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Evaluation of environmental risk factors associated with survival of generic *E. coli* in organic integrated crop-livestock systems in California and Minnesota

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Introduction: Integrated crop-livestock systems (ICLS) improve soil health and productivity but may lead to the transfer of foodborne pathogens to fresh produce from soil contaminated with the feces of grazing animals.

Methods and results: Over 2 years (2021–2022), organic ICLS field trials were conducted in California (CA) and Minnesota (MN) to examine the presence of foodborne pathogens (*Escherichia coli* O157, non-O157 Shiga toxin producing *E. coli* (STEC), and *Listeria monocytogenes*) across three treatments (fallow, cover crop without grazing, and cover crop with grazing by small ruminants) in soils and produce. A Random Forest (RF) analytical approach was used to determine potential associations between meteorological and soil chemical factors, and the generic *E. coli* (gEc) presence in soil. The persistence of gEc in soil, as an indicator of fecal contamination, was evaluated using mixed effect zero-inflated negative binomial (ZINB) models, considering top-ranked meteorological factors identified from RF analyses. One produce sample (cucumber) tested positive for non-O157 STEC (0.6%, 1/157) from grazed soil in MN, with no other foodborne pathogens detected in produce. Soil contamination by non-O157 STEC increased in 2022 (2.7%, 15/552) compared to 2021 (0%, 0/504) from both states, aligning with the increased prevalence of non-O157 STEC in post-grazed fecal samples from sheep in CA (41.7%, 5/12) and goats (42.5%, 17/40) in MN in 2022. Concentrations (Most Probable Number/100 g) of gEc in grazed soil returned to levels comparable to non-grazed or fallow treatments within 87–147 days post-graze (DPG) in both years, with a significant decrease predicted after 32 DPG. Interestingly, non-O157 STEC was detected in soil even after concentrations of gEc declined 115–147 DPG.

Discussion: Although the effects of meteorological factors and soil chemical characteristics were not as influential as treatment or sampling day effects, ZINB analyses with identified meteorological factors in grazed soil suggested that regional differences in gEc counts were likely influenced by maximum air/soil temperatures on the sampling day. Further investigation is needed to evaluate the adoption of the

NOP 90–120-day interval rules between manure application by grazing and harvest, considering a wider range of environmental regions and the potential cumulative effect of continuous ICLS in the same field on pathogen loads.

KEYWORDS

grazing, food safety, sheep, goats, STEC, cover crops, fresh produce

1 Introduction

Agroecological practices focus on building the resilience of ecosystems and supporting more sustainable food production systems (Schipanski et al., 2016; Newton et al., 2020). These practices include minimizing soil disturbance and enhancing biodiversity to build fertility, soil health, and pest control mechanisms (Rhodes, 2017; LaCanne and Lundgren, 2018). Organic and regenerative farms have increasingly coupled the use of cover crops and livestock grazing known as integrated crop-livestock systems (ICLS), which aim to enhance soil fertility and improve carbon storage and water retention (Franzluebbers et al., 2011; Hilimire, 2011; Lemaire et al., 2014; Schipanski et al., 2014). This study focused on a rotational form of ICLS in organic fresh produce production, which involves animal grazing of winter cover crops seeded between growing produce within a single field. In this system, manure deposited by the animals and remaining cover crop residue are incorporated into the soil as organic sources of fertilizer before planting produce crops.

Despite various ecological benefits, animal grazing in ICLS poses a risk of transferring foodborne pathogens to produce crops via direct contact with soil (Kudva et al., 1998; Park et al., 2012; Nazareth et al., 2021). Raw manure from livestock naturally carries enteric pathogenic bacteria including Shiga toxin-producing *Escherichia coli* (STEC, such as *E. coli* O157:H7), *Salmonella* spp., and *Listeria* spp., which can cause severe illness and even death in humans (Hutchison et al., 2005; Franz et al., 2008; Alegbeleye et al., 2018). Application of livestock manure, including biological soil amendments of animal origin (BSAAO), is recognized as one of the major sources of preharvest bacterial contamination in fresh produce production (Park et al., 2015; Sharma and Reynnells, 2016; Pires et al., 2018). These types of soil amendments are also important inputs for the United States Department of Agriculture (USDA) certified organic producers who are not allowed to use synthetic fertilizers (USDA, 2024). Certified organic farms operate under the standards established by the USDA National Organic Program (NOP) when applying BSAAO onto their fields. The NOP stipulates a time-interval of 90–120 days between the application of raw manure and the harvest of fresh produce to minimize the risk of bacterial contamination (USDA-AMS NOP, 2011). The US Food and Drug Administration (FDA) also addresses concern regarding the use of BSAAOs applied to fresh produce fields through the Produce Safety Rule of the Food Safety Modernization Act (FSMA; FDA, 2015). However, the FDA has not explicitly established the differences and rules between raw manure applications and grazing due to lack of scientific evidence. Recent studies have begun to assess the risk and persistence of bacterial contamination in raw manure-amended fields (Sharma et al., 2019; Tran et al., 2020; Ramos et al., 2021; Pires et al., 2023).

Previous studies have shown that meteorological factors (i.e., precipitation, air temperature, and wind speed) and soil characteristics (i.e., soil type, soil moisture content, and soil chemical properties) by geographical regions are risk factors for contamination of foodborne pathogens in fresh produce fields (Ivanek et al., 2009; Ongeng et al.,

2011; Strawn et al., 2013; Sharma et al., 2019; Weller et al., 2020; Litt et al., 2021; Cook et al., 2023). In particular, multiple studies reported that average precipitation (i.e., rainfall and snowfall) before soil or produce sample collection showed a significant association with the contamination and survival of STEC, *Listeria* spp., and generic *E. coli* (gEc) (Park et al., 2015; Pang et al., 2017; Litt et al., 2021; Pires et al., 2023). According to a meta-analysis compiling 42 studies investigating environmental factors regulating persistence of *E. coli* in manure-amended soils, temperature was the most influential factor affecting decline rates of *E. coli* under field conditions (Tran et al., 2020). Among soil characteristics, soil moisture content and temperature were the most determinant factors on the duration of manure-borne pathogen survival in soil (Park et al., 2016). Soil pH and temperature were also identified as critical factors influencing survival of both commensal and pathogenic *E. coli* in soil (Franz et al., 2014).

Although studies related to the application of raw manure or BSAAO in fields have been conducted in various regions, little research is present regarding the potential risk associated with directly implementing grazing in ICLS fields (Ingham et al., 2004; Nazareth et al., 2021; Goodwyn et al., 2023). Our previous observational and experimental ICLS studies conducted in California revealed that foodborne pathogen contamination of produce by grazing events was minimal, and the concentration of gEc in soil, serving as an indicator of fecal contamination, returned to the baseline levels within 120 days post-grazing (Patterson et al., 2018; Cheong et al., 2024). However, these grazing studies did not consider various agricultural and environmental factors such as meteorological elements and soil characteristics, which may influence the persistence and survival of bacterial pathogens in soil.

To provide detailed guidelines for minimizing contamination of foodborne pathogens in ICLS, more research is needed across diverse regions to clarify various environmental risk factors. In this study, we examined the risk of grazing on the contamination of soil and fresh produce by foodborne pathogens under two different environmental conditions in California (CA) and Minnesota (MN). Two-year ICLS experimental studies were conducted to evaluate the presence of foodborne pathogens (*E. coli* O157, non-O157 STEC, and *L. monocytogenes*) in soil and produce, as well as in fecal samples from grazing animals, across three different treatments (fallow, cover crop without grazing, and cover crop with grazing by small ruminants). Persistence of gEc was further assessed through monthly quantification of gEc in grazed soil, while potential risk factors related to meteorological conditions and soil health influencing the presence of gEc in soil were examined using a machine learning approach.

2 Materials and methods

2.1 Study design

Replicated field trials were established as randomized complete block designs with four replicates in organically certified fields in two

states (CA and MN) over two growing seasons (2020–2022). Fields were located at the Russell Ranch Sustainable Agriculture Facility, University of California, Davis (UC Davis; 38° 32′ 36.87″, –121° 52′ 11.89″) and at the University of Minnesota Southwest Research and Outreach Center (44° 14′ 29.4858″, –95° 19′ 1.5312″). Three treatments—fallow as a control, cover crop tilled without grazing (non-graze CC), and cover crop grazed by sheep or goats (graze CC) were randomly allocated within each block. Each block corresponded to 9 beds (13.5 m wide), and 3 beds in a row were assigned to each treatment. A middle bed (1.5 m wide) of each treatment was used for sampling, as the two side beds functioned as a buffer between treatments.

The timing of cover crop mix seeding, sheep/goats grazing, and planting of the main produce crops (spinach in 2021, and cucumber in 2022) depended on each state (CA and MN) climate and growing seasons. Both states followed the cropping sequence that is generally adopted by regional organic vegetable growers. The cover crop mix was seeded in November for the CA trial and in late August or early September for the MN trial. The cover crop mix in CA consisted of cereal rye (60 lbs./ac), crimson clover (10–12 lb./ac), and daikon radish (10 lbs/ac), while in MN the mix comprised winter rye (1 lbs/ac), berseem clover (10 lbs/ac), and daikon radish (10 lbs/ac). Grazing was initiated when the cover crops height was 25 cm. After grazing events, beds of graze CC and non-graze CC treatments were terminated, and the main crops of spinach/cucumber were transplanted/seeded in March–April in CA, and in May–June in MN.

For grazing, CA and MN trials used sheep and goats, respectively. The sheep originated from the sheep facility located at UC Davis, and goats were from a goat rental company in MN, which was located 2 h from the field. Electrical fences were installed around the areas of the graze CC treatment before sheep or goats were transported to the field. The number of grazing animal units and period of grazing were decided based on the amount of cover crop biomass available for grazing each time and location (USDA-Natural Resources Conservation Service, 2013). As a result of growing conditions, grazing was conducted once in CA but twice in MN per year. While grazing at CA, a flock of 49 sheep grazed once during March 2–4, 2021, and 25 sheep grazed once on February 17, 2022. The flock was composed of ewes (e.g., Suffolk, Hampshire, Dorset, and crossbreed) with ages from 1 to 5 years old. For MN, in the first year of the study, a herd of 40 goats grazed on November 4, 2020, and 115 goats grazed on April 28–30, 2021. In the second year, grazing took place twice and 160–170 goats grazed during October 6–8, 2021, and again on May 20, 2022. The herd comprised Spanish goat breed in age from yearlings to up to 10 years old. Sheep or goats grazed rotationally from one paddock (13.5 × 22.9 m) to another to achieve equalized levels of biomass (approximately 10 cm). Grazing animals were provided *ad libitum* water, and lights (CA) or a guard dog (MN) to protect them from wildlife when they stayed overnight at the field. The research protocol was approved by the Institutional Animal Care and Use Committees (IACUC) of the University of California, Davis (IACUC protocol #22700) and the University of Minnesota (IACUC protocol #2008-38348A).

2.2 Sample collection

A total of 1,056 soil samples were collected during the seven or eight field visits each year, including the baseline after seeding winter

cover crops, just before grazing [0 Day Post-Graze (DPG)], 7 DPG, and monthly after the grazing event until approximately 120 or 150 DPG. Figure 1 describes the specific soil sampling and grazing dates of the field trials in CA and MN, with the number of samples collected on each date. When weather conditions did not allow (i.e., heavy rain or snow), sampling was performed within 1–2 weeks from the planned DPG dates. Grazing events were conducted twice in the MN trials each year; thus, DPG dates were calculated based on the second grazing event. On each sampling day, 36 soil samples were collected as three composite soil samples per bed (12 beds × 3 composites), except for the baseline (i.e., 24 samples as two composite soil samples per bed) and 7 DPG in 2021 in MN (i.e., 60 samples as five composite soil samples per bed). Soil samples were collected from the middle bed of each treatment using a stainless-steel soil core sampling probe (15 cm deep × 2.54 cm diameter) and placed in sterile Whirl-Pak bags (Nasco, Modesto, CA). Gloves were changed and the soil core was cleaned then sanitized with 70% ethanol between each bed.

Pre- and post-grazing fecal samples were collected to assess the presence of foodborne pathogens (i.e., Non-O157 STEC, *L. monocytogenes*, and *E. coli* O157). Pre-graze fecal samples were collected from the barn floor (CA) or trailer floor (MN) before sheep or goats were transported to the field. Post-graze fecal samples were collected immediately after sheep or goats were removed from the graze CC treatment plots on the same day. A total of 36 sheep fecal samples from CA (i.e., 12 samples per each sampling) and 160 goat fecal samples from MN (i.e., 20 samples per each sampling) were collected over the two growing seasons. Pre-graze samples from the second growing season of the CA trial were not collected because the grazing event lasted less than a day. Fresh fecal samples (approximately 30 g of composite fecal material) were aseptically scooped from the barn/transporter or field using gloves and placed into a sterile Whirl-pak bag.

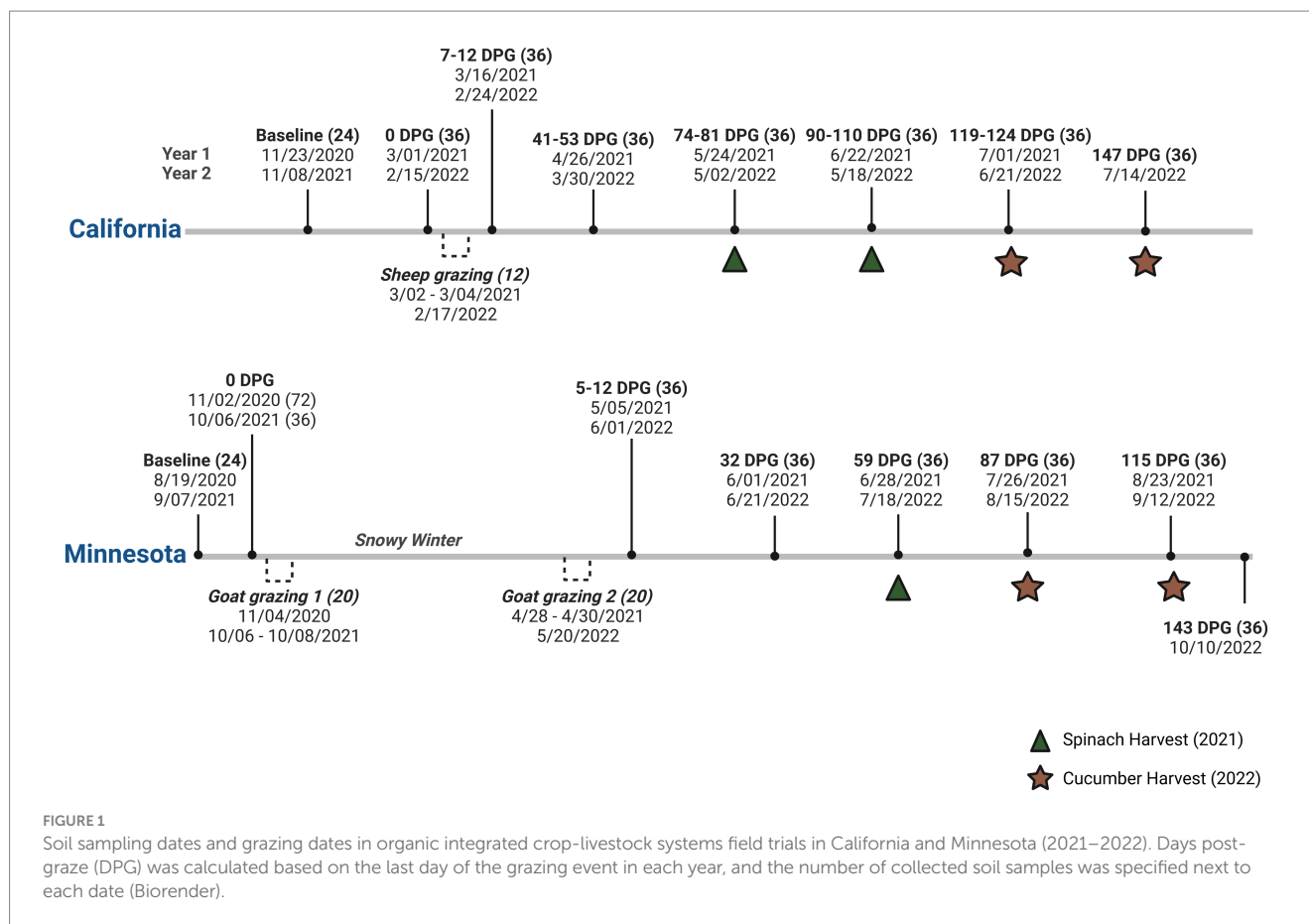
Fresh produce samples (approximately 100 g for spinach and 400–500 g for cucumber per sample) were collected once or twice each year depending on produce maturity. A total of 48 spinach and 47 cucumbers samples were harvested twice each year from CA (Figure 1). In Minnesota, severe heat and drought conditions limited spinach harvest to a total of 14 samples in the first year. In the second year, conditions were normal and a total of 48 cucumber samples were collected from the MN site. On each sampling day, up to two samples per bed (12 beds × 2 samples) of produce were collected from the middle bed of each treatment, as had been done for soil sampling.

Irrigation water (2 L) was sampled one time during each growing season directly into two sterile bottles from tap water linked to irrigation lines.

All samples were transported in a cooler or shipped in insulated containers with ice to the Pires laboratory at UC Davis within 24 h of collection, and processed within 48 h.

2.3 Sample preparation, and isolations of foodborne pathogens and generic *E. coli* (gEc)

Sample preparation, microbial analyses for the isolations of non-O157 STEC, *E. coli* O157:H7, *L. monocytogenes*, and quantification of gEc were described in our prior manuscript (Cheong et al., 2024).



In brief, each soil (30 g per sample) and fecal sample (10 g per sample) was enriched in a 24 oz. Whirl-Pak bag filled with 270 ml Tryptic Soy Broth (TSB; BD Bacto™, Heidelberg, Germany) to detect non-O157 STEC, *E. coli* O157:H7, and gEc, and Listeria Enrichment Broth (LEB; Neogen Culture Media, Lansing, MI, United States) to detect *L. monocytogenes*. To quantify the concentration of gEc (Most Probable Number, MPN/g) in the soil and produce samples, a 48-well reservoir (E&K Scientific, Santa Clara, CA, United States) was filled with 5 ml of the samples in TSB medium, with serial dilution up to 10^{-6} with 4 replications per sample. For the TSB enrichment, samples were incubated at 25 °C for 2 h followed by 42 °C for 8 h with 50 rpm shaking, then held at 6 °C with no shaking in a Multitron programmable shaking incubator (Eppendorf, Hauppauge, NY, United States). For the LEB enrichment, samples were incubated for 18 h at 30 °C with 100 rpm shaking, then held at 6 °C with no shaking.

To isolate non-O157 STEC, 1 ml of enriched TSB sample was put into a tube with 9 ml of modified enterohemorrhagic *E. coli* (mEHEC) selective media (Biocontrol, Bellevue, WA, United States), and incubated for 12 h at 42 °C. Then, mEHEC solution was streaked onto ChromSTEC agar (CHROMagar™, Paris, France) using a 10 µl inoculation loop, and incubated for 24 h at 37 °C. Presumptive positive isolates (i.e., purple colonies that fluoresced under ultraviolet light) on ChromSTEC agar were re-streaked onto secondary and tertiary ChromSTEC agar. A final pure presumptive positive colony was streaked onto Tryptic Soy Agar (TSA) to confirm as non-O157 STEC by conducting a

standard polymerase chain reaction (PCR) assay targeting the *stx1* and *stx2* genes.

To detect *E. coli* O157:H7 and *L. monocytogenes*, Immunomagnetic separation (IMS) was performed with enriched TSB and LEB samples using Dyna anti-*E. coli* O157 or Listeria beads on the automated Dyna Bead Retriever (Invitrogen, Carlsbad, CA), respectively. For *E. coli* O157:H7, an IMS product of 50 µl washed beads streaked onto both Rainbow agar (Biolog, Hayward, CA, United States) with novobiocin (20 mg/L) and tellurite (0.8 mg/L; MP Biomedicals, Solon, OH, United States), and MacConkey II Agar with sorbitol supplemented with potassium tellurite (2.5 mg/L) and cefixime (0.05 mg/L); both plates were incubated for 24 h at 37 °C. For *L. monocytogenes*, an IMS product of 30 µl washed beads streaked onto Brilliance Listeria Agar (BLA) with its selective and differential supplements (Oxoid, Hants, United Kingdom), and that of 100 µl was added into 5 ml of Fraser broth (BD, Sparks, MD); both BLA plates and Fraser broths were incubated for 48 h at 37 °C. With sequential insolation process, a final pure presumptive positive colony was streaked onto TSA, and PCR targeting the *eaeA* and *hlyA* gene was performed to confirm the presence of *E. coli* O157:H7 and *L. monocytogenes*, respectively.

To determine the presence and enumeration of gEc, 10 µl from the enriched TSB bag and 4 µl from each diluted well of the reservoir were streaked onto CHROMagar *E. coli* (ECC; CHROMagar Microbiology, Paris, France), followed by incubation for 24 h at 37 °C. After re-streaking presumptive positive colonies (i.e., blue colonies) onto secondary and tertiary ECC plates, the pure isolates on TSA were confirmed as gEc by PCR targeting the *uspA* gene.

2.4 Collection and modification of meteorological and soil health data

Meteorological data were obtained from the weather stations located approximately 1 km from the experimental sites in CA and MN. Additionally, solar radiation data for CA were retrieved from the Davis station of California Irrigation Management Information Systems (not recorded at the field weather station). The 80 meteorological variables consisted of 16 original weather variables on each soil sampling day (Table 1) and 64 calculated mean or sum variables depending on different time intervals. Different time intervals including 0–3 days, 0–7 days, 0–20 days, and 0–30 days before the soil sampling days were chosen to evaluate whether meteorological variables had immediate or long-lasting effects on the presence of gEc in soil (Ivanek et al., 2009; Park et al., 2015; Pang et al., 2017).

To investigate soil characteristics, soil sampling was conducted from each of 12 beds (i.e., 3 treatments × 4 blocks) five times in CA including the baseline (December 1, 2020) and twice during each growing season (March 29 and May 3, 2021, and April 8 and June 6, 2022), and twice (June 28, 2021, and August 9, 2022) in MN over 2 years. Soil samples (~700 g per sample) were submitted to the University of Maine Analytical Laboratory and Maine Soil Testing Service to obtain routine soil bio-physical and chemical properties. Soil chemical properties used for further analyses included 20 variables—soil pH, percentages (%) of organic matter (OM), potassium (K), magnesium (Mg), and calcium (Ca), and concentrations (ppm) of nitrate (NO₃), ammonium (NH₄), aluminum (Al), boron (B), copper (Cu), iron (Fe), Manganese (Mn), sodium (Na), sulfur (S), zinc (Zn), cation exchange capacity (CEC), and the concentrations (lb) of phosphorus (P), K, Mg, and Ca.

The moisture content of each soil sample was calculated based on the weights of wet and dry soil (g) with the Equation 1. The dry weight of soils reflects oven (105 °C) grams dry weight (gdw) of 10 g fresh weight of soil sample.

$$\text{Soil moisture content (\%)} = \left(\frac{\text{Wet weight} - \text{Dry weight of soil}}{\text{Wet weight of soil (g)}} \right) \times 100 \quad (1)$$

2.5 Statistical analysis

Proportions of foodborne pathogens (*E. coli* O157:H7, non-O157 STEC, and *L. monocytogenes*) positive in soil, produce, and fecal samples were summarized using descriptive statistics. For soil and produce samples, concentrations of gEc (log₁₀ MPN/100gdw soil) were reported as median and range (min–max) after transforming the values under the limit of quantification (LOQ = 0.089 MPN/gdw) as 0.0445 MPN/gdw (LOQ/2). To compare the median concentrations of gEc in soils across the three treatment groups on each sampling day, Kruskal-Wallis tests were used as non-parametric tests, due to non-normal distributions observed in gEc concentrations.

Two random forest (RF) model analyses (1) based on 80 meteorological factors (Table 1) and (2) 20 soil chemical factors were conducted to identify and rank risk factors associated with the presence of gEc in soils. As additional explanatory variables, state, year, treatment and sampling days as categorical variables and soil moisture content as a continuous variable were added into each model. The dataset for the

first model incorporating meteorological factors included 29 timepoints with 1,032 soil sample profiles; the dataset for the second model integrating soil characteristics factors included seven timepoints with 257 soil sample profiles. The presence of gEc data for the second model were extracted from sample profiles of the first model corresponding to the nearest dates of soil characteristics testing, because microbial analyses to investigate the soil pathogens were not conducted simultaneously with soil samplings for characteristics analyses. Each dataset was randomly split into training (80%) and testing sets (20%), and stratified sampling was performed based on the presence of gEc to make the sets balanced. The RF models were run using the randomForest package in R software (v.4.2.0), and the importance of the explanatory variables in the models was evaluated by permutation feature importance scores (Liaw and Wiener, 2002). To decrease the number of variables in the model, feature elimination was performed based on permutation feature importance scores until the out-of-bag error rate ceased to decrease, and none of the variables were highly correlated (i.e., less than 0.85) by dropping the use of variables from the same explanatory categories but within different time intervals. To run each model, ntree (i.e., number of trees) and mtry (i.e., number of randomly drawn candidate variables) were set to 500 and the square root of the number of explanatory variables, respectively. The predictive performance of each model was assessed by the remaining 20% testing sets and subsequently evaluated with a receiver operating characteristic (ROC) curve.

To evaluate the effect of sheep/goats grazing on the soil concentrations of gEc over time, particularly within the graze CC treatment (i.e., 319 samples), mixed effect zero-inflated negative binomial (ZINB) models were used after excluding one extreme outlier (i.e., 94,923 MPN/gdw). The outcome variable of gEc concentration (MPN/100gdw) was log₁₀-transformed and rounded off to the nearest integer if the data were non-zero. Explanatory variables considered in the model were state, year, and DPG. State and year were categorical variables, and the DPG variable was a spline linear continuous variable with the knot at 32 DPG because the highest concentrations of gEc was mostly observed at 32 DPG, followed by a decrease thereafter. Beds of the fields (i.e., 144 total beds) in each year of each state were added as random effects in the models to explain repeated sampling of soil over time. Final multivariable modeling was conducted with variables showing *p*-value <0.2 in univariable analyses by forward selection and included a two-way interaction between state and DPG. All the modeling was run using the *glmmTMB* (generalized linear mixed models Template Model Builder) package with *nbinom1* negative binomial distribution, in which the variance increases linearly with the mean (Brooks et al., 2017). The data collected before each growing season (i.e., November in CA and August/September in MN) as the baselines were reported separately and were not included in the modeling. Furthermore, the seven top-ranked meteorological variables from the RF analysis were individually added into the final ZINB model to evaluate their associations. All the statistical analyses were performed using R software (v.4.3.0) and the significance level was *p*-value <0.05.

3 Results

3.1 Presence of foodborne pathogens

None of the collected soil nor fecal samples from either state tested positive for *E. coli* O157:H7 during the 2 years of the study

TABLE 1 Explanatory meteorological variables considered as potential risk factors for generic *E. coli* presence in soil from organic integrated crop-livestock system field trials in California and Minnesota (2021–2022).

Original explanatory variable	Abbreviation	Unit	Calculated variables [†]
Daily average of barometric pressure	Baro_avg	inHg	Means over 4 different time Intervals—0–3 days, 0–7 days, 0–20 days, and 0–30 days before each soil sampling day
Daily average of relative humidity	RH_avg	%	
Average of air temperature at 2 m	Air_temp_avg	°C	
Maximum of air temperature at 2 m	Air_temp_max	°C	
Minimum of air temperature at 2 m	Air_temp_min	°C	
Average of soil temperature at 10 cm depth	soil_temp10_avg	°C	
Maximum of soil temperature at 10 cm depth	soil_temp10_max	°C	
Minimum of soil temperature at 10 cm depth	soil_temp10_min	°C	
Average of soil temperature at 20 cm depth	soil_temp20_avg	°C	
Maximum of soil temperature at 20 cm depth	soil_temp20_max	°C	
Minimum of soil temperature at 20 cm depth	soil_temp20_min	°C	
Wind direction*	WDir	°	
Maximum wind speed at 2 m	Wind_min	m/s	
Minimum wind speed at 2 m	Wind_max	m/s	
Solar radiation at 2 m	Sol_Rad	W/m ²	
Precipitation in a day	Precip_tot	mm	Sums over 4 different time intervals same as the above periods

*0° = wind out of the north; 180° = wind out of the south.

[†]Calculated variables; reflects the number of days at the end of the abbreviations for the original variables. For example, the average daily barometric pressure over 0–30 days preceding soil sampling would be abbreviated as Baro_avg30.

period. Table 2 summarizes the presence of non-O157 STEC and *L. monocytogenes* in the soil and fecal samples for each treatment group. Of the collected post-graze fecal samples in CA and MN, 20.8% (5/24) and 22.5% (18/80) were non-O157 STEC positive, respectively. Whereas none of the sheep fecal samples collected from CA tested positive for *L. monocytogenes*, 15.0% (12/80) of the post-graze fecal samples from goats in MN trials tested positive for *L. monocytogenes*.

In the first year (2021), none of the soil samples tested positive for foodborne pathogens. However, in 2022 trials, 1.4% (4/276) and 4.0% (11/276) of the soil samples tested positive for non-O157 STEC from CA and MN, respectively. Of four non-O157 STEC positive samples from the CA field, two from the graze CC treatment plots were collected at 0 and 147 DPG, and the other two from the non-graze CC treatment plots were also collected at 0 and 147 DPG. Of 11 non-O157 STEC positive samples from MN field, eight samples from the graze CC treatment plot were collected at 32 DPG, and the three samples from the non-graze CC treatment plots were collected at 32 and 115 DPG. In addition, one sample (0.4%, 1/276) from MN tested positive for *L. monocytogenes*, which was from the non-graze CC treatment plot at 12 DPG.

One cucumber sample (0.6%, 1/157) tested positive for non-O157 STEC collected from one graze CC treatment plot at 115 DPG in MN trials. None of the other produce samples (i.e., spinach and cucumber) from either state tested positive for foodborne pathogens (i.e., non-O157 STEC, *E. coli* O157, *L. monocytogenes*).

3.2 Presence and quantification of generic *E. coli* (gEc) in soil and produce

3.2.1 Soil

Over the 2 years of the field trials, 39.8% (86/216) and 35.7% (90/252) of soil samples from CA, and 25.4% (61/240) and 44.8% (113/252) of soil samples from MN tested positive for gEc. The concentrations of gEc (log₁₀ MPN/100gdw) in graze CC soil were similar between CA (mean = 1.35, sd = 1.07) and MN (mean = 1.49, sd = 1.16). Sampling days that showed the highest soil concentration of gEc among three treatment groups (i.e., fallow, non-graze CC, and graze CC) were between 12 and 53 DPG in the graze CC treatment in both states (Figure 2). No statistically significant differences were observed in the median concentrations of gEc (log₁₀ MPN/100gdw) among three treatment groups after 53–59 DPG in both states in the 2021 trials. In the 2022 trials, CA trial showed significant differences in the median concentrations of gEc among treatment groups until 124 DPG, whereas MN trial showed the difference at 12, 32, and 87 DPG.

Interestingly, the baseline soil samples, collected after seeding winter cover crops in the fields 1–4 months ahead of conducting grazing, showed higher proportions of gEc than soil samples collected at 0 DPG right before grazing events. In CA, proportions of gEc positive at baseline were 75.0% (36/48) although those at 0 DPG were 29.2% (21/72) for 2 years. Similarly, proportions of gEc positive in MN were 70.8% (34/48) at baseline, and 39.6% (38/96) at 0 DPG.

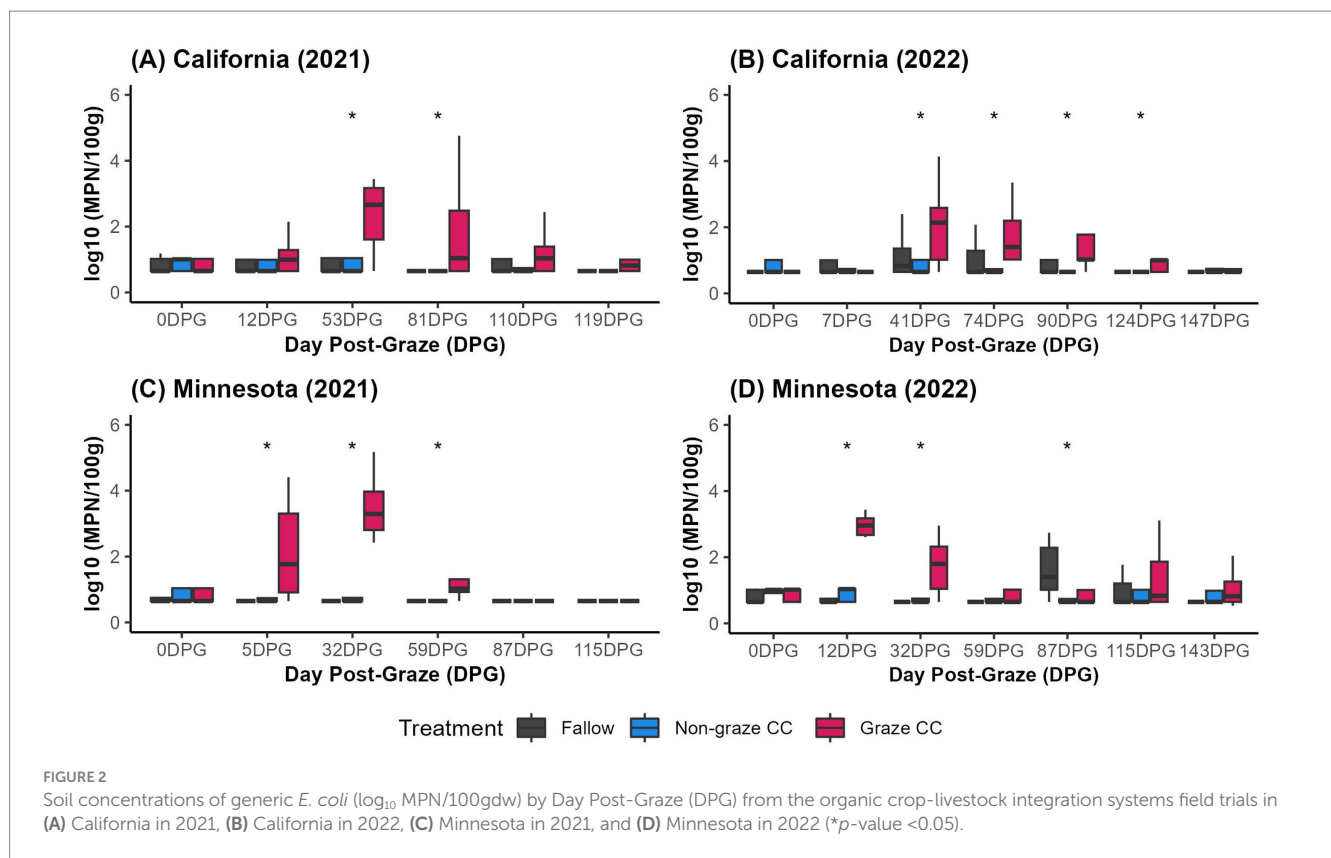
TABLE 2 Presence of foodborne pathogens (non-O157 STEC and *L. monocytogenes*) in fecal and soil samples (%) collected from the organic integrated crop-livestock system field trials (2021–2022) in California and Minnesota.

Sample Type	State	Year	Treatment	Non-O157 STEC	<i>L. monocytogenes</i>
Feces	CA (Sheep)	2021	Pre-graze	0% (0/12)	0% (0/12)
			Post-graze	0% (0/12)	0% (0/12)
		2022	Post-graze*	41.7% (5/12)	0% (0/12)
	MN (Goats)	2021	Pre-graze	2.5% (1/40)	12.5% (5/40)
			Post-graze	2.5% (1/40)	0% (0/40)
		2022	Pre-graze	27.5% (11/40)	45.0% (18/40)
Post-graze			42.5% (17/40)	30.0% (12/40)	
Soil	CA	2021	Fallow	0% (0/80)	0% (0/80)
			Non-graze CC	0% (0/80)	0% (0/80)
			Graze CC	0% (0/80)	0% (0/80)
		2022**	Fallow	0% (0/92)	0% (0/92)
			Non-graze CC	2.2% (2/92)	0% (0/92)
			Graze CC	2.2% (2/92)	0% (0/92)
	MN	2021	Fallow	0% (0/88)	0% (0/88)
			Non-graze CC	0% (0/88)	0% (0/88)
			Graze CC	0% (0/88)	0% (0/88)
		2022**	Fallow	0% (0/92)	0% (0/92)
			Non-graze CC	3.3% (3/92)	1.1% (1/92)
			Graze CC	8.7% (8/92)	0% (0/92)

None of the *E. coli* O157 was detected over 2 years.

*Pre-graze samples were not collected from the CA trial in 2022 as the grazing event lasted less than a day.

**In 2022, the total number of soil samples was 92 per treatment group as samples were collected up to 143–147 DPG.



3.2.2 Produce

A higher proportion (79.0%, 49/62) of gEc positive was observed in produce samples (i.e., spinach and cucumber) from MN compared to those from CA (5.3%, 5/95; Table 3). The median concentration of gEc (log₁₀ MPN/100grams fresh weight, gfw) in gEc-positive samples from MN was 1.78 (0.51–4.66), higher than that from CA which was 0.51 (0.51–1.78). However, the median concentrations of gEc between the types of produce were similar [i.e., spinach: 1.56 (0.51–2.38), cucumber: 1.36 (0.51–4.66)]. Among the five gEc positive samples from CA trials, two samples were from the graze CC treatment, and the three were from the non-graze CC and fallow treatments. In MN trials, out of 49 gEc positive samples, 17 were from the graze CC treatment, 17 from the non-graze CC treatment, and 15 from the fallow treatment.

3.3 Associations between presence of generic *E. coli* (gEc) in soil and meteorological/soil chemical factors

In the RF analyses, the influential explanatory variables in the final models were ranked based on ‘MeanDecreaseAccuracy’, representing the percentage of gEc presence incorrectly classified by removing each variable from the models (Figure 3). In the soil sample profile used for investigating meteorological factors, 39.6% (409/1,032) of the profile were gEc positive. The most influential meteorological factors associated with the presence of gEc in the soil, according to the final RF classifier, included maximum air temperature (Air_temp_max), maximum soil temperature at a 10 cm depth (soil_temp10_max), maximum wind speed (Wind_max) on the sampling day, average wind

TABLE 3 Proportion and median (min-max) concentrations (log₁₀ MPN/100gfw) of generic *E. coli* positive (gEc) in produce samples by sampling day [Day Post-Graze (DPG)] from the organic crop-livestock integration system field trials in California and Minnesota (2021–2022).

State	Year	Sampling day	# of gEc positive	Median (min-max) concentration of gEc in the positive samples [†] (log ₁₀ MPN/100gfw)
CA	2021	81 DPG	4.2% (1/24)	1.40 ⁺⁺
		(Spinach) 110 DPG	8.3% (2/24)	0.51 ⁺⁺
	2022	124 DPG	0% (0/23)	-
		(Cucumber) 147 DPG	8.3% (2/24)	1.14 (0.51–1.78)
MN	2021	(Spinach) 59 DPG*	64.3% (9/14)	1.78 (0.51–2.38)
		2022	87 DPG	95.8% (23/24)
	(Cucumber) 115 DPG		70.8% (17/24)	0.95 (0.95–4.66)

*Due to heat and dry conditions, spinach was collected once from the 2021 MN trial.
[†]gEc-negative produce samples were excluded for calculating the median concentration of gEc.
⁺⁺Concentrations of gEc in the positive samples were single or identical values.

direction for the 3 days before the sampling day (WDir3), average minimum air temperature for 30 days before sampling day (Air_temp_min30), and total precipitation for 7 days before the sampling day

(Precip_tot7; Figure 3A). In the soil sample profile for soil chemical factors, 45.9% (118/257) of the profile were gEc positive. The top-ranked factors associated with the presence of gEc in the soil determined by the RF classifier were CEC, percentage of Ca, organic matter, and concentration of Na, Mn, NO₃, and NH₄, along with soil moisture content (Figure 3B). In both meteorological and soil chemical models, the treatment effect was identified as the most detrimental factor affecting the presence of gEc in the soil. Although the sampling day effect was not evaluated in the soil chemical RF models due to insufficient data, it was ranked second among meteorological factors (Figure 3A). Notably, in the RF models, the rank of treatment and sampling day consistently appeared as top predictors. The order of other meteorological and soil chemical factors varied yet exhibiting similar predictive performances across the training sets. When assessing the two model performances with ROC curves, the values of area under the curve (AUC) were 0.76 for the model with meteorological factors, and 0.78 for the model with soil chemical factors.

3.4 Effects of state, sampling day (DPG), and meteorological factors on soil concentrations of generic *E. coli* (gEc) from graze CC treatment

The effect of grazing on the concentrations of gEc over time was examined within the graze CC treatment because median concentrations (log₁₀ MPN/gdw) of gEc in the fallow and non-graze CC treatments were consistently zero on each sample day across two growing seasons. Of the outcome variable, 43.5% were zero (i.e., gEc negative) with the high variance as 1.3 (mean = 1.1) in gEc count. State and year were not associated with the probability of zero count (i.e., zero-inflated component) and concentration (i.e., conditional component) of gEc in the univariable mixed-effect zero inflated negative binomial models. The DPG as a spline linear variable showed significant association with the concentration of gEc (i.e., log₁₀ MPN/100gdw rounded off to the nearest integer; $p < 0.001$). Although state alone did not exhibit the significant association, the interaction between sampling day and state showed a significant association with the concentration of gEc ($p < 0.001$).

The final multivariable model included state and sampling day variables with its interaction term (Table 4). None of the variables

showed significant associations with the probability of zero in gEc, but all the variables were significantly related to the concentrations (i.e., counts as integer) of gEc. Soil samples collected from MN had higher expected counts of gEc (RR = 3.43, 95% CI = 1.77–6.62) compared to CA. The application of a piecewise linear spline variable for the DPG variable showed a significant increase (RR = 1.07, 95% CI = 1.05–1.09) in mean gEc counts per day before 32 DPG and a significant decrease (RR = 0.98, 95% CI = 0.97–0.99) per day after 32 DPG in CA. Similarly, MN exhibited a significant increase (RR = 1.02, 95% CI = 1.01–1.03) and decrease (RR = 0.98, 95% CI = 0.97–0.99) per day in mean counts of gEc before and after 32 DPG.

When the seven top-ranked meteorological explanatory variables from the RF analyses (Figure 3A) were individually added into the final model, Air_temp_max, soil_temp10_max, Wind_max, and Air_temp_min30 were all significantly negatively associated with the concentrations (i.e., counts as integer) of gEc ($p < 0.001$). Among those four variables, two variables—Air_temp_max and soil_temp10_max—also showed significant associations with the probability of having a zero count in gEc (i.e., absence of gEc; Supplementary Table 1). The absence of gEc in the soil was significantly associated with lower Air_temp_max (OR = 0.75, 95% CI = 0.59–0.96) and soil_temp10_max (OR = 0.64, 95% CI = 0.48–0.85), which was the opposite direction of the associations when considering the concentrations of gEc in the conditional components of the models. Interestingly, when Air_temp_max and soil_temp10_max was separately added to the final model, the interaction terms between MN and DPG or (DPG–32) became non-significant. This suggests that the regional differences in the changes in the concentrations of gEc over time may be attributed to air and soil temperatures on the sampling days. After adding the meteorological variables in the final model, the changes in the coefficient of MN were larger for Air_temp_max (31%) than for soil_temp10_max (16%), which implies that air temperature may have more influence on the concentration of gEc than soil temperature (Supplementary Table 1).

4 Discussions

In this study, ICLS field trials were conducted simultaneously under two different environmental conditions in CA and MN over 2 years (2021–2022). Among the investigated foodborne pathogens in

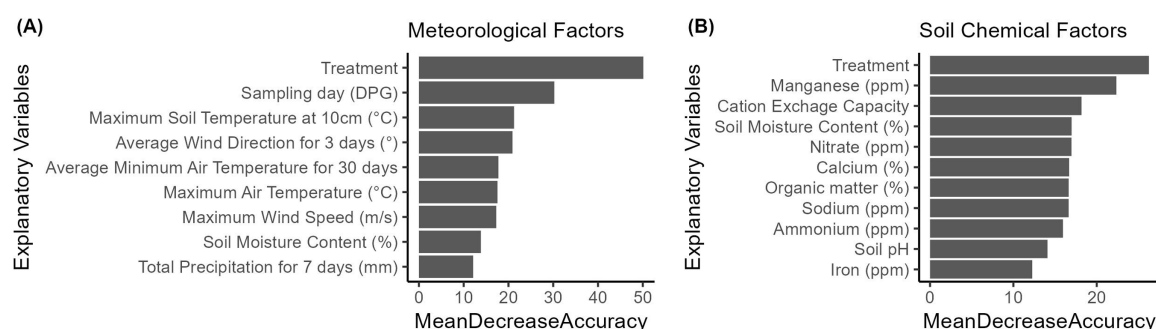


FIGURE 3 Results of random forest analyses to identify (A) meteorological and (B) soil chemical factors associated with the presence of generic *E. coli* (gEc) in the soil of the organic crop-livestock integration system field trials in California and Minnesota (2021–2022). State, year, and treatment as categorical variables and soil moisture content were added into each model, and sampling day (DPG) was additionally added into the model of (A) meteorological risk factors.

TABLE 4 Multivariable analyses using a mixed-effect zero-inflated negative binomial model with row as a random effect for the concentrations (i.e., log₁₀ MPN/100 gdw rounded off to the nearest integer) of generic *E. coli* (gEc) in soil samples collected from graze CC treatment of the organic crop-livestock integration system field trials in California and Minnesota (2021–2022).

Variable	Coefficients	Relative Risks (RR)	95% CI	p-value
Conditional component—Log (count of gEc)				
Intercept	−1.08			<0.001*
State				
CA	Reference			
MN	1.23	3.43	1.77–6.62	<0.001*
Sampling day—Day Post-Graze (DPG)				
DPG	0.07	1.07	1.05–1.09	<0.001*
(DPG−32) ⁺	−0.08	0.92	0.90–0.94	<0.001*
State × DPG				
MN × DPG	−0.05	0.95	0.93–0.98	<0.001*
MN × (DPG−32) ⁺	0.04	1.04	1.01–1.08	0.004*
Zero-inflated component—Logit (probability of zero count in gEc)				
Intercept	−19.01			0.99

*p-value < 0.05.

⁺DPG was a spline linear variable with a knot at 32 DPG, which was the day most showing the highest concentration of gEc.

produce and soils, a cucumber collected from MN trial and 1.4% of soil samples from both states tested positive for non-O157 STEC. The concentration of gEc in the graze CC soil returned to levels comparable to the non-graze CC or fallow treatments within 87–147 DPG over 2 years, showing a decrease after 32–53 DPG. Maximum air and soil temperatures on the sampling day were related to presence of gEc in the soil.

4.1 Presence of foodborne pathogens in the fresh produce, soil, and fecal samples

Only one non-O157 STEC (0.6%, 1/157) positive cucumber sample originated from the graze CC treatment at 115 DPG in MN, but none of the soil samples taken at the same time from these treatment plots were non-O157 STEC positive. In contrast, one soil sample from the non-graze CC treatment tested positive for non-O157 STEC at 115 DPG. Based on this, the positive cucumber sample found in the graze CC treatment may have been transferred from adjacent treatment plots by wind or originated from wildlife intrusions. Other studies noted that wind-driven dust or observed wildlife pose important foodborne pathogen transmission routes in fresh produce farms (Strawn et al., 2013; Pang et al., 2017). In soil, the three positive non-O157 STEC isolates were still detected at 115–147 DPG, despite none being identified between 32 and 115 DPG. The 115–147 DPG falls outside the NOP stipulated the waiting period (i.e., 90–120 days) between manure application and harvest. However, two out of three non-O157 STEC positive isolates were even collected from the non-graze CC treatment plots, making it difficult to conclude that positive isolates found in soil were solely from the feces deposited by the grazed animals. Similarly, the unexpected increase of non-O157 STEC prevalence in the soil after 120-days post-manure application has also been observed in a previous study (Ramos et al., 2021). Therefore, to distinguish the origin of the pathogen between the

isolates from soil and cucumber, further genetic investigation (e.g., serotype, virulence factors) is needed. On the other hand, an increased presence of non-O157 STEC in soil (2.7%) was observed in the second year compared to that in year one (0%). This finding aligns with the increased prevalence of non-O157 STEC in feces deposited by grazing animals during the second year (i.e., 41.7% in CA and 45% in MN), which implies that shedding status of grazing animals may affect the presence of foodborne pathogens in the soil. These were also higher prevalences compared to a previous study; the prevalence of non-O157 STEC in sheep fecal samples in the three-year ICLS study conducted in CA was 26%, which had no detection of non-O157 STEC in grazed soil (Cheong et al., 2024). In addition, fecal pathogen shedding may have been influenced by the variable number of grazing animals in the field, which was not consistent across years and was primarily determined by the density of the cover crop biomass.

The notable difference between the two states was the higher prevalence of *L. monocytogenes* (37.5%, 30/80) in goat fecal samples from MN, whereas no *L. monocytogenes* was detected in sheep fecal samples from CA over 2 years. This disparity in fecal shedding of pathogens could be due to different species of animals, stress induced by long transportation and diets of grazing animals prior to the trials, along with different environments in the two states. Studies have shown that decreasing dietary fiber or using silage, as well as exposure to stress of long transport or cold temperature, are associated with increased fecal shedding of pathogens such as *E. coli* O157 and *L. monocytogenes* in sheep, goats and cattle (Lema et al., 2002; Bach et al., 2004; Nightingale et al., 2005; Ivanek et al., 2007; Schoder et al., 2011). In this study, sheep were transported from an adjacent sheep barn located 5.5 km away from the field in CA, while goats were transported from a goat rental company located in Faribault, MN, approximately 2 h away from the field in MN. Grazing events mostly occurred in spring in CA, while in MN, they were conducted twice per growing season, once in winter (before cover crop was covered in snow) and once in spring. Diet differences could have contributed to

the shedding pattern, either because of species-specific habits or feed available to the flocks in the different states. The diet of sheep in the barn was mainly alfalfa hay with a grain rotation, although the goat diet was variable with a base of hay and other biomass that were available at the moment.

4.2 Persistence of generic *E. coli* and associations with meteorological and soil chemical factors

In previous studies conducted in CA, the presence of non-O157 STEC in soil samples collected under ICLS trials was rarely reported. [Patterson et al. \(2018\)](#) and [Cheong et al. \(2024\)](#) observed that none of the soil samples tested positive for non-O157 STEC. Accordingly, the persistence of gEc has been evaluated as a surrogate of pathogenic strains of *E. coli* in soil as other studies supported a similar survival die-off pattern and influencing factors between them ([Naganandhini et al., 2015](#); [Pang et al., 2020](#)). However, based on the observation from the present study, 10 positive non-O157 STEC isolates from soil samples were detected during the earliest and latest days of the growing seasons, including 0, 32, and 147 DPG, from the graze CC treatment, when gEc concentrations in the soil were low. Similarly, a study measuring the prevalence of *E. coli* O157 and gEc in water sources used in leafy greens farms located in CA suggested that gEc concentration was not associated with the presence of *E. coli* O157 ([Benjamin et al., 2013](#)). A meta-regression analysis noted that the dynamics of pathogenic *E. coli* in the farm environment cannot be inferred from that of gEc when soil characteristics and temperatures are taken into account ([Franz et al., 2014](#)). On the other hand, a risk factors analysis study investigating the association of non-O157 STEC presence in manured soil with weather factors reported significant positive associations with gEc concentrations in the soil ([Pires et al., 2023](#)). Thus, further studies are needed on survival of gEc and pathogenic *E. coli* under diverse environments considering intricate interactions in nature.

When modeling the gEc concentration in soil over time in this study, the piecewise linear spline variable of DPG in the ZINB model demonstrated that a significant decrease in the mean gEc counts in soil occurred after 32 DPG within the graze CC treatment for both states, although the sampling day with the highest soil concentrations of gEc varied each year in each state (i.e., 53 DPG and 41 DPG in CA each year, and 32 DPG in MN for both years). Similarly, a two-stage survival kinetic approach was used in a meta-analysis aggregating 70 studies to evaluate the survival of manure-borne pathogen after introduction into soil ([Park et al., 2016](#)); the study supported the presence of distinct patterns of pathogen decline characterized by two different slopes. In contrast to MN, in the second year of CA trial, the graze CC treatment had higher gEc concentration than the other treatments until 124 DPG. The period of elevated gEc concentration in the grazed soil lasted longer than in our previous study in CA as well, where the graze CC treatment showed higher concentrations of gEc than the other treatments only until 61–82 DPG ([Cheong et al., 2024](#)). Given that both trials in CA were conducted in the same field, and that higher gEc concentrations in the graze CC was shorter in MN, where ICLS had not been conducted in the field before, a potential cumulative effect of repeating ICLS might be present.

Therefore, the effect of continuing the ICLS over multiple years on gEc concentration in the soil and produce should be considered for future modeling.

Interestingly, despite the persistently higher concentration of gEc in the soil in CA, the presence and concentration of gEc on produce was higher in MN. Splash events after rainfall or transfer of soil pathogens to produce via irrigation (i.e., spray irrigation was used in MN fields, as opposed to drip lines in CA fields) may depend on different agricultural practices and environments ([Franz et al., 2008](#); [Litt et al., 2021](#)). Indeed, CA fields had lower average precipitation during harvest (i.e., 0–0.01 inches) in a semi-arid climate compared to MN fields (i.e., 0.64–5 inches) in a humid continental climate. According to a quantitative microbial contamination model of *E. coli* in spinach at the field level, solar radiation and rain events were identified as important factors in contamination at harvest ([Allende et al., 2017](#)). Solar radiation is linked to bacterial inactivation, while rain events are associated with the splashing of soil pathogens. Therefore, the transfer of pathogens from soil to produce and their persistence in produce may be more influenced by environmental factors than by pathogen concentrations in the soil itself.

The RF model in the present study identified treatment as an agricultural factor and sampling day (i.e., DPG) as a temporal factor, both highly ranked for the soil presence of gEc. A study evaluating factors affecting survival durations of gEc in manure-amended soils in the Mid-Atlantic United States found that combined agricultural and spatiotemporal factors were better predictors than weather variables ([Sharma et al., 2019](#)). However, the state, considered as a spatial factor in this study, was not listed among the influential factors in our final RF model. This observation corresponds to findings from a repeated cross-sectional study; the state factor was also excluded from their final multivariable model with the concentration of gEc, suggesting it may serve as a proxy for weather effects ([Park et al., 2015](#)). Similarly, in the present study, the inclusion of weather-related factors (i.e., maximum air and soil temperature) in the final ZINB model led to the interaction terms between the state and sampling day becoming non-significant.

Among meteorological factors considered for the RF analyses, maximum air and soil temperatures on the sampling day were influential factors on the soil presence of gEc as well as on the counts of gEc in the ZINB model. Similarly, a meta-analysis of 42 studies on the decline of *E. coli* in manure-amended soil revealed that temperature, including soil and air temperatures, was the most significant factor affecting persistence of gEc under field conditions among all investigated environmental factors ([Tran et al., 2020](#)), as well as the detrimental factor on longer persistence of gEc before showing decline in soil ([Park et al., 2016](#)). This aligns with the study identifying environmental factors associated with the count of gEc on spinach at preharvest in Colorado and Texas; maximum air temperature over 9 days preceding the sampling was also the factor that determined both contamination and counts of gEc in their final model ([Park et al., 2015](#)). Interestingly, our final ZINB model in the graze CC treatment by adding individual factors of maximum air and soil temperature indicated the opposite effects of maximum air and soil temperature on the probability of gEc absence and on the concentration (i.e., counts) of gEc. In short, an excess of zero counts in gEc concentrations was observed in lower temperatures, while lower concentrations of gEc-positive were observed in higher temperatures. This implies that a specific temperature threshold may exist that determines the presence of the gEc in soil, with an optimal temperature for bacterial proliferation when present.

In the present study, the linear model analysis was not performed with soil chemical characteristic factors due to the lack of sample profile dates and non-correspondence between the dates of pathogen testing and soil chemical testing. Soil moisture content, soil pH, and soil temperature have been frequently highlighted as detrimental factors to soil survival of pathogens (Lang et al., 2007; Franz et al., 2014; Xing et al., 2019; Litt et al., 2021). Specifically, higher soil moisture and pH have been linked to longer pathogen survival in soil (Lang et al., 2007; Cook et al., 2023). Although the association between the presence of gEc and soil pH or water content was not further evaluated in this study, they consistently remained as influential in either the RF models with meteorological factors or soil chemical factors. Regarding chemical nutrient factors, eight elements listed in our final RF model (e.g., Mn, CEC, NH₄, Na, OM, NO₃, Fe, and Ca) appeared as important factors influencing the presence of gEc in the soil. However, intricate interactions of soil micronutrients made it difficult to interpret the results with limited research on the topic (Chandler-Khayd et al., 2023; Cook et al., 2023). Additionally, soil type is a known important factor on soil survival of bacteria, as fine soils (e.g., clay) demonstrated prolonged survival times compared to coarse soils (e.g., sandy; Franz et al., 2008, 2014; Brennan et al., 2014), as different classification of soil types between CA and MN may have effect on the presence of gEc.

5 Conclusion

The foodborne pathogens (*E. coli* O157, non-O157 STEC, and *L. monocytogenes*) were rarely or completely not detected in soil and produce from organically certified production fields where small ruminant grazing was directly implemented in the current study. Our findings suggest that screening for the presence of foodborne pathogens in animals prior to grazing and exclusion of individuals determined positive offer a strategy to mitigate potential contamination risk in ICLS, as the presence of foodborne pathogens in soil was higher when high levels were detected in grazing animal feces. The application of the NOP 90- and 120-day interval rules between manure application by grazing and harvest should consider other management and environmental factors such as the cumulative effect of continuous ICLS in the same field, the foodborne pathogen shedding status of grazing animals, and meteorological characteristics during the growing season. Our analysis of meteorological factors in the grazed soil showed that regional differences in gEc concentrations were most likely affected by maximum air and soil temperatures on the sampling day, although the effects of meteorological factors and soil chemical characteristics were not as influential as the effects of treatment or sampling days. As regional differences on pathogen survival may be attributed to those factors, further studies will be required to consider a wider range of environmental factors across multiple regions in the future.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The research protocol was approved by the Institutional Animal Care and Use Committees (IACUC) of the University of California, Davis (IACUC protocol #22700) and the University of Minnesota (IACUC protocol 157 #2008-38348A).

Author contributions

SC: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. CC-K: Data curation, Methodology, Writing – review & editing. SW: Data curation, Methodology, Writing – review & editing. AG: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Writing – review & editing. PA: Data curation, Methodology, Writing – review & editing. MJ-R: Conceptualization, Funding acquisition, Methodology, Resources, Writing – review & editing. EE: Data curation, Writing – review & editing. LK: Data curation, Methodology, Writing – review & editing. PP: Conceptualization, Funding acquisition, Resources, Writing – review & editing. PM: Conceptualization, Funding acquisition, Resources, Writing – review & editing. AK: Writing – review & editing. FH: Conceptualization, Funding acquisition, Resources, Writing – review & editing. AS: Conceptualization, Funding acquisition, Methodology, Writing – review & editing. AP: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

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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.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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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.2024.1464018/full#supplementary-material>

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