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Pathogen reduction in minimally managed composting of bovine manure

Patricia Millner [a], David Ingram [a], Walter Mulbry [b,*], Osman A. Arikan [b,c]

[a] USDA-ARS, Beltsville Agricultural Research Center, Environmental Microbial and Food Safety Laboratory, Beltsville, MD 20705, USA
[b] USDA-ARS, Beltsville Agricultural Research Center, Sustainable Agricultural Systems Laboratory, Beltsville, MD 20705, USA
[c] Istanbul Technical University, Department of Environmental Engineering, Istanbul 34469, Turkey

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ABSTRACT

Spread of manure pathogens is of considerable concern due to use of manure for land application. In this study, the effects of four static pile treatment options for bovine manure on die-off of a generic *Escherichia coli*, *E. coli* O157:H7 surrogate, *Salmonella* Senftenberg, *Salm.* Typhimurium, and *Listeria monocytogenes* were evaluated. Bovine manure spiked with these bacteria were placed in cassettes at the top, middle, and bottom sections of four static pile treatments that reflect minimal changes in pile construction with and without straw. Temperatures were monitored continuously during the 28 day self-heating period. *E. coli* and salmonellae were reduced from 8 to 9 $\log_{10}$ CFU g^{-1} to undetectable levels (<1.77 $\log_{10}$ MPN g^{-1}) at 25–30 cm depths within 7 days in all pile sections except for the manure-only pile in which 3–4 logs of reduction were obtained. No *L. monocytogenes* initially present at 6.62 $\log_{10}$ CFU g^{-1} were recovered from straw-amended piles after 14 days, in contrast with manure-only treatment in which this pathogen was recovered even at 28 days. Decline of target bacterial populations corresponded to exposure to temperatures above 45 °C for more than 3 days and amendments of manure with straw to increase thermophilic zones. Use of straw to increase aeration, self-heating capacity, and heat retention in manure piles provides producers a minimal management option for composting that enhances pathogen die-off and thereby reduces risk of environmental spread when manure is applied to land.

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1. Introduction

Livestock and poultry manure are known sources of bacterial pathogens such as *Campylobacter jejuni*, *Escherichia coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes*, (Bicudo and Goyal, 2003; Ferens and Hovde, 2011; Mc Carthy et al., 2011; US EPA, 2005) that can be disseminated from sources via waterways, runoff, vectors, fomites, and the air (Dungan, 2010; Millner, 2009; Ravva et al., 2011). Spread of manure pathogens has also been implicated in foodborne illness outbreaks associated with consumption of fresh produce (Braden and Tauxe, 2013; Sapers and Doyle, 2009), consequently there is concern about the efficacy of composting with regard to reducing or eliminating fecal bacterial pathogens.

Thermophilic composting of manure is recognized as a well-established practice that, when properly conducted and monitored, generates a product that has significantly reduced numbers of pathogens compared to that in raw manure, reaching undetectable or nearly undetectable concentrations (Haug, 1993). Nonetheless, many producers simply stack manure in piles until weather and time permit direct land application on-farm or export off-farm because they do not have the time, staff, or economic incentive to compost manure according to prescribed protocols while meeting other demands of operating livestock and poultry production systems. Stacked manure piles often self-heat because, in the central core, the heat of microbial respiration does not escape as quickly as it is generated (Burton and Turner, 2003; de Bertoldi et al., 1996). However, insufficiently self-heated areas of the piles may harbor viable pathogens that can persist or even regrow (Elving et al., 2009), but 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.

* Corresponding author. Address: USDA-ARS, Beltsville Agricultural Research Center, Sustainable Agricultural Systems Laboratory, Building 308, BARC-East Beltsville, MD 20705, USA. Tel.: +1 301 504 6417.

E-mail address: walter.mulbry@ars.usda.gov (W. Mulbry).

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We hypothesized that through a minimal management process configuration, pathogens in bovine manure stockpiles can be reduced substantially. The objective of this study was to evaluate the efficacy of several options for minimal management of on-farm composting using a commonly available amendment, straw, to modify and insulate bovine manure piles to enhance self-heating temperatures to reduce concentrations of a nonpathogenic *E. coli* strain that is described as a thermotolerant surrogate for *E. coli* O157:H7, *Salmonella enterica* serovar Senftenberg, *Salm. enterica* serovar Typhimurium, and two strains of *L. monocytogenes*. Because the bottom areas within manure piles can be significantly cooler than middle or top sections due to heat loss to cold base materials, we tested use of an insulative layer of straw below the pile. A total of four treatments were included to span a range of manure pile management options – from simple manure stockpiles (no amendment) to amending manure stockpiles with straw (to increase aeration and thereby microbial respiration) and adding insulative layers to blanket and/or cushion the base of the pile to enhance the amount of the self-heating mass.

2. Materials and 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 eight 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, two replicate manure piles were placed directly on the concrete floor; Treatment 2 (straw base), two replicate manure piles were placed on 15 cm 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 15 cm 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 15 cm bases of straw, and covered with a 15 cm blanket of straw (Fig. 1). Piles were constructed in separate three-sided open-top concrete bins with leachate collection in the concrete floor; bins were located in an unheated, three-sided pole barn. Characteristics of the manure and straw amended manure are shown in Table 1.

2.2. Preparation of inoculated manure samples

2.2.1. E. coli, Salmonella, and Listeria

Four bacterial strains were used to inoculate manure: (1) *E. coli* (USDA-ARS-Eastern Regional Research Center-ERRC 991232), a spontaneous mutant resistant to 50 μg ml^{-1} rifampicin (Rif) and slightly more thermotolerant than toxigenic strains of *E. coli* O157:H7 (Eblen et al., 2005); (2) *S. enterica* serovar Typhimurium (ATCC 53648); (3) *S. enterica* serovar Senftenberg (Dr. Jianghong Meng 336, University of Maryland, College Park, MD), a spontaneous mutant resistant to 50 μg l^{-1} nalidixic acid (Nal); and (4) two *L. monocytogenes* (Dr. Joanne van Kessel, USDA, ARS) strains Li118 and Li300 isolated from bulk milk tank filters. Cultures were stored at −80 °C in trypticase soy broth (TSB, Difco, Becton Dickinson, (D-BD) Sparks, MD) containing 30% (vol/vol) glycerol and were resuscitated in TSB overnight, then streaked onto TSB agar plates; single colonies were grown and also maintained at 4 °C on TSB agar for up to 5 days.

Isolates were grown statically at 37 °C until stationary phase, 20 h, in TSB with 50 μg l^{-1} Rif for *E. coli*, and Nal for *S.* Senftenberg, and without antibiotics for both strains of *L. monocytogenes* and *S.* Typhimurium. Cell cultures in TSB were centrifuged and triple washed with sterile phosphate buffered saline (PBS), before final resuspension in 10 ml PBS. Cell preparations were $\sim 10^8$ CFU ml^{-1} as verified by plating on MacConkey-Rif (MAC-Rif; D-BD) agar for *E. coli*, xylose-lysine tergitol-Nal (XLT4-Nal; D-BD) for *S.* Senftenberg, XLT4 for *S.* Typhimurium, and Modified Oxoid agar (MOX; Oxford medium base with Modified Oxford antimicrobic supplements, Difco) for *L. monocytogenes*. All plates were incubated for 24 h at 37 °C.

2.2.2. Inoculation of manure

Inocula were conditioned on manure (in the process described as follows) in order to avoid abrupt substrate shifts and to allow isolates to attach to, and become associated with, solid manure matrices. First, fresh manure from a mature bull fed a high forage diet was diluted 1:10 with deionized distilled water, and autoclaved in 100 ml aliquots 25 min at 121 °C (15 psi). When cooled, the diluted manure aliquots were inoculated separately with two loops of 24 h colonies of the respective bacterial cultures, and then incubated at 35 °C, 24 h on a rotary shaker (180 rpm). Next, four individual 100 g aliquots of dried dairy manure solids, (<10% water at 105 °C for 18 h), previously steamed on 3 successive days were

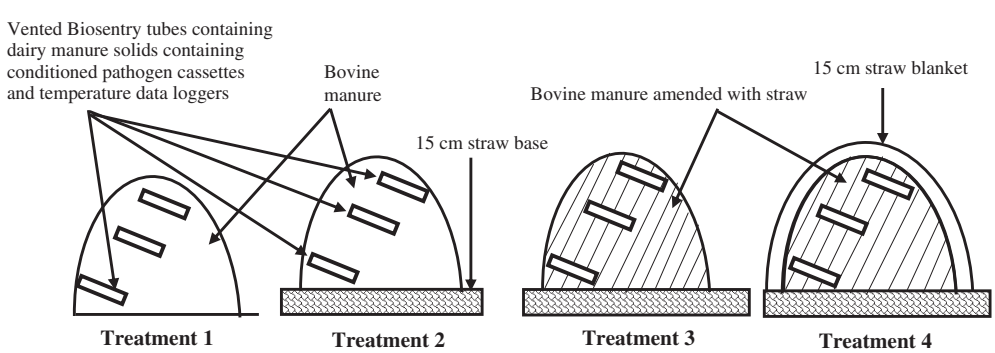

Fig. 1. Diagram of manure treatments and approximate locations of manure-containing cassettes within the manure piles. Treatment 1, non-amended manure pile; treatment 2, manure pile placed on 15 cm thick straw base; treatment 3, manure amended with straw (3:1 v/v) and placed on a straw base; treatment 4, manure amended with straw (3:1 v/v), placed on a straw base, and covered with a 15 cm thick layer of straw. A duplicate of BioSentry tubes with manure-containing cassettes and temperature dataloggers were also incubated outside of piles at ambient temperature.

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

Parameter	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 and 2.
[c] Used in treatments 3 and 4.

inoculated (1:1) separately with 24 h manure cultures. The inoculated manure solids were mixed thoroughly in a sterile plastic bag and then incubated 48 h at 35 °C, before storage at 4 °C for 96 h until used to spike calf-pen manure. Next, equal portions (300 g) of each of the four manure-based inocula preparations were mixed together thoroughly by massaging in a sealed plastic bag. Finally, the multistrain preparation (1200 g) was then mixed with 2000 g of the same calf-manure used to construct the piles. Preliminary assays showed no detectable indigenous salmonellae, but naturally present fecal coliforms were 10^6 CFU g^{-1} dw (dry weight) in this calf-manure. Final concentrations of inocula aliquots were confirmed by plating on MAC-Rif for *E. coli* O157:H7, XLT4 for *Salm.* Typhimurium, XLT4-Nal for *Salm.* Senftenberg, and MOX for *L. monocytogenes*. Although the inocula populations (ranged 10^6–10^9 CFU g^{-1}dw manure) were much greater than typical for bovine manure, they were used to ensure that 5-log units of population decrease could be measured, as suggested by Erickson et al. (2009).

Aliquots (25 g wet weight) of pathogen-spiked manure solids compost feedstock were placed in vented plastic cassettes (Simport Macrosette, Beloeil, Quebec, Canada) prior to being loaded into vented pipes (hereafter termed BioSentry tubes). BioSentry tubes were constructed of 5 cm diameter × 0.48 cm (wall thickness) × 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 pile material and 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 the top and middle of each pile, and 20 cm below the surface at the bottom section in each pile. Two additional BioSentry tubes with manure (uninoculated) and temperature dataloggers were placed on the concrete floor next to the manure piles and were incubated at ambient temperature. Since we expected pathogen die-off to occur during the first four weeks when pile temperatures are highest, on days 7, 14, and 28, tubes were removed from the piles and one set of manure-cassettes (along with the temperature data-loggers) was removed. After data downloading, temperature dataloggers were reset and reloaded into the BioSentry tubes and the tubes were reinserted into their designated sections in each pile. Manure from the cassettes was removed in the laboratory and immediately processed for analysis of moisture content and viable microbial concentrations. Sample cassette, tube loading, and microbial enumerations were conducted in a BL-2 laboratory.

2.3. Sampling and microbial enumeration

Samples removed from cassettes in the laboratory were immediately placed into a sterile stomacher bag fitted with a filter mesh (Seward Limited, London, UK), and diluted 1:10 (w/v) with sterile 0.1% buffered peptone water with 0.25% sodium pyruvate (BPW-P) prior to blending with a stomacher blender (Seward Medical, London, UK) at 200 rpm for 1 min. Sample suspensions were serially diluted (1:10) in BPW-P, and 0.1 ml from each dilution tube was plated in duplicate using a WASP2 spiral plating apparatus (Microbiology International, Frederick, MD) on the surface of MAC-Rif, XLT4, XLT4-Nal, and MOX agar plates. Serial dilutions (duplicate 0.1 ml portions of each dilution) of the samples described above were also spiral-plated onto trypticase soy agar (TSA, Difco) plates and incubated at 37 °C for 72 h for enumeration of aerobic heterotrophic bacteria and onto MAC and MAC-Rif agar plates with incubation at 44.5 °C to enumerate fecal coliforms and *E. coli*, respectively. For salmonellae, the serial dilutions of samples were spiral-plated onto XLT4 and XLT4-Nal and plates were incubated at 37 °C for 24 h. For *Listeria*, serial dilutions of samples were spiral-plated onto MOX and plates were incubated at 37 °C for 48 h.

In addition, microplate-MPN (most probable number) enumeration with six 1:2-dilutions and eight replicates per dilution, was performed in sterile plastic plates on eight 200 μl aliquots of primary dilution samples. The microplate-MPN enrichment step used TSB with novobiocin (20 μg l^{-1}) for the inoculated *E. coli* and *Salmonella* followed by incubation at 37 °C for 24 h. Fecal coliforms, aerobic heterotrophs, and *Listeria* were not assayed by the MPN procedure. Enriched liquid (20 μl) from each turbid well was spot-dropped and streaked onto MAC-Rif, XLT4, XLT4-Nal agar plates, then incubated at 37 °C for 24 h. Streaks were examined for characteristic colony morphology; randomly selected presumptive positive colonies for *E. coli* and salmonellae were picked and streaked for purity and tested by Triple Sugar Iron agar (Difco), Motility-Indole-Lysine medium (MIL, Difco) and slide agglutination with *Salmonella* O antisera (Difco) and Enterotube (Roche) for *E. coli*.

Samples (5 ml) were also enriched in 2x Buffered Listeria Enrichment Broth (Difco) to recover viable *Listeria* after incubation at 37 °C 48 h prior to streaking onto MOX agar plates with 48 h incubation at 37 °C. Five suspected *Listeria* colonies per presumptive positive sample, brown-greenish and surrounded by black halo on MOX agar, were transferred to TSA containing 0.6% yeast extract (TSA-YE, Difco) and incubated at 37 °C 24–48 h. Characteristics used to confirm isolates on MOX Agar as *L. monocytogenes* included presence of catalase, hemolysis on horse blood Columbia agar bilayer plates (Remel, Lenexa, KS, USA), fermentation of xylose and rhamnose, oxidase, and umbrella-shaped growth in motility in SIM Medium (Sulfur Reduction Test, Indole Production, Motility) (Oxoid, Fisher Scientific, Pittsburgh, PA).

Subsamples were oven dried at 105 °C 24 h and dry weight per wet weight were calculated and moisture content (%) was averaged for each section on each sample date. Microbial counts were corrected for moisture content prior to $\log_{10}$ transformation and the calculation of means and standard errors. Microbial counts are expressed as colony-forming units (CFU) g^{-1} dw or MPN g^{-1} dw. The limit of detection for direct plated samples was 2.77 $\log_{10}$ CFU g^{-1} dw, and for MPN analyses the limit of detection was 1.66 $\log_{10}$ MPN g^{-1} dw.

3. Results

3.1. Effect of manure treatments on pile temperature profiles

As shown in Fig. 2, average temperatures of the piles (top, middle and bottom of replicate treatments) and within-pile temperature profiles were substantially influenced by the pile configuration-composition treatment. Samples from all treatment

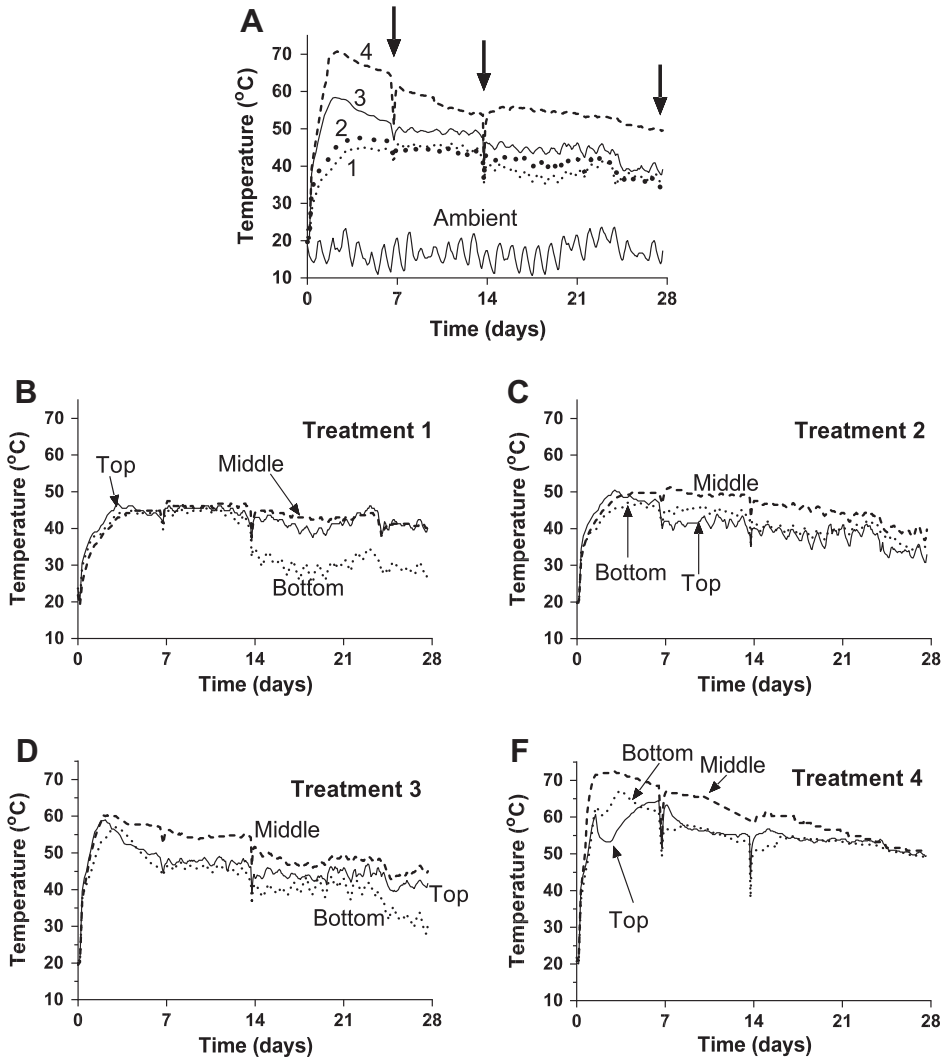

Fig. 2. Means of whole- and within-pile temperatures from treatments. Values are means from three locations within duplicate piles (treatments 1–4). Dataloggers were briefly removed from piles on days 7, 14 and 28 (arrows graph A) to download data. Graph A, mean whole-pile temperatures for each treatment. Ambient temperatures are from dataloggers placed next to the pile in the barn. Graphs B–E, within-pile temperatures for each treatment. Treatment 1, non-amended manure pile; Treatment 2, manure pile placed on 15 cm thick 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 15 cm thick layer of straw.

piles except Treatment 1 (unamended, bovine manure piled without straw base or straw cover) were exposed to mean temperatures in excess of 45 °C for at least 24 h. Ambient temperatures during the experiment ranged from 11 to 24 °C (mean, 17 °C) (Fig. 2, panel A). Mean temperatures within piles rose quickly and reached maximal values within 2–3 days (Fig. 2, panel A). Treatment 1 (pile directly contacting the concrete floor) reached a maximal mean temperature of 45 °C by day 3, whereas treatment 2 (15 cm layer of straw between the pile and concrete floor) achieved a slightly increased peak mean temperature of 47 °C by day 3. In contrast, mean temperatures of treatments 3 and 4 (manure amended with straw, placed on straw layer, without and with an overlying straw covering, respectively) achieved peak temperatures of 58 °C and 70 °C, respectively, by day 2. Mean pile temperatures for all treatments gradually declined after reaching maxima. By day 28, mean pile temperatures of treatments 1–3 were comparable (36–38 °C), but considerably less 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 sections up to day 14 when the bottom datalogger was removed for data retrieval. However, after data retrieval, the BioSentry tube was apparently relocated to a much cooler position at the bottom of the pile (close to the cool floor) (Fig. 2, panel B). Overall, within-pile temperature patterns for middle pile section of treatments 2–4 (Fig. 2, panels C–E) were similar to each other and ~5 °C greater than bottom and top section temperatures.

3.2. Effect of pile construction on bacterial populations

Concentrations of indigenous fecal coliforms and aerobic heterotrophic bacteria were 5.53 ± 0.15 and 5.80 ± 0.04 CFU $\log_{10}$ g^{-1} -dw, respectively, prior to inoculation of the bovine manure with *E. coli,* salmonella, and *L. monocytogenes*, and the populations increased substantially after inoculation (Table 2). Overall, *E. coli* and salmonellae populations decreased substantially in samples at all sections in the self-heating manure piles during the 28-day composting study, except for bottom section samples in treatment 1 (the non-amended pile) (Table 2, Fig. 3). Fecal coliforms initially present in bovine manure and *E. coli* ERRC 991232 initially recovered from the inoculated bovine manure at 8.84 and 9.06 $\log_{10}$ CFU g^{-1} dw, respectively, were reduced to 7.32 and 7.37 $\log_{10}$ CFU g^{-1} dw, respectively, in the low temperature bottom

Table 2
Effect of treatment and time on concentrations (Log_{10} mean ± SE) of fecal coliforms, *E. coli*, heterotrophic bacteria, and presence of the *Listeria* in bottom, middle and top compost pile locations.

Treatment[**]-sample section	Days of composting				Days of composting			
	0[†]	7	14	28	0	7	14	28
	Fecal coliforms (CFU g^{-1} dw)				*E. coli* ERRC (CFU g^{-1} dw/MPN g^{-1} dw)			
1-B[*]	8.85 ± 0.04	7.32 ± 0.09	5.80 ± 0.11	5.24 ± 0.20	9.06 ± 0.05	7.37 ± 0.09	5.74 ± 0.17	5.53 ± 0.19
1-M		4.04 ± 0.13	3.89 ± 0.07	4.88 ± 0.08		4.04 ± 0.13	3.89 ± 0.07	4.88 ± 0.08
1-T		4.15 ± 0.12	3.81 ± 0.01	4.94 ± 0.09		4.15 ± 0.12	3.81 ± 0.01	4.94 ± 0.09
2-B		1.22 ± 1.73	2.85 ± 1.23	3.41 ± 0.15		<2.77/<1.66	<2.77/<1.66	<2.77/<1.66
2-M		<2.77[‡]	<2.77	3.15 ± 0.16		<2.77/<1.66	<2.77/<1.66	<2.77/<1.66
2-T		<2.77	<2.77	3.50 ± 0.11		<2.77/<1.66	<2.77/<1.66	<2.77/<1.66
3-B		<2.77	4.11 ± 0.01	<2.77		<2.77/<1.66	<2.77/<1.66	<2.77/<1.66
3-M		<2.77	<2.77	<2.77		<2.77/<1.66	<2.77/<1.66	<2.77/<1.66
3-T		<2.77	<2.77	<2.77		<2.77/<1.66	<2.77/<1.66	<2.77/<1.66
4-B		4.95 ± 0.66	<2.77	4.74 ± 1.59		<2.77/<1.66	<2.77/<1.66	<2.77/<1.66
4-M		<2.77	<2.77	<2.77		<2.77/<1.66	<2.77/<1.66	<2.77/<1.66
4-T		<2.77	<2.77	<2.77		<2.77/<1.66	<2.77/<1.66	<2.77/<1.66
	Heterotrophic bacteria (CFU g^{-1} dw)				*Listeria monocytogenes* [§] (CFU g^{-1} dw or presence)			
1-B	9.76 ± 0.05	9.60 ± 0.05	8.79 ± 0.01	9.22 ± 0.19	6.62 ± 0.23	+	+	+
1-M		9.22 ± 0.19	9.38 ± 0.15	9.47 ± 0.12		–	–	–
1-T		9.27 ± 0.13	9.32 ± 0.05	9.44 ± 0.05		–	–	–
2-B		8.04 ± 0.66	8.60 ± 0.42	9.32 ± 0.13		+	–	–
2-M		7.55 ± 0.04	9.88 ± 0.06	9.11 ± 0.02		–	–	–
2-T		7.37 ± 0.31	9.70 ± 0.16	10.28 ± 0.03		–	–	–
3-B		8.88 ± 0.04	9.72 ± 0.09	9.70 ± 0.16		–	–	–
3-M		7.72 ± 0.03	9.74 ± 0.03	9.94 ± 0.07		–	–	–
3-T		8.88 ± 0.12	10.19 ± 0.07	10.00 ± 0.03		–	–	–
4-B		4.95 ± 0.62	9.76 ± 0.02	9.43 ± 0.06		–	–	–
4-M		7.42 ± 0.01	9.81 ± 0.07	9.06 ± 0.22		–	–	–
4-T		8.47 ± 0.02	10.57 ± 0.07	9.07 ± 0.19		–	–	–

[*] Letters indicate sample locations from which mean values were derived, B. bottom; M: middle; T: top.
[†] Day 0 values shown are for inoculated manure.
[‡] Detection limits: 2.77 log_{10} CFU g^{-1} dw by direct plating, and 1.66 log_{10} MPN g^{-1} dw by microplate MPN.
[§] '+'indicates detection 0.4 CFU g^{-1} dw (=0.5 × 0.8 CFU g^{-1} dw, the detection limit), and '–' indicates no detection, respectively, of *L. monocytogenes* in enrichment broths with 5 ml of 1:10 sample suspensions.
[**] Treatment 1, non-amended manure pile; treatment 2, manure pile placed on 15 cm thick 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 15 cm thick layer of straw.

section samples of treatment 1 by day 7 (Fig. 3A). Populations of fecal coliforms in the middle and top sections of the stacked manure in treatment 1, while approximately 3 log_{10} units less than those from the bottom section in the piles, were still detectable at all time points (Table 2). In contrast, populations of fecal coliforms in the middle and top pile sections in treatments 2, 3, and 4 were undetectable at day 7 (Table 2). Pile temperatures in treatment 1 were not sustained long enough at >45 °C by day 7 to inactivate fecal coliforms (Haug, 1993). Substantial depopulation of fecal coliforms and *E. coli* occurred in bottom sections of treatment 2, 3, and 4 (Fig. 3B–D) and were below the detection limits by direct plating and MPN procedures in the middle, and top sections of treatments 3 (straw base and straw-manure mix) and 4 (straw base, straw cover, and straw-manure mix) at day 7 (Table 2). However, fecal coliform populations gradually increased in bottom section of treatment 2 (Fig. 2), and by day 28, were detected in the middle and top sections as well (Table 2). Overall, the lowest mean concentrations of fecal coliforms at top, middle, and bottom sections by day 28 were recorded for treatment 4 (1.53 log_{10} CFU g^{-1} dw) and this corresponded to the greatest sustained overall pile temperatures at top, middle, and bottom sections (Fig. 2). Only treatments 3 and 4, i.e., piles in which manure was mixed with straw and with insulative layers, achieved fecal coliform populations by day 28 that were below the limit of 3 log_{10} CFU g^{-1} for composted biosolids (US EPA, 2003). This value is also used by many agencies and producers for composts produced from a variety of residuals that do not contain biosolids.

Concentrations of *E. coli* ERRC and salmonellae were undetectable at 7, 14, and 28 days for the bottom sections (with the coolest mean temperatures) in treatment 2 piles, and all sections in treatments 3 and 4. The latter two treatments correspondingly achieved overall high temperatures (>50 °C) for more than 3 days, which are sufficient to inactivate these bacteria (Burge et al., 1982; Haug, 1993). *Salmonella* Senftenberg, unlike *Salmonella* Typhimurium, survived for 28 days at the bottom section of treatment 1 decreasing from an initial concentration of 8.43 ± 0.44 to 5.02 ± 0.06 log_{10} CFU g^{-1} dw. *Salmonella* Typhiumurium populations decreased from 8.55 ± 0.86 to 5.33 ± 0.21 by day 14. Both salmonellae strains were not detected by direct plating or by MPN by day 7 at all other pile sections in treatments 2, 3, and 4 (Fig. 1).

Populations of aerobic heterotrophic bacteria decreased somewhat to 7.3–8.8 log_{10} CFU g^{-1} dw at 7 days in treatments 2, 3, and 4 in the middle and top sections before increasing to final concentrations at 28 days that were slightly above initial levels.

L. monocytogenes were detected only in bottom sections of pile treatments 1 and 2 on day 7 and in the bottom section of treatment 1 on days 14 and 28 (data not shown). All other sections and assay dates in straw-amended treatments 2, 3, and 4 corresponded to pile temperatures >45 °C for 3 days and resulted in undetectable levels of *L. monocytogenes.*

Along with temperature, moisture content has a major effect on bacterial survival in composting conditions. The average moisture content of manure in the piles in this study was 71% on day 0 and was maintained at nearly this level in treatments 1 and 2 until day 28. In contrast, for treatments 3 and 4, the average moisture content decreased to 64–67% and 51–65%, respectively, by day 28. None of these moisture contents were within a range that is reported to limit the growth or survival of the bacterial indicators and pathogens in this study.

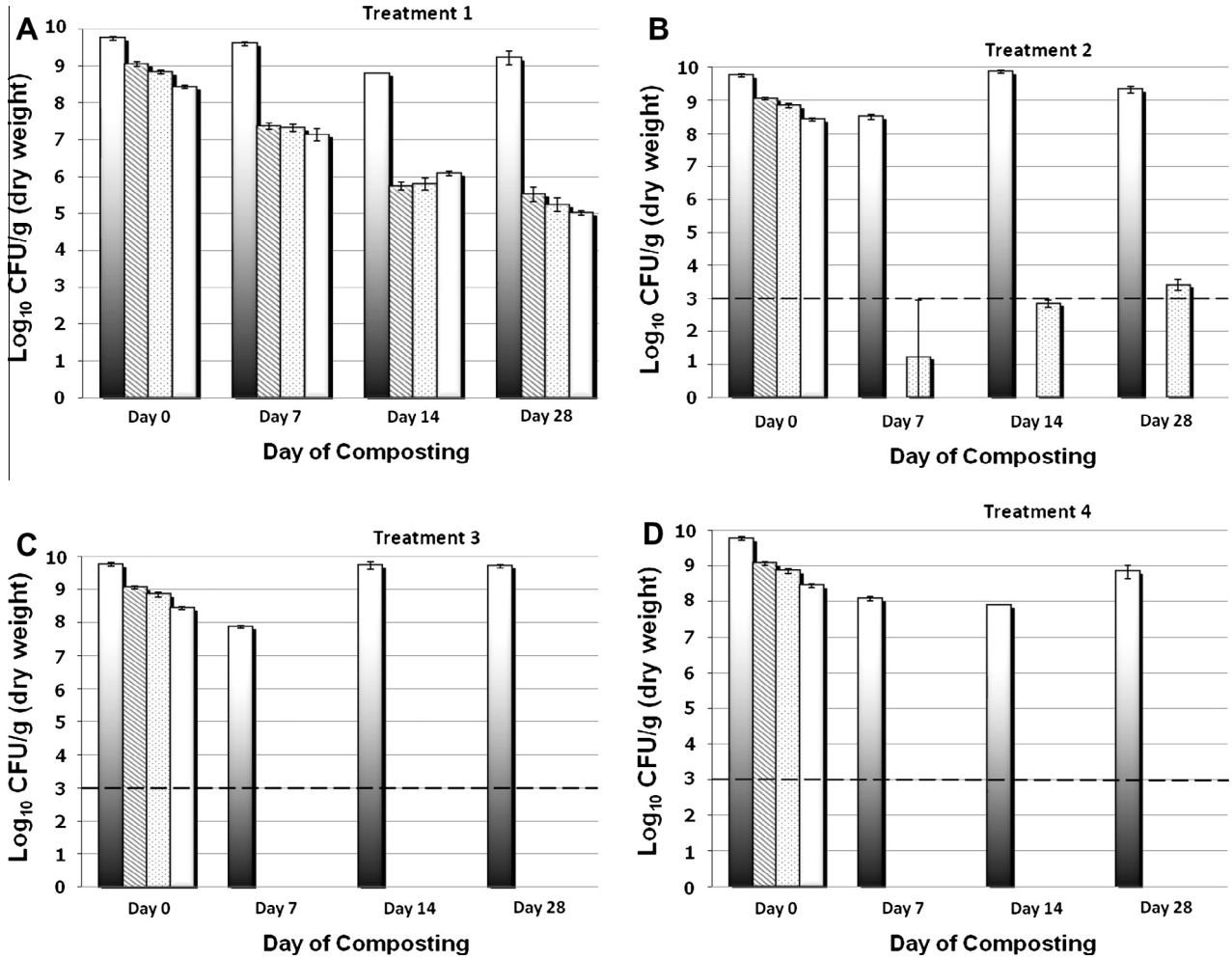

Fig. 3. Survival of bacteria in bottom portion (the lowest temperature portion where pathogen-die-off is least likely) of manure piles during 28 days of self-heating. (A). Treatment 1 (non-amended manure pile); (B). Treatment 2 (manure pile placed on 15 cm thick straw base); (C). Treatment 3 (manure amended with straw (3:1 v/v) and placed on a straw base); (D). Treatment 4 (manure amended with straw (3:1 v/v), placed on a straw base, and covered with a 15 cm thick layer of straw). Mean concentrations of heterotrophic bacteria ( ), fecal coliforms ( ), *E. coli* ERRC ( ), and *Salm.* Senftenberg ( ). Dashed line indicates the US EPA 3.0 $\log_{10}$ upper limit for fecal coliforms for Class A compost. Values are means from duplicate piles. Error bars indicate standard error values.

4. Discussion

The management strategies selected for evaluation were chosen because they use materials readily available in livestock or poultry operations and require minimal changes to existing manure handling practices in terms of operator time and durable inputs. Treatments comprising a progression from ordinary stacked manure piles to piles with enhanced porosity and insulative barriers (straw mix and cover layers) had a corresponding progression of self-heated temperature responses in the bottom, middle, and top sections within the piles. Data from this study show that pile configurations and insulation can extend the thermophilic zones, particularly in the colder bottom and 'toe' portions of piles, during active self-heating periods. The combination of base and cover insulation along with straw incorporation in the feedstock mix also increased the duration and extent of the high temperature, thermophilic phase during the 0–28 day composting period. Surface ambient temperatures and layers just below the outer surface of piles that are not constructed with an insulative layer will not typically heat sufficiently to destroy pathogens or indicator microbial populations (de Bertoldi et al., 1996; Haug, 1993; Shepherd et al., 2011). However, desiccation, ammonia production, and other factors associated with natural attenuation can result in substantial reduction in populations of microbial indicators (e.g., fecal coliforms and *E. coli*) or pathogens. Nonetheless, pathogens may persist at low concentrations in the exterior and base of a stacked compost pile that lacks bottom and exterior insulative materials (de Bertoldi et al., 1996; Haug, 1993; Shepherd et al., 2011). Turning exterior portions into the self-heating pile interior aids exposure of all portions of the mass to destructive temperatures. For this reason, piles either need to be turned periodically or configured to ensure that all raw manure is exposed to time–temperature conditions that will meet acceptable pathogen reduction limits (U.S. EPA, 2000, 2003).

Recent advances in manure management provide additional options for large producers to economically handle manure in an environmentally superior manner (Vanotti et al., 2009). However, stockpiling manure is still common even with increased pressure to reduce gaseous emissions. Although on-farm manure composting is a well-described approach for stabilization of nutrients and reduction of pathogens and odors, results shown here for using straw to amend/insulate manure piles, as well as those using finished compost as cover (Berry et al., 2013; Shepherd et al., 2011) provides animal producers a strategy for minimal management of manure piles that can further reduce pathogens. The exposure time and temperature required to inactivate *Salmonella* in

composting matrices has been reported as 2–3 days at 55 °C in biosolids (Burge et al., 1982; Zaleski et al., 2005), and 1.13 days at 50 °C, and 2.5 days at 38 °C with initial inoculated concentrations of 3.3–8.2 $\log_{10} g^{-1}$ (Ahmed and Sorensen, 1995). Vinneras (2007) reported a decrease from 7.4 CFU $\log_{10} g^{-1}$ to 5 CFU $\log_{10} g^{-1}$ over 5 days for *S.* Typhimurium in a compost reactor treating a mixture of fecal matter and food waste which slowly self-heated to 65 °C over 12 days.

Decimal reduction times for *E. coli* O157:H7 and *Salm.* Typhimurium in a bench-scale cattle manure study ranged from 3.6–8.9 to 1.7–8.4 days respectively, for 37 °C exposures (Himathongkham et al., 1999). Die-off from exposure for 3 days at >45 °C in our outdoor pile study is reasonable given the multiple stressors present in the self-heating manure piles. Ammonia is a stressor likely involved in the rapid decline of *E. coli* and salmonellae during early phases of manure composting, and piles mixed with straw volatilize significant amounts (14–84 mg m^{-3}) of NH_3 in the first 3–7 days of composting (unpublished data, Mulbry). Bovine manure has been reported to release major quantities of ammonia, either stored, composted, or land-applied (Meisinger and Jokela, 2000; Liang et al., 2006). Exposure of heat-stressed cells to NH_3 in manure is likely to increase lethal stress resulting from combined high manure concentrations of CO_3^{-2} and NH_3 (Park and Diez-Gonzalez, 2003). Although *Salmonella* is usually more persistent than *E. coli* in non-host conditions (Winfield and Groisman, 2003), our *Salm.* Senftenberg strain persisted longer than the *Salm.* Typhimurium strain, but less than the *E. coli* strain. The *Salm.* Typhimurium strain used here decreased to undetectable levels even in the bottom sections of treatment 1 piles indicating its greater vulnerability to environmental stressors present in an unamended, poorly self-heating area of the manure pile.

In conclusion, our data show that use of straw to insulate the base and exterior of bovine manure piles and to incorporate into the feedstock mixture can extend the duration of the self-heating period prior to its gradual decline to mesophilic (<45 °C) temperatures over 28 days. In addition, these low input practices can expand the thermophilic sections such that the tendency for the bottom areas to remain cool are diminished to the point that fecal coliforms, *E. coli*, salmonellae and *Listeria* populations can be reduced very substantially during 28 days. These results are consistent with those reported by other researchers who have reported on use of surface covering of piles (Shepherd et al., 2011) and pile base and surface covering in cold winter environments (Berry et al., 2013). The insulative barrier at the base of the manure pile can be particularly helpful when ambient base temperatures are very low relative to pile temperatures. However, for static pile composting or manure stacking, turning of piles is a well-documented means of stimulating another cycle of self-heating (Haug, 1993) that will aid further depopulation of fecal indicator bacteria and pathogens such as salmonellae, and during subsequent curing phase fosters much needed stabilization of manure for beneficial uses (de Bertoldi et al., 1996). Data here show that minimally-managed composting of animal manure that includes use of base and cover insulative layers as well as a porous feedstock mixture can self-heat adequately to reduce pathogens substantially. Nonetheless, in general, to achieve a product of value in horticultural applications and to avoid adverse environmental effects from the process or product, the stability and maturity of the final compost needs to be considered. Overall factors for consideration include careful siting of the composting operation, management of leachate/runoff and the process appropriately to avoid odor emissions, and provision for an extended curing period to ensure stability and maturity and additional pathogen reduction. The source of manures and their variability in terms of age, any pre-treatment, including storage or intentional anaerobic digestion prior to inclusion in compost feedstocks are likely to impact the bacteria pathogen load and therefore should also be taken into account when planning and designed a minimal management approach for composting manure.

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Mention of a trade name, vendor, proprietary product, or specific equipment is not a guarantee or a warranty by the USDA, and does not imply an approval to the exclusion of other products or vendors that also may be suitable.

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