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**Research Paper**

# Differential Survival of *Escherichia coli* and *Listeria* spp. in Northeastern U.S. Soils Amended with Dairy Manure Compost, Poultry Litter Compost, and Heat-Treated Poultry Pellets and Fate in Raw Edible Radish Crops

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## ABSTRACT

Composted or heat-treated biological soil amendments of animal origin (BSAAOs) can be added to soils to provide nutrients for fresh produce. These products lower the risk of pathogen contamination of fresh produce compared with the use of untreated BSAAOs; however, meteorological conditions, geographic location, and soil properties can influence the presence of pathogenic bacteria or their indicators (e.g., generic *Escherichia coli*) and allow potential for produce contamination. Replicated field plots of loamy or sandy soils were tilled and amended with dairy manure compost (DMC), poultry litter compost (PLC), or no compost (NoC) over two field seasons and noncomposted heat-treated poultry pellets (HTPPs) during the second field season. Plots were inoculated with a three-strain cocktail of rifampin-resistant *E. coli* (r*E. coli*) at levels of 8.7 log CFU/m$^2$. Direct plating and most-probable-number methods measured the persistence of r*E. coli* and *Listeria* spp. in plots through 104 days postinoculation. Greater survival of r*E. coli* was observed in PLC plots in comparison to DMC plots and NoC plots during year 1 ($P < 0.05$). Similar trends were observed for year 2, when r*E. coli* survival was also greater in HTPP-amended plots ($P < 0.05$). Survival of r*E. coli* depended on soil type, and water potential and temperature were significant covariables. *Listeria* spp. were found in NoC plots, but not in plots amended with HTPPs, PLC, or DMC. Radish data demonstrate that PLC treatment promoted the greatest level of r*E. coli* translocation compared with DMC and NoC treatments ($P < 0.05$). These results are consistent with findings from studies conducted in other regions of the United States, and they inform northeast produce growers that composted and noncomposted poultry-based BSAAOs support greater survival of r*E. coli* in field soils. This result has the potential to affect the food safety risk of edible produce grown in BSAAO-amended soils as a result of pathogen contamination.

## HIGHLIGHTS

- *E. coli* survived better in soil amended with poultry- versus dairy-based amendments.
- Compost amendment affected soil moisture, which indirectly affected *E. coli* survival.
- *E. coli* dispersal on radishes was greater with poultry litter than with dairy manure compost.

Key words: Biological soil amendments of animal origin; *Escherichia coli*; *Listeria*; Produce safety rule

Biological soil amendments of animal origin (BSAAOs) are materials including manure or other nonfecal by-products that include cattle manure, poultry litter, swine slurry, or horse manure *(28)*. BSAAOs play an important role in providing nutrients to improve soil and produce quality; they are also a potential source of pathogenic microorganisms *(24)*. A primary focus of the Food Safety Modernization Act (FSMA) is to prevent foodborne illness. Exercising caution, the U.S. Food and Drug Administration (FDA) recommended 120 or 90 days between BSAAOs and consumption of produce with the edible portion having or not having direct soil contact, respectively *(30)*. Since then, the FSMA has adopted the compost guidelines established by the U.S. Department of Agriculture National Organic Program (NOP) standards for handling BSAAOs. Therefore, the FDA recommends application of FSMA-compliant compost to soils instead of raw manure. The NOP requires thermophilic compost to reduce pathogens in compost. Specifically, thermophilic-phase conditions are achieved with (i) static composting in an oxygenated environment that achieves 55°C (131°F) for 3 days, followed by curing with proper insulation, or (ii) turned composting in an

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aerobic environment that achieves 55°C (131°F) for 15 days throughout five turnings, followed by curing *(25, 29)*. Current FDA guidance also suggests that no interval (0 days) is needed between the application of treated (including composted and heat-treated) BSAAOs to crops, revising its previous 45-day application interval for composts that are treated properly, with the understanding that compost is a BSAAO that provides a reduced public health risk compared with the use of raw (untreated) manure *(28, 30)*.

Foodborne pathogens of concern found in BSAAOs include enterohemorrhagic *Escherichia coli, Salmonella, Campylobacter jejuni, Cryptosporidium parvum,* and *Listeria monocytogenes (7, 24)*. The FDA established microbiological limits for detectable amounts of bacteria (including *L. monocytogenes, Salmonella* spp., fecal coliforms, and *E. coli* O157:H7) for processes used to treat biological soil amendments, including manure *(29)*. The Produce Safety Rule specifies that biological amendments that undergo a physical (thermal), chemical, or combined process must comply with the microbial standards under Final Produce Rule §§112.54(b) and 112.55(b) subparts, where it is specified that biosolids must contain less than 1,000 most probable number (MPN) per gram of fecal coliforms for BSAAOs that are treated and that no *L. monocytogenes* may be detected in any 5-g (or 5 mL for a liquid) analytical sample. *Salmonella* spp. cannot be detected above 3 MPN/4 g of total solids dry weight (gdw), and *E. coli* O157:H7 cannot be detected above 0.3 MPN/g of analytical portion *(29)*.

The FDA seeks data to better understand the association between human illness and produce consumption from growing areas amended with composted and noncomposted BSAAOs that are potentially contaminated with enteric pathogens (*E. coli* O157:H7 or *Salmonella*). Therefore, the impact of different agricultural or ecological conditions and interventions that include use of a time interval or intervals between application of composted and postinoculation BSAAOs and harvest of edible crops needs to be evaluated *(31)*. The present study was conducted to compare the survival of nonpathogenic *E. coli* and indigenous *Listeria* spp. in soils amended with (i) dairy manure and poultry litter composts, (ii) composted and noncomposted poultry manure–based amendments, and (iii) assessment of their impact on edible produce related to food safety risk.

## MATERIALS AND METHODS

**Field experimental design.** Two field trials were conducted in South Burlington, VT (44°26′37.4″N, 73°11′23.2″W), from May to September in 2016 (year 1) and 2017 (year 2) using replicated field plots (2 by 1 m). The Lilac field (field A) consisted of a Hinesburg B fine sandy loam soil (sand 60%, silt 10%, and clay 30%) with a pH of 6.4, an organic matter content of 2.9%, and a 3 to 8% slope. The Wheelock field (field B) consisted of an Adams B loamy sand soil (sand 40%, silt 40%, and clay 20%) with a pH of 6.3, an organic matter content of 2.6%, and a 5 to 12% slope *(12, 16)*. No shade was present over these fields.

Treatments were arranged in a randomized complete block design. Year 1 and 2 treatments were replicated four and five times per treatment, respectively, for $n = 16$ and $n = 20$ plots. Individual 2-$m^2$ plots (2 by 1 m) were separated by 1.5-m (5-ft) alleyways to avoid border interference. These plots were tilled and amended with the following treatments: (i) no compost and no rifampin-resistant *E. coli* (r*E. coli*; negative control); (ii) no compost with r*E. coli* (positive control for *E. coli*); (iii) dairy manure compost and r*E. coli* (DMC); (iv) poultry litter compost and r*E. coli* (PLC); and (v) heat-treated poultry pellets with r*E. coli* (HTPPs) treatment (only in year 2) *(16)*. Composts and HTPPs tested negative for resident *E. coli* and *Listeria* spp. before application to plots *(16)*. For all treatments, composts were first added to soils, followed by r*E. coli* inoculation and subsequent tilling. The r*E. coli* inoculum ($10^6$ CFU/mL) *(22)* was dispensed using a battery-powered backpack sprayer (Hudson Never Pump, Eldora, IA) at a rate of 1 L per plot. The rototiller blades were sanitized with 75% ethanol between tiling each treatment as specified by Neher et al. *(16)*. PLC, DMC, and HTPPs were applied at a rate of 30,038.8 kg/ha (13.4 tons/acre), 15,063.2 kg/ha (6.7 tons/acre), and 14,940 kg/ha (6.7 tons/acre), respectively. These application rates were added based on total nitrogen (N) per hectare and are comparable to recommendations for vegetable production (100 lb of N per acre, converted to tons per acre and then to metric tons per hectare). Composts were spread evenly on plot surfaces. Detailed nutrient analysis and microbial community properties are presented elsewhere *(16)*.

**r*E. coli* inoculum preparation.** The r*E. coli* inoculum was prepared *(17, 25)* with three strains of generic, nonpathogenic, r*E. coli* (TVS 353, TVS 354, and TVS 355), as noted in previous field studies *(16, 22)*. Use of r*E. coli* strains in this experiment allowed differentiation from generic *E. coli* (gEc) in soils. Tomás-Callejas et al. *(27)* initially isolated the r*E. coli* strains, which were provided by the Environmental Microbial Food Safety Laboratory at the Beltsville Agriculture Research Center in Beltsville, MD. TVS 353 was isolated from irrigation water, TVS 354 was isolated from romaine lettuce surfaces, and TVS 355 was isolated from lettuce production soil in the Salinas Valley area *(5)*. Individual colonies of each r*E. coli* strain, grown on tryptone bile X-glucuronide (TBX) agar (Neogen Corp., Lansing, MI) supplemented with 80 μg/mL rifampin (TBXR; Sigma-Aldrich, St. Louis, MO), were inoculated separately into tryptic soy broth containing 80 μg/mL rifampin (TSBR) and incubated at 37°C for 24 h.

**Preparation of dairy manure extract.** Manure was collected from a local dairy farm and was added at a 1:10 dilution of double-distilled water ($ddH_2O$; 100 g of manure to 900 mL of $ddH_2O$) in a large (2-L) Nalgene bucket. Manure was massaged manually to remove large intact material. This solution was stirred for 5 min. Sanitary cheesecloth was used to hand squeeze solids out of the extract to be used, collecting approximately 75% the input water volume. This extract was transferred to a clean carboy of equal parts $ddH_2O$ for 3 L of diluted extract (1:2) per carboy, which was then sterilized by autoclaving at 121°C for 60 min.

**Preparation of the bacterial inoculum.** Three strains of r*E. coli* (TVS 353, TVS 354, and TVS 355) were cultured separately in 100 mL of TSBR at 37°C with agitation for 24 h. Each 100-mL culture was added to the 3-L carboy containing dairy manure extract, shaken, incubated at 37°C, and then stored at 4°C for no longer than 48 h. *E. coli* levels were enumerated by plating 100 μL of the cultures or serial dilutions onto TBXR and incubating plates at 37°C for 24 h. Appropriate volumes of each strain were diluted in sterile water to bring the level of each strain to $3.33 \times 10^5$ CFU/mL. The level of the final inoculum (all three strains) was ca. $1 \times$

$10^6$ CFU/mL, and 1 L was sprayed per plot using a backpack sprayer (Hudson Never Pump), resulting in a total inoculum of 2.5 $\times$ $10^8$ CFU/$m^2$ *(16)*.

**Sampling procedure.** Soil samples were collected 0, 1, 3, 7, 14, 28, and 56 days postinoculation (dpi) and a subsequent month thereafter. Three core soil samples removed from random locations within each treatment plot on each sampling day were combined into a single sterile Whirl-Pak bag. Each core sample was removed from a depth 15 cm below the soil surface, and sample stakes were placed at the sampled areas to ensure that soil would not be resampled in that location. Samples were transported to the laboratory and hand massaged for 30 s to homogenize each subsample thoroughly. Thirty grams of each composite soil sample was removed and suspended in 120 mL of sterile buffered peptone water (BPW) to achieve a 1:5 dilution (w/w). These diluted samples were homogenized before further processing. The percentage of moisture was determined gravimetrically (grams of water per gram of dry soil) for each soil sample that was collected to standardize enumeration based on soil moisture.

**Enumeration and microbial analysis of samples.** Enumeration and enrichment methods were similar to those described by Lekkas et al. *(12)* and Sharma et al. *(22)*. Soil samples from control plots (no r*E. coli* inoculum applied) were processed for enumeration and presence or absence using TBX to detect for indigenous non–r*E. coli*. Enumeration methods were adjusted through time to accommodate lower thresholds of detection as *E. coli* survival declined. Initially, homogenates of soil samples (100 µL) were plated on each of two plates of TBXR to quantify r*E. coli* survival in soil after inoculation. As r*E. coli* survival declined to fewer than 20 CFU/100 µL (the limit of detection), the method was modified to add 1 mL of soil homogenates per TBXR plate. Likewise, we shifted to an MPN method when r*E. coli* survival dropped below 20 CFU/mL. In all cases, plates were incubated at 42°C for 24 h to determine number of CFU per gram of soil.

**MPN method.** For MPN enumeration, 1 mL of homogenate was transferred into 1 mL of double-strength TSBR in the first row of a 48-well plate (8 rows by 6 columns by 5-mL well). The MPN dilution series began with a 1:2 dilution (0.5) and subsequent serial dilutions of $10^{-1}$, $10^{-2}$, $10^{-3}$, $10^{-4}$, and $10^{-5}$. Subsequent rows contained 1.8 mL of single-strength TSBR. Serial dilutions were completed by aliquoting 200 µL of sample and mixing in each well. Blocks were covered with a breathable Easy plate (VWR, Bridgeport, NJ) membrane and incubated at 42°C for 24 h. Each well was then plated on TBXR (r*E. coli*) or TBX (gEc) plates and incubated for 24 h at 42°C. Each dilution was replicated eight times within a plate. MPN statistical computation was completed using an MPN calculator (https://mostprobablenumbercalculator.epa.gov/mpnForm) to determine MPN values for r*E. coli*.

**Bag enrichment.** If r*E. coli* levels from homogenates of soils fell below detection levels of 0.24 MPN/gdw *(22),* then bag enrichment was used to enrich r*E. coli* in BPW. The bag enrichment method followed the protocol described under "Sampling procedure" with massaging to achieve homogeneity. This sample was then placed into an incubator for 24 h at 42°C, plated onto TBXR (r*E. coli*) plates, and incubated for another 24 h at 42°C.

***Listeria* spp. identification.** *Listeria* presence in non–compost-amended soils and compost-amended soils was determined using enrichment methods established by D'Amico and Donnelly *(3)*. Buffered *Listeria* enrichment broth (BLEB) was used for the primary enrichment of soil samples for *Listeria* spp. detection. Thus, 10 g of soil samples was incubated in 90 mL of BLEB for 4 h to enable injured or stressed cells to resuscitate, after which acriflavine (3 mL/L), cycloheximide (5 mL/L), and nalidixic acid (8 mL/L) were added. A secondary enrichment of morpholine propanesulfonic acid–BLEB (MOPS-BLEB) broth was also used. Once the MOPS-BLEB medium was made and cooled to room temperature, acriflavine (3 mL/L), cycloheximide (5 mL/L), and nalidixic acid (8 mL/L) were added to the media before adding 100 µL of the homogenate sample. Subsequently, 100 µL of the mixture was plated onto CHROMagar *Listeria* (DRG International, Springfield, NJ) and incubated for 24 h at 37°C. Confirmation of *Listeria* spp. was completed using CHROMagar *Listeria* identification agar to confirm *L. monocytogenes* from suspect colonies on CHROMagar *Listeria*. Isolated colonies were assayed subsequently using the DuPont Qualicon BAX Q7 system (Hygiena DuPont Qualicon, Wilmington, DE) to detect the presence of *Listeria* spp. Any presumptive *L. monocytogenes* confirmed through culturing methods were isolated and ribotyped using the DuPont Riboprinter Microbial Characterization System.

**Radish sampling methods.** Radishes were chosen as an edible crop for assessment of translocation of pathogens from manure-amended plots during both years. The radish variety was Ping Pong, an organic round white variety (Johnny Seeds, Albion, ME). Radish seeds were planted at 0 dpi in experimental plots by hand broadcasting across all treatments and were allowed to grow to maturity (28 days). Weeds were also allowed to grow within plots to better imitate the plant rhizosphere dynamic on soil communities. Radishes were chosen randomly and aseptically removed from plots between 32 and 53 days after planting and then were transferred to a sterile Whirl-Pak bag *(15)*. Sterile scissors were used to remove the radish leaves, and a 30- to 55-g subsample of only edible roots was added aseptically to a sterile Whirl-Pak bag containing 99 mL of BPW and then hand massaged or shaken. This method was used to enhance removal of bacteria from the radish surface. MPN methods were used to quantify r*E. coli* from each sample. When levels fell below the detection limit by MPN, bag enrichment was completed to determine the presence or absence of r*E. coli* on radishes.

**Soil microclimate monitoring.** Soil temperature and moisture were covariables recorded hourly in each field at 10-cm depths throughout both field trials using the Campbell Scientific 10X dataloggers (Logan, UT). Soil temperatures and water potential were quantified using Thermister probes and Watermark probes, respectively. Water potential (–kPa) was measured to evaluate any association between moisture and biological activity. A –kPa of 0 demonstrates saturated soil, whereas increasingly negative values correspond with increasingly drier soils.

**Statistical analyses.** The effects of compost treatment on the survival of r*E. coli* in soil or on radish surfaces were determined by one-way repeated measures analysis of covariance with soil temperature and water potential as covariables. Linear regression was performed to quantify the ability of compost treatments to predict the survival of *E coli* in soil or radish surfaces. Analysis of variance (ANOVA) and Bonferroni post hoc tests were performed to compare slope and intercept coefficients among treatment combinations. Chi-square analysis was used to determine the significance for bag enrichment results. IBM SPSS Statistics Chi 24 software was used for the analysis of covariance, correlation,

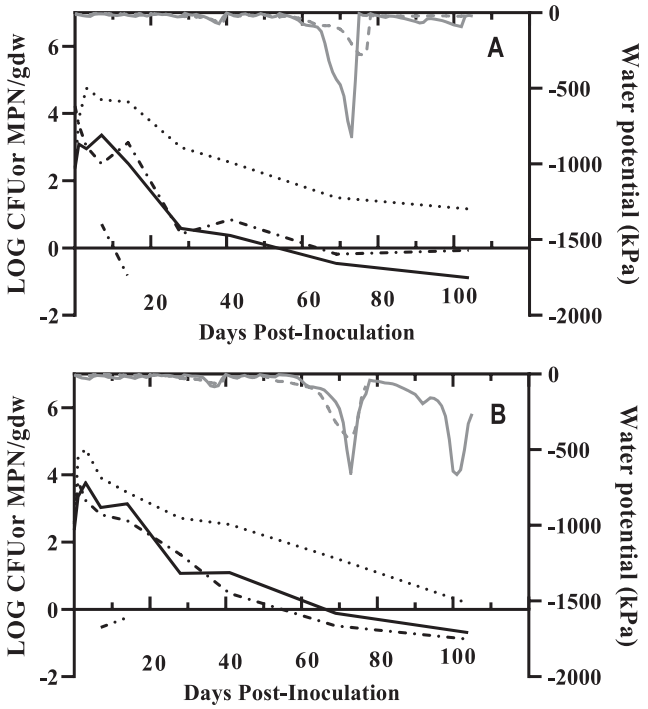


FIGURE 1. *Abundance of rifampin-resistant* E. coli *through time (left* y *axis) and water potential through time (gray lines, right* y *axis) in 2016. Mean abundance (*n = *5) is expressed as either CFU or the most probable number per gram of dry soil. Two separate fields (A and B) are illustrated as different panels, with treatments depicted by line style (solid,* E. coli *without compost; dot-dash,* E. coli *with dairy manure compost; dotted,* E. coli *with poultry litter compost; double dot-dash in the low left, no* E. coli *and no compost). Water potential was measured at both a 2-cm depth (solid line) and a 10-cm depth (dashed lines). Values of 0 represent saturation, and increasingly negative values represent progressively drier soil conditions.*

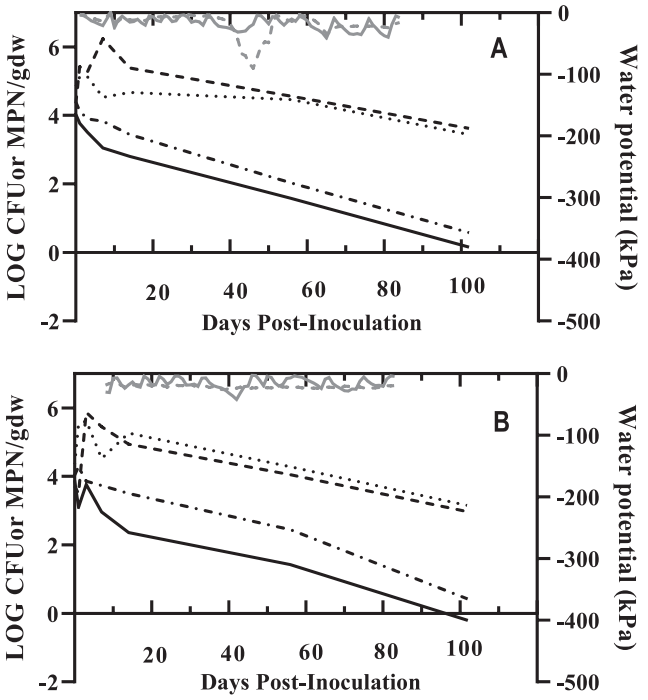


FIGURE 2. *Abundance of rifampin-resistant* E. coli *through time (left* y *axis) and water potential through time (gray lines, right* y *axis) in 2017. Mean abundance (*n = *4) is expressed as either CFU or the most probable number per gram of dry soil. Two separate fields (A and B) are illustrated as different panels, with compost treatments depicted by line style (solid,* E. coli *without compost; dot-dash,* E. coli *with dairy manure compost; dotted,* E. coli *with poultry litter compost; dashed,* E. coli *with non-composted, heat-treated poultry pellets). Water potential was measured at both a 2-cm depth (solid line) and a 10-cm depth (dashed lines). Values of 0 represent saturation, and increasingly negative values represent progressively drier soil conditions.*

and chi-square analyses. Tests for normality were performed before running statistical analyses.

## RESULTS

**Effects of compost on r*E. coli* survival in soils.** Regardless of soil composition or treatment, r*E. coli* levels declined through time. Generally, survival of r*E. coli* remained greater in soils amended with HTPPs and PLC than in DMC and NoC plots (Figs. 1 and 2). Initial r*E. coli* populations ranged from 2.5 to 4.5 log CFU/gdw across all treatments when inoculated on day 0. Populations of r*E. coli* increased slightly at 3 dpi in both fields and then declined during year 1 and year 2 trials over 104 and 102 dpi, respectively. During year 1, PLC, DMC, and NoC treatments demonstrated declines of 1.21 to 1.07, 4.29 to 3.42, and 3.29 to 3.05 log CFU/gdw, respectively. Year 2 trials showed similar trends, in which HTPP, PLC, DMC, and NoC treatments demonstrated declines of 1.69 to 1.24, 4.36 to 2.52, 3.85 to 3.82, and 4.88 to 4.74 log CFU/gdw, respectively. During year 1, at 104 dpi, inoculated r*E. coli* populations were higher in PLC plots compared with DMC plots and NoC plots ($P < 0.01$; Fig. 1). Similar trends were observed for year 2, when at 102 dpi, inoculated r*E. coli* survival was higher in HTPP plots and PLC plots compared with DMC plots and NoC plots ($P < 0.01$; Fig. 2). During both years, r*E. coli* levels in PLC-amended soils in field A (Hinesburg B sandy loam soil) did go below the detection threshold, whereas *E. coli* levels in field B (Adams B loamy sand soil) did not. Although both PLC and HTPP treatments allowed slower rates of decline of r*E. coli* in soil compared with NoC and DMC treatments, levels of r*E. coli* in soils amended with each treatment varied with the year of the study (Table 1).

A total of 38 soil samples fell below a detection threshold of r*E. coli* and required bag enrichment beyond 102 dpi (year 1) to 104 dpi (year 2). Of these 38 samples, 19 and 14 samples were NoC and DMC treatments, respectively. Of these NoC and DMC samples that fell below the detection threshold and required bag enrichment, 17 samples (89.5%) and 12 samples (85.7%) were positive for r*E. coli* (Table 2). Most PLC and HTPP samples allowed direct enumeration of r*E. coli*; therefore, only four PLC samples and one HTPP sample required bag enrichment. In contrast, no samples (0%) and one sample (100%) fell below the detection threshold and required bag enrichment in PLC and HTPP samples, respectively.

TABLE 1. *Linear regression equations of rifampin-resistant* E. coli *enumeration results from soil and radish samples processed during year 1 and year 2 summer trials*

| Independent variable | Treatment | Dependent variables, $y =$ Slope (a) | Intercept (b) |
|---|---|---|---|
| Soil: log CFU/gdw and MPN/gdw[a] | NoC A[b] | −0.037 ± 0.003* | 3.101 ± 0.101* |
| | DMC B | −0.032 ± 0.003* | 3.330 ± 0.099* |
| | PLC C | −0.027 ± 0.002* | 4.208 ± 0.098* |
| | HTPPs D[c] | −0.021 ± 0.004* | 4.736 ± 0.147* |
| Radishes[d]: log MPN/sample[a] | NoC A | −0.008 ± 0.027 | 0.105 ± 1.103 |
| | DMC A | −0.027 ± 0.016 | 1.421 ± 0.682* |
| | PLC B | 0.051 ± 0.016* | −0.732 ± 0.682 |

[a] Linear models: one-way ANOVA, Bonferroni alpha (* $P < 0.05$). gdw, grams dry weight; MPN/gdw, most probable number per gram dry weight; NoC, no compost; DMC, dairy manure compost; PLC, poultry litter compost; HTPPs, heat-treated poultry pellets.
[b] Different letters indicate statistically significant differences between treatments.
[c] HTPP-amended soils were not part of the study during year 1.
[d] No radishes were planted in HTPP-amended soils during year 1.

**Effects of environmental factors on enumeration and bag enrichment r*E. coli* results in soils.** Survival of r*E. coli* depended on soil type, and water potential and temperature were significant covariables that influenced r*E. coli* levels (CFU and MPN per gram). These environmental factors associated with HTPPs and PLC significantly contrasted with DMC and NoC treatments ($P < 0.01$; Tables 3 and 4), confirming the importance of collecting these measures as covariables.

***Listeria* spp. recovery from soil and crop samples.** No *Listeria* spp. were detected in any compost soil treatment sample tested, with the exception of a single *L. innocua* isolate detected in a loamy field (field B) control plot (without compost and without r*E. coli*).

TABLE 2. *Chi-square analysis of rifampin-resistant* E. coli *detection by bag enrichment (BE) from soil and radish samples processed during year 1 and year 2 summer trials and the day BE was first performed*

| Independent variable | Treatment[a] | Presence (+)/ total samples (%) | First sampling day requiring BE |
|---|---|---|---|
| Soil: BE/gdw[b] | NoC | 17/19 (89.5) | 108 (year 1)<br>56 (year 2) |
| | DMC | 12/14 (85.7) | 69 (year 1)<br>56 (year 2) |
| | PLC | 0/4 (0) | 109 (year 1)<br>No BE (year 2) |
| | HTPPs[c] | 1/1 (100) | 112 (year 2) |
| Radishes[d]: BE/radish sample | NoC | 21/27 (77.8) | 38 (year 1) |
| | DMC | 2/6 (33.3) | 32 (year 2) |
| | PLC | 4/6 (66.7) | |

[a] NoC, no compost; DMC, dairy manure compost; PLC, poultry litter compost; HTPPs, heat-treated poultry pellets.
[b] BE data were found to be statistically significant; * $P = 0.001$. Chi-square analysis was used to determine statistical significance.
[c] HTPP-amended soils were not part of the study during year 1.
[d] No radishes were planted in HTPP-amended soils during year 1.

**Effects of composts and environmental factors on *E. coli* contamination of edible crops.** The PLC treatment promoted greater survival of r*E. coli* on radish than did DMC and NoC treatments (Fig. 3). A significantly greater amount of r*E. coli* was recovered from radishes grown in PLC-amended soils on 46 and 53 dpi, compared with 38 and 32 dpi during years 1 and 2, respectively ($P < 0.05$; Fig. 3).

Radish samples required bag enrichment until the final harvest on 102 dpi (year 1) or 104 dpi (year 2; Table 2). Of the 27 NoC radish samples that fell below the detectable threshold and required bag enrichment, 21 samples (77.8%) were positive for r*E. coli*. However, of the six samples that required bag enrichment for both DMC and PLC treatments, only two samples (33.3%) and four samples (66.7%) were positive for r*E. coli,* respectively.

## DISCUSSION

In the present study, there is a reduced risk of r*E. coli* survival in dairy- versus poultry-based compost amendments and a reduced risk in composted versus heat-treated poultry manure amendments. Edible produce grown in soils amended with NOP-approved composted products pose minimal risk of foodborne pathogens surviving. Results of the present study provide realistic field conditions and are congruent with previous findings that were more conservative in their methodological approach *(7, 10, 11, 15, 16, 22)*. However, many of these studies applied large doses (ca. $10^8$/g) of pathogens or indicator organisms to soils, which may not represent conditions encountered by growers during routine growing practices *(10)*. Survival studies determined that dairy manure amendments are a primary source of *E. coli* O157:H7 and promote pathogen survival for 42, 56, and 70 days at 37, 22, and 5°C, respectively *(10)*. Similarly, in loamy or sandy soil amended with dairy manure, *E. coli* levels declined by 3 to 3.5 log between day 0 and day 56, respectively *(12)*. Little difference was observed in *E. coli* O157:H7 persistence based on compost type alone. However, higher levels of gEc and attenuated *E. coli* O157:H7 (2.84 to 2.88 log CFU/g [dry weight]) were recovered from poultry litter–amended soils compared with

TABLE 3. *Linear regression equations of rifampin-resistant* E. coli *enumeration results (log CFU/gdw and MPN/gdw) from soil samples processed and independent variable interactions during year 1 and year 2 summer trials*[a]

| Independent variable | Treatment | Dependent variables, $y =$[b] | |
|---|---|---|---|
| Water potential at a 10-cm depth (−kPa) | NoC A[c] | 0.007 ± 0.002* | 2.430 ± 0.120* |
| | DMC A | 0.011 ± 0.002* | 2.697 ± 0.119* |
| | PLC B | 0.008 ± 0.002* | 3.587 ± 0.116* |
| | HTPPs C[d] | −0.043 ± 0.041 | 4.044 ± 0.349* |
| Temp at a 10-cm depth (°C) | NoC A | −0.055 ± 0.034 | 3.409 ± 0.711* |
| | DMC A | −0.74 ± 0.034* | 4.001 ± 0.706* |
| | PLC B | −0.097 ± 0.033* | 5.411 ± 0.704* |
| | HTPPs C | −0.098 ± 0.046* | 6.025 ± 0.807* |

[a] gdw, grams of dry weight; MPN/gdw, most probable number per gram dry weight; NoC, no compost; DMC, dairy manure compost; PLC, poultry litter compost; HTPPs, heat-treated poultry pellets.
[b] Linear models and interactions: one-way ANOVA, Bonferroni alpha (* $P < 0.05$).
[c] Different letters indicate statistically significant differences between treatments.
[d] HTPP-amended soils were not part of the study during year 1.

levels found in dairy manure–amended soils (0.29 to 0.32 log CFU/gdw) or unamended soils (0.25 to 0.28 log CFU/gdw) *(22)*. These findings confirm other studies that report longer periods of *E. coli* persistence in soils amended with PLC compared with DMC *(16)*.

Although our study did not focus on pathogens such as *E. coli* O157:H7, results of this study demonstrate that r*E. coli* strains have similar survival trends in soils amended with composts and HTPPs *(8, 14, 17, 19, 21)*. Specifically, survival of *E. coli* (TVS 355) is extended in soils containing HTPPs compared with poultry litter amendments in specific seasons, but rainfall and soil moisture content were often important drivers of *E. coli* survival durations in soils *(13)*. In addition, heat-treated and pelletized BSAAOs may have fewer competing microbial species compared with composted poultry litter or dairy manure, which may support greater survival of *E. coli* in soils *(2)*.

Extended survival of gEc and *E. coli* O157:H7 in BSAAOs may be attributed to higher availability of nutrients (e.g., N and phosphorous) accentuated in poultry litter amendments *(23)*. N is a strong driver of *E. coli* survival *(4),* and in manure, 60 to 80% of N is typically in an organic form (i.e., urea and protein) *(11)*. Soil moisture and temperature tend to affect the availability of N, especially in sandy soils *(9, 16),* where $NH_4$-N is converted to $NO_3$-N through nitrification. *E. coli* prefers the $NH_4$-N form, especially when bioavailability is high *(4)*. However, N in the form of $NO_3$-N is more likely to disseminate

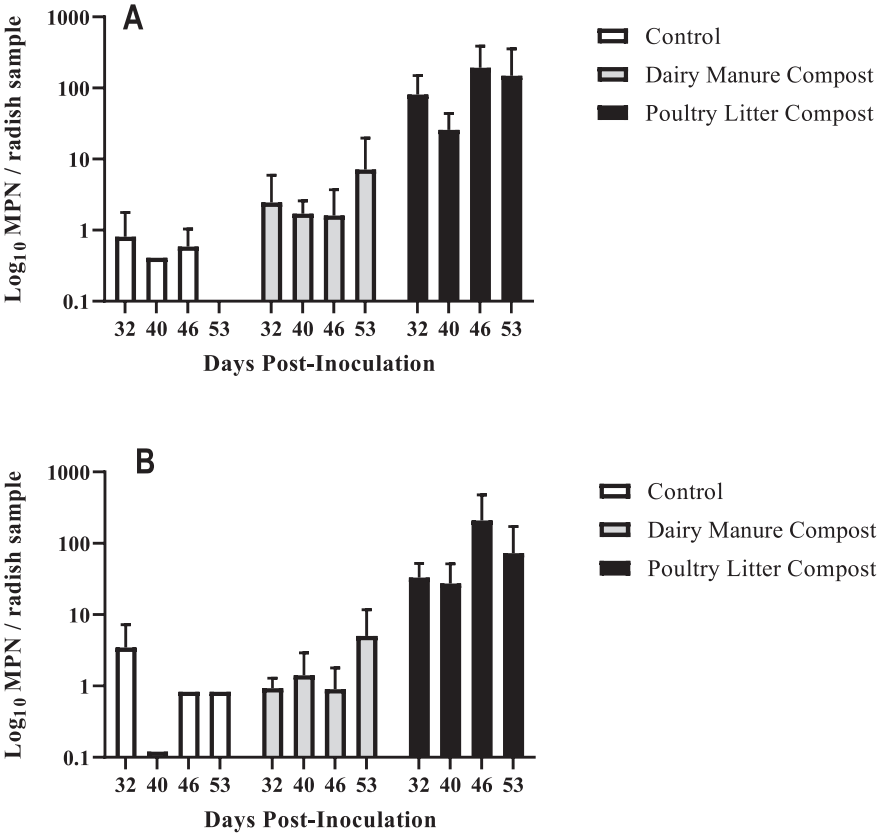


FIGURE 3. *Abundance of rifampin-resistant* E. coli *per radish sample as a function of postinoculation in 2016. Abundance is expressed as the most probable number per gram of radish crop (roots). Two separate fields (A and B) are illustrated as different panels with compost treatments depicted by fill patterns (white, control; gray, dairy manure compost; black, poultry litter compost). Bars represent means (*n = 4*).*

TABLE 4. *Linear regression equations of rifampin-resistant* E. coli *enumeration results (log MPN/gdw) from radish samples processed and independent variable interactions during year 1 and year 2 summer trials*[a]

| Independent variable | Treatment | Dependent variables, $y =$ | |
|---|---|---|---|
| Water potential at a 10-cm depth (−kPa)[b] | NoC A[c] | 0.002 ± 0.008 | −0.172 ± 0.320 |
| | DMC A | 0.014 ± 0.006* | 0.620 ± 0.206* |
| | PLC B | −0.014 ± 0.006* | 1.086 ± 0.206* |
| Temp at a 10-cm depth (°C)[b] | NoC A | −0.033 ± 0.063 | 0.439 ± 1.284 |
| | DMC A | −1.181 ± 0.045* | 4.056 ± 0.943* |
| | PLC B | 0.155 ± 0.045* | −1.809 ± 0.943 |

[a] MPN/gdw, most probable number per gram dry weight; NoC, no compost; DMC, dairy manure compost; PLC, poultry litter compost.
[b] Linear models and interactions: one-way ANOVA, Bonferroni alpha (* $P < 0.05$).
[c] Different letters indicate statistically significant differences between treatments.

because it is more readily available to bind to clay and other organic materials *(16)*. This may explain why total N availability persists for longer periods in poultry litter composts compared with dairy composts. Higher nutrient content in poultry litter amendments is likely contributing to the extended survival of gEc and *E. coli* O157:H7 *(24)*. Furthermore, $NO_3$ concentration measured on day 30 of a 120-day field trial predicts *E. coli* survival duration in plots amended with PLC, HTPPs, poultry litter, or chemical fertilizers *(13)*.

*E. coli* survival on produce may also vary based on environmental conditions, such as temperature, humidity, and season, which are likely associated with *E. coli* survival on preharvested produce *(13, 18, 31)*. Pathogen concentration, plant and leaf age, physical damage of the growing crop, and epiphytic bacteria are all correlated with produce contamination *(18)*.

Excess moisture promotes *E. coli* survival through the creation of an anaerobic environment that allow a facultative anaerobe such as *E. coli* to continue metabolic respiration *(26)*. Therefore, *E. coli* can take advantage of the competitive circumstances and thrive in the absence of indigenous obligate aerobes *(16)*. As moisture declines and temperature increases during the summer months, *E. coli* cannot retain this competitive survival advantage and abundance falls below detectable thresholds. Less negative water potential values (wetter soils) allow the release and diffusion of nutrients, which likely affect the microbial community by enabling a community shift from strictly aerobes to facultative aerobes *(16)*. In years with less rainfall, *E. coli* survival durations in HTPP- and PLC-amended soils were shorter than in years with more rainfall *(13)*. The association of organic matter, particle size, and distribution all affect die-off rates of *E. coli* as soil moisture exceeds equilibrium in a system when other extrinsic factors (i.e., temperature) are constant *(32)*. The variation in slope between sites and lack of shade may have affected *E. coli* survival because of impacts on moisture retention, causing a slower *E. coli* mortality *(33)*.

We emphasize differences in water potential between plots containing HTPPs and DMC. A controlled experiment demonstrated that HTPP-amended soils had a greater water-holding capacity than DMC- or NoC-amended soil *(20)*. The porosity of the material is fine with many micropores (neck diameter $< 0.6$ μm) relative to the total pore space. Pore size distribution is critical for balancing the growth of microorganisms while maintaining adequate aeration *(1)*. Micropores have a high surface area and, as a result, adsorb water with greater force. The adsorption exhibited by these micropores inhibits water infiltration and drainage during and after irrigation events, an action vital for exchanging oxygen and other gases in and out of soil pore spaces.

The type of soil that may be mixed with the compost also affects the survival and persistence of pathogens through time. In this study, *E. coli* demonstrated longer persistence in clay soils than in sandy or loamy soils, consistent with prior reports *(5, 6, 12, 22)*. Therefore, sandy soils are less favorable for survival of pathogens that are facultative anaerobes.

In conclusion, meteorological conditions, geographic location, and soil properties affect the survival of *E. coli* in compost-amended soils. Although poultry litter compost is ideal for crop utilization because it provides a large pulse of N, this study is consistent with other studies conducted in other regions of the United States that show that composted poultry litter–based BSAAOs and HTPPs support greater numbers and longer periods of persistence in field soils of *E. coli* than composted dairy–based BSAAOs. Furthermore, heat-treated poultry pellets pose a greater food safety risk for raw edible crops than composted BSAAOs. Shiga toxin–producing *E. coli* causes many outbreaks related to produce, and this research suggests that regulators and farmers consider alternative practices when harvesting produce intended for consumption.

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