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Factors associated with foodborne pathogens and indicator organisms in agricultural soils

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Soil can be a route for contamination of fresh fruits and vegetables. While growers routinely manage soil nutrient levels, little research exists on the synergistic or antagonistic effects of soil nutrients on foodborne pathogens. Data on foodborne pathogen prevalence in unamended soils is also relatively limited in literature. This study evaluated foodborne pathogen prevalence (*Salmonella*, *Listeria monocytogenes*) and concentration of indicator bacteria (total coliforms, generic *Escherichia coli*) in agricultural soils, and characterized associations between soil properties (e.g., macro- and micro-nutrient levels) and each microbial target. Three Virginia produce farms, representing different regions and soil types, were sampled four times over 1 year (October 2021–November 2022). For each individual farm visit, composite soil samples were collected from 20 sample sites (25 m^2) per farm per visit for microbial and nutrient analysis ($n = 240$). Samples (25 g) were processed for *Listeria* spp. and *Salmonella* using a modified FDA BAM method; samples (5 g) were enumerated for generic *E. coli* and total coliforms (TC) using Petrifilm. Presumptive *Listeria* spp. and *Salmonella* isolates were confirmed by PCR using the sigB and invA genes, respectively. Soil nutrients from each sample were tested and evaluated for their association with each microbial target by Bayesian Mixed Models. *Salmonella* prevalence was 4.2% (10/240), with 90% (9/10) recovered on Farm C. *Listeria* spp. and *L. monocytogenes* prevalence were 10% (24/240) and 2.5% (6/240), respectively. When samples were positive for generic *E. coli* (107/240), the average concentration was 1.53 ± 0.77 log10 CFU/g. Soil pH was positively associated with *L. monocytogenes* [Odds Ratio (OR) = 5.5] and generic *E. coli* (OR = 4.9) prevalence. There was no association between *Salmonella* prevalence and any evaluated factor; however, *Salmonella* was 11.6 times more likely to be detected on Farm C, compared to other farms. Results show pathogen prevalence was relatively low in unamended soils, and that factors influencing prevalence and concentration varied by microbial target and farm.

KEYWORDS

***Salmonella*, *Listeria*, unamended soil, preharvest, produce safety, soil nutrients, prevalence, management practices**

Introduction

In the last two decades, fresh produce has remained a major vehicle associated with foodborne outbreaks and recalls (Lynch et al., 2009; Callejón et al., 2015; Wadamori et al., 2017; Carstens et al., 2019). For example, a large retail grocery market voluntarily recalled three micro greens, sweet pea leaves, and cat grass products when the supplier found the soil in which these products were grown tested positive for *Salmonella* (Food and Drug Administration, 2022). While no illnesses were associated with this voluntary recall, due to products being grown in contaminated soil, there was the potential for *Salmonella* contamination (Food and Drug Administration, 2022).

Managing the risk of produce contamination from soil is complicated due to the ability of foodborne pathogens to survive in soil for extended periods. Studies have demonstrated that *Salmonella* (Chandler and Craven, 1980; Holley et al., 2006; You et al., 2006; Underthun et al., 2018; Jechalke et al., 2019; Bardsley et al., 2021) and *L. monocytogenes* (Jiang et al., 2004; McLaughlin et al., 2011) can survive in agricultural soils for up to 1 year. For instance, *Salmonella* persisted in amended soils for 129 days, with survival differing by soil type (clay-loam > sandy-loam) and irrigation regimen (weekly > daily) (Bardsley et al., 2021). Factors including soil properties (e.g., moisture), meteorological events (e.g., rainfall), and management practices (e.g., tilling) have been shown to influence the likelihood of detection and survival of foodborne pathogens in soils (Danyluk et al., 2008; Ivanek et al., 2009; McLaughlin et al., 2011; Strawn et al., 2013a; Park et al., 2014; Weller et al., 2015; Bardsley et al., 2021; Ramos et al., 2021). For example, a 2013 study in New York (United States) showed rainfall 72 h prior to sampling increased the likelihood of detecting *Salmonella* in poorly drained soils (Strawn et al., 2013a). A longitudinal study in California (United States) evaluating four foodborne pathogens (*Salmonella*, *L. monocytogenes*, *Escherichia coli* O157:H7, and non-O157 Shiga-toxin producing *E. coli*) in amended agricultural soils found that prevalence was pathogen-specific and dependent on biological soil amendment, soil type, environmental conditions, and region (Ramos et al., 2021).

Growers routinely test agricultural soil for macro- and micro-nutrients, and other soil properties, to gather data to assist in nutrient management plans and fertility practices (Maguire et al., 2005). A nationwide genomic atlas study observed that the micronutrient molybdenum was associated with increased *Listeria* spp. prevalence in undisturbed soils from national parks, refuges, or non-agricultural areas (Liao et al., 2021). While previous studies have explored foodborne pathogen prevalence in agricultural soils (Moshtaghi et al., 2003; Rodriguez et al., 2006; Strawn et al., 2013a; Harrand et al., 2020; Ferguson et al., 2023; Murphy et al., 2023), minimal research exists on understanding how soil properties (e.g., macro- and micro-nutrients) impact foodborne pathogen prevalence in unamended agricultural soils. Since the survival and persistence of microorganisms can be impacted by the availability and composition of nutrient sources (Pike et al., 2019); as well as, prior work showing the association between *Listeria* spp. prevalence and molybdenum, understanding the influence of macro- and micro-nutrients on foodpathogens in soil is of interest.

Indentifying how soil properties interact (synergistically and/or antagonistically) with foodborne pathogens may elucidate information to predict potential risky periods for contamination in agricultural soils (i.e., when soil may test positive for foodborne pathogens). To address research gaps, the present observational study was performed to generate baseline data to inform key soil properties influencing microbial targets in agricultural soils. The aims were to determine the prevalence of *Salmonell*a and *L. monocytogenes* in unamended agricultural soil, and to investigate if associations exist between soil properties and each microbiological target (*Salmonell*a, *L. monocytogenes*, generic *E. coli*, and total coliforms).

Materials and methods

Experimental design

A longitudinal field study was performed on three Virginia farms; each farm represented a different growing region (i.e., Blue Ridge Highlands, Piedmont, Coastal Plain; distance between farms ranged from 338 to 591 km). Within each farm, 20 unique 0.2 ha (5 m x 5 m) sites were selected based on the produce crops grown (e.g., crops that potentially or frequently contact the ground), the feasibility of soil sample collection, and diversity in management practices and topography (Supplementary Table S1). Global Positioning System (GPS) coordinates for each sample site center were recorded and used to ensure the same site was sampled at each visit. Each farm was sampled four times between October 2021 and November 2022 capturing a full growing season. In total, 240 composite soil samples were collected for this study (4 sampling visits, 3 farms, 20 sample sites).

An initial questionnaire, informed by prior field studies (Park et al., 2013; Strawn et al., 2013b) was modified and administered orally to farm management to collect information regarding historical land-use and field-level management practices (e.g., irrigation, worker/equipment presence) for each sample site, prior to the first sampling event on each farm (Supplementary Table S2). Additionally, an observational survey detailing characteristics and management practices was completed for each sampling site during each visit (Supplementary Table S3).

Sample collection and preparation

Sampling was performed following previously described protocols (Strawn et al., 2013a; Weller et al., 2015). Briefly, nitrile gloves were worn for sample collection and changed between each field sample site. Within each sample site, sub-samples from five locations (each of the four corners, and centroid) were collected using sterile scoops (Fisher Scientific, Hampton, NH, United States). Soil was collected from the rhizosphere layer (i.e., the zone surrounding the plant roots), up to approximately 15.2 cm below the surface, and deposited into sterile re-closable 3.79 L storage bags (Fisher Scientific, Hampton, NH). All samples were transported on ice and then stored at 4 ± 2°C for processing within 24 h.

For foodborne pathogen testing, two 25 g composite soil samples were prepared, one for each pathogen, by combining 5 g portions of each of the 5 sub-samples into a sterile filtered Whirl-Pak bag (Nasco, Madison, WI, United States). An additional 1 g from each sub-sample (5 g total) were pooled into a Whirl-Pak bag for enumeration of generic *E. coli* and total coliforms. Composite samples (150 g) were prepared by combining 30 g portions of each of the five sub-soil

samples into a brown paper bag (Fisher Scientific, Hampton, NH, United States). Soil samples were held at 21 ± 2°C until visibly dry (approximately 2 weeks) to determine soil moisture content (Maguire et al., 2005). Air-dried soil samples were shipped and analyzed at Waters Agricultural Laboratories (Camilla, GA, United States) for macro- and micro-nutrients. Samples were analyzed under the Mehlich 3 extraction method (Mehlich, 1984). A list of soil nutrients and properties that were collected (and transformed) can be found in Table 1.

Soil microbial analysis

Listeria detection and isolation was performed using a modified version of the United States Food and Drug Administration's (FDA) *Bacteriological Analytical Manual* (BAM) (Hitchins et al., 2022). Briefly, soil samples were diluted 1:10 by adding 225 mL of buffered *Listeria* enrichment broth (BLEB; Oxoid, Basingstoke, Hampshire) to each sample (25 g) and incubated at 30 ± 2°C for 4 h. After 4 h of incubation, *Listeria* selective enrichment supplement (Oxoid, Basingstoke, Hampshire) was added to each sample and incubated at 30 ± 2°C. At 24 and 48 h of incubation, 50 µL of each enrichment was streaked for isolation onto Modified Oxford Agar (MOX; Becton Dickinson, Franklin Lakes, NJ, United States) and *L. monocytogenes* Plating Medium (LMPM; R&F Products, Downers Grove, IL, United States) and incubated at 30 ± 2°C for 48 h and 35 ± 2°C for 48 h, respectively. Up to three presumptive *Listeria* colonies per time and plating agar combination (e.g., MOX at 24 h and LMPM at 48 h) were sub-streaked onto reversed agar combinations (e.g., MOX plated onto LMPM and LMPM plated onto MOX) and incubated for 48 h at each agar's respective temperatures. Presumptive positive *Listeria* colonies were confirmed by a *sigB* PCR assay and species identified by partial *sigB* gene sequencing, as previously described (Nightingale et al., 2007).

Salmonella detection and isolation were performed using a modified version of the procedure outlined in the United States FDA BAM (Andrews et al., 2011). Briefly, soil samples were diluted 1:10 by adding 225 mL of Buffered Peptone Water (BPW) to each 25 g composited sample. Samples were incubated at 35 ± 2°C for 24 h. Following incubation, 1.0 and 0.1 mL were transferred to 9.0 and 9.9 mL of Tetrathionate (TT; Oxoid; Basingstoke, Hampshire) and Rappaport Vassiliadis (RV: Oxoid, Basingstoke, Hampshire) broth, respectively. Enrichment broths were incubated at 35 ± 2°C and 42 ± 2°C for 24 and 48 h, respectively. From each broth, 50 µL was placed onto Xylose Lysine Tergitol-4 (XLT-4; Neogen, Lansing, MI, United States) and Hektoen Enteric (HE; Neogen Lansing, MI, United States) agars and incubated at 35 ± 2°C for 24 h. Up to three presumptive *Salmonella* colonies per enrichment and plating agar combination (e.g., TT-XLT-4, RV-XLT-4) were sub-streaked onto reversed agar combinations (e.g., XLT-4 plated onto HE and HE plated onto XLT-4) and incubated at 35 ± 2°C for 24 h. Presumptive positive *Salmonella* colonies were confirmed by a PCR assay for the *invA* gene (Kim et al., 2007). One isolate per *Salmonella*-positive sample was serotyped by Kauffmann–White classification at the National Veterinary Laboratory Services (Ames, IA, United States).

Generic *E. coli* and total coliform enumeration were performed as previously described (Berry and Miller, 2005). Briefly, a 5 g composite sample was placed in a filtered Whirl-Pak bag diluted in 45 mL of 2% BPW. Following serial dilution, 1 mL of each dilution was plated onto *E. coli*/coliform Petrifilm (3 M, Saint Paul, MN, United States) in duplicate. Petrifilm was incubated at 37 ± 2°C for 48 h, and anlyzed according to manufacturers instructions to enumerate *E. coli* and total coliforms. Counts were log transformed ($\log_{10}$ CFU/g).

Meteorological data collection

Meteorological data including average daily air temperature (°C), total precipitation (mm), and relative humidity (%) for the 5 days prior to sampling were obtained for each farm and sampling visit. Meteorological data were collected from the nearest Virginia Agricultural Research and Extension Center to each farm using the Virginia Tech WeatherSTEM platform.[1]

Statistical analysis

Analyzes were performed in RStudio version 4.2.2 (R Foundation for Statistical Computing, Vienna, Austria). Descriptive statistics were performed on all variables to characterize trends across farms and sampling visits, and summary statistics were calculated for soil nutrient data. Extreme outliers in soil nutrient data were identified using the Rosner Test (Rosner, 1975, 1983), and biologically implausible outliers were removed. Due to strong skew, some soil properties were $\log_{10}$ transformed (Table 1).

Bayesian mixed models were implemented using the *brms* package (Bürkner, 2017a,b) to identify factors associated with: (i) the isolation of *Listeria* spp., *L. monocytogenes*, and *Salmonella*; (ii) if $\log_{10}$ generic *E. coli* levels were above versus below the limit of detection; and (iii) *E. coli* concentration in samples with enumerable levels. For the logistic models, a Bernoulli distribution with a logit link function was used. For the linear models, an identity link was used. The model outcome was a function of a random effect of site nested in farm, a random effect of month, and a fixed effect of year in addition to a fixed effect for the factor of interest (Supplementary Table S4).

All models were fit using the *brms* package with uninformative priors, 5,001 iterations per chain, thinning set to 10, and 3 chains (Bürkner, 2017a,b). The Maximum *a posteriori* estimate (MAP) is the most probable value of the posterior distribution for the effect estimate for the linear models and odds ratio for the logistic models. The presence and strength of an association between the outcome and factor of interest were assessed using the probability of direction (PD), and region of practical equivalence (ROPE) values (Makowski et al., 2019a,b). In addition to assessing associations between soil microbial quality and environmental parameters, models were also implemented to determine if there were substantial differences in soil microbial quality between farms. Since there were no *Listeria* spp. positive samples from Farm C, and no *Salmonella* positive samples from Farm A, models were run (i) using data from all farms, and (ii) using only data from farms where there was at least one positive sample for the microbial target. A factor was considered associated with the odds of detecting a microbial target if ROPE was ≤0.025 and the probability

1 https://en.weatherstem.com/data

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TABLE 1 Summary statistics of soil properties by farm and sampling visit.

Soil factor		Farm A				Farm B				Farm C			
		Oct. 21	June 22	Aug. 22	Oct. 22	Oct. 21	July 22	Aug. 22	Oct. 22	Nov. 21	June 22	Sept. 22	Nov. 22
Ca (mg/kg)*[a]	Mean	1107.2	1021.8	931.7	944.9	1159.9	1096.3	1198.8	1191.3	505.9	475.0	662.9	117.5
	SD	382.0	341.7	353.5	306.9	420.2	296.8	403.9	523.6	278.9	188.7	457.8	97.9
	Outliers[b]	0	0	0	0	0	1	0	0	0	0	1	0
Mg (mg/kg)*	Mean	224.5	238.1	208.2	207.1	140.2	125.6	157.9	145.3	69.8	69.1	93.2	98.1
	SD	83.8	68.5	73.1	78.4	45.2	40.4	47.7	47.8	33.1	27.6	53.9	49.1
	Outliers	0	1	0	0	0	0	0	0	0	0	1	1
P (mg/kg)*	Mean	78.4	71	75.4	73.4	140.2	223.3	88.9	69.3	110.2	134.4	142.1	133
	SD	63	49.5	65.4	62.4	45.2	80.9	45.4	41.4	54.7	56.6	64.7	44.9
	Outliers	0	1	0	0	0	1	0	0	0	0	0	0
K (mg/kg)*	Mean	204.3	215.8	258.8	258.8	231	251.2	284.8	245.3	130.5	129.1	204.6	198.4
	SD	115.0	75.1	97.1	126.6	71.9	80.7	126.6	114.5	70.5	60.7	120.3	92.7
	Outliers	0	1	0	0	0	0	1	1	0	0	0	0
Na (mg/kg)*	Mean	10.3	17.5	10.9	13.8	12.7	17.7	13.7	14.1	11.8	14.8	11.1	15.2
	SD	4.10	9.23	3.78	7.16	6.13	6.46	2.98	6.96	4.12	2.41	1.97	7.62
	Outliers	0	6	0	0	1	0	0	0	0	0	2	1
S (mg/kg)*	Mean	16.9	14.3	13.7	11.7	16.2	14.8	16.5	13.3	13.9	18.6	18.3	20.8
	SD	13.4	9.18	7.61	8.98	7.94	4.61	3.60	9.35	9.44	11.3	9.32	10.7
	Outliers	0	1	0	0	1	0	0	0	0	0	4	4
Al (mg/kg)*	Mean	586.2	630.5	582.8	582.3	449.3	516.4	461.5	447.7	655.8	752.2	733.9	693.8
	SD	87.0	137.5	91.3	92.7	52.8	70.4	55.8	60.8	124.6	64.5	141.6	76.3
	Outliers	0	0	0	0	0	0	0	0	0	1	0	0
Co (mg/kg)*	Mean	1.24	1.46	1.18	1.19	3.78	3.55	3.91	3.16	1.06	1.08	1.2	1.15
	SD	0.64	1.15	0.80	0.92	1.61	1.53	1.56	1.35	0.60	0.43	0.45	0.34
	Outliers	0	0	0	0	0	0	0	0	1	0	0	0
Fe (mg/kg)*	Mean	144.7	147.4	134.4	135.2	131.5	129.9	124.1	113.4	147.2	10.9	153.5	149.8
	SD	46.4	38.7	42.6	41.8	20.0	27.3	25.1	23.6	56.2	6.22	43.7	29.1
	Outliers	0	0	0	0	0	0	0	0	2	0	0	0
Mn (mg/kg)*	Mean	102.0	117.4	110.0	101.5	146.9	144.1	124.9	116.8	17.1	21.8	23.6	23.2
	SD	41.4	46.9	43.1	37.2	62.3	67.2	62.3	49.1	9.42	12.4	13.2	8.19
	Outliers	0	0	0	0	0	0	0	0	0	0	0	0
Mo (mg/kg)*	Mean	0.16	0.06	0.40	0.05	0.15	0.05	0.40	0.07	0.14	0.04	0.38	0.06
	SD	0.06	0.10	0.04	0.02	0.05	0.03	0.07	0.03	0.05	0.04	0.05	0.02
	Outliers	0	0	0	0	0	0	0	0	2	0	0	0
Zn (mg/kg)*	Mean	2.23	1.82	0.29	2.61	7.65	8.06	0.31	6.64	2.49	2.50	0.25	4.42
	SD	1.82	1.27	0.22	2.95	2.97	3.03	0.09	2.47	1.78	0.85	0.23	2.02
	Outliers	0	1	1	0	0	1	0	1	1	0	5	0
TN (%)[c]	Mean	0.31	0.27	0.17	0.27	0.37	0.29	0.21	0.27	0.23	0.21	0.09	0.20
	SD	0.10	0.05	0.05	0.04	0.04	0.05	0.04	0.06	0.07	0.04	0.04	0.04
	Outliers	2	1	0	0	0	0	0	0	0	0	0	0
TC (%)[d]	Mean	1.87	2.15	1.69	2.26	2.27	2.35	2.19	2.33	1.17	1.42	0.91	1.49
	SD	0.48	0.46	0.61	0.59	0.34	0.52	0.59	0.62	0.56	0.44	0.45	0.28
	Outliers	0	1	0	0	0	0	0	0	0	0	0	0

(Continued)

TABLE 1 (Continued)

Soil factor		Farm A				Farm B				Farm C			
		Oct. 21	June 22	Aug. 22	Oct. 22	Oct. 21	July 22	Aug. 22	Oct. 22	Nov. 21	June 22	Sept. 22	Nov. 22
OM (%)*[e]	Mean	2.17	2.32	1.93	2.29	2.70	2.52	2.41	2.48	1.11	0.93	1.02	1.17
	SD	0.61	0.52	0.44	0.73	0.55	0.49	0.75	0.60	0.65	0.39	0.41	0.33
	Outliers	0	1	4	0	0	0	0	0	0	0	1	0
pH*	Mean	6.19	6.41	6.10	6.22	6.35	6.19	6.24	6.58	6.03	5.91	6.00	5.95
	SD	0.31	0.62	0.39	0.36	0.54	0.63	0.54	0.53	0.52	0.55	0.40	0.27
	Outliers	0	1	0	0	0	0	0	0	0	0	0	0
Soil Moisture	Average (%)	15.0	27.6	14.8	12.8	17.1	22.7	8.15	17.3	15.1	13.4	7.05	7.26

[a]Soil nutrients denoted by (*) were $\log_{10}$ transformed for Bayesian mixed model analyzes.
[b]All individual farm and sampling visit observations were out of 20 (total 240); outliers removed were not considered in summary statistics.
[c]TN: Total Nitrogen (%).
[d]TC: Total Carbon (%).
[e]Organic Matter (%).

of direction (PD) was ≥0.95; for all factors where these thresholds were not met, this was considered as failing to find evidence of an association.

Results

Soil analysis and meteorological data

For each farm and sampling visit, values for calcium (mg/kg), magnesium (mg/kg), phosphorous (mg/kg), potassium (mg/kg), sodium (mg/kg), sulfur (mg/kg), aluminum (mg/kg), copper (mg/kg), iron (mg/kg), manganese (mg/kg), molybdenum (mg/kg), zinc (mg/kg), total nitrogen (%), total carbon (%), organic matter (%), and pH were assessed. Summary statistics for all soil nutrients and properties across farms and sampling visits can be found in Table 1. Furthermore, values for air temperature (°C), dew point (°C), relative humidity (%), and cumulative rainfall (mm) for the 5 days prior to each sampling visit by farm can be found in Supplementary Table S5, or soil type in Supplementary Table S7.

Foodborne pathogen and indicator bacteria prevalence

The overall prevalence of *Listeria* spp. and *L. monocytogenes* in soil across the three farms were 10.0% (24/240) and 2.5% (6/240), respectively (Table 2). Farm B had the highest prevalence of both *Listeria* spp. (18.8%; 15/80) and *L. monocytogenes* (6.3%; 5/80), while no samples from Farm C were positive for *Listeria*. On Farm B, the five soil samples positive for *L. monocytogenes* represented four unique sampling sites, with one site testing positive during two separate sampling visits (visit 1 and 4; both during the fall separated by approximately 1 year). The overall prevalence of *Salmonella* in soil across the three farms was 4.2% (10/240; Table 2). Ninety percent (9/10) of *Salmonella*-positive soil samples were recovered on Farm C, with the majority recovered during the first sampling visit (6 of 9). These nine *Salmonella*-positive soil samples from Farm C represented eight unique sampling sites, with one site testing positive during two separate sampling visits (visit 1 and 2). The site with repeated *Salmonella* positive soil samples was directly adjacent to a pond used for irrigation. Irrigation on Farm C was weather dependent and applied by drip under plastic mulch. One soil sample was positive for *Salmonella* from Farm B during sampling visit 4. Farm A yielded no *Salmonella*- positive samples across all four visits.

Generic *E. coli* levels fell below the lower limit of detection (1 $\log_{10}$ CFU/g) in over half of the soil samples collected in this study (55%; 133/240). The greatest prevalence of generic *E. coli* was found from soils collected on Farm B (67.5%; 54/80); followed by Farm A (43.8%; 35/80) and Farm C (22.5%; 18/80; Table 2). For the 107 soil samples where generic *E. coli* were above the limit of detection, the average concentration across all farms and sampling visits was 1.53 ± 0.77 $\log_{10}$ CFU/g (Supplementary Table S6). While not significantly different by farm, Farm B had the highest generic *E. coli* levels among positive samples at 1.61 ± 0.86 $\log_{10}$ CFU/g, followed by Farm A (1.48 ± 0.70 $\log_{10}$ CFU/g) and Farm C (1.43 ± 0.62 $\log_{10}$ CFU/g), respectively. Total coliform levels were detectable in all 240 samples collected and had an average concentration of 4.21 ± 0.94 $\log_{10}$ CFU/g (Supplementary Table S6). Similar to generic *E. coli*, Farm B had the highest total coliform levels (4.66 ± 0.63 $\log_{10}$ CFU/g), followed by Farm A (4.55 ± 0.78 $\log_{10}$ CFU/g) and Farm C (3.42 ± 0.85 $\log_{10}$ CFU/g), respectively.

Associations between foodborne pathogens and factors

Since no soil sample from Farm C was positive for *Listeria*, only data from Farms A and B were used when assessing how odds of isolating *Listeria* spp. and *L. monocytogenes* differed across farms. There was no evidence (PD = 0.92; ROPE = 0.11) of an association between *Listeria* spp. and farms when using data from Farms A and B. Only two factors evaluated in the present study were substantially associated with a change in odds of *Listeria* spp. detection (PD ≥ 0.95 and ROPE ≤0.025); (i) if farm equipment and (ii) if farm workers were present in the field during sampling (Table 3). When farm equipment (e.g., tillage equipment, tractors) and farm workers (employees of the operation) were present during the time of sampling, the odds of detecting *Listeria* spp. was approximately 9 [MAP = 8.98, 95% Credibility Interval (CrI) = 1.63, 38.75] and 8

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TABLE 2 Prevalence of *Listeria* spp., *L. monocytogenes*, *Salmonella*, and *Escherichia coli* by farm and visit.

Sampling visit	No. of samples	Frequency (Percent)			
		Listeria spp[a]	*L. monocytogenes*	*Salmonella*	*E. coli*[b]
Farm A					
Visit 1	20	1 (5.0)	0 (0.0)	0 (0.0)	11 (55.0)
Visit 2	20	3 (15.0)	0 (0.0)	0 (0.0)	6 (30.0)
Visit 3	20	4 (20.0)	1 (5.0)	0 (0.0)	7 (35.0)
Visit 4	20	1 (5.0)	0 (0.0)	0 (0.0)	11(55.0)
Total	80	9 (11.3)	1 (1.3)	0 (0.0)	35 (43.8)
Farm B					
Visit 1	20	4 (20.0)	1 (5.0)	0 (0.0)	13 (65.0)
Visit 2	20	4 (20.0)	1 (5.0)	0 (0.0)	8 (40.0)
Visit 3	20	4 (20.0)	0 (0.0)	0 (0.0)	16 (80.0)
Visit 4	20	3 (15.0)	3 (15.0)	1 (0.0)	17 (85.0)
Total	80	15 (18.8)	5 (6.3)	1 (1.3)	54 (67.5)
Farm C					
Visit 1	20	0 (0.0)	0 (0.0)	6 (30.0)	8 (40.0)
Visit 2	20	0 (0.0)	0 (0.0)	1 (5.0)	0 (0.0)
Visit 3	20	0 (0.0)	0 (0.0)	2 (15.0)	6 (30.0)
Visit 4	20	0 (0.0)	0 (0.0)	0 (0.0)	4 (20.0)
Total	80	0 (0.0)	0 (0.0)	9 (11.3)	18 (22.5)
Overall total	240	24 (10.0)	6 (2.50)	10 (4.2)	107 (44.6)

[a]*Listeria* spp. prevalence includes *L. monocytogenes*.
[b]Samples above the Limit of Detection (1 $\log_{10}$ CFU/g).

(MAP = 7.72, CrI = 2.03, 64.17) times more likely, respectively (Table 3). As for *L. monocytogenes,* soil pH was the only evaluated factor substantially associated with a change in *L. monocytogenes* detection odds, with each one-unit increase in pH, there was a 5.5 times increase in the odds of *L. monocytogenes* detection (MAP = 5.48, CrI = 0.75, 345.39; Table 3).

Since no *Salmonella* was detected on Farm A, only data from Farms B and C were used when assessing the odds of detection. *Salmonella* was 11.6 (CrI = 1.36, 1155.27) times more likely to be recovered on Farm C, compared to Farm B (Table 3). The average concentration of generic *E. coli* in samples positive and negative for *Salmonella* were 1.35 ± 0.73 and 1.54 ± 0.78 $\log_{10}$ CFU/g, respectively. There was no evidence of an association between the odds of *Salmonella* detection and all other factors considered in this study (e.g., weather, soil nutrients, management factors), including both the presence and concentration of generic *E. coli* or total coliforms.

Odds of indicator bacteria were associated with soil nutrients and properties

Since *E. coli* levels fell below the lower limit of detection (1 $\log_{10}$ CFU/g) for 55% (133/240) of soil samples, a hurdle model was implemented in order to separately understand factors associated with the presence and concentration of generic *E. coli.* Odds of generic *E. coli* detection differed by farm, with the odds of detection being 0.29 and 0.05 times less likely in Farm A and Farm C, compared to Farm B (Table 4). However, when the concentration of *E. coli* was examined, farm did not substantially impact levels (PD: 0.69–0.79; ROPE: 0.15–0.24). Three soil nutrients and two soil properties examined in this study were substantially associated with increased odds of detecting generic *E. coli*: calcium (MAP = 4.18, CrI = 0.89, 29.10), iron (MAP = 3.44, CrI = 0.93, 45.29), magnesium (MAP = 7.12, CrI = 1.53, 104.32), organic matter (MAP = 7.81, CrI = 0.75, 97.69), and pH (MAP = 4.87, CrI = 2.31, 12.22; Table 4). Organic matter had the greatest effect on generic *E. coli* detection with a one-unit change in organic matter (%) resulting in a 7.81 increase in odds of generic *E. coli* detection. Three soil nutrients were substantially associated with decreased levels of generic *E. coli*: iron (MAP = 1.71, CrI = −3.22, −0.15), sodium (MAP = −1.31, CrI = −2.21, −0.30), and sulfur (MAP = −0.73, CrI = −1.66, −0.04). A one-unit change in iron (mg/kg) resulted in the largest decrease in generic *E. coli* levels with a −1.71 $\log_{10}$ CFU/g change (Table 4).

Total coliform concentrations were 1.51 $\log_{10}$ CFU/g lower on Farm C, as compared to Farm B (CrI = −2.04, −1.05; Table 4). No substantial difference in total coliform levels were observed between Farm B and Farm A (PD = 0.88, ROPE = 0.27). Four soil nutrients and two soil properties were substantially positively associated with total coliform levels: calcium (MAP = 0.49, CrI = 0.06, 1.02), iron (MAP = 0.38, CrI = 0.05, 0.78), manganese (MAP = 0.75, CrI = 0.41, 1.04), magnesium (MAP = 0.80, CrI = 0.23, 1.33), organic matter (MAP = 1.09, CrI = 0.48, 1.61), and soil pH (MAP = 0.36, CrI = 0.14, 0.55; Table 4). Similar to *E. coli* detection, organic matter had the greatest effect with a one-unit change in organic matter (%) resulting in an increase in total coliforms of 1.09 $\log_{10}$ CFU/g.

TABLE 3 Factors associated with the odds of isolating *Listeria* spp., *L. monocytogenes*, and *Salmonella* according to Bayesian mixed models.

Covariate	MAP[a]	CrI[b]	PD[c]	ROPE[d]
Listeria spp.[e]				
Magnesium (mg/kg)	0.08	0.00, 2.74	0.92	0.02*
Farm Equipment (Y/N)	8.98	1.63, 38.75	0.99*	0.00*
Farm Workers (Y/N)	7.72	2.03, 64.17	0.99*	0.00*
L. monocytogenes				
Farm B (Reference = Farm A)	5.16	0.51, 296.81	0.94	0.03*
Aluminum (mg/kg)	$5.98{\cdot}10^{4}$	$0.01, 1.52{\cdot}10^{13}$	0.91	0.01*
Organic Matter (%)	16.73	$0.01, 1.13{\cdot}10^{5}$	0.79	0.02*
pH	5.48	0.75, 345.39	0.98*	0.01*
Temperature Day 0 to 5 BSC[f] (°C)	0.78	0.21, 1.16	0.96*	0.24
Relative Humidity 0 to 5 BSC (%)	1.62	1.00, 4.18	1.00*	0.05
Total Precipitation Day 0 to 5 BSC (mm)	1.01	0.99, 1.03	0.97*	1.00
Salmonella [g]				
Farm C (Reference = Farm B)	11.55	1.36, 1155.27	0.98*	0.00*
Sodium (mg/kg)	17.86	0.08, 2964.83	0.87	0.02*
Aluminum (mg/kg)	1.02	0.00, 22171.31	0.55	0.02*
Total Precipitation Day 0 to 5 BSC (mm)	1.01	0.99, 1.04	0.96*	1.00

[a]Maximum a posteriori estimate. For covariates that are categorical, the MAP is interpreted as the change in odds due to the change in level. For continuous covariates, the MAP is interpreted as the change in odds relative to a one-unit change in the covariate.
[b]95% Credibility Interval.
[c]Probability of Direction: values ≥0.95 are marked with*.
[d]Region of Practical Equivalence: values ≤0.025 are marked with*.
[e]Since no soil sample from Farm C were positives for *Listeria*, only data from Farms A and B were used when assessing how odds of isolating *Listeria* spp. and *L. monocytogenes* differed across farms.
[f]Before Sample Collection.
[g]Since no soil sample from Farm A were positives for *Salmonella*, only data from Farms B and C were used when assessing how odds of isolating *Salmonella* differed across farms.

Salmonella serotypes

From the 10 *Salmonella*-positive samples, five different *Salmonella* serovars were identified. *Salmonella* isolates from Farm B (visit 4) were identified as III 42:z10:e,n,x,z15 (Table 5). The remaining *Salmonella* isolates, all from Farm C, yielded the following serovars: Enteritidis (1), Florida (2), Javiana (3), and Newport (3). *S. enteritidis*, *S. Florida*, and *S.* Javiana were all isolated from visit 1 on Farm C, while *S.* Newport was only isolated during visits 2 and 3 (summer timeframe).

Discussion

The current study was performed to investigate foodborne pathogen prevalence (*Salmonella*, *Listeria monocytogenes*) and concentration of indicator bacteria (total coliforms, generic *Escherichia coli*) in agricultural soils; as well as, characterize associations between soil properties (e.g., macro- and micro-nutrient levels) and each microbial target. The goal was to understand how certain soil nutrients and/or properties may synergistically and/or antagonistically affect pathogen prevalence and indicator concentrations in soil. The prevalence of *Salmonella*, *Listeria* spp., and *L. monocytogenes* recovered in unamended soils was low, and also similar to a previous study conducted in Virginia, which also found low *Salmonella* and *L. monocytogenes* prevalence in the agricultural soils (0.5–1.8%) (Murphy et al., 2023). Interestingly, the prevalence of foodborne pathogens in unamended soils in the study reported here is lower than pathogen prevalence in amended soils reported in previous work (Gu et al., 2019; Hailu et al., 2021; Ferguson et al., 2023; Pires et al., 2023). For example, a previous study in Ohio (United States) noted that pathogen prevalence (*E. coli* O157, *Salmonella*, *L. monocytogenes*, and *Campylobacter*) was higher in manure-amended soils (84%), compared to non-amended soil samples (15.9%; $p < 0.05$) on farms (Hailu et al., 2021). Due to the low prevalence of foodborne pathogens in unamended soils, future studies may opt to investigate pathogen associations with soil nutrients and/or properties may utilize laboratory and/or greenhouse based experiments.

Management practices influenced *Listeria* prevalence while region influenced *Salmonella* prevalence

When farm equipment (e.g., tillage equipment, tractors, etc.) and farm workers were present in the field at the time of sampling, the odds of isolating *Listeria* spp. increased substantially. While we failed to find an association between soil disturbances by animal intrusions and *Listeria* spp. prevalence, these results suggest that soil disturbances by field management practices may impact *Listeria* spp. prevalence. This aligns with previous studies which have demonstrated that management practices that disturb the soil and allow the subsurface to become exposed increase the probability of microorganism detection (Vivant et al., 2013; Strawn et al., 2013b). In contrast, an

TABLE 4 Factors associated with odds of generic *E. coli* detection and levels of generic *E. coli* and total coliforms according to Bayesian mixed models.

Covariate	MAP[a]	CrI[b]	PD[c]	ROPE[d]
E. coli (P/A)				
Farm (Reference = Farm B)				
Farm A	0.29	0.09, 0.75	0.99*	0.00*
Farm C	0.05	0.00, 0.32	1.00*	0.00*
Calcium (mg/kg)	4.18	0.89, 29.10	0.98*	0.01*
Iron (mg/kg)	3.44	0.93, 45.29	0.97*	0.02*
Magnesium (mg/kg)	7.12	1.53, 104.32	0.99*	0.00*
Organic Matter (%)	7.81	0.75, 97.69	0.96*	0.02*
pH	4.87	2.31, 12.22	1.00*	0.00*
E. coli ($\log_{10}$ CFU/g)				
Iron (mg/kg)	−1.71	−3.22, −0.15	0.98*	0.00*
Phosphorus (mg/kg)	−0.54	−1.24, 0.07	0.96*	0.04
Sodium (mg/kg)	−1.31	−2.21, −0.30	0.99*	0.00*
Sulfur (mg/kg)	−0.73	−1.66, −0.04	0.96*	0.02*
Total Carbon (%)	−0.30	−0.60, −0.03	0.97*	0.06
Total Nitrogen (%)	−1.56	−3.91, 0.99	0.89	0.02*
Total Coliforms ($\log_{10}$ CFU/g)				
Farm (Reference = Farm B)				
Farm A	−0.19	−0.43, 0.10	0.88	0.27
Farm C	−1.51	−2.04, −1.05	1.00*	0.00*
Calcium (mg/kg)	0.49	0.06, 1.02	0.98*	0.02*
Iron (mg/kg)	0.38	0.05, 0.78	0.99*	0.01*
Magnesium (mg/kg)	0.80	0.23, 1.33	0.99*	0.00*
Manganese (mg/kg)	0.75	0.41, 1.04	1.00*	0.00*
Organic Matter (%)	1.09	0.48, 1.61	1.00*	0.00*
pH	0.36	0.14, 0.55	1.00*	0.00*
Temperature Day 0 to 5 (°C)	−0.06	−0.13, −0.01	0.98*	0.89

[a]Maximum a posteriori estimate. For covariates that are categorical, the MAP is interpreted as the change in odds due to the change in level. For continuous covariates, the MAP is interpreted as the change in odds relative to a one-unit change in the covariate.
[b]95% Credibility Interval.
[c]Probability of Direction: values ≥0.95 are marked with*.
[d]Region of Practical Equivalence: values ≤0.025 are marked with*.

additional study found that within an organic field in Maryland (United States), *Listeria* spp. populations decreased following tilling events (Reed-Jones et al., 2016). However, since tillage has been shown to affect the microbial structure of soil, leading to the loss of total carbon, total nitrogen, and the breakdown of organic matter (Jackson et al., 2003), more research is needed on the effects of soil disturbances on bacterial detection, specifically *Listeria*.

The study presented here demonstrated that intrastate region influenced *Salmonella* prevalence in Virginia soils with *Salmonella* detection more likely to occur in soils from the Coastal Plain region (Farm C). Additionally, the *Salmonella* serovar isolated from Farm B was not isolated from Farm C. These results suggest that diversity of *Salmonella* may differ by region; however, this finding may be an artifact of the small number of positive *Salmonella* samples in the reported study. This is supported by previous work that has shown *Salmonella* serovar diversity differs across regions, including interstate regions (Gorski et al., 2011; Strawn et al., 2014; Murphy et al., 2023). These findings are of interest as produce grown and packed in the Coastal Plain region of Virginia have been implicated in *Salmonella* outbreaks associated with fresh produce (Greene et al., 2008; Bell et al., 2015). Previous research in this region of Virginia has also shown repeated isolation of *Salmonella* from environmental sources including sediment, water, soil, and wildlife (Greene et al., 2008; Gruszynski et al., 2014; Angelo et al., 2015; Bell et al., 2015; Truitt et al., 2018; Gu et al., 2019; Murphy et al., 2023). It has been hypothesized that *Salmonella* may be introduced to Coastal Plain soils by wildlife within the production environment (Greene et al., 2008; Gruszynski et al., 2014; Bell et al., 2015; Truitt et al., 2018; Gu et al., 2019). A 2013 study conducted within the Coastal Plain region of Virginia revealed that 8.8% (23/262) of wildlife samples were positive for *Salmonella*, with the majority originating from avian species (65.2%; 15/23) followed by reptiles (30.4%; 7/23) and mammalian species (4.3%; 1/23) (Gruszynski et al., 2014). Surveys in this study collected from Farm C (costal plain region) listed expected animal

TABLE 5 *Salmonella* serovars by farm and sampling visit.

Farm	Sampling visit	Serotype	No. of isolates
B	Visit 4	III 42:z10:e,n,x,z15	1
C	Visit 1	Florida	2
		Enteritidis	1
		Javiana	3
	Visit 2	Newport	1
	Visit 3	Newport	2

intrusion from avian, rodent, and reptile species. While all farms in this study mentioned wildlife control measures were in place, it was noted the difficulty to maintain these barriers and/or controls. Therefore, a continued understanding of how regional factors, such as wildlife in the surrounding area, contribute to *Salmonella* prevalence could be of value to growers when evaluating the risks associated with their production environments.

Increases in pH resulted in higher prevalence of *Listeria monocytogenes* and generic *Escherichia coli* and increased concentrations of total coliforms

The prevalence of both *L. monocytogenes* and generic *E. coli*; as well as, the concentration of total coliforms were positively associated with increased pH levels. Farm B soil, which had the highest prevalence of both *L. monocytogenes* and generic *E. coli*, and the highest concentration of total coliforms, had the highest pH values at 6.34 ± 0.57 (range 5.30–7.60). Conversely, Farm C had the lowest pH values (5.97 ± 0.44), with no *L. monocytogenes*-positive samples, the lowest generic *E. coli* prevalence, and the lowest total coliform concentrations. While *L. monocytogenes* can grow in both neutral and acidic soil conditions (Locatelli et al., 2013; Linke et al., 2014; Ferguson et al., 2023), a previous study conducted across 2 years from 12 areas in Austria found that from 467 soil samples collected, *Listeria* isolation was significantly associated with soils that had a neutral pH (7.44; 3.43–9.90; Linke et al., 2014). As for *E. coli*, greater survival rates have been observed in soils with a moderately neutral to alkaline pH (6.0–8.5), compared to acidic (Reddy et al., 1981; Sjogren, 1994; Wang et al., 2014; Emch et al., 2020). In Virginia, soils tend to be acidic and require lime additions every few years as the pH slowly drops, with lime applications based on soil testing with a target pH of 6.2 for most produce fields (Maguire and Heckendorn, 2022). As this practice may contribute to changes in soil pH, more research is needed to better understand the relationship between soil pH, management practices, and pathogen prevalence.

However, one limitation of the study presented here is data was not collected for when fertilization of sites occurred (e.g., when were additions/applications of nitrogen, phosphorous); as well as, information on exact fertilization sources. This additional data could assist with elucidating potential associations between foodborne pathogens and macro- and micro-nutrients. It is known that crops will uptake soil nutrients for their metabolisms (Näsholm et al., 2009; Bindraban et al., 2015); thus understanding how and at what concentrations foodborne pathogens may require macro-and micro-nutrients could assist in understanding survival and persistence behavior within agricultural soils.

Indicator organisms may not be sufficient to predict pathogen prevalence in soil

Total coliforms and generic *E. coli* are frequently used as indicators for sanitary conditions and the presence of fecal bacteria; however, since both have known ecological and environmental niches other than fecal sources, their presence is not always indicative of fecal contamination (Cox et al., 1988; Luo et al., 2011). Testing for foodborne pathogens is often more labor, time, and cost-intensive, so testing for *E. coli* and total coliforms is often still beneficial, under certain circumstances (e.g., flooding). Previous studies have investigated the use of indicator bacteria, including generic *E. coli* and total coliforms, as indicators of foodborne pathogens in soils, and have found varying results (Natvig et al., 2002; Holvoet et al., 2014; Reed-Jones et al., 2016; Hruby et al., 2018; Emch et al., 2020). Results from this study showed *E. coli*, both prevalence and concentration, and total coliforms were not associated with *Salmonella*, *Listeria* spp., or *L. monocytogenes* prevalence. Therefore, the use of indicator bacteria for predicting times and locations of increased likelihood of pathogen prevalence in soil may not be appropriate. This result may be due to the limited sample size, complexities in soil properties, and/or environmental conditions, but in the present study indicator bacteria were not associated with *Salmonella* or *L. monocytogenes* prevalence.

However, the use of indicator organisms for pathogen prediction under specific soil conditions and situations may be useful, such as after a flooding event (Castro-Ibáñez et al., 2015; Bergholz et al., 2016; Callahan et al., 2017). Flooding events pose a large risk for growers both from a food safety, quality, and operational perspective. A previous study found that, following a flooding event, bacteria could be recovered from soils up to 9 m from the threshold of the flood (Callahan et al., 2017), while an additional study observed irrigation water samples collected 1 week after a flooding event had elevated levels of both coliforms and generic *E. coli* (Castro-Ibáñez et al., 2015). These findings demonstrate a flood event may lead to increased levels of biological contamination in the environment (e.g., soil, irrigation water). Recent flooding events in other major growing regions of the United States have emphasized this risk, attracting attention from the United States FDA; as well as, trade organizations, such as Western Growers Association and the California Leafy Greens Marketing Agreement (LGMA) (California Leafy Greens Marketing Agreement, 2023; Food and Drug Administration, 2023; Timmins, 2023). Specifically, the California LGMA requires soil testing following a flooding event that suggests growers should wait a minimum of 60 days after a flooding event to begin planting and that fecal coliform levels should be <100 MPN/g in soils (California Leafy Greens Marketing Agreement, 2021; California Leafy Greens Marketing Agreement, 2023). While the research presented here demonstrates that routine testing of soils for indicator organisms may not be able to provide insight into pathogen prevalence, it may be helpful for growers in mitigating risks during unforeseen events or conditions (e.g., flooding), or trending/monitoring when indicator organism levels adjust to baseline levels.

Conclusion

The interface between foodborne pathogens and factors influencing agricultural soils within the production environment is complex. The

findings of the study showed the prevalence of *Salmonella*, *L. monocytogenes*, and generic *E. coli* was low in unamended agricultural soils, especially compared to previous pathogen prevalence data in amended soils. The recovery of *Salmonella* and *L. monocytogenes* varied substantially by region indicating regional produce safety guidance/best practices may be appropriate. Additionally, the concentration of indicator bacteria and *Listeria* spp. were also impacted by soil nutrients, soil properties and field management practices. While conducting additional soil testing for generic *E. coli* and other indicator bacteria may be useful tools during catastrophic flooding events, data from this study and others, demonstrated indicator bacteria presence and levels were not sufficient to predict pathogen prevalence in soil. This study also offered insights into how growers may potentially utilize soil testing (a practice already routinely preformed by growers for soil health and fertility) to identify how foodborne pathogens and/or indicator bacteria may be present in agricultural soils.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

CC: Data curation, Formal Analysis, Investigation, Writing – original draft, Writing – review & editing. CD: Writing – review & editing, Conceptualization. DW: Writing – review & editing, Formal Analysis, Visualization. CM: Formal Analysis, Writing – review & editing, Data curation, Writing – original draft. AH: Writing – review & editing, Investigation. MP: Writing – review & editing. RB: Writing – review & editing. SR: Writing – review & editing, Conceptualization, Data curation, Investigation. RM: Data curation, Investigation, Writing – review & editing. MD: Writing – review & editing, Conceptualization, Funding acquisition, Methodology. LS: Conceptualization, Funding acquisition, Methodology, Writing – review & editing, Data curation, Formal Analysis, Investigation, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2023.1269117/full#supplementary-material

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