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Minimally managed composting of beef manure at the pilot scale: Effect of manure pile construction on pile temperature profiles and on the fate of oxytetracycline and chlortetracycline

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ABSTRACT

Oxytetracycline (OTC) and chlortetracycline (CTC) are broad-spectrum antibiotics used in livestock production. Although laboratory-scale studies have shown that extractable concentrations of these compounds decrease over time within treated and untreated manures and soils, there is relatively little information from farm-scale experiments. The objective of this study was to determine the effect of different levels of management on manure pile temperature profiles and on the fate of OTC and CTC in manure from therapeutically treated calves. Four treatments were designed to span a range of management options – from simply piling up the manure to amending it with straw to increase aeration and adding insulating layers of straw. Replicate samples of antibiotic-containing calf manure were held at ambient temperature or placed in three locations within replicate 3 m^3 piles of beef manure. During the 28-day incubation period, concentrations of buffer-extractable OTC and CTC/ECTC (the summed concentrations of CTC and its epimer 4-epi-chlortetracycline (ECTC)) in manure samples incubated at ambient temperature (11–24 °C) decreased 75% (from 18 to 4.6 mg kg^{-1} dry weight (DW)) and 90% (from 192 to 16 mg kg^{-1} DW), respectively. Concentrations of the CTC metabolite iso-chlortetracycline (ICTC) decreased 90% (from 37 to 3 mg kg^{-1} DW). OTC and CTC/ECTC concentrations in samples incubated for 28 days within a non-amended manure pile decreased 91% and >99%, respectively. During that period, the manure pile temperature ranged from 36 °C to 45 °C. Manure piles insulated with a blanket of straw and/or amended with straw (3:1, v/v) attained temperatures up to 70 °C and contained very low levels of OTC, CTC/ECTC, and ICTC (ranging from <0.1 to 0.4 mg kg^{-1} DW) after 28 days.

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1. Introduction

Raw manure can be a significant source of pathogens and antibiotics (Sarmah et al., 2006). When animal producers periodically remove manure from pens, housing units, barns or sheds, the manure is often stockpiled until time and circumstances are available to land apply the material. Depending on soil saturation and freeze conditions as well as availability of work crews, stockpiles may remain for several months, for example in winter before being applied to pasture or cropland in spring. Manure stockpiles typically have a tendency to self-heat when piled in sufficient volume (at least 3 m^3) so as to retain the heat of microbial respiration. However, the steep temperature gradient from the self-heated thermophilic core zones of stockpiles to the ambient exterior areas leaves a substantial portion of the stockpiled mass unaffected by the thermophilic stabilization that occurs in the core. Pathogens may persist in the outer or cool pile areas and antibiotic residues from feeding may also persist in these same zones because of low or minimal microbial decomposition. To the extent that manure stockpiles contain pathogens and antibiotics, then a portion of the land-applied material will contribute to pathogen transfer to water, soil, and crops (Nicholson et al., 2005), and antibiotic residues will contribute to potential for antibiotic persistence and development of antibiotic resistance among native microbial communities (Kelley et al., 1998; Rysz and Alvarez, 2004; Sapkota et al., 2007; Witte, 1998). Therefore, it is important to implement appropriate management practices that minimize the risk of disseminating pathogens and antibiotics from the stockpiled manure.

Previous laboratory-scale composting studies have shown that thermophilic temperatures and aerobic conditions increase antibiotic removal rates (Sarmah et al., 2006). However, there is little comparable information on the fate of antibiotic residues from farm-scale experiments using bovine manure. Although on-farm manure composting is a well-described approach for stabilization

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of nutrients and reduction of pathogens and odors (US Composting Council, 2000), few producers have the time, staff or economic incentive to compost their manure according to prescribed protocols for producing products that can be distributed to the public for unrestricted use. However, with a few simple modifications, on-farm manure stockpiles could be manipulated to promote a more extensive zone of destructive temperature, more like that achieved by intensively managed composting practices. By attending to the conformation of the pile and utilizing on-farm resources like old straw, old hay, sawdust, or existing cured compost, an increased zone of aerobic thermophilic decomposition can be achieved. Through a minimal management process configuration it is likely that pathogen and antibiotic concentrations in manure stockpiles can be reduced substantially if not significantly from the starting concentrations. The objective of the study reported here was to evaluate the efficacy of a series of minimal management options for composting manure on-farm to reduce concentrations of the widely used antibiotics oxytetracycline (OTC) and chlortetracycline (CTC) (Fig. 1). Four treatments were designed to span a range of management options – from simply piling up the manure to amending it with straw (to increase aeration) and adding insulating layers of straw.

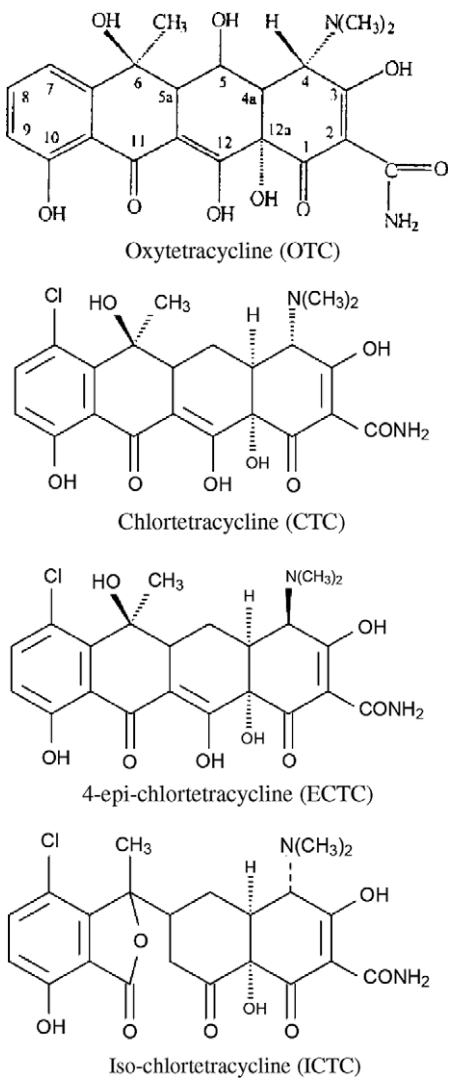

Fig. 1. Chemical structures of OTC, CTC, ECTC (an epimer of CTC) and ICTC (a metabolite of CTC).

2. Methods

2.1. Experimental design

2.1.1. Manure collection and treatment design

Beef manure, consisting of feces and urine from beef calves mixed with sawdust bedding was scraped from concrete pens and temporarily stored in a covered area prior to being transported to the USDA's Composting Research Facility at the Beltsville Agricultural Research Center (Beltsville, MD, USA). Water was added to the manure–bedding mixture (hereafter simply termed "manure") and mixed with a front-end loader to achieve approximately 70% moisture content prior to subdividing it into seven separate conically-shaped piles. Each pile had a volume of approximately 3 m^3, dimensions of 2.8 × 1.5 m (diameter × height) and mass of approximately 1500 kg. Four treatments were included in the experiment: treatment 1, one manure pile was placed directly on the concrete floor; treatment 2 (straw base), two replicate manure piles were placed on 6" bases of straw; treatment 3 (straw base, amended with straw), two replicate manure piles were mixed with straw (3:1, v/v) to increase aeration within the piles and the amended piles were placed on 6" bases of straw; treatment 4 (straw base, amended with straw, straw blanket), two replicate manure piles were mixed with straw (3:1, v/v), the amended piles were placed on 6" bases of straw, and covered with a 6" blanket of straw. The piles were constructed in separate concrete bins each with a leachate collection trough embedded in the concrete floor. Bins were located outdoors but under cover in a three-sided pole barn. Table 1 shows characteristics of the manure and straw-amended manure.

2.1.2. Preparation of cassettes containing manure with antibiotics

Collection and characteristics of the OTC and CTC-containing beef calf manures used in this study have been described previously (Arikan et al., 2007; Arikan, 2008). The concentration of buffer-extractable OTC in the fresh manure from OTC-treated calves was 225 ± 15 mg kg^{-1} DW (Arikan et al., 2007). An aliquot of the OTC-containing manure was stored at 4 °C for 25 months until its use in this study. During this storage period, the concentration of extractable OTC in the manure declined to 18 ± 2 mg kg^{-1} DW. The concentrations of extractable CTC/ECTC (the summed concentrations of CTC and its epimer 4-epi-chlortetracycline (ECTC)) and the CTC metabolite iso-chlortetracycline (ICTC) in the fresh manure from CTC-treated calves were 208 ± 10 and 33 ± 15 mg kg^{-1} DW, respectively (Arikan, 2008). An aliquot of the CTC-containing manure was stored at −20 °C for 11 months until its use in this study. During this storage period, the concentration of extractable CTC/ECTC in the manure declined (to 192 ± 14 mg kg^{-1} DW), but the concentration ICTC remained constant (37 ± 4 mg kg^{-1} DW).

Table 1
Characteristics of beef manure and straw-amended beef manure[a].

	Manure[b]	Manure–straw mixture (3:1, v/v)[c]
pH	8.7	8.9
EC (mS cm^{-1})	1.75	1.66
Moisture content (%)	69	67
Density (g l^{-1})	550	475
C content (% DW)	12.4	13.9
N content (% DW)	0.54	0.60
P content (% DW)	0.34	0.28
C/N	23	23

[a] Values are from single measurements of composite samples taken on day 0.
[b] Used in treatments 1, 2, and samples incubated at ambient temperature.
[c] Used in treatments 3 and 4.

Aliquots (30 g wet weight) of the two antibiotic-containing manures were placed in vented plastic cassettes (Tissue Path IV cassettes, Fisher Scientific) prior to being loaded into sections of plastic pipe (hereafter termed BioSentry tubes). BioSentry tubes were constructed of 2" (5 cm) diameter × 3/16" (0.48 cm) (wall thickness) × 10" (25 cm) long polyvinylchloride (PVC) pipe with fiberglass screen covering each end. Each BioSentry tube had forty holes (0.6 cm diameter) equidistantly arranged around the circumference of the tube to allow gas exchange between bulk pile material and the cassette contents. Each BioSentry tube contained a temperature datalogger (WatchDog 100 datalogger, Spectrum Technologies, Plainfield, IL or Tidbit datalogger, Onset Computer Corp., Pocasset, MA). BioSentry tubes were placed approximately 30 cm beneath the surface at three locations in each pile: near the base, in the middle, and near the top (Fig. 2). Two additional BioSentry tubes with manure-containing cassettes and temperature data-loggers were placed on the concrete floor next to the manure piles and were incubated at ambient temperature. On days 7, 14 and 28, tubes were pulled out of the treatment piles and one set of manure-containing cassettes (along with the temperature data-loggers) was removed. After data downloading, the temperature data-loggers were reloaded into the BioSentry tubes and the tubes were reinserted into their respective locations within each manure pile. Manure from the cassettes was removed in the laboratory and immediately processed for analysis of moisture content and antibiotic concentrations.

2.1.3. Analysis of OTC, CTC/ECTC, and ICTC

2.1.3.1. Extraction. Manure subsamples were extracted in duplicate for OTC, CTC/ECTC and ICTC analyses using amethod described previously (Cappone et al., 1996; Arikan et al., 2007). Briefly, 1 g subsamples were extracted three times with 3 ml of 0.1 M Na_2EDTA–McIlvaine buffer by vortexing for 30 s followed by sonication for 3 min in a 100 W sonication bath (Bronson Ultrasonics, Danbury, CT). After each extraction, the extracts were subjected to centrifugation (500*g*, 5 min, 5 °C), the supernatants were pooled, again subjected to centrifugation (1650*g*, 20 min, 5 °C), filtered through Whatman glass microfiber (grade GFB) filter paper, and passed through cartridges (Waters Sep-Pak C-18 for OTC and Waters 60 mg HLB Oasis for CTC/ECTC and ICTC) after the cartridges had been prewashed with 5 ml methanol and 10 ml 0.1 M Na_2EDTA–McIlvaine buffer. After the extracts were loaded, the cartridges were flushed with 20 ml distilled water, followed by sample elution using 8 ml of 0.01 M methanolic oxalic acid. The eluents were concentrated under a flow of N_2 to a volume of 0.5 mL. Distilled water (0.5 ml) was added to each tube of concentrated eluent, the tubes were mixed by vortexing (30 s), and the contents were transferred to amber autosampler vials. Demeclocycline (2 μg) was added to each sample as an internal standard prior to analysis by LC–MS/MS.

2.1.3.2. LC–MS/MS analysis. The analyses of OTC, CTC, ECTC and ICTC were performed using LC–MS/MS as previously described (Arikan et al., 2006; Arikan, 2008) using a Waters 2690 XE (Waters Corp., Milford, MA) separations module with an Xterra MS C_{18} column (150 mm × 2.1 mm i.d., 5 μm) (Waters Corp., Milford, MA) at 50 °C for OTC and 45 °C for CTC, ECTC and ICTC. The flow rate was 0.25 ml min^{-1} and the injection volume was 10 μl. A mobile-phase gradient was necessary to separate the compounds. For OTC analysis, the solvent compositions were: (A) methanol–water (5:95, v/v) with formic acid (308 μl/l) added; (B) methanol–water (95:5, v/v) with formic acid (308 μl/l) added. The solvent program was: 0–6 min 89% A, 11% B; 6–11 min, a linear gradient to 50% A, 50% B; 11–21 min, 50% A, 50% B; 21–22 min, linear gradient back to initial conditions (89% A, 11% B). The column was stabilized for 13 min prior to the next analysis. For CTC, ECTC and ICTC analyses, the solvent compositions were: (A) 1% formic acid–methanol (70:30, v/v); (B) water; and (C) methanol. The solvent program was: 0–1 min, 50% A, 50% B, 0% C; 1–12 min, a linear gradient from initial conditions to 70% A, 0% B, 30% C; 12–20 min, 42% A, 0% B, 58% C; 20–22 min, 0% A, 0% B, 100% C; 22–25 min, 0% A, 0% B, 100% C; 25–27 min, a linear gradient back to initial conditions (50% A, 50% B). The column was allowed to stabilize for 10 min prior to the next analysis. Calibration curves were generated using results from 10 μl injections of standards ranging from 0.1 to 10 mg l^{-1}. Atmospheric pressure ionization-tandem mass spectrometry was performed on a benchtop triple quadrupole mass spectrometer (Quattro LC from Micromass Ltd., Manchester, UK) operated in positive electrospray ionization mode. Analyte concentrations were calculated by the internal standard method using demeclocycline as an internal standard (Zhu et al., 2001). Peak integration and quantitation were performed automatically using MassLynx 3.5 software (Waters Corp., Milford, MA). According to European regulations, CTC levels in foodstuffs of animal origins are calculated as the summed levels of the antibiotically-active CTC and ECTC. Therefore, results from CTC and ECTC analyses were summed and are reported as CTC/ECTC values.

2.1.3.3. Recoveries. To determine extraction efficiencies, duplicate samples of unmedicated manure were spiked with stock solutions to obtain samples with 1 and 10 mg kg^{-1} (wet weight) of OTC, CTC, ECTC and ICTC. Samples were thoroughly mixed and then extracted as described above prior to LC–MS/MS analysis. Average recoveries of 1 mg kg^{-1} spikes were slightly higher than recoveries of 10 mg kg^{-1} spikes (Table 2). The moisture content of the manure samples was 69%. Thus, the concentrations in the spiked 1 and 10 mg kg^{-1} (wet weight) samples expressed on a dry weight basis were 3.2 and 32 mg kg^{-1} DW, respectively. Average recoveries of OTC, CTC, ECTC and ICTC were 71%, 76%, 66% and 63%, respectively.

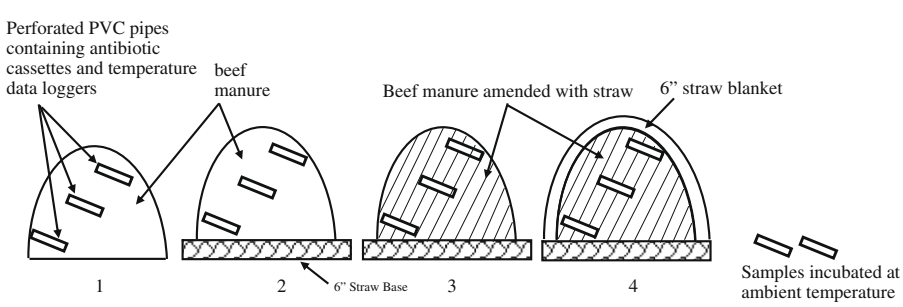

Fig. 2. Schematic drawing of manure treatments and approximate locations of manure-containing cassettes within the manure piles. Treatment 1, manure pile without further treatment; treatment 2, manure pile placed on straw base; treatment 3, manure amended with straw (3:1, v/v) and placed on a straw base; treatment 4, manure amended with straw (3:1, v/v), placed on a straw base, and covered with a layer of straw. In addition, duplicate BioSentry tubes with manure-containing cassettes and temperature data-loggers were incubated at ambient temperature. Each manure pile was approximately 3 m^3 in volume and contained approximately 1500 kg of manure.

Table 2
Recovery of OTC, CTC, ECTC and ICTC in beef manure.

Compound	% mean recovery (95% confidence limits)[a] Spike level (mg kg^{-1} wet weight)[b] 1	10
OTC	71 (63–79)	70 (61–79)
CTC	79 (75–83)	73 (69–77)
ECTC	72 (64–80)	61 (52–69)
ICTC	66 (60–72)	59 (54–64)

[a] Values are means from duplicate samples.
[b] The corresponding concentrations in the spiked samples expressed on a dry weight basis are 3.2 and 32 mg kg^{-1} DW.

3. Results

Manure pile temperatures are influenced by factors affecting bacterial metabolism (manure nutrient content, moisture content and bulk density) and by factors affecting heat loss from the pile (size and conformation of pile, presence or absence of insulating layers). The treatments were designed to span a range of management options – from simply piling up the manure to amending it with straw and adding insulating layers of straw. The first level of treatment was to place a non-amended manure pile on a concrete floor within a three-sided pole barn (Fig. 2). Increasing levels of treatment included: placing manure pile on a 6" layer of straw to reduce heat loss from the base of the pile (treatment 2), amending the manure with straw to increase aeration within the pile (treatment 3), and covering the manure piles with a 6" layer of straw to reduce heat loss from the top of the pile (treatment 4). Treatment 1 was not replicated; treatments 2–4 were conducted in duplicate. We predicted that piles of straw-amended beef manure would attain higher temperatures than piles of non-amended manure because improved aeration would lead to improved bacterial metabolism. We also predicted that bottom locations within the manure piles would be significantly cooler than middle or top locations because of heat loss to the cool floor. Adding straw layers below and on top of the manure pile would decrease heat loss and would thereby raise and unify temperatures within the piles. With regard to concentrations of antibiotics within the manure, we predicted that increased temperatures within the manure piles would

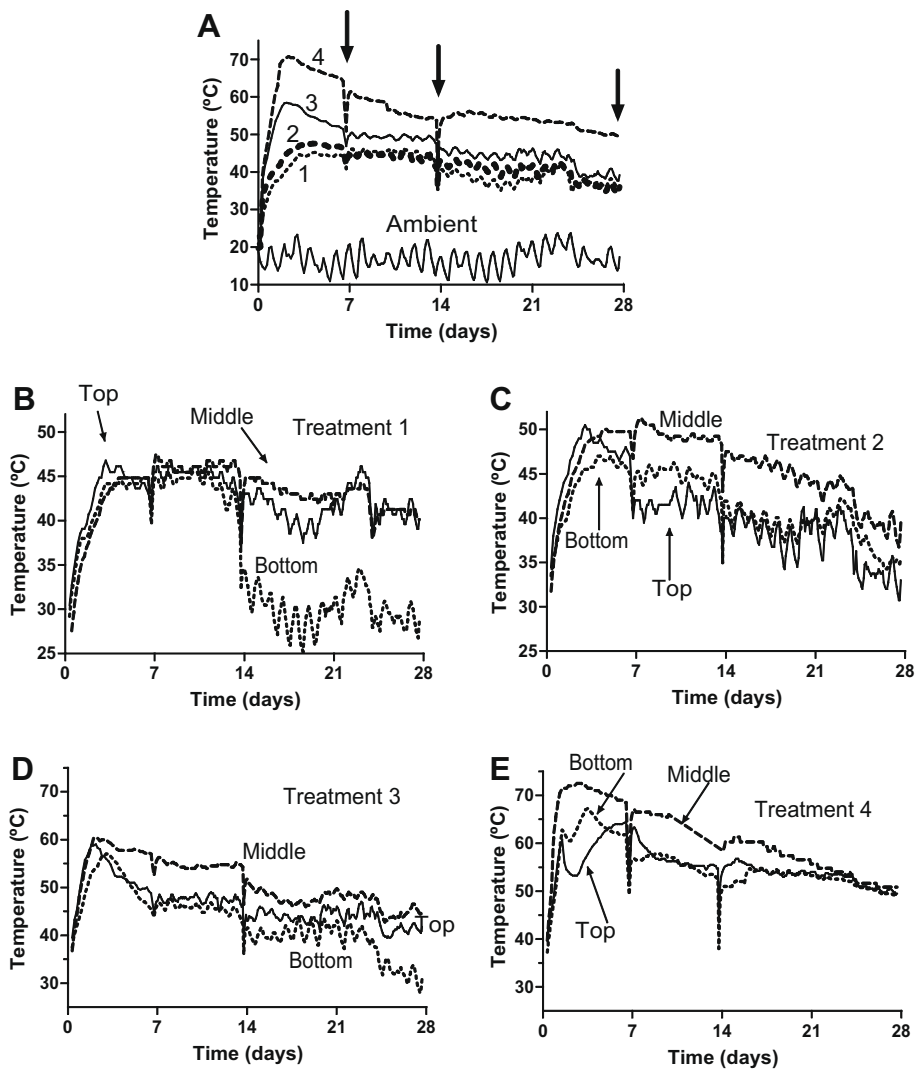

Fig. 3. Mean whole-pile and mean within-pile temperature profiles from manure pile treatments during the 28 day experiment. Panel A, mean whole-pile temperature profiles for each treatment. Values are the means of results from three locations within a single manure pile (treatment 1) or within duplicate manure piles (treatments 2–4). Ambient temperature values are from dataloggers placed within BioSentry tubes located on the floor of the three pole barn. Panels B–E, within-pile temperature profiles for each treatment. Values are from single readings from three locations (top, middle and bottom) within one manure pile (treatment 1) or are the means of readings from three locations in duplicate manure piles (treatments 2–4). Temperature data-loggers were temporarily removed from manure piles on days 7, 14 and 28 (shown by arrows in panel A) in order to download temperature results.

lead to increased adsorption and lower concentrations of extractable antibiotic residues.

3.1. Effect of manure treatments on pile temperature profiles

Mean manure pile temperatures (averaged values from top, middle and bottom locations from replicate treatments) and within-pile temperature profiles were significantly influenced by treatment. During the incubation period (April 13–May 10, 2006), ambient temperature in the covered building fluctuated from 11–24 °C (with a mean of 17 °C) (Fig. 3, panel A). Mean temperatures within the manure piles rose quickly above ambient temperatures, and reached their maximum values within 2–3 days of incubation (Fig. 3, panel A). The mean temperature of treatment 1 (manure pile placed directly on the concrete floor) increased to a maximum of 45 °C by day 3. The mean temperature of treatment 2 (manure pile placed on a 6" layer of straw) followed a similar profile and increased to a slightly higher maximum of 47 °C by day 3. The mean temperatures of treatments 3 and 4 (manure amended with straw, placed on straw layer, without and with an overlying straw blanket, respectively) increased to maximums of 58 °C and 70 °C, respectively, within two days. In all treatments, mean pile temperatures gradually declined after reaching their maximum values. At the end of the 28 day incubation period, mean pile temperatures of treatments 1–3 were nearly identical (36–38 °C) and were much lower than the mean temperature of treatment 4 (50 °C).

The within-pile temperature profile for treatment 1 showed very similar temperatures from the three within-pile locations until day 14 when the bottom data-logger was removed for data retrieval. After data retrieval, the BioSentry tube was apparently relocated to a much cooler position within the bottom of the pile (presumably closer to the cool floor) (Fig. 3, panel B). In general, the within-pile temperature profiles for treatments 2–4 (Fig. 3, panels C–E) showed bottom and top location temperatures to be very similar and approximately 5 °C lower than middle pile temperatures.

3.2. Effect of pile construction on concentrations of extractable OTC, CTC/ECTC, and ICTC

During the 28 day incubation period concentrations of OTC, CTC/ECTC, and ICTC decreased 75–91% within samples incubated at ambient temperatures and decreased >99% within the four manure treatments (Fig. 4, Table 3). The rates of residue removal for the first 14 days of incubation were similar for treatments 1 and 2 and were lower than the initial removal rates shown in treatments 3 and 4 (Fig. 4). Concentrations of the metabolite ICTC transiently increased in some samples after 7 days of incubation.

Within the samples incubated for 28 days at ambient temperature, extractable concentrations of OTC decreased 75% from 18 to 5 mg kg^{-1} DW, CTC/ECTC concentrations decreased 91% from 192 to 16 mg kg^{-1} DW, and ICTC concentrations decreased 91% from 37 to 3 mg kg^{-1} DW (Fig. 4, Table 3). Within the samples from the non-amended manure piles (treatments 1 and 2), extractable concentrations of OTC, CTC/ECTC, and ICTC (0.3–1.6, 1.2–1.4, and <0.1 mg kg^{-1} DW, respectively) were 3–10 fold lower at the end of the experiment than the samples held at ambient temperature. In comparison, within the samples from the straw-amended manure piles with the highest temperatures (treatments 3 and 4), extractable concentrations of OTC, CTC/ECTC, and ICTC were even lower (0.3–0.4, 0.1–0.3, and <0.1 mg kg^{-1} DW, respectively) at the end of the experiment (Table 3). Although there were within-pile variations in concentrations of extractable residues, we observed no consistent trend with regard to residue concentrations as a function of location within the manure piles (not shown).

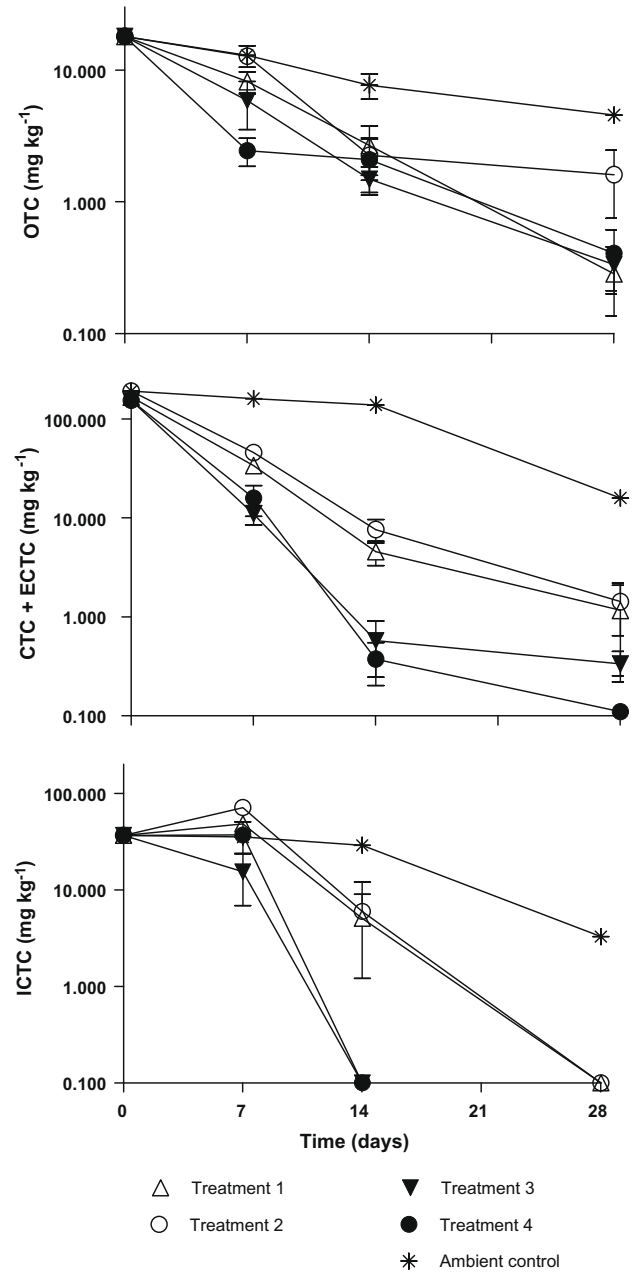

Fig. 4. Extractable concentrations of OTC, CTC/ECTC, and ICTC as a function of treatment during the 28 day experiment.

4. Discussion

Temperature profiles for the manure treatments in this study are comparable to results reported in previous studies. Sommer and coworkers measured the temperatures of a cone-shaped pile (3.1 m diameter, 1.1 m height) of manure from a beef cow feedlot as part of a gas emissions study (Sommer et al., 2004). At 10 cm depth, the manure pile temperature reached 11–49 °C after 2 days and ranged from 50–60 °C from 3–6 days. Chadwick and coworkers measured core pile temperatures in 7–13 m^3 piles of beef manure. Temperatures reached 65 °C within several days, then gradually declined to 55 °C on day 20 and 45 °C on day 40 (Chadwick, 2005). Temperature profiles of large-scale windrows of beef manure show similar peak temperatures but longer time intervals of self-heating and cooling (Hao et al., 2001; Larney et al., 2003; Michel et al., 2004; Storteboom et al., 2007).

Table 3
Concentrations (mean ± SE) (in mg kg^{-1} DW) of OTC, CTC/ECTC, and ICTC during the incubation period.

Treatment	1	2	3	4	Ambient temperature
Day					
OTC					
0	18.0 ± 1.9				
7	12.7 ± 0.3	8.2 ± 1.6	5.9 ± 2.3	2.5 ± 0.6	12.9 ± 2.4
14	2.3 ± 0.8	2.7 ± 1.1	1.5 ± 0.4	2.1 ± 0.9	7.7 ± 1.7
28	1.6 ± 0.8	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.2	4.6 ± 0.3
$T_{½}$ (days)	7.5	4.7	4.7	5.6	14.0
CTC/ECTC					
0	192 ± 14				
7	46.0 ± 2.7	33.8 ± 4.9	10.9 ± 2.4	15.9 ± 5.4	160 ± 7
14	7.6 ± 2.0	4.5 ± 1.2	0.6 ± 0.3	0.4 ± 0.2	139 ± 3
28	7.6 ± 2.0	4.5 ± 1.2	0.6 ± 0.3	0.4 ± 0.2	139 ± 3
$T_{½}$ (days)	4.0	3.8	3.0	2.6	7.8
ICTC					
0	36.8 ± 4.0				
7	71.5 ± 6.8	48.6 ± 6.7	15.4 ± 8.5	37.2 ± 13.4	35.5 ± 1.7
14	6.0 ± 6.0	5.1 ± 3.9	DL[a]	DL	29 ± 0.5
28	DL	DL	DL	DL	3.3 ± 0.4
$T_{½}$ (days)	ND[b]	ND	ND	ND	ND

[a] DL, value below detection limit (0.1 mg kg^{-1} DW).
[b] ND, not determined.

The fate of tetracyclines in soil, sediments, and in manure treated by composting or anaerobic digestion at a laboratory-scale has been the subject of numerous studies (reviewed in; Sarmah et al., 2006; Arikan et al., 2007). In general, tetracyclines adsorb strongly to organic matter and, consequently, extractable concentrations of tetracyclines and their metabolites decline over time in organic matrices. In this study, the half-life values of OTC and CTC/ECTC in beef manure incubated at ambient temperature (11–24 °C) were approximately 14 and 8 days, respectively. These ambient temperature half-life value are lower than the 30-day value reported from a study in which manure from OTC-treated calves was stored outdoors in a 3–4 ton pile (De Liguoro et al., 2003) and are much lower than the >40 day values from laboratory scale incubation studies at 25 °C using sterile and non-sterile manure that had been treated with OTC or CTC (Arikan et al., 2007; Arikan et al., 2009). At present, we have no explanation for our lower values.

We predicted that increased temperatures within the manure piles would lead to increased adsorption and lower concentrations of extractable antibiotic residues. Our results showed that there was a rapid reduction of extractable concentrations of OTC and CTC/ECTC within the first seven days of all manure treatments. The half-life values of OTC ranged from 5 to 8 days between the different treatments and decreased with increasing mean whole-pile temperatures. These values compare very well with results from a previous laboratory-scale composting experiment using the same OTC-containing calf manure (Arikan, 2008). In that experiment, the temperature of a composted manure–straw–woodchip mixture ranged from 65–70 °C within the first 6 days of treatment and the calculated half-life for OTC was approximately 3 days. In the present experiment, treatment 4 (manure amended with straw and insulated with straw blanket) showed a similar temperature profile over the first 7 days and yielded a calculated half-life for OTC of 4 days. Our values are lower than those recently reported in a pilot-scale treatment study characterizing the fate of CTC, OTC, tylosin and monensin in spiked horse manure, and OTC and CTC in dairy and beef feedlot manure (Storteboom et al., 2007). In that study, the half-life value for OTC was 10–18 days in composted dairy manure and was 15–31 days in composted feedlot manure. It is likely that the different results are due to the differences in the compositions and moisture contents of the different manures used in the these studies.

The half-life values of CTC/ECTC varied very little (ranging from 2–4 days) between the different treatments in our study but decreased with increasing mean whole-pile temperatures. These half-life values are comparable to those reported by Pruden and coworkers (Storteboom et al., 2007) in the pilot-scale manure treatment study described above. In that study, the half-life value for CTC was 5–8 days in composted horse manure, 6–7 days in composted dairy manure and 13 days in composted feedlot manure. The values presented here also compare very well with more recent results from a laboratory-scale composting experiment using CTC-containing calf manure (Arikan et al., 2009). In that study, CTC-containing manure from treated calves was collected and an aliquot was sterilized by irradiation. Results showed that concentrations of extractable CTC/ECTC decreased slowly in sterile and non-sterile manure samples during incubation at 25 °C (half-life values of 40–47 days) and decreased rapidly during incubation at 55 °C (half-life values of 4–5 days). Since there were no significant differences in the rate of antibiotic disappearance from sterile and non-sterile manure samples, the decreased concentrations appear to be entirely due to temperature dependent abiotic adsorption (Arikan et al., 2009). Although we did not include comparable sterile samples in the current study, it is likely that the decrease in extractable residues shown here is also due to absorption rather than biodegradation.

We do not have any information on the long-term environmental fate of absorbed OTC or CTC residues in composted manure. However, results from two previous composting studies using OTC- and CTC-containing manures suggest that non-extractable antibiotic residues are not bioavailable to the manures' microbial flora (Arikan et al., 2007; Arikan et al., 2009). In those studies, total heterotrophic and OTC- or CTC-resistant bacteria in the respective manures were enumerated before and after composting. Results from both studies showed greatly increased numbers of total heterotrophic organisms after composting and decreased numbers of OTC- or CTC-resistant organisms after composting of the respective antibiotic-containing manures. Although these results suggest that non-extractable residues are not bioavailable in the short-term, we have no comparable information on extractability or bioavailabilty of these residues after the composted mixtures are applied to soil. Nonetheless, in the absence of more definitive information, we believe that absorbed antibiotic residues are much less likely to promote the selection or spread of antibiotic resistant bacteria compared to extractable antibiotic residues in untreated manures.

Our results demonstrate that extractable concentrations of OTC and CTC/ECTC decrease >90% and >99%, respectively, in stockpiled manure that self heats to 40–45 °C for 28 days. Although manure piles amended with straw attained higher temperatures and more rapid decreases in antibiotic concentrations, there is, at present, no compelling justification for producers to expend additional resources needed to achieve the more rapid rates of antibiotic removal. However, the ability of manure piles to self-heat is clearly dependent on manure moisture content as well as its composition. In the present experiment, as in most composting studies, water was added to stockpiled manure to achieve a moisture content of roughly 50–80%. Since stockpiled manure can dry very quickly and is not typically monitored for moisture content, additional experiments are needed to establish the range of moisture content needed to consistently achieve this relatively low level of self-heating.

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