



Research Paper

Risk Factors Associated with Generic *E. coli* Survival in Soils from Certified Organic Farms

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ABSTRACT

Untreated manure is commonly used as a biological soil amendment of animal origin in organic production but may introduce foodborne pathogens into the farm environment. In this multiregional study, generic *E. coli* was used as an indicator of fecal contamination to identify factors associated with its concentration in manure-amended soils. The analysis included samples from 19 certified organic farms in California, Maine, Minnesota, and Maryland collected over two growing seasons. A zero-inflated linear mixed model was used to assess the association between generic *E. coli* concentration in soil, and farm-related practices, soil and environmental factors. Generic *E. coli* concentration in the soil declined in the first 60 days after manure application (β : -1.00 , CI_{95} : -1.17 , -0.83) before a secondary increase through Day 180. Manure management practices, including use of different types of animal manure and manure application rates, were significantly associated with generic *E. coli* concentration in soil. Soil nutrients (phosphorus (β : 0.22 , CI_{95} : 0.15 , 0.30)) and micronutrients (zinc (β : 0.03 , CI_{95} : 0.01 , 0.04) and manganese (β : 0.02 , CI_{95} : 0.01 , 0.02)), elevated moisture content (β : 0.13 , CI_{95} : 0.07 , 0.19), and *Salmonella* (β : 1.79 , CI_{95} : 1.31 , 2.23) or *Listeria monocytogenes* (β : 0.24 , CI_{95} : 0.03 , 0.44) also showed positive association with generic *E. coli* concentration in soil. Lower wind speed (β : -0.12 , CI_{95} : -0.19 , -0.06), increased precipitation (β : 0.03 , CI_{95} : 0.01 , 0.05), and increased UV index (β : 0.24 , CI_{95} : 0.15 , 0.34) were found to be associated with higher *E. coli* concentration in manured soil. Overall, the persistence of generic *E. coli* in manure-amended soil was multifactorial. Effective microbial risk management should incorporate considerations for animal origin of manure, management, environmental, and meteorological factors alongside current USDA National Organic Program standards (90–120 days) regarding the interval of application of raw manure to harvest of the edible portion of food crops.

Certified organic production often incorporates biological soil amendments of animal origin, such as untreated manure, as organic fertilizer to improve nutrient availability, soil health, and enhance crop growth and yield (Rayne & Aula, 2020). Despite such benefits,

untreated manure may contain foodborne pathogens such as Shiga toxin-producing *Escherichia coli* (STEC) and *Salmonella* spp. (Chen et al., 2019; Ferguson et al., 2023; Lengacher et al., 2010; Pandey et al., 2024). Once applied to the soil, foodborne pathogens can

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survive for prolonged periods, posing increased food safety risks to crops compared to unamended soil (Bardsley et al., 2021; Limoges et al., 2022; Litt et al., 2021; McKenzie-Reynolds et al., 2025; Murphy et al., 2024). Previous studies have established that the survival of such foodborne pathogens is driven by a set of multifactorial contributors, including manure management (e.g., animal origin, storage conditions), adjacent lands, soil-specific properties (e.g., moisture content), and environmental characteristics (e.g., rainfall, temperature) (McKenzie-Reynolds et al., 2025; Sharma et al., 2019). Together, these considerations emphasize the need for a comprehensive evaluation of how farm practices, environmental conditions, and manure applications interact and impact microbial contamination of the soil and preharvest food safety.

Despite substantial research on the survival of pathogens and indicators of fecal contamination following manure application, translating findings into broadly applicable guidance remains challenging. Reported survival patterns and associated risk factors have often been derived from studies conducted within a single geographic region (e.g., multiple sites within the same climatic zone), limited farm cohorts, or controlled inoculation settings rather than open-field observational conditions (Appolon et al., 2026; Cook et al., 2023; Goodwyn et al., 2023). Because microbial persistence is known to vary over time and across environments (Cheong, Jay-Russell, et al., 2024b), findings from such narrowly scoped studies may not be generalized elsewhere, limiting the translation from research findings into mitigation strategies for growers, extension professionals, and stakeholders. This gap is further compounded by methodological challenges that real-world agricultural datasets frequently include missing observations (e.g., field and record-keeping constraints) and an excess of zeros driven by nondetects or limits of quantification (Guseva et al., 2022; Kaul et al., 2017). Failing to account for missing data and such structural and sampling zeros can underestimate the associations and distort inference on key predictors (Lopucki et al., 2022; Nakagawa & Freckleton, 2008). Addressing these limitations requires multiregional data, coupled with analytical approaches that account for inherent data clustering, missingness, and excess zeros in agricultural field studies.

To bridge the knowledge and methodological gaps, the objective of this study was to identify the risk factors associated with generic *E. coli* (as the pathogen indicator) survival in untreated manure-amended soil from certified organic farms across four regions in the United States over two growing seasons. By analyzing multiregional hierarchical data and implementing statistical methods appropriate to handle missing observations and zero-inflation, the overall goal was to inform more targeted risk mitigation strategies across the states and support on-farm decision-making to reduce the risks associated with microbial contamination in manure-amended soil in organic production.

Methods

Study design. Details on farm selection, field sample collection, and microbiological analyses used in this study are described previously (Ramos et al., 2021). In brief, a multiregional cross-sectional study was conducted in 19 organic farms across California (CA; $n = 9$), Minnesota (MN; $n = 5$), Maine (ME; $n = 4$), and Maryland (MD; $n = 1$) over two growing seasons in 2017 and 2018. In 2017, 16 farms were enrolled in the study. In 2018, there were 18 farms. Farm inclusion criteria were: (1) USDA National Organic Program (NOP)-certified organic farms; (2) growing at least one type of the following produce: leafy greens (e.g., lettuce, spinach), fruits and vegetables (e.g., melons, squash, tomatoes, peppers), and root vegetables (e.g., carrots); (3) applying untreated manure, and/or other organic fertilizers (e.g., compost, fish emulsion); (4) voluntary participation, including biological sample collection and farm information disclosure.

Questionnaire. The questionnaires were distributed to farm owners/managers at the end of each growing season (Supplementary Material 1). There were five major categories in the questionnaire: (1) crop production (types of produce grown) and farm demographic (e.g., state, coverage of farms); (2) soil-related information, such as soil type and previous land use; (3) manure application and management, such as animal origin of manure, application timing and rate, methods of application and storage; (4) agriculture water-related information, such as water source and irrigation method; (5) animal-related information, such as presence of wildlife around crop fields and cattle operation proximity from the farms.

Sample collection and microbiological analysis. Soil samples were collected once before manure application (Day 0A) and once immediately after (Day 0B), followed by sampling at 30-day intervals thereafter till 180 days after manure application. Each soil sample consisted of five subsamples, collected from a depth of 15 cm (6 inches) of soil layer. A composite of five untreated manure or compost samples was also collected, and each weighed 90–100 g. Irrigation water samples were collected from the water source between Day 90 and Day 120 after manure application. Generic *E. coli* in soil was enriched in Tryptic Soy Broth (TSB), serially diluted to 10^{-6} with four replicates, and plated onto CHROMagar ECC for enumeration. Generic *E. coli* was quantified as Most Probable Number (MPN) per gram of dry weight (gdw) (EPA MPN Calculator, 2020). To detect the presence of foodborne pathogens (*E. coli* O157:H7, non-O157 STEC, *Salmonella*, and *Listeria monocytogenes*) in soil and manure, samples were enriched in broth (TSB, Listeria Enrichment Broth, and Buffered Peptone Water), immunomagnetically separated (*E. coli* O157:H7 and *L. monocytogenes* only; Invitrogen/Dynal, Carlsbad, CA, USA), isolated on selective media (modified enterohemorrhagic *E. coli*; Biocontrol, Bellevue, WA, USA; ChromSTEC; CHROMagar™, Paris, France; Fraser Broth; BD, Sparks, MD; Brilliance Listeria Agar; Oxoid, Hants, UK; Rainbow Agar O157; Biolog, Hayward, CA, USA; CT-SMAC; BBL™ Sorbitol MacConkey II Agar with Cefixime and Tellurite; Tetrathionate broth and Rappaport-Vassiliadis broth; BD, Sparks, MD, USA; Xylose lysine deoxycholate Tergitol-4; BD Difco, Le Pont de Claix, France). Presumptive positive colonies from culture-based isolation were confirmed by Polymerase Chain Reaction (PCR) assay, screening for *eaeA* (*E. coli* O157:H7), *stx1* and *stx2* (non-O157:H7 STEC), *hlyA* (*L. monocytogenes*), and *invA* (*Salmonella*) (Kawasaki et al., 2010). Generic *E. coli* in irrigation water was enumerated using standard membrane filtration techniques with a tenfold volume series (100 mL to 1 μ L). The concentration was reported in Colony Forming Unit (CFU) per 100 mL. Detailed microbiological processing methods were described in Ramos et al. (2021) and the limit of quantification (LOQ) of generic *E. coli* in soil was 0.089 MPN/gdw, as reported in Cheong, Chandler-Khayd, et al. (2024a).

Data management. Soil samples with a generic *E. coli* concentration below the LOQ were considered to be zero, accounting for 39.0% (946/2,427) of the total. To model the outcome of interest using a zero-inflated linear mixed model, the generic *E. coli* concentration was log-transformed by applying $\log_{10} [\text{MPN} + 1]$. The outcome was then defined as $\log_{10} (\text{MPN} + 1)$ per gdw.

Explanatory variables were categorized into six groups: (1) spatiotemporal characteristics (e.g., farm state, sampling day, sampling season); (2) soil-related information from the questionnaire (e.g., soil type) and laboratory results (e.g., soil moisture content and chemical properties); (3) manure application information from the questionnaire (e.g., animal origin of manure) and presence of foodborne pathogens in manure samples; (4) agricultural water information from questionnaire (e.g., source of irrigation water) and microbiological results (e.g., generic *E. coli* concentration in water); (5) animal-related information from the questionnaire; and (6) meteorological variables (e.g., Ultraviolet index, corrected precipitation) from NASA POWER (NASA, 2024) by matching farm location and sampling date. For each meteorological variable, three aggregated values were

computed: the average on the day of soil sampling, the average over the last three days prior to sampling, and the average over the last seven days prior to sampling. Soil chemical properties such as soil pH, available nutrients (e.g. phosphorus, zinc, and others) were measured by regional laboratories and normalized by centering at the mean and scaling by standard deviation.

Responses from questionnaires with multiple responses were re-categorized as (1) indicator variables (yes or no/presence or absence) such as the use of cattle-originated manure, presence of wildlife in the proximity of the crop fields; or (2) multilevel variables with several subcategories (e.g., manure application season).

Statistical analyses. To minimize data loss and maximize statistical power, two strategies were applied to address observations with missing values: (1) observations that missed continuous variables (e.g., phosphorus, generic *E. coli* concentration in irrigation water) were imputed using the mean within the corresponding state $\times$ season stratum (simple mean imputation); (2) observations that missed categorical variables (indicator or multi-level) were recoded into a created sublevel 'missing'. Feature selection using random forest was performed, and an increase in Mean Square Error (MSE) was evaluated to rank and screen for important variables. Variables with an increase in MSE greater than 10% were considered as candidate predictors for model selection (Lee et al., 2022). A zero-inflated linear mixed model (ZILMM), with farm ID nested within state as the random effect and a constant zero-inflation probability, was used as to assess the association between generic *E. coli* concentration in soil and candidate predictors (Cheong, Jay-Russell, et al., 2024b; Zhang & Yi, 2020). This two-part approach separately modeled (1) the probability of a sample being zero, via a logistic component, and (2) the magnitude of log-transformed concentrations among detected samples, via a linear component.

To avoid the potential multicollinearity problem among correlated variables, once one meteorological variable was selected into the model, other variables of the same type but measured over different time windows (e.g., temperature averaged over 3 versus 7 days) were excluded from the remaining candidate predictors. Starting with an empty ZILMM, a manual forward model selection was applied using Akaike Information Criterion (AIC) as the criterion to assess model fit. The order of adding candidate predictors to the current model was based on the ranking from feature selection. All candidate predictors were assessed, and the final model was selected based on model diagnostics. All statistical analyses and figure plotting were implemented in RStudio (R Core Team, 2022). Data cleaning and visualization were performed using packages 'tidyr' (Wickham et al., 2024), 'dplyr' (Wickham et al., 2023), 'ggplot2' (Wickham, 2016), 'broom.mixed' (Bolker et al., 2024), and preinstalled 'base'. Feature selection using random forest was ranked with the package 'randomForest' (Cutler & Wiener, 2022). Zero-inflated linear mixed modeling and model selection were conducted using packages 'glmmTMB' (Brooks et al., 2025) and preinstalled 'stats'.

Results

Sample collection and generic *E. coli* concentration in soil.

Across the two growing seasons, 2,427 soil samples were collected from 19 farms. Among those, nine farms were in California ($n = 1,064$), five in Maine ($n = 465$), four in Minnesota ($n = 702$), and one in Maryland ($n = 196$). Each farm was visited 6 to 8 times per growing season, with 1 to 24 soil samples collected per visit. The number of samples collected was comparable from Day 30 to Day 120, with fewer samples collected on Day 150 and Day 180 due to the harvest cycle. Of the 2,427 samples, 946 (39.0%) had generic *E. coli* concentrations below the LOQ. Among the samples with detectable concentrations, the average generic *E. coli* concentration was $1.33 \log_{10}$ MPN/gdw. As shown in Figure 1, soil samples from California on

Day 0A presented the highest baseline generic *E. coli* level (0.53 log MPN/gdw) and the greatest variability ($SD = 0.75 \log$ MPN/gdw). Immediately after manure application (Day 0B), generic *E. coli* levels increased independently of farm location, while farms in Minnesota presented the most distinguished peak. Following the peak, generic *E. coli* levels on Day 30 showed a state-varying decline. Beginning on Day 90, generic *E. coli* levels demonstrated a more consistent pattern across the states.

Feature selection with random forest ranking. After considering generic *E. coli* concentration in soil before manure application ($n = 263$) as the baseline concentration, 2,164 data points were included in the analyses. A total of 134 explanatory variables were screened, and the top 31 with an increase in MSE of over 10% were retained as candidate predictors for further analyses. As shown in Figure 2 (showing the top 15 variables; all 31 variables are listed in Supplementary Material 2), the most important variables included soil moisture, sample-related variables (e.g., sampling days after manure application, sampling season), soil characteristics (e.g., baseline generic *E. coli* concentration in soil before manure application), and manure management variables (e.g., manure storage location, manure application season). Other meteorological variables, such as wind speed, temperature, and precipitation over different time frames, were also ranked highly.

Risk factors associated with generic *E. coli* concentration in manure-amended soil. Based on the variable importance ranking, a ZILMM was built to identify the risk factors associated with generic *E. coli* concentration in manure-amended soil. After incorporating Day 0A as the baseline data, 1,934 observations were included and analyzed in a ZILMM with farm ID as the random effect to account for clustering. The estimated variance of farm-level generic *E. coli* concentration in soil was 0.11 log MPN/gdw and the residual variance was 0.72, indicating greater variability within farms than between farms. The intercept of the zero-inflated component of the model was -2.04 ($CI_{95}: -2.33, -1.74$; logit scale $p < 0.001$), indicating a significant, low probability (11.5%) of detecting zero observations when setting all predictors at their reference levels.

Generic *E. coli* concentration in soil varied significantly by state and the time interval between manure incorporation and sampling day (Fig. 3). Compared with the observed means, the adjusted marginal predictions from the zero-inflated linear mixed model showed a different pattern of between-state separation after controlling for other covariates in the model. In the adjusted model, Maryland ($\beta: 1.37$, $CI_{95}: 0.53, 2.20$) and Minnesota ($\beta: 0.83$, $CI_{95}: 0.32, 1.34$) had overall higher predicted generic *E. coli* concentrations compared to California, whereas Maine did not differ significantly from California ($\beta: 0.29$, $CI_{95}: -0.20, 0.79$) (Table 1). Sampling day remained strongly associated with concentration after accounting for other covariates. As shown in Table 1 the lowest coefficient on Day 60 indicated the greatest reduction in the concentration of generic *E. coli* relative to the end of study period ($\beta: -1.00$, $CI_{95}: -1.17, -0.83$). The adjusted coefficient of Day 180 ($\beta: -0.60$, $CI_{95}: -0.82, -0.39$) was higher than that of Day 60, reflecting a secondary increase in generic *E. coli* concentration in soil, and these results were statistically significant.

In the conditional model with fixed effects, soil, manure, and meteorological factors were identified as significant factors influencing generic *E. coli* concentration in soil. Increased soil moisture, higher zinc, phosphorus, and manganese levels in soil, and presence of *Salmonella* and *L. monocytogenes* were significantly associated with increased generic *E. coli* concentration in soil. Every one percent increase in soil moisture was associated with 0.13 log MPN/gdw increase in generic *E. coli* concentration ($\beta: 0.13$, $CI_{95}: 0.07, 0.19$). Similarly, each one-unit (ppm) increase in zinc ($\beta: 0.03$, $CI_{95}: 0.01, 0.04$) and manganese ($\beta: 0.02$, $CI_{95}: 0.01, 0.02$) concentration was associated with a 0.03 and 0.02 log MPN/gdw increase of generic *E. coli* in soil, respectively. Presence of foodborne pathogens in soil, such as *Salmonella* ($\beta: 1.79$, $CI_{95}: 1.31, 2.23$) and *L. monocytogenes* ($\beta: 0.24$, $CI_{95}: 0.03, 0.44$) were

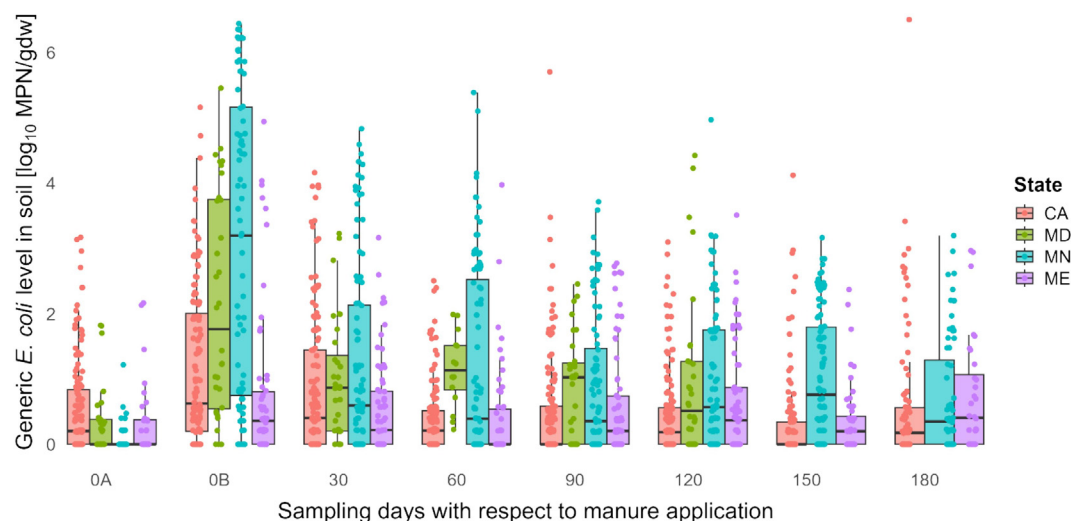


Figure 1. Distribution of generic *E. coli* concentration ($\log_{10}$ MPN/gdw) in manure-amended soil by sampling day and state; boxplots summarize distributions, and jittered dots represent individual samples (color-coded by state) ($n = 2,427$). Sampling day 0A represents soil samples collected before manure application, and 0B represents samples collected immediately after manure application on the same day as 0A.

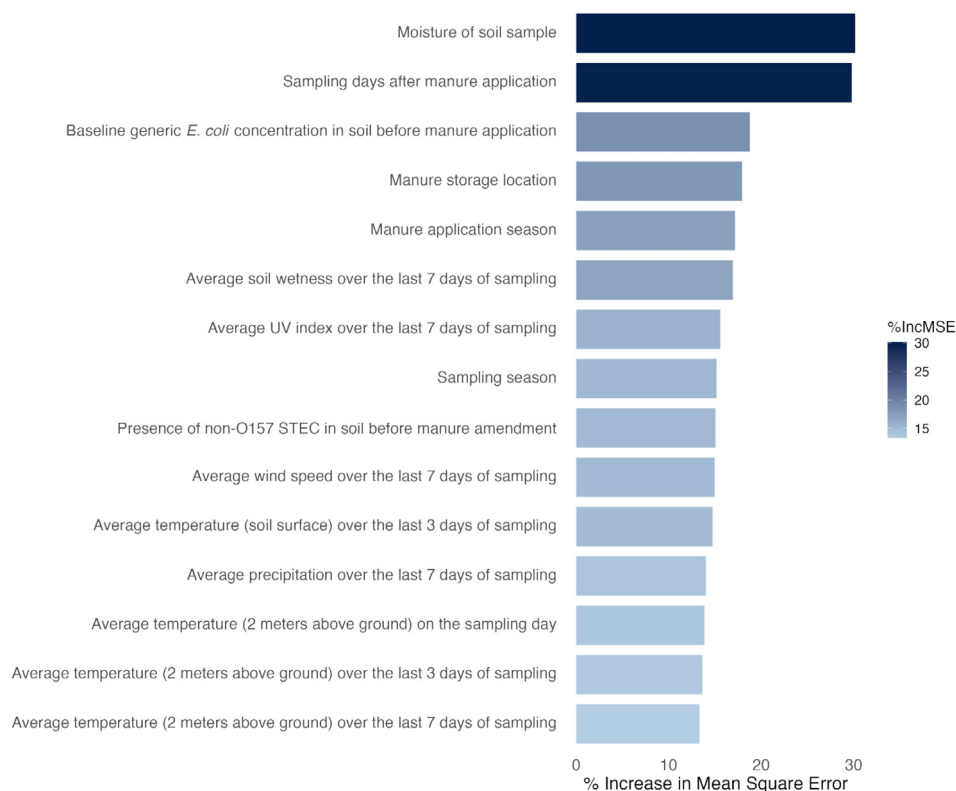


Figure 2. Ranking of agricultural and meteorological variables of generic *E. coli* concentration in manure-amended soil. Importance was quantified by the percentage change of Mean Square Error from a random forest permutation test; longer and darker bars denote greater influence ($n = 2,164$). The top 15 variables were listed in this Figure 2. The complete list of 31 variables is provided in Supplementary Material 2.

found associated with higher generic *E. coli* concentration, while the presence of generic *E. coli* in soil before manure application was negatively associated with the outcome ($\beta: -0.26$, $CI_{95}: -0.40, -0.13$).

In terms of manure use, exclusive use of poultry-originated manure or other nonmanure organic soil amendment (e.g., bone meal, seaweed emulsion, green waste) significantly increased the generic

E. coli concentration in soil compared to the exclusive use of cattle-originated manure ($\beta: 1.12$, $CI_{95}: 0.97, 1.34$ for poultry and $\beta: 1.88$, $CI_{95}: 0.94, 2.81$ for other nonmanure fertilizer). Additionally, mixed use of manure with diverse animal origins was associated with a slight increase in generic *E. coli* concentration in soil ($\beta: 0.24$, $CI_{95}: 0.01, 0.47$). A higher rate of manure application ($\beta: -1.05$, $CI_{95}: -1.45$,

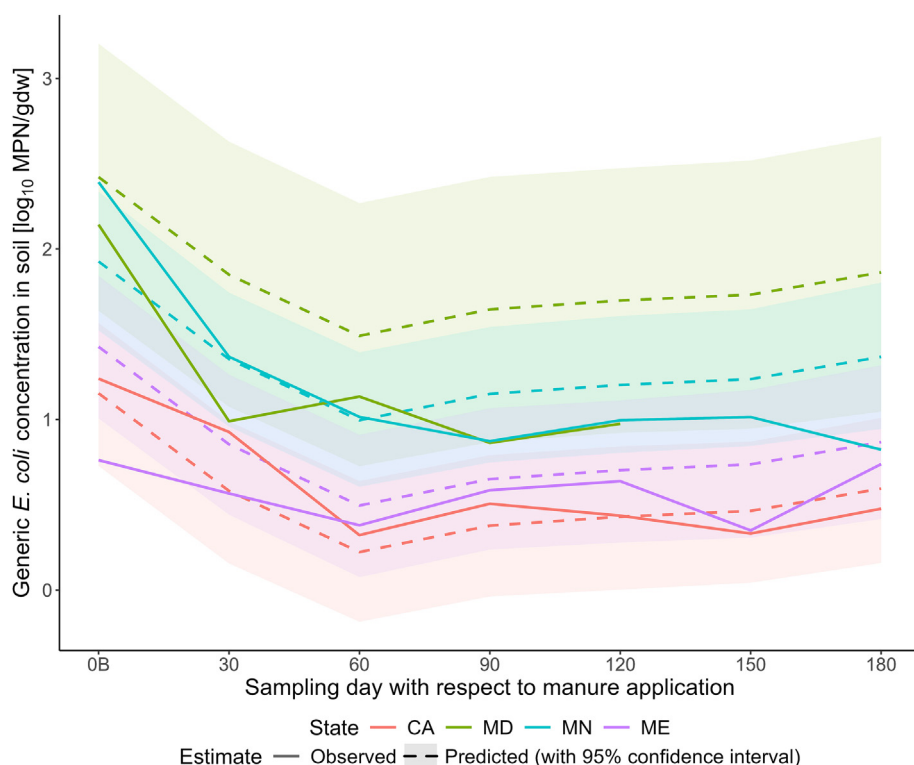


Figure 3. Observed and adjusted prediction of generic *E. coli* concentrations in manure-amended soil by state and sampling day. Solid lines show the observed mean of generic *E. coli* concentration in soil ($\log_{10}$ MPN/gdw) at each sampling day (Day 0B to Day 180) for California (CA), Maine (ME), Maryland (MD), and Minnesota (MN); no soil samples were collected from Maryland on Day 150 or Day 180. Dashed lines and shaded ribbons show state-specific marginal predictions and 95% confidence intervals from zero-inflated linear mixed model with farm as a random effect, adjusted for animal origin of manure, manure application rate, baseline presence of generic *E. coli* in soil before manure application, *Salmonella* and *L. monocytogenes* in soil (set to 'Reference' level), soil characteristics (moisture content, zinc, phosphorus, and manganese; set to respective mean values) and meteorological variables (wind speed, UV, and precipitation; set to respective mean values).

−0.64) or use of other nonmanure organic soil amendments (β : −1.26, CI_{95} : −1.63, −0.90) were associated with lower generic *E. coli* concentration in soil (Table 1).

As shown in Table 1, meteorological factors including higher UV radiation (β : 0.24, CI_{95} : 0.15, 0.34) on the soil sampling day and heavier average precipitation seven days prior to soil sampling (β : 0.03, CI_{95} : 0.01, 0.05) were significantly associated with a 24% and 3% increase in generic *E. coli* concentration in soil, respectively. In contrast, higher average wind speed two meters above the ground three days prior to the soil sampling day (β : −0.12, CI_{95} : −0.19, −0.06) was significantly associated with an approximately 12% decrease in generic *E. coli* concentration.

Discussion

This study investigated the risk factors associated with generic *E. coli* concentration in soil from certified organic farms across the U.S. after untreated manure applications. Animal origins of manure and manure application rate significantly impacted the generic *E. coli* concentrations in amended soil. Additionally, the concentration of generic *E. coli* in soil varied significantly across states and the time interval between manure application (or incorporation) and crop harvest. Soil characteristics, including moisture content, nutrient profiles (zinc, phosphorus, and manganese), and background microbial population, were significant predictors as well. Likewise, meteorological factors including wind speed, UV radiation, and precipitation significantly influenced the generic *E. coli* concentrations in soil.

In our study, we found that the survival pattern of *E. coli* in manure-amended soil was spatiotemporally distinct across geographic regions, consistent with previous literature. For example, Sharma et al. (2019)

evaluated the survival of nonpathogenic *E. coli* (and attenuated O157:H7) in soils amended with untreated poultry litter, horse manure, and dairy manure across three sites in Maryland and Pennsylvania, reporting location as one of the key factors of survival duration. Likewise, Kharel et al. (2025) evaluated the survival of *E. coli* in treated poultry litter at two field locations in Florida and Georgia, reporting a significant location $\times$ treatment $\times$ time interaction. This location-dependent effect was not only evident in organic fertilizer-amended organic farms but also in integrated crop-livestock systems. Cheong, Chandler-Khayd, et al. (2024a) reported generic *E. coli* declining more rapidly in California compared to Minnesota in postgrazing organic soil. These findings indicate that the incorporation of animal manure into agricultural soil could drive site-specific microbial responses, which were associated with soil characteristics (abiotic and biotic) and microbial communities. For example, Liang et al. (2011) demonstrated that survival of inoculated *E. coli* O157:H7 in soil declined progressively as the microbial community diversity reduced. Similarly, Shawver et al. (2024) reported that soil microbial community structure was impacted by soil type, soil moisture, and manure treatments across various soil conditions.

Apart from the effect of region, the time interval between manure application and soil sampling remained a key factor in *E. coli* survival. The substantial decline of concentration in the first 60 days after manure application, followed by a slight secondary increase and a modest decline tail, was a consistent pattern. McKenzie-Reynolds et al. (2025) reported a comparable initial decline followed by growth on Day 90 after manure application and a secondary decline until Day 150 over two growing seasons. Nevertheless, the apparent change from observed concentration by sampling day (Fig. 1) to conditional coefficients from the ZILMM model in this study (Fig. 3) reflected

Table 1

Multivariable analysis results for generic *E. coli* concentration in manure-amended soil samples from multiregional certified organic farms (2017–2018) using a zero-inflated linear mixed model. The model includes two components: a conditional component, modeling generic *E. coli* concentration in soil ($\log_{10}$ MPN/gdw) among detected values, and a zero-inflated component, modeling the probability of nondetect (logit scale)

Variable	Category	β^+ (95% CI)	p-value
Conditional component: generic <i>E. coli</i> concentration in soil ($\log_{10}$ MPN/gdw)			
Intercept	–	1.17 (0.55, 1.78)	<0.001
Sampling spatiotemporal characteristics			
State ^a	California	Ref	Ref
	Maryland	1.37 (0.53, 2.20)	0.001
	Maine	0.29 (–0.20, 0.79)	0.247
	Minnesota	0.83 (0.32, 1.34)	0.001
Sampling day after manure application ^a	Day 0B	Ref	Ref
	Day 30	–0.62 (–0.78, –0.45)	<0.001
	Day 60	–1.00 (–1.17, –0.83)	<0.001
	Day 90	–0.84 (–0.99, –0.68)	<0.001
	Day 120	–0.78 (–0.94, –0.62)	<0.001
	Day 150	–0.74 (–0.92, –0.57)	<0.001
	Day 180	–0.60 (–0.82, –0.39)	<0.001
Soil characteristics			
Soil moisture [%] ^b	–	0.13 (0.07, 0.19)	<0.001
Zinc (Zn) [ppm] ^b	–	0.03 (0.01, 0.04)	<0.001
Phosphorus (P) [unitless] ^b	–	0.22 (0.15, 0.30)	<0.001
Manganese (Mn) [ppm] ^b	–	0.02 (0.01, 0.02)	<0.001
Presence of generic <i>E. coli</i> in soil before manure application ^c	No	Ref	Ref
	Yes	–0.26 (–0.40, –0.13)	<0.001
Presence of <i>Salmonella</i> in soil sample ^c	No	Ref	Ref
	Yes	1.79 (1.31, 2.23)	<0.001
Presence of <i>L. monocytogenes</i> in soil sample ^c	No	Ref	Ref
	Yes	0.24 (0.03, 0.44)	0.024
Manure application			
Animal origin of manure ^a	Cattle	Ref	Ref
	Poultry	1.12 (0.97, 1.34)	<0.001
	Horse	–0.18 (–0.43, 0.07)	0.164
	Small ruminant	0.50 (–0.31, 1.31)	0.223
	Mix	0.24 (0.01, 0.47)	0.039
Manure application rate [tons/acre] ^a	Nonmanure fertilizer	1.88 (0.94, 2.81)	<0.001
	0 to 5	Ref	Ref
	5 to 15	–1.05 (–1.45, –0.64)	<0.001
	Nonmanure fertilizer	–1.26 (–1.63, –0.90)	<0.001
Meteorological variables			
Average wind speed 2 m above ground over the last 3 days of soil sampling [m/s] ^b		–0.12 (–0.19, –0.06)	<0.001
Average of precipitation over the last 7 days of soil sampling [mm] ^b		0.03 (0.01, 0.05)	0.007
Average UV radiation on the day of soil sampling [unitless] ^b		0.24 (0.15, 0.34)	<0.001
Zero-inflated component: probability of nondetect of generic <i>E. coli</i> in soil (logit)			
Intercept	–	–2.04 (–2.33, –1.74)	<0.001

^a variable is assessed as a multilevel categorical variable, with the reference level (denoted “Ref” in the table) as the baseline against which β coefficients for the other levels are compared.

^b variable is assessed as a continuous variable.

^c variable is assessed as an indicator variable, with ‘No’ assigned as the reference level (denoted “Ref” in the table).

⁺ For the conditional component, β represents the regression coefficient indicating the estimated change in generic *E. coli* concentration in soil ($\log_{10}$ MPN/gdw) associated with each variable, relative to the reference level for categorical variables. For the zero-inflated component, β represents the regression coefficient on the logit scale, indicating the estimated change in the logit of nondetect associated with each variable, relative to the reference level for categorical variables.

the potential confounding effects from other covariates such as soil characteristics and short-term meteorological factors. Taken together, the decline in generic *E. coli* concentration and accounting for other risk factors (e.g., geographic location, animal origin of manure, soil properties, and meteorological conditions) should be taken into consideration when following the current USDA NOP standards of 90/120-day application intervals (USDA, 2011) as a mitigation for contamination of fresh produce. However, significant effects of geographic location, animal origin of manure, soil properties, and generic *E. coli* survival indicate that those should be taken into consideration in addition to time intervals. A risk-based approach accounting for heterogeneity across production environments alongside current NOP application interval standards will allow a systems-based approach and support adaptive decision-making in diverse organic production systems and regions.

Abiotic soil characteristics, including moisture content and nutrient compositions (zinc, phosphorus, manganese), contributed to the generic *E. coli* survival in manure-amended soil. The positive association between moisture content and generic *E. coli* in soil aligned with earlier controlled studies showing that soil moisture can be a key factor associated with *E. coli* survival. For example, Williams et al. (2015) reported that moisture and pH affected short-term suppression of *E. coli* O157:H7 under laboratory settings, and that field suppression varied by region and season, with evidence that soil moisture interacted with soil organic matter in field settings. In contrast, Underthun et al. (2018) concluded that soil type and temperature explained the survival of *Salmonella* and *E. coli* in soil, suggesting that the effect of moisture may depend on a broader soil-climate context. Nutrient composition also influenced generic *E. coli* survival in soil, with elevated concentrations observed in phosphorus-, manganese-,

and zinc-rich soils. This result is consistent with the observed significant positive correlation between available phosphorus and prolonged survival of *E. coli* O157:H7 in soil (Zhang et al., 2023). Additionally, positive associations were observed between micronutrients (manganese and zinc) and *E. coli* survival, potentially through their influence on soil bacterial community structure and function (Shepherd & Oliverio, 2024), indicating that trace elements can affect microbial interactions that may indirectly affect pathogen survival. In contrast, Richter et al. (2025) documented reduced survival of STEC in long-term dairy manure-amended soils, suggesting that nutrient enrichment may also enhance microbial competition or antagonism under certain management conditions. Taken together, the observed associations among soil moisture, nutrient profiles, and generic *E. coli* survival in manure-amended soil suggest that abiotic soil characteristics influence microbial survival through complex and interacting pathways. These findings highlight the need to consider nutrient management and manure application practices that balance crop productivity goals and mitigation of microbial risk in agricultural soils.

In addition to nutrient profile differences, animal origin of manure and application rates served as independent predictors of generic *E. coli* survival in soil, reflecting inherent differences in microbial population, including pathogens, across animal origins. Once applied to soil, animal origin influences both prevalence and survival patterns of foodborne pathogens in soil. Hailu et al. (2021) reported higher prevalence of pathogens (e.g., *E. coli* O157 and *L. monocytogenes*) in dairy cattle compared to poultry manure-amended farms, indicating animal origin-specific microbial risks. Similarly, Sharma et al. (2016) demonstrated that survival patterns of enteric pathogens in soil varied across poultry litter, dairy manure, and composted amendments, highlighting the role of animal origin in influencing survival of pathogens following land application. Manure application rate was also a significant predictor of generic *E. coli* concentration, with higher manure volumes being associated with lower *E. coli* levels in our study. However, Çekiç et al. (2017) observed the opposite pattern in a Florida field trial, observing extended *E. coli* survival in heavily manured soils compared to light or medium manure application rates. This discrepancy may reflect methodological differences: our multiregional, observational study relied on self-reported data subject to potential recall bias, while Çekiç et al. (2017) conducted a controlled single-site field trial with standardized measurements. These findings collectively emphasize the importance of considering both the animal origin and application rate when assessing microbial risk in manure-amended soil, with the distinct effects of different manure sources suggesting the need for animal origin-specific management strategies in organic farming.

Meteorological factors, including precipitation, wind speed, and UV exposure, significantly influenced generic *E. coli* concentration in manure-amended soil. We observed a negative association between wind speed and generic *E. coli* concentration, which aligned with findings from Pang et al. (2018), who observed that wind speed up to three days prior to sampling was negatively associated with generic *E. coli* count in farm samples (produce, soil, and dairy manure). Pang et al. (2018) also investigated precipitation effects and found that associations were time-dependent, with two-day versus 30-day averages showing contrasting results. We observed a positive association between seven-day average precipitation prior to soil sampling and *E. coli* concentration, suggesting this time-dependent effect may peak at specific averaging intervals, with intermediate averaging periods (such as seven days) capturing a distinct precipitation effect, compared to shorter (two-day) or longer (30-day) intervals. Unexpectedly, our results identified a positive association between UV index on the sampling day and generic *E. coli* concentration, contrasting with established observations that UV radiation reduced *E. coli* O157 levels or accelerated decay rates on exposed produce (Bozkurt et al., 2021; Brandl et al., 2023). However, this apparent contradiction may be potentially attributed to sampling methodology rather than biological

inconsistency: the manure-amended soil samples in our study were collected from a six-inch depth, effectively shielded from direct UV penetration. Therefore, the positive association identified between UV index and *E. coli* concentration in our study does not challenge biological expectations but emphasizes the complexity of the impact of meteorological factors in subsurface soil after manure application. Overall, these meteorological factors showed time-dependent, complex effects on microbial persistence across different agricultural conditions. Spatiotemporal differences, especially meteorological differences, should be considered to mitigate microbial contamination risk in organic production, and further research is needed to clarify how environmental stressors interact across temporal frames and geographic regions.

The significant risk factors identified across this multiregional study provide evidence of how temporal, spatial, environmental, and management factors interact to influence generic *E. coli* survival in organic agriculture. Despite these valuable insights, several limitations should be acknowledged. First, while generic *E. coli* is employed as a contamination indicator in regulatory and commodity metrics (California LGMA, 2023; FDA, 2024), recent studies have investigated its reliability for assessing environmental contamination. Murphy et al. (2024) found that generic *E. coli* survived detectably longer than *E. coli* O157:H7 and non-O157 pathogenic *E. coli*, suggesting its use may overestimate pathogen survival in manure-amended soils. Similarly, Weller et al. (2020) reported a positive correlation between generic *E. coli* and STEC markers but limited correlation with *Salmonella* and *L. monocytogenes*. Nevertheless, observed positive correlations between pathogens (*Salmonella* and *L. monocytogenes*) and generic *E. coli* in our study support generic *E. coli* as a suitable indicator in manure-amended soil, with *Salmonella* correlating with nearly a 2-log increase in *E. coli* concentrations. This robust cooccurrence pattern between generic *E. coli* and pathogens suggests these organisms share similar environmental responses and survival dynamics influenced by manure origin (Bardsley et al., 2021; Ferguson et al., 2023; Murphy et al., 2023). Given the low occurrence and high detection costs of target foodborne pathogens such as STEC (Chen et al., 2019; Lengacher et al., 2010), generic *E. coli* monitoring remains a commonly used and pragmatic, cost-effective screening tool to evaluate microbial risk in agricultural environments (Nowicki et al., 2021). Second, the geographic imbalance in sample distribution, with California samples comprising most of the dataset and Maine substantially underrepresented, may have introduced selection bias and residual confounding that limits the generalizability of findings to other regions. Third, the hierarchical sampling scheme, involving repeated sampling from identical plots nested in identical farms over time, resulted in shared baseline microbial characteristics. This data structure constrained within-farm variability in preamendment generic *E. coli* presence in soil, which may have affected the precision of associations between the other predictors and the outcome. However, the statistical approach used in the present study accounted for repeated sampling and unbalanced sampling across states and farms.

Conclusion

This multiregional study provides epidemiological evidence that generic *E. coli* survival in manure-amended organic soils is influenced by multiple interacting risk factors rather than time since manure application alone. Generic *E. coli* declined most rapidly during the first 60 days after manure application, with slower reductions thereafter. However, survival also varied by manure source, geographic location, soil moisture, and soil nutrient composition, indicating that risk assessments should consider environmental and management factors in addition to application timing. From a practical perspective, organic producers and food safety professionals should consider the animal origin of the manure when selecting soil amendments and the

environmental conditions that may influence microbial survival after application. These findings provide a foundation for developing farm-specific risk assessments and decision-support tools to guide the best risk mitigation practices. Such evidence-based, adaptive approaches would enhance food safety beyond what temporal standards alone can achieve while supporting the continued viability and public trust in organic production systems.

Declaration of generative AI in scientific writing

The author(s) used ChatGPT for statistical code troubleshooting and Claude for language editing to improve clarity and readability. All outputs were reviewed and edited by the author(s), who took full responsibility for the final content of the publication.

CRediT authorship contribution statement

Kefang Nie: Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Data curation. **Sejin Cheong:** Writing – review & editing, Methodology. **Jerome N. Baron:** Writing – review & editing, Methodology, Data curation. **Michele T. Jay-Russell:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Thais De Melo Ramos:** Writing – review & editing, Methodology, Investigation, Data curation. **Peiman Aminabadi:** Writing – review & editing, Methodology, Data curation. **Patricia D. Millner:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Annette Kenney:** Writing – review & editing, Investigation, Data curation. **Fawzy Hashem:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Paulo H. Pagliari:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Mark Hutchinson:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Beatriz Martínez-López:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Elizabeth A. Bihn:** Writing – review & editing, Funding acquisition, Conceptualization. **Donna P. Clements:** Writing – review & editing, Conceptualization. **Jessica B. Shade:** Writing – review & editing, Funding acquisition, Conceptualization. **Amber R. Sciligo:** Writing – review & editing, Funding acquisition, Conceptualization. **Alda F.A. Pires:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

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

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Appendix A. Supplementary material

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