Halling-Sorensen et al., 2005).

The trend in the dissipation of chlortetracycline in the raw manure-amended soils was similar to that of tylosin; however, initial dissipation rates were markedly lower (Fig. 1). At day 7, 62% to 72% of chlortetracycline remained in the three soils, while around 50% of tylosin was transformed by day 7 (Fig. 1). Continuous dissipation of chlortetracycline in soils has also been observed in laboratory and field experiments (Halling-Sorensen et al., 2005; Carlson and Mabury, 2006; Zhang and Zhang, 2010; Fang et al., 2014). In a field study using manure-borne antibiotics, 50% reduction of chlortetracycline was observed in both a sandy loam and sandy soil within 20-34 days and 28-42 days, respectively (Halling-Sorensen et al., 2005).

In contrast to the above antibiotic dissipation patterns, the levels of pirlimycin in all raw manure-amended soils first increased 1.98-2.70 times from day 0 to day 3, rapidly decreased from day 3 to day 29, and then remained relatively constant thereafter until day 120 (Fig. 1). The initial spike in pirlimycin concentration was most notable in the sandy loam soil (Table 1). Previous research has shown that pirlimycin administrated to dairy cows can be conjugated in the liver and gastrointestinal tract to form pirlimycin-sulfoxide, pirlimycin-sulfone, pirlimycin-adenylate, pirlimycin-uridylate, and pirlimycin sulfoxide-adenylate (Hornish et al., 1998). These conjugates are subsequently excreted into the feces and urine at substantial levels, up to 50% of the total secreted pirlimycin. Therefore, we hypothesize that the initial observed rise in concentration was likely due to deconjugation of pirlimycin conjugates back to pirlimycin.

3.1.2 Compost-amended soils

Although a significant proportion (62%-99%) of manure-borne antibiotics can be transformed during composting (Ray et al., 2017), to the best of our knowledge no prior study has examined whether the residual antibiotics in finished compost (e.g., Table S2) are subject to further dissipation after application to soil.

The initial concentrations of sulfamethazine in the three soils ranged from 1.1-1.6 μg kg^{-1} and from 0.35 to 0.80 μg kg^{-1}, after amending with static and turned composts, respectively (Table 1). One prior study indicated that the highest concentration of chlortetracycline in soils nearby a swine manure composting facility was 0.85 μg kg^{-1} (Awad et al., 2014). This highlights the importance of understanding the fate of low concentrations of antibiotics in compost-amended soils. In contrast to raw manure-amended soils, no dissipation of sulfamethazine was observed in the compost-amended soils over the 120-day period (Fig. 1), indicating that it may not have been bioavailable to soil microorganisms. This was consistent with the observation that no dissipation of sulfamethazine occurred after day 7 in raw manure-amended soil (Fig. 1). Strong adsorption to compost could be a key factor limiting the bioavailability of sulfamethazine and contributing to its observed persistence.

The initial concentrations of tylosin in soils after application with static compost and turned compost ranged from 1.3 to 1.8 μg kg^{-1} and from 0.27 to 1.2 μg kg^{-1}, respectively (Table 1). Rapid dissipation was observed within the first 7 days of microcosm incubation, resulting in 48-54% and 28-68% of the initially added tylosin remaining in all three soils for static compost-amended soil and turned compost-amended soil, respectively (Fig. 1). By day 57, tylosin was below detection in all soils applied with compost.

Initial concentrations of chlortetracycline ranged from 7.5 to 11 μg kg^{-1} and from 4.7 to 6.4 μg kg^{-1} (Table 1) after application of static compost and turned compost to soil, respectively. Compared to tylosin, chlortetracycline dissipation was much slower during the first week. By day 7, 82-92% and 82-106% of the initially added chlortetracycline remained in all three soils for static compost-amended soils and turned compost-amended soils, respectively (Fig. 1). After 120 days, chlortetracycline was still above the detection limit, with concentrations ranging from 1.8 to 3.1 μg kg^{-1} and from 1.1 to 2.4 μg kg^{-1} in static and turned compost-amended soil, respectively (Table 1).

3.2 Antibiotic dissipation rates in the soil microcosms

3.2.1 Raw manure-amended soils

Single-phase first order kinetics did not adequately describe the dissipation of the target antibiotics in raw manure-amended soils in this study, as the coefficients of determination (R^2) varied largely from 0.27 to 0.93 upon fitting the data to a single-phase first order kinetic model. When fitting their dissipation using bi-phasic first order kinetics using the Hockey-Stick model (Sarmah and Rohan, 2011), R^2 values ranged from 0.94 to 0.99. This model consists of two sequential first-order kinetics with the integrated equation shown below:

$$C_t = C_o e^{-k_1 t_b} \text{ for } t<t_b, \quad C_t = C_o e^{-k_1 t_b} e^{-k_2 (t-t_b)}, \text{ for } t>t_b$$

Where C_t is the compound concentration (μg kg^{-1}) at time t (d) after application, C_o is the initial concentration (μg kg^{-1}), k_1 is the rate constant (d^{-1}) until $t = t_b$. The time at which rate constant changes from k_1 to k_2 is denoted by t_b (breakpoint). The breakpoints for sulfamethazine, tylosin, and chlortetracycline were 7 days, 3 days, and 29 days, respectively. For pirlimycin, due to the initial deconjugation of it conjugates, its C_o is defined as the peak concentration detected at day 3, with a corresponding breakpoint of 29 days (26 days after day 3).

The antibiotic dissipation rate constants in the raw manure-amended soils are shown in Table 2. Because the dairy manure matrix is distinct from that of beef cattle manure, the dissipation of pirlimycin is discussed separately. For the three soils, the first phase dissipation rate constants (k_1) ranked in the order of tylosin > sulfamethazine > chlortetracycline (Table 2). It has been shown that antibiotic degradation is typically catalyzed by different extracellular hydrolytic enzymes (protease, lipase, and cellulose) released by microorganisms, mainly in the aqueous phase of soil systems (Thiele-Bruhn, 2003). Therefore, the overall dissipation rate of antibiotics is largely affected by their hydrophilicity (Otker and Akmehmet-Balcioglu, 2005; Wegst-Uhrich et al., 2014). Accordingly, the observed order of dissipation rate constants was consistent with the order of the water solubility of these three compounds: tylosin (5000 mg L^{-1}) > sulfamethazine (1500 mg L^{-1}) > chlortetracycline (600 mg L^{-1}) (Table S1).

The dissipation rate constants of these three antibiotics in the second phase (k_2) were much lower than those in the first phase (k_1) (Table 2). Availability-adjusted first order kinetic models assume that antibiotic availability in soils decreases exponentially with time, largely due to sorption, and have been applied in prior studies with decreasing dissipation rates (Wang et al., 2006; Stoob et al., 2007; Pan and Chu, 2016). Sorption of antibiotics is an important factor affecting the dissipation rate (Otker and Akmehmet-Balcioglu, 2005; Wegst-Uhrich et al., 2014) and thus the partitioning coefficient (k_d) is a key parameter used in estimating the migration potential of aqueous-phase contaminants in contact with solid soil components. Median k_d values of sulfamethazine and tylosin reported in prior literature were 3 and 100 L kg^{-1}, respectively

(Wegst-Uhrich et al., 2014), while the k_d values of chlortetracycline ranged from 1208-2386 L kg^{-1} (Sarmah et al., 2006). This suggests that soil sorption tendency (loss of availability) follows the order of chlortetracycline > tylosin > sulfamethazine. In the current study, the rate constants for the second phase (k_2) were in the order of tylosin > chlortetracycline > sulfamethazine, which is not consistent with the assumption of loss of bioavailability of the antibiotics in soils due to adsorption. Antibiotics examined in previous studies were spiked into the systems (Wang et al., 2006; Stoob et al., 2007; Pan and Chu, 2016), while our study utilized manure from antibiotic-treated animals. Partitioning coefficients of antibiotics in manure are likely different from those described for soils (Loke et al., 2002). Also, in prior studies spiking antibiotics, it is likely that the interactions of antibiotics with manure or soil components did not achieve a steady state before dissipation began. Therefore, the bioavailability of the antibiotics in the soils could decrease over time. On the other hand, the antibiotics in our study are more likely to have reached equilibrium with the manure matrix after passing through the digestion system. After application of manure to the soils, it is likely that desorption begins to dominate sorption in the second dissipation phase, at which point aqueous antibiotic dissipation is near completion. The desorption of chlortetracycline could be retarded due to the lowest dissipation rate of the released chlortetracycline (smallest k_1). By contrast, the desorption of tylosin could be accelerated due to the fastest dissipation rate of the released tylosin (highest k_1). Lack of dissipation of sulfamethazine in the second phase may due to a fraction of sulfamethazine that is irreversibly sorbed to the manure.

Pirlimycin dissipation rate constants in all raw manure-amended soils were higher during the first phase (day 3 to 29) compared to the second phase (day 29 to 120) (Table 2). Similarly, dissipation of clindamycin (a lincosamide antibiotic) in biosolids followed a biphasic pattern, with faster dissipation during the first phase followed by relatively stabilized second phase (Wu et al., 2009).

3.2.2 Compost-amended soils

Since sulfamethazine concentrations remained stable in compost-amended soils over the 120 days, no rate constants could be estimated. By contrast, tylosin was below detection limit by day 57. Therefore, for compost-amended soil, a concentration at half of the detection limit (0.12 μg kg^{-1}) of tylosin was assumed beyond day 57, with simple first order kinetics used to fit the curve from day 0 to day 57, with R^2 ranged from 0.67-0.99. . For chlortetracycline, simple first order kinetics were applied to fit the curves for soils applied with both compost types, with R^2 ranging from 0.88 to 0.97. In compost-amended soils, the rate constants of tylosin in all three soils were greater than those for chlortetracycline, which is consistent with the trend for these two antibiotics in raw manure-amended soils. Curve fitting was not conducted for pirlimycin because it was below the detection limit in all compost-amended soils over the duration of the study (Table 2).

3.3 Antibiotic dissipation half-lives in the soil microcosms

The half-lives of the four target antibiotics in the current study are shown in Table 3. In the raw manure amended soils, the half-lives of tylosin, chlortetracycline, and pirlimycin in the second phase were 14, 4.6, and 16 times as long as those in the first phase (Table 3), respectively, indicating that manure-borne antibiotics could persist in soil at low concentrations for a long period of time. The observed bi-phasic dissipation patterns suggest that a portion of the manure-borne antibiotics is immediately bioavailable and transformed rapidly after manure application. The remaining portion appears to be released slowly from the manure as dissipation continues.

The BIOWIN model in EPI Suite™ (USEPA., 2012) was applied to predict half-lives specifically with respect to primary biodegradation, estimating values of 8.67 days for sulfamerazine and 15 days for tylosin and chlortetracycline. The EPI Suite™-predicted half-lives are similar to the dissipation half-lives measured for the raw-manure amended soils, while, for most of the cases, significantly shorter than the second-phase dissipation half-lives and the single-phase half-lives for the compost-amended soils (Table 3). This suggests that the initial antibiotics are more bioavailable in the raw manure-amended soil and their dissipation is most likely biologically-driven. By contrast, in the later phase or in the compost-added soils these compounds became more recalcitrant and their dissipation is more likely affected by a complex array of biological, chemical, and physical factors.

Varied half-lives of antibiotics had been reported in the literature (Table 3) Lertpaitoonpan (2008)noted that longer half-lives of sulfamethazine were observed with higher initial spiked concentrations and suggested that microbial activity may be inhibited by higher antibiotic concentrations (Lertpaitoonpan, 2008). However, the highest concentration of sulfamethazine determined in the present study, 47 $\mu g\ kg^{-1}$ (Table 3), is far below these previous studies. An effective concentration (EC_{10} values) of 13,000 $\mu g\ kg^{-1}$ sulfamethazine was required to influence microbial respiration in rice paddy soils (Liu et al., 2009). Besides, even dissipation of antibiotics within the same class can vary. For example, six commonly used antibiotics were spiked into a sandy loam soils with an initial concentration of 2,000 $\mu g\ kg^{-1}$for a 120-day microcosm study to examine their dissipation (Martinez-Carballo et al., 2007). Among them, the half-lives for four structurally-related macrolides, including tylosin, erythromycin, oleandomycin, and roxithromycin ranged from 8 days to >120 days.

The half-life of pirlimycin in 0.1 N NaOH (pH 12.5) solution and in pure water (under UV exposure) were 5 and 6.7 days and thus comparable to those estimated for the first phase of dissipation in the raw manure amended soil current study.

In summary, the half-lives of antibiotics in soils reported in the literature appears system-specific and guidance may need to be system specific and incorporate safety factors, assuming the longest observed dissipation rates.

3.4 Effect of amendment and soil type on dissipation of manure-borne antibiotics in soils

Potential interactive effects of manure amendment type and soil type on antibiotic dissipation were examined, but none were found (Table 4). Overall, composting appears to be a promising approach for reducing antibiotic input to soils before manure land application. At the same nitrogen application rates, the initial antibiotic concentrations were much lower in compost than in manure-amended soils and remained low throughout the study period, with a much lower end-point concentration (Table 1). However, lower initial concentrations can translate to slower subsequent dissipation rates, as was observed for the compost-amended soils relative to the first phase dissipation rates in the raw manure-amended soils (Table 2). As suggested by the comparison of the EPI Suite™-predicted half-lives and the measured half-lives, antibiotics in compost are less bioavailable comparing to raw manure because most of the available fraction is transformed and the residual fraction becomes more recalcitrant during composting. Our prior study observed decreasing dissipation rate of antibiotics during manure composting (Ray et al., 2017). Notably, static versus turned compost did not result in significantly different dissipation patterns or rates in soils (Table 3), which may be related to the high similarity of the small-scale compost conditions (Ray et al., 2017).

Statistically significant differences were not observed for dissipation of manure-borne antibiotics among different types of soil receiving manure application (Table 3 and Table S4). Soil properties, such as pH, organic matter content, and clay content theoretically could affect the partition coefficient of antibiotics (Gao and Pedersen, 2010; Wegst-Uhrich et al., 2014) and, therefore, affect the dissipation of antibiotics in soils. In particular, hydrophobic interactions between chemicals and the organic matter is considered to be a predominant mechanism of sorption (Zhang et al., 2010). However, these interactions and factors might not be applicable for manure-borne antibiotics because different from antibiotics that are spiked into soil systems, the manure-borne antibiotics enter into the soils are likely in various complexed forms with manure matrixes, most likely with the organic matter in manure. As a result, soil physic-chemical properties might become less important, as observed by others (Sassman et al., 2007; Bailey et al., 2016).

3.5 Environmental implication

More so than toxicity, a main concern regarding land application of antibiotic-containing manure is the potential to select for antibiotics resistance and gene transfer, resulting in accumulation in soils (Knapp et al., 2010; Knapp et al., 2011). Selection pressure has been reported to occur at very low antibiotic concentrations, as suggested by susceptible/resistant bacteria competition tests (Gullberg et al., 2011; Sandegren, 2014). Minimal selective antibiotic concentrations (MSCs), which could be several hundred-fold below the minimal inhibitory concentrations

(MICs) of susceptible bacteria, have been reported to be capable of enriching for resistant bacteria (Gullberg et al., 2011).Here, antibiotic resistance selection potential was assessed for the initial and final 120-day concentrations of antibiotic residues in the microcosms. Using the method described by Bengtsson-Palme and Larsson (2016) (Bengtsson-Palme and Larsson, 2016) which assume the concentrations of antibiotics that inhibit growth of some bacteria will by consequence have selective effects on the community level, the estimated MSCs of targeted antibiotics instead of toxicity thresholds were applied to standard risk quotients.

The initial sulfamethazine levels were at the upper end of “medium” in raw manure-amended soils (Fig. 2). After 120 days, although sulfamethazine RQ values in the raw-manure amended soils decreased, the concentrations levels were still in the “medium” category for antibiotic resistant selection. In composted-amended soils, sulfamethazine RQ values remained <1 throughout the 120-day incubation period (Fig 2). The initial tylosin levels were at the lower end of “high” in raw manure-amended soils and at or close to “medium” in compost amended-soils, with RQ values ranging from 0.1-1 or close to 0.1, respectively (Fig 2). In contrast to sulfamethazine, the potential for tylosin to select for antibiotic resistance decreased from initial “high” or “medium” levels to “low” for all soils after 120-day incubation. The potential for chlortetracycline to select for antibiotic resistance remained “high” for all the soils during the 120-day incubation. Pirlimycin was detectable only in manure-amended soils. Similar to tylosin, the potential of pirlimycin for antibiotic resistance selection decreased from the lower end of “high” or upper end of “medium” levels to “low” after 120 days (Fig 2).

The result from this study suggest that composting manure reduces the potential for antibiotic resistance selection relative to raw manure application to soils. Further, the results support the conceptual benefits of a wait period prior to harvest, especially for raw manure-amended soil. However, 120 days may not be sufficient for some antibiotics to reduce their potential to a "low" risk level for antibiotic resistance selection potential. After incubation for 120 days, the concentrations of antibiotics in raw manure-amended soils were still significantly higher than those in the compost-amended soils ($p<0.001$) (Table S5 and Table S6). The persistence of antibiotics in manure-amended soils and their potential for resistance selection imply that identification of appropriate manure management practice prior to land application warrants attention.

4. Conclusions

The study employed a controlled, replicated microcosm approach to understand the effect of composting and soil type on the dissipation of manure-borne antibiotics in soils amended with raw manure or compost. Manure-borne antibiotics, including sulfamethazine and chlortetracycline, can persist in soils at low concentrations for extended periods (120-day). Extended persistence of these antibiotics in soils indicates the possibility of antibiotic accumulation in soils with repeated input of antibiotics with manure application over time. Dissipation of antibiotics in raw manure-amended soils was significantly faster than in compost-amended soils, but composting reduced initial inputs of antibiotics and generally resulted in lower levels by 120 days. Soil type did not have a measurable influence on the fate of manure-borne antibiotics, likely because the complex interactions between antibiotics and manure components in the animals' digestive system and during composting reduce the relevance of soil properties in affecting antibiotic fate. Thus, manure management practices for reducing antibiotic inputs may be widely applicable to various soil types. Further, composting may be advantageous for reducing antibiotic inputs to soil systems, while enforcing a wait period prior to crop harvest may provide additional benefits for reducing the chances of contributing to selection and spread of resistant bacteria.

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Table 1. Initial (C_o) and final (120-day) concentrations of antibiotics (μg kg^{-1}) in three different soils amended with raw manure, static compost, or turned compost.

Amendment types	Soil types	Sulfamethazine		Tylosin		Chlortetracycline		Pirlimycin		
		Initial	Final	Initial	Final	Initial	Final	Initial	Day 3†	Final
Raw manure	sandy loam	47±9.9	18±3.5	8.4±2.6	0.70±0.07	57±14	17±2.2	4.6±0.64	12±1.4	0.79±0.08
	silt loam	29±4.8	12±2.3	8.6±1.1	0.51±0.03	80±13	19±2.4	10±1.8	19±1.9	0.47±0.21
	silty clay loam	32±7.3	13±3.6	10±2.3	0.87±0.07	46±11	9.3±1.0	13±1.6	26±1.7	0.63±0.05
Static compost	sandy loam	1.6±0.35	1.5±0.03	1.8±0.35	BDL	11±0.38	3.1±0.14	BDL	BDL	BDL
	silt loam	1.1±0.22	1.2±0.05	1.8±0.40	BDL	7.5±0.52	1.8±0.11	BDL	BDL	BDL
	silty clay loam	1.5±0.58	1.4±0.06	1.3±0.20	BDL	8.9±0.50	1.8±0.16	BDL	BDL	BDL
Turned compost	sandy loam	0.80±0.12	0.78±0.38	0.27±0.14	BDL	4.7±1.2	1.1±0.52	BDL	BDL	BDL
	silt loam	0.35±0.03	0.43±0.03	0.63±0.30	BDL	6.4±1.9	2.4±1.5	BDL	BDL	BDL
	silty clay loam	0.35±0.08	0.39±0.08	1.2±0.45	BDL	5.9±0.57	2.1±0.50	BDL	BDL	BDL

†The initial concentrations of pirlimycin in the raw manure amended soils are the peak concentrations determined at day 3 of the microcosm incubation.

BDL: concentrations which are below method detection limits (0.13, 0.25, 0.59, and 0.54 μg $kg^{-1}$for sulfamethazine, tylosin, chlortetracycline, and pirlimycin, respectively)

Table 2. Dissipation rate constants (k) and goodness of curve fittingof different antibiotics in three soils amended with raw manure, static compost, or turned compost during the 120-day microcosm incubation study

Amendment types	Soil types	Sulfamethazine			Tylosin			Chlortetracycline			Pirlimycin		
		k_1	k_2	R^2	k_1	k_2	R^2	k_1	k_2	R^2	k_1	k_2	R^2
Raw manure†	sandy loam	0.116±0.029	ND	0.94	0.261±0.054	0.016±0.001	0.96	0.028±0.005	0.005±0.002	0.95	0.085±0.004	0.007±0.001	0.99
	silt loam	0.092±0.017	ND	0.96	0.192±0.038	0.023±0.001	0.98	0.030±0.004	0.006±0.002	0.94	0.104±0.010	0.008±0.001	0.90
	silty clay loam	0.039±0.021	ND	0.94	0.238±0.052	0.016±0.001	0.96	0.028±0.005	0.009±0.002	0.95	0.127±0.001	0.005±0.001	0.99
Static compost‡	sandy loam	ND		NA	NA		0.99	0.012±0.001		0.94	NA		
	silt loam	ND		NA	NA		0.97	0.013±0.001		0.96	NA		
	silty clay loam	ND		NA	NA		0.97	0.014±0.001		0.98	NA		
Turned compost‡	sandy loam	ND		NA	0.011±0.005§		0.85	0.011±0.001		0.96	NA		

	silt loam	ND	NA	0.019±0.011§	0.69	0.007±0.001	0.88	NA
	silty clay loam	ND	NA	0.032±0.008§	0.67	0.008±0.001	0.94	NA

† Dissipation of antibiotics followed bi-phasic first order kinetics in raw manure-amended soils

‡ Dissipation of antibiotics followed single phase first order kinetics in the compost-amended soils

§ The dissipation curves of tylosin in the compost-amended soil are fitted to a single phase first kinetic from day 0 to day 57, half of the method detection limit (0.12 μg kg^{-1}] are used to represent the concentrations of tylosin at day 57.

ND: no dissipation (k values are close to 0];

NA: not available due to below detection limit of pirlimycin in the compost-amended soils.

Table 3. The half-lives of antibiotics in soils in this study and literatures

Antibiotics	Initial concentrations (μg kg^{-1})	Samples	Half-lives (days)	References
Sulfamethazine	29-47†	manure amended-sandy loam/-silt loam/-silty clay loam	6-18 (1^{st} phase) ND (>120, 2^{nd} phase)	This study
	1.1-1.5†	static compost amended-sandy loam/-silt loam/-silty clay loam	ND (>120)	
	0.35-0.80†	turned compost amended-sandy loam/-silt loam/-silty clay loam	ND (>120)	
	1,000 to 1,000,000‡	silt loam/sandy loam	18.6	(Accinelli et al., 2007)
	500 to 100,000‡	sandy loam	1.3-5.9	(Lertpaitoonpan, 2008)
	500 to 100,000‡	manure amended-sandy loam	1.2-6.6	
	200‡	manure amended-sandy loam/-clay loam	ND (>28)	(Bailey et al., 2016)
	100‡	clay loam soil	24.8	(Pan and Chu, 2016)
Tylosin	8.4-10†	manure amended-sandy loam/-silt loam/-silty clay loam	2.7-3.7 (1^{st} phase) 41-44 (2^{nd} phase)	This study
	1.3-1.8†	static compost amended-sandy loam/-silt loam/-silty clay loam	15-17	

	0.27-1.2†	turned compost amended-sandy loam/-silt loam/-silty clay loam	22-63	
	1142‡	sandy loam	6.1	(Carlson and Mabury, 2006)
	1408‡	manure amended-sandy loam	4.5	
	2000‡	sandy loam	8	(Schlusener and Bester, 2006)
	50000‡	sandy loam	7	(Hu and Coats, 2007)
	50†	manure amended-loamy sand	67	(Halling-Sorensen et al., 2005)
	25†	manure amended-sandy	49	
Chlortetracycline	57-80†	manure amended-sandy loam/-silt loam/-silty clay loam	23-25 (1st phase) 75-144 (2nd phase)	This study
	7.5-11†	static compost amended-sandy loam/-silt loam/-silty clay loam	49-58	
	4.7-6.4†	turned compost amended-sandy loam/-silt loam/-silty clay loam	61-104	
	754‡	sandy loam	21	(Carlson and Mabury, 2006)
	705‡	manure amended-sandy loam	24	
	5000‡	loam	31.9	(Zhang and Zhang, 2010)
	5000‡	manure amended-loam	37.3	
	20000‡	silt loam	5.5	(Fang et al., 2014)

	20-30†	manure amended-loamy sand	25	(Halling-Sorensen et al., 2005)
	20-30†	manure amended-sandy	34	
Pirlimycin	12-26†	manure amended-sandy loam/-silt loam/-silty clay loam	5.5-8.2 87-142	This study

† Anitibotics in naturally excreted form when manure-based amendments are applied to soil

‡ Antibiotics directly spiked into the soil systems

Table 4. *P* values of two-way ANOVA of the effect of manure amendment type and soil type on antibiotics dissipation and multiple pair comparisons of effect of manure amendment types on antibiotic dissipation

Factors	Sulfamethazine	Tylosin	Chlortetracycline	Pirlimycin
Amendment type	***<0.0001***	*0.53*	***0.01***	*NA*
Soil Type	*0.05*	*0.36*	*0.72*	*0.18*
Amendment Type × Soils	*0.12*	*0.11*	*0.81*	*NA*
Pair comparisons of amendment type	Sulfamethazine	Tylosin	Chlortetracycline	Pirlimycin
Raw manure vs. Static compost	***<0.0001***	*0.95*	*0.17*	*NA*
Raw manure vs. Turned compost	***<0.0001***	*0.51*	***0.01***	*NA*
Static compost vs. Turned compost	*0.06*	*0.71*	*0.44*	*NA*

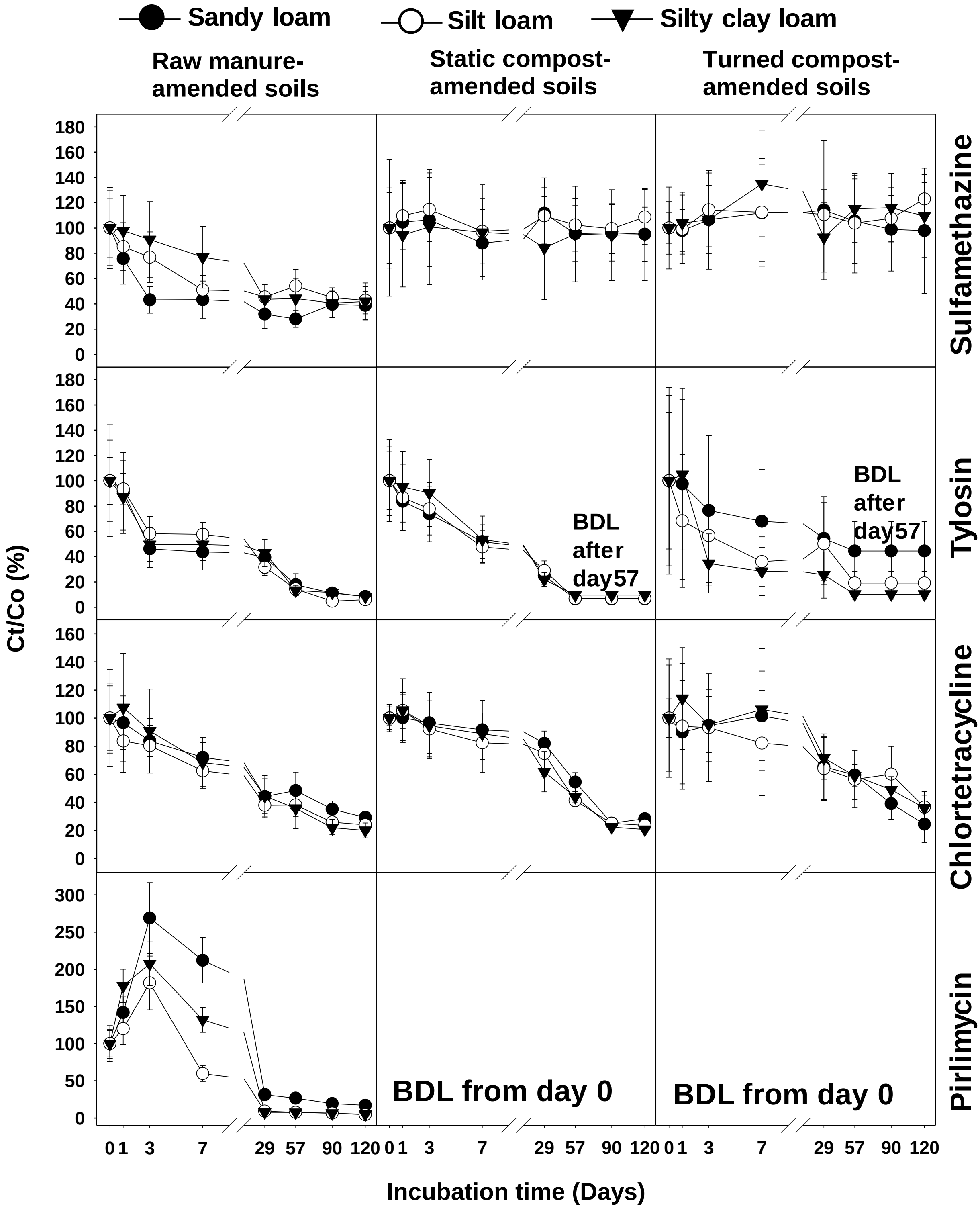
NA: not available due to below detection limit of pirlimycin in the compost-amended soils

Figure captions

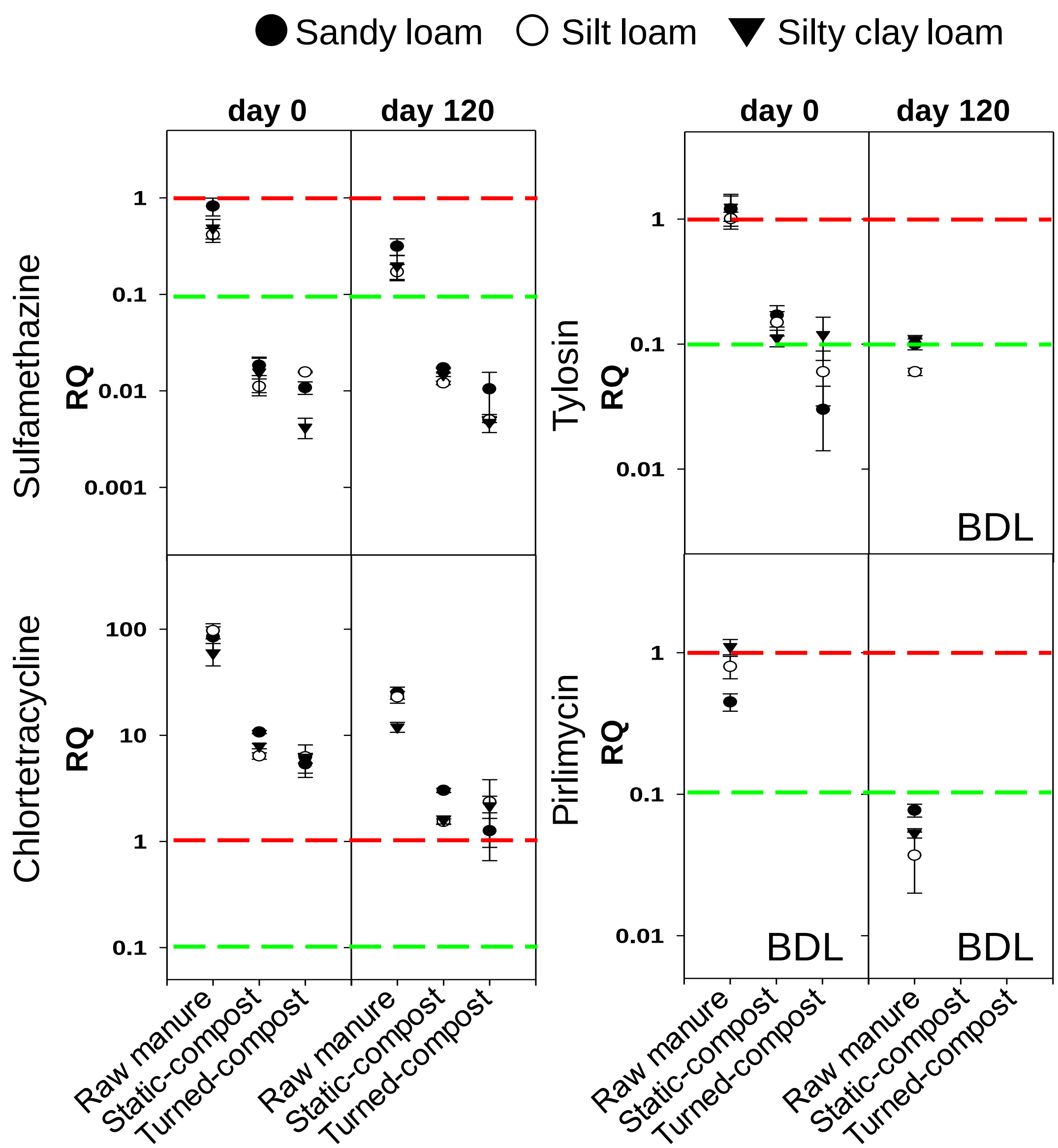
Figure 1. Dissipation of sulfamethazine, tylosin, chlortetracycline, and pirlimycin in sandy, silt, and silty clay loam soils amended with raw manure, static compost, and turned compost. Error bars represent standard deviations from replicate microcosms (n=3].

Figure 2. The antibiotic resistant selection potential risk quotient (RQ] values of sulfamethazine, tylosin, chlortetracycline, and pirlimycin in soils applied with raw manure, static compost, and turned compost

Figure 1.


Sandy loam
Silt loam
Silty clay loam
Raw manure-amended soils
Static compost-amended soils
Turned compost-amended soils
Sulfamethazine
Tylosin
Chlortetracycline
Pirlimycin
BDL after day57
BDL after day57
BDL from day 0
BDL from day 0
Ct/Co (%)
Incubation time (Days)
0 1 3 7 29 57 90 120

628 Figure 2.



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