Synthesizing the evidence for the effects of agricultural practices on food safety in the preharvest produce-growing environment

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About the core author team

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Cover image: An aerial view of the century experimental field station, UC Davis Russell Ranch Sustainable Agriculture Facility, California, USA.

About this synthesis:

The purpose of this document is to summarize published data concerning the effects of pre-harvest farming practices on the growth and survival of foodborne pathogens in produce fields. We present a summary of scientific evidence, reviewing the effectiveness of on-farm preharvest management practices that may help growers predict and mitigate risks associated with foodborne pathogens. This synopsis includes five categories of management practices:

1. Soil Management
2. Non-crop vegetation management
3. Wildlife management
4. Landscape risk factors
5. Agricultural water management

Of the five categories, four pertain to on-farm management (soil, non-crop vegetation, wildlife, and agricultural water management) and one pertains to broader risk factors (landscape). The efficacy of these management and food safety risk factors can be measured using various metrics. With the help of our Science Advisory Team (SAT), we have defined a list of metrics (Table 1) for all selected management practices. These metrics are shown in bold text throughout the synopsis (usually included in summary title), and the key messages for the selected metrics are grouped by each farm management practice.

How we summarized the scientific evidence:

Through broad literature reviews and consultation with an expert panel of scientists, we systematically summarized research concerning the effects of local practices and landscape context on selected foodborne pathogens. First, a science advisory team was organized to lead the synthesis, composed of: Drs. Daniel Karp, Naresh Devarajan, Daniel Weller, and Matthew Jones. As a team, we decided on the relevant search terms to capture the scientific evidence from 3 public electronic databases: PubMed, Web of Science, and PubMed Central (PMC).

Searches were conducted (Jul, 2019) using standardized search terms, iteratively developed in consultation with the science advisory team to comprehensively return as much relevant literature as possible (Figure 1A). Briefly, search terms included: selected foodborne pathogens, specific regions, produce types regulated under the Food Safety Modernization Act (FSMA), key words related to on-farm/pre-harvest conditions, and terms related to four distinct farm management or landscape categories: as stated above.

Studies were selected for inclusion based on the following criteria (Figure 1B):

1) Studies must quantify effects of on-farm, pre-harvest management practices or risks imposed through farms being situated in alternative landscape contexts.
2) Studies must report original data (i.e., no reviews or purely theoretical models).
3) Studies must focus on *E. coli* (generic or pathogenic), *Salmonella* spp., *Listeria* spp., or *Campylobacter* spp.
4) Studies should focus on "FSMA regulated crops" (excluding sprouts) in produce fields.
5) Studies must be performed in the United States or Canada,
6) Studies must be published in peer-reviewed journals.

In total, we reviewed 2,489 abstracts. All abstracts were screened by at least two members of the science advisory board. As a team, we finalized 123 papers that passed our selection criteria. Selected papers were then assigned to broad categories: soil management, non-crop vegetation management, wildlife management, landscape factors, and agricultural water management. Next, selected management categories

were sub-categorized according to the specific farming practices evaluated (Table 1; multiple practices were often examined within a single paper).

We then systematically summarized the methods and results reported in each paper through writing a series of standardized one-paragraph summaries (one for each management practice evaluated in each published paper). Each paragraph reports: the experiment type, the management intervention, the study period, the growing region, the study year, the focal pathogen, the experiment type, the farm type, the sample size, and the effect size. In addition, we include a short description of the methods and management-relevant results. A member from the science advisory team drafted all the summaries, which were then edited by at least one other member of the scientific advisory board.

Table 1. List of practices and metrics for which evidence is summarized in this synopsis

	Farm management practice	Target	Metric
Soil Management	Add compost to soil	Selected Foodborne Pathogen or General indicator bacteria	*E. coli* O157:H7, *Salmonella*, *Listeria*, and *Campylobacter* or Fecal indicator bacteria, Generic *E. coli*
	Add untreated manure to soil		
	Add biosolids to soil		
	Farm fields organically		
	Use soil tillage		
	Grow low risk crop types		
	Add mulch as a ground cover		
	Grow crops using a trellis production system		
Non-crop vegetation management	Add treatment wetlands		
	Add hedgerows or vegetative buffer strips		
	Add winter cover crops		
	Remove weeds in produce fields		
Wildlife management	Add riparian fencing		
	Integrate livestock in produce fields		
	Decrease the presence of wildlife in produce fields		
Landscape factors	Increase distance between produce fields and open/closed livestock areas		
	Increase distance between produce fields and water sources/wetlands		
	Increase distance between produce fields and non-grazed natural lands		
	Increase distance between produce fields and urban areas		
Agricultural Water Management	Irrigate with low-risk irrigation source		
	Choose low-risk irrigation type		

Fig. 1. A systematic approach for evaluating food-safety practices: A) example of search terms for soil management practices, B) paper selection criteria.

1. Soil management:

1.1. Add compost to soil (7 studies)

- **Animal-based compost implementation options (2 studies):** Two replicated, experimental studies conducted in the USA (1, 2) found that compost added to the soil had no major impact on generic *E. coli*, *E. coli* O157:H7, and *Salmonella*. Of the 2 studies, one study compared plots with and without organic compost and found no difference in generic *E. coli* and *E. coli* O157:H7 prevalence. The other study compared various ratios of dairy manure compost amendments and found no major impact of amount of cow manure compost used on *E. coli* O157:H7 and *Salmonella* survival.
- **Comparing compost sources (4 studies)**: Four replicated, experimental, studies from the USA, in Vermont (3), Georgia (4, 5), and Maryland (6), found that composts from different sources varied in the amount of pathogens that they supported. Of the four studies, two found higher prevalence of *Salmonella* and generic *E. coli* in plots where poultry manure compost was added, compared to plots amended with dairy manure compost. Another study found that the time-to-non detect for *E. coli* O157:H7 did not differ between soils amended with poultry and dairy manure compost. The final study found that *Salmonella* survived longer in soils amended with heat-treated poultry pellets than in unamended soils.
- One study (7) reported that fields located in proximity to compost piles was associated with microbial contamination of leafy greens.

a) ***Generic and pathogenic E. coli prevalence in compost amended versus unamended organic farms:*** A replicated, observational study (1), surveyed generic and pathogenic *E. coli* prevalence in Central California lettuce fields and found that the addition of organic compost had no major impact on the prevalence of generic and pathogenic (Shiga-toxin-producing) *E. coli*, when comparing fields receiving and not receiving organic compost. **Methods:** Field experiments were conducted in certified organic farms, in the spring (N=14 sites) and summer (N=15 sites) 2014. At each site, sub plots (5 X 10 m) were demarcated, and lettuce was planted within vegetable fields (lettuce N=19, broccoli N=7, celery N=3). Each sub-plot was divided in half (5 X 5 m), where one half received the regular field management used by the growers (heat-treated, pelleted and/or liquid fertilizers) and the other half received supplemental treated organic compost (1-2 months prior planting) in addition to heat-treated pelleted or liquid fertilizers. Treated organic compost was a cow, chicken and green manure blend (applied at 25 tons/hectare). Each sub-plot was planted with lettuce. Prevalence of generic and pathogenic *E. coli* was determined by culture-based methods. **Results:** In this study, all samples tested negative for pathogenic *E. coli,* while one sample tested positive for generic *E. coli* under each treatment group (compost vs no compost). This study reports addition of organic compost improved lettuce yields and increased soil organic matter content.

b) ***E. coli* O157:H7 *and Salmonella survival and internalization into lettuce tissue with commercial cow manure compost applications:*** A replicated, controlled, experimental growth chamber study (2) examined *E. coli* O157:H7 and *Salmonella* survival on and internalization by lettuce in Georgia, USA, and found that the amount of compost added to the soil surface did not affect pathogen survival or internalization into lettuce tissues. **Methods:** Lettuce seedlings were transplanted to pots (10.2 cm diameter) containing either 0:5, 1:5, or 2:5 (wet-weight) cow manure compost, and top soil mixtures (250 g per pot) and placed in a growth chamber. Water contaminated with either *E. coli* O157:H7 or *Salmonella* at one of two concentrations (low=10^3; high=10^6 CFU/ml) was applied to each pot either 3 days (30 mL per seedling) or 30 days after adding the compost (50 mL per mid-age plant). Four plants were collected from each treatment group, on days 6, 33, and 60 post-transplantation. Separate from this inoculation step and beginning 30-d after transplanting, water-stressed plants were irrigated with 40ml of water (instead 75 ml) on alternative days up to 2-weeks prior to soil contamination. Rhizosphere soil samples and lettuce samples (leaves and roots) were analyzed to enumerate total (internalized and surface) and internalized pathogen concentrations. **Results:** All leaf samples and internalized root samples were 100% negative for *E. coli* O157:H7 and *Salmonella* spp. Addition of compost mixtures [0:5, 1:5, or 2:5 (wet-weight) compost/soil] did not significantly affect the *E. coli* O157:H7 and *Salmonella* spp., concentrations in rhizosphere soil samples across all sampling time periods. Pathogens in lettuce roots were not significantly affected by the addition of soil amendments and the reduced water rate resulted in an 0.8 log reduction in *E. coli* O157:H7. This study reports that addition of compost had no effect on the survival of pathogens in the soil and did not promote the internalization of pathogens into plant tissues.

c) ***Generic E. coli die-off in soils amended with dairy versus poultry-based compost:*** A replicated, controlled, experimental study (3) in 2015 that compared generic *E. coli* die-off in soils from dairy manure-amended, poultry litter-amended and unamended fields in Vermont, USA. The study found that generic *E. coli* levels were consistently lower in the dairy compared to poultry-based composts amended fields. Dairy-manure based composts are less likely to support generic *E. coli* (TVS 353, 354 and 355) survival than poultry-based composts. **Methods:** Two field experiments were conducted, each of which encompassed fifteen 1x2 m plots. Five treatments were employed for each experiment (i) *E. coli* inoculum with poultry litter compost, (ii) *E. coli* inoculum with dairy windrow compost, (iii) *E. coli* inoculum with dairy vermicompost, (iv) *E. coli* inoculum without compost, and (v) an untreated control with neither inoculum or compost. Poultry composts were applied at 13.4 tons $acre^{-1}$ (i.e., 2.7 kg $plot^{-1}$) and dairy manure-based composts at 6.72 tons $acre^{-1}$ (i.e., 1.36 kg $plot^{-1}$). Fields were manually contaminated with 1L *E. coli* inoculum (2.5 x 10^8 CFU/m^2) per plot. Spinach was planted in in each plot. Plots were not irrigated as precipitation met or exceeded crop demand. Soil composite samples (N=1 per field) were collected per treatment at each of the following timepoints: 0, 1, 3, 6, 10, 15,

23, 29, 37, 49, 63, 78, 105, and 161 days post-inoculation. *E. coli* levels were enumerated in each sample. **Results:** Generic *E. coli* had similar survival trends and responses to experimental treatments across both field sites. There was an initial exponential decay, and then a stabilization at 50 days post--inoculation, followed by a second rapid decay until eventual extinction in all treatments except those with poultry-based compost. In plots amended with poultry-based composts where *E. coli* increased initially and then declined exponentially until 105 days post inoculation, after which the concentrations stabilized and did not go below detectable levels. Moreover, *E. coli* concentrations were always higher in the poultry manure-based compost treatments compared to the dairy manure-based compost and control treatments.

d) ***E. coli O157:H7 survival in poultry versus dairy compost*:** A replicated, controlled, experimental study (4) examined *E. coli* O157:H7 die-off in Georgia, USA fields following amendment with poultry or dairy manure composts, and found that *E. coli O157:H7* survived for similar periods of time in the poultry and dairy manure amended soils. **Methods:** Three composts contaminated with *E. coli* O157:H7 (10^7 CFU/g) were used: poultry manure compost, dairy cattle manure compost, and alkaline-pH-stabilized dairy cattle manure compost. A fourth treatment, irrigation water contaminated with *E. coli* O157:H7 (10^5 CFU ml^{-1}), was also used. A split block design was used included 5 treatment groups (3 with each of three composts, one without compost (the control), and one without compost but with contaminated water). Twenty-five plots, each 1.8 X 4.6 m, were used for each crop. Each treatment (N=5) had 5 replicate plots for each crop. Compost was added at 4.5 metric tons/hectare, 24 hours before lettuce and parsley seedlings were transplanted. Three weeks after planting, a one-time treatment of contaminated irrigation water (2L/plot) was hand-sprayed onto the soil of each of the five plots within the contaminated water treatment. Samples (soil and produce) were collected at selected time intervals (soil day 0 to 238, and produce day 21 to 182) from each plot. *E. coli* O157:H7 levels in each sample were then were enumerated. **Results:** Survival of *E. coli* O157:H7 was not affected by the type of compost, or the use of contaminated water. *E. coli* O157:H7 was detected within soil samples collected from lettuce and parsley fields for 154 and 217 days, respectively. *E. coli* O157:H7 was detected on crop samples for 77 and 177 days on lettuce and parsley, respectively. Following the application of contaminated source (compost or irrigation water), *E. coli* O157:H7 persisted in soil for >5 months regardless of produce type. No significant difference was found in the persistence of *E. coli* O157:H7 based on compost type alone.

e) ***Salmonella survival in soil amended with poultry versus dairy compost:*** A replicated, controlled, experimental study (5) that examined *Salmonella* survival in field soils amended with poultry and dairy manure composts in Georgia, USA, found that *Salmonella* survived longer in soil amended with poultry or dairy manure compost compared to soil amended with alkaline-pH-stabilized dairy manure compost. **Methods:** Three compost types were used: 1) poultry manure compost, 2) dairy cattle manure compost, and (3)

alkaline-pH-stabilized dairy cattle manure compost. Each compost and one treatment of irrigation water were inoculated with *Salmonella enterica* serovar Typhimurium at 10^7 CFU g^{-1} and 10^5 CFU ml^{-1}, respectively. A split block design was followed for each crop, and included 5 treatment groups (1 per compost type, one without compost, and one without compost but contaminated by water inoculated with *Salmonella*). Each treatment was replicated 5 times for each crop. Plots measured 1.8 X 4.6 m. Compost was added at 4.5 metric tons/hectare, 24 hours before planting either carrot or radish. Shortly after planting, a one-time treatment of 2 L of contaminated irrigation water was hand-sprayed onto the soil of the contaminated water treatment plots. Samples (soil and produce) were collected at selected time intervals (approx. from 0 to 260 days) from each plot and enumerated for *Salmonella*. **Results**: *Salmonella* survived longer in plots where carrots were grown (203 days) compared to radishes (84 days). *Salmonella* counts on carrots decreased over time (initially (0 day): 1.8 to 3.83 $\log_{10}$ CFU g^{-1}; at harvest (231 days): 1.0 to 1.2 $\log_{10}$ CFU g^{-1}) but remained unchanged on radishes (initially (0 day): 1.53-2.56 $\log_{10}$ CFU g^{-1}; at harvest (91 days): 1.0-2.5 $\log_{10}$ CFU g^{-1}). *Salmonella* survived for at least 203 days in the alkaline-pH-stabilized dairy manure treatment and the contaminated irrigation water treatment but survived 231 days in the dairy manure and poultry manure compost treatments. No *Salmonella* was detected in control samples at any time point. In conclusion, *Salmonella* survival was greatest in soil amended with poultry or dairy compost and lowest in soil containing alkaline-pH-stabilized dairy cattle manure compost.

f) ***Salmonella survival in heat treated poultry pellet amended soil vs unamended soil:*** A replicated, controlled, experimental study (6) compared *Salmonella* Newport survival in soils amended with poultry pellets to soils that were unamended, and found that *Salmonella* Newport survived longer, and was more likely to transfer to spinach, the crop grown in the soils, in amended compared to unamended soils. **Methods:** Field soil collected in Maryland, USA, was added to 28 box planters. Heat-treated poultry pellets [nutrient content of 3-2-3 (N-P-K)], were distributed evenly across the soil surface of 14 planters at a rate of 5 tons per acre (106.5 g per planter). In 12 of the amended and 12 of the unamended planters, the soil surface was sprayed with 175 ml of inoculum of wild type *Salmonella* Newport (8.81 log10 CFU/ml) and a Δ*rpoS* mutant *S*. Newport strain (8.48 log CFU/ml). Spinach was planted 24 h of after inoculation, and irrigated with sterile deionized water. Composite soil samples were collected and pathogen concentrations enumerated 0, 7, 8, 14, 21, 28, 49, 50, 51, 63, 77, and 91 days post-inoculation. On days 28, 35, and 63 post-inoculation soil was sprinkled on the spinach leaves to ensure contact between the spinach plant and inoculum. In total, 336 soil samples were collected (12 per day), and 216 produce samples were collected (54 before and 162 after sprinkling with soil). **Results:** Levels of *Salmonella* Newport wild type and Δ*rpoS* mutant in both poultry pellet-amended (7.35±0.26 log CFU/g dry weight) and unamended (7.40±0.14 log CFU/g dry weight) soil on day 0 were similar ($P>0.05$). At day 7, both wild type (5.66±0.29 log CFU/g dry weight) and Δ*rpoS* mutant (5.21±0.19 log CFU/g dry weight) counts in unamended soil were

significantly lower than in the amended soil [wildtype (6.87±0.16 log CFU/g dry weight); Δ*rpoS* mutant (6.93±0.26 log CFU/g dry weight)] ($P<0.05$). Pathogen levels in unamended soil declined rapidly and reached the limit of detection by day 35, while pathogen levels in the amended soil remain detectable at 91 days post-inoculation. Die-off (log10 CFU/g per day) was significantly faster ($P<0.05$) in unamended compared (between 0.33 and 0.36) to amended soils (between 0.12 and 0.13), but did not differ between the wild type and mutant *Salmonella* strains. The presence versus absence of spinach did not influence the die-off rate in amended or unamended soil ($P>0.05$). By enumerating *Salmonella* levels on leaves collected before sprinkling with soil, it was determined that *Salmonella* naturally transferred from the soils to the spinach leaves in the amended soils (3 out of 18, and 2 out of 18 samples were positive for the wild type and mutant strains, respectively) and the unamended soils (2 out of 18 and 0 out of 18 were positive for wild type and mutant, respectively). Within two hours of sprinkling the leaves with soil, all samples tested positive for *Salmonella*; however, only spinach grown in amended planters was positive 48 hours later (11 and 7 of 18 were positive for wild type and mutant, respectively).

g) ***Salmonella, STEC, and generic E. coli prevalence in produce fields near compost piles:*** A 2-year replicated, observational study (7) was conducted in 2012 to 2013 on farms (N=32) in Maryland, Delaware, and New Jersey, USA. The study, found that landscape structure, such as proximity to compost piles was associated with microbial contamination of leafy greens. **Methods:** Field samples were collected from 32 farms (15 conventional and 17 organic) in fall 2012 and spring 2013. Sample types included: foliage of leafy greens (N=369 leaves, some of which touched the ground or were soiled), irrigation water (N=124 from well, pond, or river water), sediment (N=13 from pond or river), field soil (N=60), and compost (N=11). Generic *E. coli* levels was assayed via. culture methods; presence of *Salmonella* (*invA*) and STEC (*stx1* and *stx2*) were determined using qPCR amplification, followed by culture-based isolation for PCR positive samples. **Results:** STEC gene prevalence (qPCR) was 0.3% (2/577) across all samples. *Salmonella invA* prevalence was 4.2% (24/577) across all samples (leafy greens N=15, water N=6, compost N=1, and soil/sediment N=2). *Salmonella* presence was culture confirmed in 9 of 24 samples (leafy greens N=8 and pond sediment N=1). Overall, 10% of samples were positive for generic *E. coli*: leafy greens (6%), irrigation water (18%), soil (10%), sediment (38%), and compost (27%) samples. Among the leaf samples that touched the ground or were soiled, higher average generic *E. coli* levels were associated with leafy greens near compost piles (N=6, 0.35 log CFU/g), followed by plants collected in flooded areas (N=7, 0.29 log CFU/g), and plants below power lines or on edge of fields (N=24, 0.08 log CFU/g).

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1.2. Add untreated manure to soil (17 studies)

- **Non-composted animal-based manure (12 studies):** Seven replicated, experimental studies (1-6) from the USA (Virginia, North Carolina, Iowa, Illinois), and Canada (Ontario, Quebec), as well as five observational studies (7-11) from the USA (Colorado, Texas, New York, Minnesota and Wisconsin), found higher prevalence's of pathogens, generic *E. coli*, and/or other biotic contaminants in plots amended with non-composted manure, compared to plots amended with conventional fertilizers or unamended plots, in some or all comparisons.

- **Animal-based manure source (2 studies):** Two replicated, experimental studies (3, 12) from the USA found poultry-litter amended soils supported generic *E. coli, Salmonella,* and *E. coli* O157:H7 at higher concentrations compared to other animal-based manures.

- **Manure implementation to harvest (4 studies):** One replicated experimental study (13) from the USA (Washington State) found no major impact on food safety of red raspberry when plots were amended with non-composted fresh dairy manure at least 4-months before harvest. Two replicated, experimental studies (14, 15) from the USA (Wisconsin and Pennsylvania) found that generic *E. coli* and *Salmonella* persisted in soil for more than 100-days when amended with non-composted fresh dairy manure.

- **Manure age (2 studies):** Two observational studies (9, 11) from the USA (Minnesota and Wisconsin) found aging manures for 6-12 months before use may reduce produce contamination with generic *E. coli* at the preharvest level.

- **Refrain from irrigating after applying soil amendments (2 studies):** Two replicated, controlled, experimental studies from Canada (16, 17) found that application of untreated animal-based manure increased generic *E. coli* concentration in runoff water.

a) ***Salmonella prevalence in poultry litter, poultry litter ash, and conventional fertilizer:*** A 4-year replicated, controlled, experimental study (3) conducted in Delmarva tomato fields found a higher prevalence of *Salmonella* in poultry litter amended soils compared to soils amended with poultry litter ash, or conventional fertilizer. **Methods:** Four field experiments were performed between 2012 and 2015 using a split-pot design. The plots used in the split-pot were: fresh poultry litter, poultry litter ash, and conventional fertilizer. Amendments were added 1 week before transplanting 7-week old tomatoes into the field, to achieve a standard rate of P_2O_5 (100 kg / hectare); specifically, 25 Kg of fresh poultry litter, 4 kg of poultry litter ash, or 2 Kg of conventional fertilizer (Triple Super Phosphate) were applied. Each plot was 12.5 m x 0.6 m, and contained ~30 tomato plants. Plants were drip irrigated daily (~6L water/sub plot). Tomato rhizosphere samples were collected from

either 24 sub-plots (Jul to Nov, 2012-2013; 8 per treatment) or 36 sub-plots (Jul to Nov 2014-2015; 12 per treatment) and tested for *Salmonella* spp. via culture-based methods. **Results:** In fresh poultry litter, *Salmonella* ranged from <2.1 to 2100 MPN/kg (wet weight basis), whereas no samples were *Salmonella* positive in poultry litter ash samples. *Field Trial 1:* Initially, in plots amended with fresh poultry litter, *Salmonella* spp. was found in 100% of samples from the tomato rhizosphere (130 – 450 MPN/Kg; 100 % detection rate, N=24), whereas no samples tested positive in plots amended with poultry litter ash (N=24) or conventional fertilizer (N=24). In poultry litter plots, *Salmonella* concentrations decreased over the growing season. *Field Trial 2*: All samples (N=72) tested negative for *Salmonella* regardless of amendment used. *Field Trial 3:* As in field trial 1, *Salmonella* was not detected in rhizospehere samples from the poultry litter ash or conventional fertilizer treatments, but was found on tomato rhizosphere samples (8.8 - 54 MPN/Kg; 58.3 – 75 % detection rate, N=36) collected from the poultry litter treatment. *Salmonella* prevalence decreased over the growing season. *Field Trial 4*: Again, *Salmonella* was not detected in any samples from plots amended with poultry litter ash or conventional fertilizer plots. In poultry litter amended plots, *Salmonella* was found on the tomato rhizosphere (4.6 - 10 MPN/Kg; 25 – 41.7 % detection rate, N=36), in the poultry litter, on the produce itself, but never on the tomato fruits. The authors concluded that soil amended with poultry litter ash posed fewer food safety risks than soil amended with fresh poultry litter.

b) ***E. coli persistence in soil amended with fresh bovine manure:*** A replicated, experimental study (14) conducted in 2003 examined *E. coli* persistence in carrot, radish, and lettuce fields in Wisconsin, USA, found that *E. coli* persisted in soils for more than 100 days after manure application. **Methods:** This study included three separate fields, each with different soil types: loamy sand, silt loam, and silty clay loam. Each field consisted of 44, 3.05 x 3.05 m plots. Fresh manure (≤3 days old) was added to each plot at a rate of 67.2 metric tons (wet weight) per ha. Plots varied in the date of manure application, the time of planting, when manure was tilled into the soil (after planting, prior to planting, not tilled in), and whether the manure was amended with chopped oat straw or not. Plots were irrigated as needed. Each plot was manually planted with carrot, radish, or lettuce. Composite soil samples from within the plot were collected at biweekly intervals, while produce samples were collected at thinning and harvest. *E. coli* levels in all soils were enumerated using the 3M Petrifilm *E. coli*-coliform plate count method. **Results:** Overall, the levels of *E. coli* introduced into the plots during manure amendment decreased rapidly following manure application. Despite the lack of statistically significant (i) differences between soil types, or (ii) timing of tillage effect, *E. coli* persisted for an empirically longer time in the silty clay loam when compared to the other two soils. Low levels of *E. coli* persisted in manure-fertilized soil for > 100 days after manure application in all three soil types. Across all three fields, *E. coli* was detected in 13% (5/38), 2% (1/48), and 25% (6/24) of carrot, lettuce, and radish samples, respectively. For 9 of the *E. coli* positive produce

samples, the mean level of *E. coli* detected was <1.0 log CFU/g. For the remaining 3 *E. coli* positive produce samples, *E. coli* levels were between 1.1 and 1.2 log CFU/g. The authors concluded that waiting ≥120 days following manure application to harvest did not guarantee the absence of manure-borne bacteria on the harvested produce.

c) ***Pathogen prevalence in farms that apply and do not apply manures:*** A 2-year replicated, controlled, experimental study (13) conducted between 2017 and 2018 in a raspberry field in Washington, USA, found that despite a one-time application (4 months before harvest) of dairy manure (raw/derived) fertilizers no raspberries were positive for foodborne pathogens. **Methods:** In a 4.79-acre commercial red raspberry field, individual plots (22.86 x 3.05 m) consisting of one row of raspberry plants each were randomized to contain four replicates per each of the following fertilizer treatments: i) standard synthetic fertilizer, ii) lagoon raw manure, iii) anaerobically digested liquid effluent, iv) compost, and v) dairy manure-derived fertilizers including ammonium sulfate and phosphorous solid. Fertilizers were applied 4-months before harvest. The plots were drip irrigated with well water. Sample types included fertilizer samples (five samples per fertilizer), soil (sample from each plot before and after fertilization, and 1-month before and after harvest), foliar (approx. 50 samples), and fruits (3samples per plot, one day before harvest). Samples were enumerated for fecal indicator bacteria (generic *E. coli* and total coliforms), Shiga toxin-producing *E. coli* (STEC), *Salmonella,* and *Listeria monocytogenes*. **Results:** All samples were negative for STEC and *L. monocytogenes*, while all foliar and fruit samples were 100% negative for *Salmonella.* Generic *E. coli* was not detected in any fruit samples, and present at levels below 0.4 $\log_{10}$ MPN/g in foliar samples. Generally, in soil samples, generic *E. coli* counts were below 1.0 $\log_{10}$ CFU/g throughout the season. Some fertilizers tested positive for *Salmonella*; specifically, 1 out of 6 samples in 2017 (phosphorous solid) and 2 out of 6 samples in 2018 (digested liquid effluent and straight lagoon raw manure) were *Salmonella* positive. All expect one soil sample (digested liquid effluent treatment) were negative for *Salmonella*. Sporadically, soil samples collected after the application of fertilizer tested positive for *Salmonella* spp., (< 12 MPN/g): 2017 (phosphorous solid and straight lagoon raw manure treatments), 2018 (synthetic fertilizer, ammonium sulfate, anaerobically digested liquid effluent, and straight lagoon raw manure treatments). All soil samples collected 1-month before and after harvest were negative for all microbial targets. Overall, this study did not find evidence of increased food safety risks at harvest following fertilization 4 months before harvest.

d) ***Generic E. coli prevalence on farms that apply and do not apply manures:*** A replicated, observational study (9) conducted in the Midwestern US between 2003-2004 found that manure amendment was associated with an increased prevalence of generic *E. coli* in produce collected from commercial farms. **Methods:** At the beginning of each harvest season, a questionnaire was mailed to participating farmers. Based on the survey, selected farms were classified as semi-organic (N= 30), organic (N=14), or conventional (N=19). During the harvest season, produce samples were collected and *Escherichia coli*

determined by enrichment. A total of 847 (178 organic, 372 semi-organic, and 297 conventional), and 1182 (295 organic, 539 semi-organic, and 348 conventional) produce samples were collected for the years 2003 and 2004, respectively. **Results:** Between ~44-50% of the conventional growers, 70-100% of the semi-organic, and 100% of the organic growers used manure as fertilizers. Proper composting techniques were followed by 25-32% of the conventional growers, 64-71% of the semi-organic growers, and 90-100% of the organic growers. 30-40% of the organic growers, 40-50% of the semi-organic growers, and 90-98% of the conventional growers aged manures >6 months before application. Application of manures was associated with an increased likelihood of *E. coli* detection semi-organic (Odds Ratio [OR]=12.9, 95% CI=2.9–56.3, P-value=0.0001) and organic (OR=13.2, 95% CI=2.2–61.2, P-value=0.0001) farms. Specifically, cattle-based manures were associated with an ~7-fold increase in the likelihood of detection ~7-fold (OR= 7.4, 95% CI=1.6–36.8, P-value=0.003). While aging manures for >6 months was associated with a decreased likelihood of *E. coli* detection (OR= 4.2 95% CI = 1.7–12.3, P-value=0.005), no effect of timing of manure application was reported. In summary, generic *E. coli* prevalence was elevated when manure was used but the magnitude of this increase was reduced substantially by aging the manure (>6 months).

e) ***Salmonella survival in swine manure following application:*** A 2016 randomized, experimental, controlled study (4) that sampled manure, and soil following manure application, from thirteen commercial swine farms in North Carolina and Iowa, USA, found that application of swine manure increased *Salmonella* prevalence, which subsequently persisted for ≥21 days after the manure was spread. **Methods:** The Iowa farms used a deep pit slurry system to store and treat manure; slurry from the pits were then transferred to the field via an injection method. The North Carolina farms used an anaerobic lagoon system, and sprayed the manure onto fields using overhead irrigation. Soil and manure samples were longitudinally collected from the thirteen commercial farms (6 in North Carolina, 7 in Iowa) over five samplings: before and immediately after manure application, as well as 7, 14, and 21 days after manure application. At each visit, soil (total N=1300) and manure (N=130) samples were collected, and tested for *Salmonella* presence using culture-based enrichment methods. **Results:** *Salmonella* prevalence was significantly higher in manure samples (50/130, 38.46%) compared to soil samples (139/1300; 10.69%, P < 0.0001). *Salmonella* was only isolated from a single soil sample (from North Carolina) collected before manure application. In total, 21.33% (128/600) of soil samples from North Carolina and 1.57% (11/700) of soil samples from Iowa tested positive for *Salmonella*. On average, *Salmonella* prevalence in the soil samples was highest immediately following manure application but decreased from day 0 to day 21 (except on one farm in North Carolina). The study concluded that application of untreated swine manure may play a role in the dissemination of *Salmonella* to arable lands and that once introduce *Salmonella* can persist on land for at least 21 days.

f) ***Generic E. coli prevalence in farms that apply and do not apply manure:*** A replicated, observational, cross-sectional study (7) assessed the microbial safety of spinach grown by commercial farms in Colorado and Texas, USA in 2010 and 2012, and found that fertilization with manure increased the odds of harvesting spinach contaminated by generic *E. coli*. **Methods:** Spinach samples (N=955) collected from selected farms in Colorado (N=4) and Texas (N=8) were enumerated for *E. coli*. Information on the farm-related management and environmental factors were obtained from the growers (questionnaire on farm management and environmental factors) and the National Resources Information (NRI) databases (weather and landscape factors). Variables were evaluated using a mixed-effect logistic regression model. **Results:** Generic *E. coli* was detected in 6.6% (63/955) of spinach samples. In a multivariate, mixed-effect, logistic regression model with farm and date as random effects, the odds of spinach contamination with generic *E. coli* was significantly higher on farms that applied manure fertilizer (OR=52.2, P= 0.008) compared to farms that did not apply manure.

g) ***Bacterial prevalence in dairy manure, swine manure, and conventional fertilizer:*** A 2-year replicated, controlled, experimental study (1) conducted between 2011 and 2012 in Ontario, Canada found very few significant effects of manure amendments (conventional fertilizer vs. dairy manure vs. swine manure) on bacterial concentrations in soil samples and vegetable samples at harvest. **Methods:** Manure (dairy and swine) was acquired from local farms and applied to fields in 2011 (dairy: 6900 gal/acre; swine: 3600 gal/acre) and 2012 (8500 gal/acre for both dairy and swine). While control plots received only inorganic fertilizers, fields reeving manure also received inorganic fertilizers. In 2011, both control and treatment (swine and dairy) fields were divided into 20 (4X6 m) plots and planted with 4-replicates of five crops (tomatoes, radishes, carrots, cucumbers, and peppers). In 2012, fields were divided into 16 (4X6 m) plots and planted with 4-replicates of four crops (tomatoes, radishes, carrots, and lettuce). Plots were irrigated with well water. Composite soil samples (at days 0, 7, 30 and at harvest) and vegetable samples (at harvest) were collected and levels of the following target were enumerated: fecal coliforms, *Escherichia coli*, *Enterococcus* species, *Clostridium perfringens*, *Aeromonas* species, *Yersinia*, *Campylobacter*, *Salmonella*, and *Listeria* spp. **Results:** While all raw manure samples (N=10) were negative for *Listeria monocytogenes*; *Campylobacter* was detected only in 2012; and *Salmonella* was detected only in 2011. Except *C. perfringens*, all other bacterial populations declined significantly (P<0.05) in swine manure (N=2) after storage for 22 days. *Listeria* spp. and *S. enterica* were never detected in soil. In vegetable samples, *Campylobacter* spp., *Listeria* spp., and *S. enterica* were never detected. Overall, soil amendment did not appear to affect the prevalence of foodborne pathogens in soil or on produce.

h) ***Generic E. coli and Salmonella prevalence and survival in liquid hog manure vs. conventional fertilizer:*** A 3-year replicated, randomized, controlled, experimental study (5) was conducted between 1998 and 2000, in a pickling cucumber field Quebec, Canada,

and found that application of liquid hog manure sometimes increased generic *E. coli* concentrations in soils but did not affect *E. coli* or *Salmonella* in cucumber samples. **Methods:** On a sandy loam soil, 4 fertilization treatments were evaluated over 2-years: in treatment 1 and 2, 100% mineral fertilizer (115 kg/ha), 100% liquid hog manure (115 kg/ha) was applied before plating. In treatment 3 and 4 fertilizer was applied at two stages of plant growth: mix 1 (80 kg/ha mineral fertilizer was applied before seedlings were planted + 35 kg/ha mineral fertilizer at mid-growth stage), and mix 2 (80 kg/ha liquid hog manure was applied before seedlings were planted + 35 kg/ha mineral fertilizer at mid-growth stage). Each treatment was applied to four (4X10 m) plots. In year 3, two treatments (mix 1 and 2) were also implemented in loamy sand soils. Fertilizers were applied before seedlings were planted, except in mix 1 and 2 treatments where 35kg/ha mineral fertilizer was applied at the mid-growth phase. Composite soil samples were collected from each plot before fertilization and 2, 4, 6, and 8 weeks after fertilization. At harvest, cucumber samples (washed N=10 and unwashed N=10) were collected from each plot. Generic *Escherichia coli* and *Salmonella* levels in each sample were enumerated. **Results:** Concentrations of *E. coli* in the liquid hog manure were below the detection limit in Year 1, 5.43 log CFU/g in Year 2, and 6.11 log CFU/g in Year 3. *Salmonella* were detected in year 3 liquid hog manure and soil samples. *E. coli* and *Salmonella* were never detected in cucumber samples (N=320) or in year-1 soil samples (N=80). In the second year, soil samples collected after the manure spreading were positive for *E. coli* (average at the time of harvest: 100% liquid hog manure = 1.87 CFU/g and mix 2 = 1.70 log CFU/g). In the third year, *E. coli* concentrations were 1.87 log CFU/g in sandy loam and 2.12 log CFU/g in loamy sand following fertilization. The authors reported a linear decrease in *E. coli* concentrations in soil after the spreading of manure (R^2 = 0.82 to 0.99 in sandy loam and 0.94 in loamy sand). The number of days required for *E. coli* to reach 0 ($\alpha = 0.10$) ranged from 37 to 97 in sandy loam (year 1 and 2) and 50 to 124 in loamy sand (year 3). In year 3, *Salmonella* persisted 54 and 27 days in loamy sand and sandy loam, respectively. As a result, this study recommended a 100-day delay between liquid hog manure application and harvest.

i) ***E. coli, Salmonella and Listeria prevalence in broccoli fields amended with liquid hog manure:*** A 2-year replicated, controlled, experimental study (2) between 2011 and 2012 in a broccoli field in Quebec, Canada, found a significantly higher prevalence of generic *E. coli* in plots amended with liquid hog manure compared to conventional fertilizers. **Methods:** Field experiments were performed on a 0.45-ha field. The study used a split-plot factorial design consisting of two either a soil amendment (liquid hog manure) or a control (mineral amendment), and one of three irrigation frequencies (0, 1, or 2 times). Soil amendments were added 1 week before planting to achieve a standard rate of P_2O_5 (30 kg / hectare). Each plot was then subdivided with half receiving irrigation water that was contaminated with bovine or pig fecal slurry and half not receiving contaminated water. Following irrigation, broccoli (collected on days 1, 3 and 5) and soil samples (collected on

day-5) were collected and tested for the presence of *Listeria monocytogenes* and *Salmonella* via culture methods. The presence of pathogenic *E. coli* O157, Shiga toxin-producing, and enteropathogenic pathotypes were identified using molecular approaches based on detection of the *rfbE, stx*$_1$*, stx*$_2$, and *eae* genes, respectively. Generic *E. coli* levels were also enumerated. In **Results:** Mean generic *E. coli* counts in liquid hog manure were 5.6 and 5.2 log CFU/g in 2011 and 2012. *Salmonella* serovar Typhimurium DT 104 was positive in manure samples in 2011 but not in 2012. Manure and slurry samples were 100% negative for *L. monocytogenes* and *E. coli* (carrying *rfbE, stx*$_1$*, stx*$_2$, and *eae* genes). Generic *E. coli* levels in the contaminated irrigation water ranged between 2.6 and 3.5 log CFU/100ml. All broccoli samples (N=144) were below the detection limit for generic *E. coli* (enumeration method), however, following enrichment method, 15 and 51% of the broccoli samples were generic *E. coli*-positive in 2011 and 2012, respectively. *E. coli* was more frequently recovered in broccoli samples fertilized with manure (19%) than in those fertilized with mineral fertilizers (11%). *Salmonella* and pathogenic *E. coli* markers were not detected in any of the broccoli samples. *L. monocytogenes* was detected in one broccoli sample. *E. coli* counts from manure-fertilized plots (36 CFU/g) were significantly (P = 0.0006) higher than plots that received mineral fertilizers (1 CFU/g). *L. monocytogenes*, *Salmonella*, and pathogenic *E. coli* were not detected in soil samples, except in 2012, *L. monocytogenes* (6/24) were positive in both treatment (N=2) and control (N=4) soil samples.

j) ***Salmonella persistence in dairy manure and manure amended soils:*** A 2006 replicated, study (15) that used soil collected from an agricultural field in Pennsylvania, USA, found that *Salmonella* serovar Newport could persist for 184, 332, and 405 days in manure, manure-amended nonsterile soil, and manure-amended sterilized soil, respectively. **Methods:** Fresh dairy manure was obtained from six dairy cows. Conestoga silt loam, fine-loamy, mixed, and mesic Typic Hapludalf soil was collected from an agricultural field at a experimental farm that was planted with silage corn and barley (winter cover crop), and that had received no manure/fertilizers for the previous 6 years. Bulk soil was divided into two portions, sterile soil (autoclaved for 15 mins) and non-sterile soil. Manure and manure-amended soils were added to containers and inoculated with two strains (0306-91 and 0007-33) of *Salmonella* serovar Newport (~10^{10} CFU/ml). Sterile water was added periodically to maintain the soil moisture content at 80%. Samples (manure and amended soils) were enumerated for *Salmonella* serovar Newport on selected days until the pathogen could no longer be detected. **Results:** Concentrations of *Salmonella* in the inoculated manure and manure-soil mixtures were 7.2 and 6.86 $\log_{10}$CFU/gram at day-0, respectively. In manure samples, an initial increase (day 0-3) in concentration was observed for both strains (317% for strain 0306-91 and 319% for strain 0007-33). Following this initial increase, the concentration of both strains decreased from day 0 to day 49 with concentrations dropping below the detection limit between day 35 to 49. However, the strains persisted in the manure until day 184 (enrichment method). Manure-amended soil

samples followed a similar trend as inoculated manure samples, an initial increase from day 0 to day 1 and steady decrease until day 107 (non-sterile soil with manure) and day 158 (sterile soil with manure). *Salmonella* cells persisted until day 332 post-inoculation in non-sterile soil with manure and until day 405 in sterile soil with manure.

k) ***E. coli prevalence in poultry, dairy, and horse manure:*** A 4-year, replicated, controlled, experimental study (6) conducted between 2011 and 2015 in three field sites in the Mid-Atlantic region of the USA, found that *E. coli* survived longer in poultry litter amended soils compared to horse manure amended soils, unamended soils, and dairy manure amended soils. **Methods:** In Maryland and Pennsylvania, USA, 12 different field trials were performed in 2011 to 2014, spring and fall. Plots (2 m^2) were cultivated under a randomized complete block experimental design, which included five soil amendment treatments: i) poultry litter, ii) dairy manure solid, iii) dairy manure liquid, iv) horse manure, and v) unamended fields (no manure). Soil amendments were added before the beginning of the study, to achieve an application rate equal to 5 tons/acre (poultry litter 2.27 kg, /2 m^2, horse manure 2.27 kg/2 m^2, dairy manure liquid 38 liters/2 m^2, and dairy manure solid 2.27 kg/2 m^2). Three rifampin-resistant nonpathogenic *E. coli* strains (TVS 353, 354, and 355) and two rifampin-resistant attenuated *E. coli* O157:H7 strains (PTVS 154, and 155) were used to prepare low inocula (1 x 10^4 CFU/ml) and high inocula (1 X 10^6 CFU/ml). Plots were hand sprayed with 1 L of either low or high inocula. Plots were examined at different depth (surface and tilled). Composite soil samples were collected from selected plots in 2011 (2 fields, 1 season), 2012 (3 fields, 2 seasons), 2013 (3 fields, 2 seasons), and 2014 (3 fields, 1 season) over the course of each season, and the level of inoculation strain was enumerated until levels fell below the limit of detection. **Results:** Models (six candidate nonlinear regression curves, ANCOVA, and random forest models) were used to predict *E. coli* survival by estimating dpi100mort values (*i.e.*, the number of days post-inoculation in which *E. coli* values reached 0.11 log MPN/g dry weight). Results from these models indicate that no single factor can explain the majority of the observed dpi100mort experimental variability; indeed, a combination of factors were needed to accurately assess the dpi100mort values. Spatiotemporal factor (e.g., site, year, and season) more significantly influenced the survival of *E. coli* than other agricultural factors (management practices, manure type, soil depth). Soils amended with poultry litter and horse manure (to a lesser extent) were able to support extended survival of *E. coli* when compared to dairy manure solids or liquids. Survival of nonpathogenic *E. coli* and attenuated *E. coli* O157 in manure amended soils influenced by spatiotemporal factors and the soil amendment type.

l) ***Salmonella and Listeria prevalence on farms that apply and do not apply manure:*** A 2012, observational study (8) conducted on farms (N=21) in Western, Central, and Eastern New York, USA found higher prevalence of *Salmonella* and *Listeria monocytogenes* in manure amended fields than in fields where manure had not been applied. **Methods:** A questionnaire was mailed to selected farms in western (N=5), central (N=12), and eastern

(N=4) New York and farm visits were performed over a 5-week period (June and July, 2012). One composite soil sample, and one drag swab were collected from each field, while irrigation water samples (N=23), and non-irrigation water samples (N=51) located within 50 m from a sampled field were sampled opportunistically. All samples (263 soil, 263 drag swab, and 74 water samples) were tested via an enrichment method for *Salmonella* and *Listeria monocytogenes*. **Results:** *Salmonella* and *L. monocytogenes* were detected in 6.1% (16/263) and 17.5% (46/263) of fields sampled, respectively. Prevalence of *Salmonella* and *L. monocytogenes* in terrestrial samples (N=526 *i.e.*, soil and swab samples) were 3.4% (18/526) and 9.7% (51/526), respectively. Fields where manure was applied within a year of sample collection had higher odds of *Salmonella* detection (odds ratios [OR]=19.0; 95% confidence interval [CI]=4.9, 77.0) and *L. monocytogenes* (OR=7.0; 95% CI=3.1, 15.4) than fields where manure had not been applied. Fields that were cultivated ≤7 days prior to sample collection were approx. 6 times more likely to detect *Salmonella* (OR = 6.3; 95% CI = 1.6, 23.0) and 8 times more likely to detect *L. monocytogenes* (OR = 8.1; 95% CI=3.3, 19.6) compared to fields where soil was not cultivated for at least 30 days. Similar results were reported with multivariable analysis, where application of manure to a field within a year prior to sample collection was associated with a higher likelihood of *Salmonella* (OR = 16.7; 95% CI = 3.0, 94.4) being detected in a field than in fields where manure had not been applied.

m) ***Generic E. coli prevalence in spinach from organic and conventional farms:*** A 2-year replicated, observational study (10) conducted from 2010 to 2011 in spinach fields in Western Colorado and Southwestern Texas, USA found that generic *E. coli* was significantly more prevalent on spinach grown in manure amended soil (15.6%) compared to soil amended with chemical fertilizers (4.8%). **Methods:** A total of 955 spinach samples were collected from 1-6 fields per visit from each of 12 spinach farms [Colorado (N=4) and Texas (N=8)]. Five spinach samples were collected per field and generic *E. coli* levels in each sample were enumerated. At each farm visit, a questionnaire was also administered to obtain information on farm management and environmental factors. **Results:** The prevalence of generic *E. coli* was 6.6% (63/955) across all spinach samples. Spinach samples collected from farms that added manure to fields had higher proportion of generic *E. coli* positive samples than did farms not using it (15.6% versus 4.8%). Based on univariant analysis manure application increased the risk of generic *E. coli* contamination (OR = 7.9; 95% CI 1.6, 39.4; P=0.011) when compared to fields amended with chemical fertilizers. Additionally, the prevalence of generic *E. coli* in spinach samples was lowest when the time since last manure spreading was >200 days (OR = 0.08; 95% CI 0.01, 0.90; P=0.041) when compared to fields that added manure ≤200 days. Similarly, manure aged for > 13 weeks reduced the risk of generic *E. coli* contamination (OR= 156.6; 95% CI 0.2, 114,716.7; P=0.133) than manure aged ≤ 13 weeks. Among spinach samples collected from fields amended with manure, samples from poultry manure amended fields had a significant higher odd of contamination than samples from fields amended with cattle

manure (OR= 11.4; 95% CI 1.1, 123.5; P=0.045). However, the final multivariant model did not include manure application, time, age, and source as a risk factor of generic *E. coli* prevalence, suggesting that univariate analyses may have been confounded.

n) ***Salmonella, E. coli O157:H7, and generic E. coli prevalence in organic and conventional farms:*** A replicated, observational study (11) was conducted during the 2002 growing season in Minnesota, USA. The study found that soil amended with manure and or compost that was aged less than 12 months had an increased (19 times) generic *E. coli* prevalence than farms that used older (aged >1 year) manure/compost. **Methods:** Following enrollment of farms (N=40) in the study, farms were classified as either organic (including certified, N=8, or non-certified N=24), or conventional (N=8 farms) based on responses to a grower survey. The participating organic farms used aged or composted manure, while four of the conventional farms reported using composted manure in addition to chemical fertilizer. Among the organic farms, 18 used 6 to 12-month-old manure and/or compost, while six farms used manure/compost aged for >1 year and 2 farms used manure/compost aged for less than 6 months. Soil amendments were applied to the fields in spring (N=14 farms), in fall (N=5 farms), in spring and fall (N=3 farms) and in summer (N=1 farm). The type of manure applied included, chicken litter (N=15 farms), cattle (N=12 farms), sheep (N=8 farms), and horse (N=4 farms). Sixteen farms used a mix of several manure types. A total of 476 (certified organic N=117) and 129 produce samples were collected from the participating farms. Prevalence of *Salmonella* and, two pathogenic *E. coli* markers (the *stx1* and *stx2* genes), as well as generic *E. coli* levels were determined via culture-based methods. **Results:** All samples tested negative for *E. coli* O157:H7. *Salmonella* was isolated from two organic produce samples. Generic *E. coli* was detected in 8% of all samples (average 3.1 log MPN/g). Generic *E. coli* positive samples in conventional, certified-organic and noncertified-organic produce were 1.6, 4.3 and 9.7%, respectively. The generic *E. coli* prevalence in farms (both organic and conventional) that used aged manure or compost (> 1year) was not statistically significant (P>0.05). However, farms that used manures aged between 6 to 12 months had significantly (OR=23.8, P<0.05) more generic *E. coli* vs those from farms that used materials more than 1-year old. Source and age of manure/compost was a significant factor: for example, the prevalence of generic *E. coli* was 2.4-times greater in produce grown on farms using cattle manure vs farms using other types of manure.

o) ***Generic E. coli and E. coli O157:H7 prevalence in poultry litter, liquid dairy manure, horse manure, and conventional fertilizer:*** A 2-year replicated, controlled, experimental study (12) conducted between 2012 and 2013 in greenhouses in Maryland, USA found that poultry litter amended soils supported generic *E. coli* and *E. coli* O157:H7 at higher concentrations compared to liquid dairy manure amended soils, horse manure amended soils, and unamended soils. **Methods:** Three studies were performed using a mixed *E. coli* culture with 3-rifampin resistant nonpathogenic *E. coli* strains (TVS 353, 354, and 355) and two rifampin-resistant attenuated *E. coli* O157:H7 strains (PTVS 154 and 155). *Study*

1: Small containers (389cm^3) were filled with 1.5 kg of silty loam soil (N=60) or sandy loam soils (N=60). Four replicates of each container received one of three treatments: unamended, liquid dairy manure (3ml/container), or poultry litter (7 g/container). *E. coli* mix (3ml of 1x10^{4}CFU/ml) was added to each container on day 0. Containers were irrigated with distilled water (200 ml/24 h) and stored in a greenhouse. *E. coli* levels in samples was enumerated on each sampling day. *Study 2*: Large containers (1,194 cm^2) were filled with silty loam (N=12) or clay loam soil (N=12). Four replicates of each container received either no manure, poultry litter (590g/container), or horse manure (735g/container). *E. coli* mix (328ml of 1x10^{6}CFU/ml) was added to each container on day 0. Containers were irrigated once per week. *E. coli* was enumerated on each sampling day. *Study 3*: Large and small containers were prepared as mentioned above. Small containers received 3 treatments: unamended, poultry litter (5.2 g per container), and horse manure (6.5 g per container), in addition to 3 ml of *E. coli* inoculum (1 X 10^6 CFU/ml). Large containers received 3 treatments: unamended, poultry litter (590 g per container), and horse manure (735 g per container), in addition to 328 ml of *E. coli* inoculum (1 X 10^6 CFU/ml). Small and large containers were stored for 56 days, with samples taken 0, 7, 14, 28, and 56 days post inoculation. **Results:** *Study1:* Soils amended with poultry litter supported significantly ($p<0.05$) more generic *E. coli* and attenuated *E. coli* O157 (generic: 2.89 log CFU/gram dry weight (gdw); O157: 2.84 log CFU/gdw) relative to dairy liquid manure (generic: 0.32 log CFU/gdw; O157: 0.29 log CFU/gdw) and unamended soils (generic: 0.28 log CFU/gdw; O157: 0.25 log CFU/gdw). *Study 2:* Generic *E. coli* concentrations were significantly greater ($p<0.05$) in poultry amended soil (3.72 log CFU/gdw) than horse manure amended (0.13 log CFU/gdw) or unamended (0.09 log CFU/gd w) soil. Again, *E. coli* (attenuated) O157 results were similar to generic *E. coli*. *Study 3:* Generic *E. coli* concentrations were significantly ($p<0.05$) greater in poultry amended soils (clay loam: 5.16 log CFU/gdw; silty loam: 5.08 log CFU/gdw) versus unamended (clay loam: 3.24 log CFU/gdw; silty loam: 1.77 log CFU/gdw) and horse manure amended (clay loam: 3.18 log CFU/gdw; silty loam: 1.02 log CFU/gdw) soils. In summary, regardless of container size or soil type, *E. coli* persisted more in poultry litter amended soils than liquid dairy manure, horse manure, and unamended soils.

p) ***Generic E. coli prevalence in runoff water generated from fresh poultry manure amended soils:*** A 3-year replicated, controlled, experimental study (17) from 2001 to 2003 in a potato field in New Brunswick, Canada, found that application of fresh poultry manure increased the *E. coli* concentrations in the runoff water. Methods: Field experiments were conducted in 7 plots (10x30 m) in one field, all with gravel loam soil. Four plots had a 11% slope and 3 plots had 8% slope. The plots were planted with potatoes in June (2001), or May (2002, 2003). Plots received one of four treatments: a no manure control, or application of fresh poultry manure (4 Mg/ha) in late fall, pre-planting. Runoff and sediment from the plots were collected using a "collector system" located at the lower end of each plot. Samples were collected within 12 h of a runoff-generating event (with annual

average rainfall 834mm or snowmelt 300mm) and *E. coli* levels were enumerated. Results: Fresh poultry manure contained 2.2 X 1010 MPN *E. coli*/Mg. E. coli concentrations in the run-off water from the control and treatment plots varied seasonally and ranged from 0 to 16,500 MPN/100 mL in 8% slope and 0 to 92,100 MPN/100 mL in 11% slope. In general, snowmelt runoff had lower concentrations of *E. coli* (<10 MPN/100mL) than rainfall runoff water. Plots receiving manure had significantly (P<0.10) higher *E. coli* concentrations than control plots. Mean annual flow-weighed runoff concentrations of *E. coli* were: 3288, 3950, 1442, 4879 MPN/100mL for control, fall, pre-planting, and pre-hilling treatments, respectively, in plots with 11% slope and 475, 902, and 1594 MPN/100mL for control, fall, and pre-hilling treatments, respectively, in plots with 8% slope. Addition of manure increased *E. coli* concentrations in runoff water from 8-11% slope by 20-230%. Plots amended with fresh poultry manure increased the E. coli concentrations in runoff water by 20 to 230%.

q) ***Generic E. coli prevalence in rain runoff water from dairy manure amended soil:*** A three year replicated, randomized, experimental study (16) from 1992 to 1994 in a corn field in the Elora Research Farm, Ontario, Canada, found that rainfall after manure applications increased movements of a bio-tracer (nalidixic acid resistant generic *E. coli*) from fields to surface waters via tile drains. **Methods:** Four experiments were conducted between 1992-94, in a 0.9 ha section of a field with a 4% slope. Liquid cow manure was applied at 56,000 L/ha twice a year (spring: before planting and fall: after harvest). The average rate of bio-tracer application to soils was 7.6 x 103 to 1.3x105 CFU/g wet weight and the depth of manure application was 2.9 to 6.9 mm. Before and after manure application, soil core samples (up to 900 mm) were collected and flow rates into tile drains were determined. Water samples were collected from tile lines. Sample cups were added to the field to measure spreading rates. Samples were enumerated for the bio-tracer (nalidixic acid resistant generic *E. coli*). Results: Prior to application of manure, bio-tracers were never detected in tile drain samples or soil core samples. Following manure application, bio-tracer concentrations in tile drain water ranged from 1 to 1400 CFU/100ml. Manure application depths had no impact on these results. However, rainfall influenced bio-tracer concentrations in the tile drain water: in spring 1994, 8.6mm of rain after 24 hours of manure application elicited bio-tracer concentrations of >1000 CFU/100ml. Similarly, in fall 1992, 27 mm of rain after 5 days of manure application, elicited bio-tracer concentrations of 100 CFU/100ml. Soil core samples collected after manure application (24-48 h), revealed that bio-tracers penetrated the soil to 900mm. After 20 days, bio-tracers in soil cores were reduced to 1% of the initial amount. In summary, rain following manure application increased bio-tracer contamination of tile drain water and soils.

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1.3. Add biosolids to soil (3 studies)

- **Biosolids implementation options (2 studies):** Two replicated, experimental, studies (1, 2) in Canada (Ontario, Debert) found no differences in pathogen and generic *E. coli* levels between plots amended with biosolids and plots amended with conventional fertilizers, in at least some of the statistical tests conducted. Of the 2 studies, one study compared plots with municipal biosolid and sewage sludge versus plots with conventional fertilizers and found no significant difference in multiple bacterial populations on vegetables at harvest. The other study, compared soil amended with N-Viro biosolids to conventional fertilizers in a blueberry field, and found no effects on pathogenic *E. coli* and *Salmonella* prevalence.
- **Comparing Biosolid sources (1 study):** One observational study (3) across Ontario, Canada found pathogen levels were lower in paper mill biosolids compared to sewage sludge. However, pathogens were higher in paper mill biosolids compared to commercially available compost.

a) ***Prevalence of pathogenic E. coli and Salmonella in fields fertilized with N-Viro biosolids:*** A 2-year replicated, controlled, experimental study (1), conducted between 2008 and 2009 in a wild blueberry field in Nova Scotia, Canada, did not detect *E. coli* or *Salmonella* in N-Viro biosolids and soil, reflecting the absence of these pathogens in the biosolids. **Methods:** On a sandy loam acidic soil, 4 treatments were evaluated for 2-years: irrigated N-Viro amended (11.13 kg/plot), rainfed N-Viro amended (11.13 kg/plot), irrigated conventional fertilizer amended (32 kg N ha^{-1}), and rainfed conventional fertilizer amended (32 kg N ha^{-1}). Each treatment was applied to 4 plots (N=16, 9x3 m) separated by a 3m buffer. Soil samples were collected from the treatment plots at specific time intervals: before fertilization and at harvest (3rd week of July 2008 and 2009). Soil water samples were collected in 4-phases, using suction cup lysimeters, that were installed at 20 and 40 cm depths in each plot. Pathogenic *E. coli* and *Salmonella* levels were enumerated in each sample using a culture-based method. **Results:** All samples (soil and biosolid) tested negative for *E. coli* and *Salmonella* across all 4 treatment plots.

b) ***Bacterial prevalence in municipal biosolids and sewage-based sludge:*** A 2-year replicated, controlled, experimental study (2) conducted between 2011 to 2012 in Ontario, Canada found no significant effects of municipal biosolid and sewage sludge amended on the abundance of multiple bacteria, including pathogen. **Methods:** Sewage sludge and dewatered municipal biosolids were acquired from a local source and broadcasted onto treatment plots in the field in 2011 (municipal biosolids: 10.8 wet tones/ha) and 2012 (sewage sludge: 28.6 wet tones/ha). Control plots received only inorganic fertilizers (NPK at 224 kg/ha in 2011 and 336 kg/ha in 2012). In 2011, control and treatment blocks were divided into 20 (4X6 m) plots and planted with 4-replicates of tomato, radish, carrot, cucumber, and pepper. In 2012, fields were divided into 16 (4X6 m) plots and planted with

4-replicates of tomato, radish, carrot, and lettuce. Plots were irrigated with well water via overhead irrigation. Levels of the following microbes in composite soil samples (at day 0, 7, 30, and at harvest) and vegetable samples (at harvest) were enumerated: fecal coliforms, *Escherichia coli*, *Enterococcus* species, *Clostridium perfringens*, *Aeromonas* species, *Yersinia* species, *Campylobacter* species, *Salmonella* species, and *Listeria* species. **Results:** In 2011 (municipal biosolids), *E. coli* and all pathogens were sometimes detected by enrichment but were always below the quantification limit (20 CFU/plate). All pathogens were quantifiable with the exception of *Salmonella* and *L. monocytogenes* in 2012 (sewage sludge). Enterococci were >10 times less abundant in biosolids than sewage sludge. Fecal coliforms, *E. coli*, enterococci, and *Yersinia* spp. levels in amended and untreated control plots were not significantly different. *Campylobacter* species, *Salmonella* species, and *Listeria* species were never detected. *Clostridium perfringens* was detected significantly more frequently in amended soil than in unamended control soil, with an odds ratio of 48:1 (chi square = 38.96; $P < 0.0001$). Overall, soil amendments had no significant impact on the prevalence of bacterial populations on vegetables at harvest.

c) ***Pathogen prevalence in paper mill biosolids, compost, sewage pellets, and soil:*** A 2-year replicated, controlled, observational study (3) was conducted between 2005 and 2006 in Ontario, Canada and reported (i) lower prevalence of foodborne pathogens in paper mill biosolids compared to sewage biosolids, and (ii) pathogen prevalence was generally higher or similar in paper mill biosolids compared to commercially available compost and soil. **Methods:** Once in summer and once in winter, pulp paper and biosolids (N=93) were sampled from paper mills in Ontario (N=12) and Quebec (N=1), Canada. Out of 93 samples, 24 were collected from mills with sanitary waste input systems and 63 from mills without sanitary input systems. Three composite samples were collected from retail commercial composts (N=21 from 7 bags), potting soil mixes and potting top soil (N=12, from 4 bags), outdoor soil samples (home garden and public parks N=12), and sewage biosolid granules (N=15) from mesophilic anaerobically digested municipal sewage biosolids. Levels of the following microbes were enumerated in each sample: *E. coli*, *Clostridium perfringens*, *Salmonella* spp., and *Campylobacter*. After DNA extraction, *Salmonella* spp., *Shigella* spp., *Campylobacter jejuni* and *Campylobacter coli*, *Cryptosporidium parvum*, and *Giardia intestinalis* were detected by qualitative polymerase chain reactions. **Results:** *E. coli* (55% positive) and enterococci (83% positive) were more prevalent in paper mill biosolids than in soil (*E. coli* 8% and enterococci 33%) and compost (*E. coli* 14% and enterococci 29%) but less frequently when compared to sewage biosolids (*E. coli* and enterococci 100%). Prevalence of *Clostridium perfringens* was less frequent in paper mill biosolids (28%) than in soil (71%) and compost (76%) but 100% positive in sewage biosolids. Similarly, *Salmonella* (9%, mean 2.6 CFU/g), *Shigella* (6%, mean 7 CFU/g), and *C. parvum* (6%, mean 9 CFU/g) were detected at lower frequencies in paper mill biosolids than sewage biosolids (50%, 0%, and 17%, respectively), but at higher or equivalent frequencies compared with soils (0 to 4%) and composts (0 to 5%). *Salmonella*

was culture positive only in paper mill and sewage biosolids, but was not detected in other sample types. *Giardia* (mean = 30 cysts/g) were higher in paper mill biosolids, but was not significantly (p=0.185) more prevalent in paper mill biosolids samples (19%) than compost (0%) and soil (8%) samples. Overall, pathogen levels in paper mill biosolids were consistently lower than sewage biosolids but were not significantly different from commercially available soil or compost.

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1.4. Farm fields organically (8 studies)

- **Pathogen prevalence (5 studies):** Four observational studies (1-4) from the USA (Maryland, Delaware, New Jersey, Colorado, Texas, and Minnesota) found mixed effects of organic versus conventional soil management on pathogen prevalence on produce and soils. Specifically, one study (4) found higher prevalence of generic *E. coli* in conventional compared to organic farms, two studies (2, 5) found higher prevalence of generic *E. coli* in non-certified organic compared to certified organic and conventional farms, and two studies (1, 3) found no effects of organic status.
- **Pathogen survival (3 studies):** Three studies found mixed effects of pathogen survival in conventional versus organic soils in the USA (Arizona, California, and Florida) (6-8). Specifically, two experimental lab studies (6, 7) from the USA (Arizona, California) found *E. coli* persisted longer in organic soils compared to conventionally managed soils, in at least some of the statistical tests conducted. In contrast, one experimental greenhouse study (8) found *Salmonella* persisted longer in tomato plants grown in conventional than organic soil.

a) ***Salmonella, STEC, and generic E. coli prevalence on organic and conventional farms:*** A 2-year replicated, observational study (1) from 2012 to 2013 at farms (N=32) in Maryland, Delaware, and New Jersey, USA, found levels of STEC, *Salmonella*, and generic *E. coli* did not significantly differ between organic versus conventional farming systems. **Methods:** Field samples were collected from 32 farms (15 conventional and 17 organic) in fall 2012 and spring 2013. Sample types included: foliage of leafy greens (N=369 leaves, some of which touched the ground or were soiled), irrigation water (N=124 from well, pond, or river water), sediment (N=13 from pond or river), field soil (N=60), and compost (N=11). Generic *E. coli* was assayed via. culture methods; presence of *Salmonella* (*invA*) and STEC (*stx1* and *stx2*) were determined using qPCR amplification, followed by enrichment methods for PCR positive samples. **Results:** Prevalence of STEC genes (qPCR) was 0.3% (2/577) across all samples; however, STEC was not isolated from either of these positive samples. Prevalence of *Salmonella invA* was 4.2% (24/577) across all samples (leafy greens N=15, water N=6, compost N=1, and soil/sediment N=2), with 58% and 42% of *Salmonella*-positive samples coming from organic and conventional farm samples, respectively. In leafy greens, 6.2% (23/369) were positive for generic *E. coli*. Organic and conventional systems did not differ in generic *E. coli* concentrations ($P = 0.646$) or numbers of positive samples [X^2 (1) = 0.05, $P = 0.826$]. Field soil samples (in both the years) yielded no significant relationships between generic *E. coli* prevalence and farming system type [X^2 (1) = 3.69 ($P = 0.055$)].

b) ***Salmonella enterica, STEC, and generic E. coli prevalence on organic versus conventional farms:*** A replicated, observational study (4) from July to September 2012, at tomato farms in Maryland, Delaware and New Jersey, USA, found that generic *E. coli* counts on tomatoes and soil from conventional farms were marginally higher than from

organic farms. **Methods:** Based on a grower survey, tomato farms (N=24) in Maryland, Delaware, and New Jersey, USA were classified as organic certified/non-certified (N=12) or conventional (N=12). During the harvest season, tomato fruit, soil, compost, and water samples were collected from the fields and levels of STEC, *Salmonella enterica*, and indicator bacteria (generic *E. coli* and total coliforms) were enumerated. *Salmonella* (*invA*) and STEC (*stx1* and *stx2*) markers were detected using PCR amplification; presumptive PCR-positive were culture confirmed by enrichment. A total of 422 samples were collected from 24 farms: 259 tomato samples (130 conventional and 129 organic), 45 soil samples, 9 pond sediment samples, 7 compost samples and 102 water samples (N=40 wells, N=17 ponds, N=4 creeks and streams, and N=41 end of irrigation system lines). 23 farms used drip/trickle irrigation, and 1 farm used overhead sprinkler irrigation. **Results:** Few samples were positive for *stx* genes (3/422 samples, 0.7%): 2 from surface irrigation water samples (*stx*2) and 1 from tomato fruit (*stx*1 and *stx*2). Similarly, only 5/422 (1.18%) of the samples were positive for *Salmonella inv*A: 2 tomato samples, 2 water samples, and 1 soil sample. All samples were negative for STEC and *Salmonella* following enrichment. Compost samples (N=7) were 100% negative for generic *E. coli*. Among soil samples, 8.9% (4/45) were positive for generic *E. coli*. Generic *E. coli* was not significantly (P=0.611) more prevalent in soil from conventional farms (3/24) than organic farms (1/21). Generic *E. coli* prevalence on tomatoes did not differ between organic and conventional farms [X^2 (2) = 4.60, p = 0.100]. However, the total number of all samples (regardless of type) that tested positive for generic *E. coli* was higher on conventional than organic farms [organic 2/129 vs conventional 9/130, X^2 (1) = 4.60, p = 0.032]. Moreover, generic *E. coli* counts were marginally higher on conventional than organic farms (organic 0.03 vs conventional 0.13 log CFU/g, p = 0.0813).

c) ***Salmonella, E. coli O157:H7, and generic E. coli prevalence in organic and conventional farms:*** A replicated, observational study (2), conducted in 2002 in Minnesota, USA, found that generic *E. coli* prevalence was higher on non-certified organic farms compared to certified organic or conventional farms. **Methods:** Following enrollment of farms (N=40) in the study, farms were classified as either organic (including certified organic, N=8, and non-certified farms, N=24), or conventional (N=8 farms) based on responses to a grower survey. The participating organic farms used aged or composted manure, while four of the conventional farms reported using composted manure in addition to chemical fertilizer. Among the organic farms, 18 used 6 to 12-month-old manure and/or compost, while six farms used manure/compost aged for >1 year and 2 farms used manure/compost aged for less than 6 months. Soil amendments were applied to the fields in spring (N=14 farms), in fall (N=5 farms), in spring and fall (N=3 farms), and in summer (N=1 farm). The type of manure applied included: chicken litter (N=15 farms), cattle manure (N=12 farms), sheep manure (N=8 farms), and horse manure (N=4 farms). Sixteen farms used a mix of several manure types. A total of 476 (certified organic N=117) and 129 produce samples were collected from organic (N=32) and conventional (N=8) farms.

Prevalence of *Salmonella,* two pathogenic *E. coli* markers (the *stx1* and *stx2* genes), and generic *E. coli* levels were tested via culture method. **Results:** All samples tested negative for *E. coli* O157:H7. *Salmonella* was isolated from two organic produce samples. Generic *E. coli* was detected in 8% of all samples collected from 13 organic farms and 2 conventional farms; the average concentration in these positive samples was 3.1 log MPN/g. Generic *E. coli* positive samples in conventional, certified-organic and noncertified-organic produce were 1.6, 4.3 and 9.7%, respectively. Noncertified organic produce had significantly ($P<0.05$) higher (6x times) generic *E. coli* counts than conventional produce samples. The detection of generic *E. coli* from certified organic produce samples (4.3%) was 3-times greater (odds ratio = 2.9) than conventional produce (1.6%), but not significant ($P=0.094$). However, generic *E. coli* detection was significantly ($P<0.05$) less likely (odds-ratio = 2.8) in samples collected from certified organic farms compared to non-certified organic farms. Among organic samples, produce type was significantly ($P<0.05$) associated with the likelihood of detecting generic *E. coli.* For example, organic lettuce contributed to 22.4% of generic *E. coli* positive samples when compared to other produce samples.

d) ***Generic E. coli prevalence in spinach from organic and conventional farms:*** A 2-year replicated, observational study (3) conducted from 2010 to 2011 in spinach fields in Western Colorado and the Southwestern Texas, USA found that generic *E. coli* did not significantly differ between spinach grown in conventional versus organic soils. **Methods:** A total of 955 spinach samples were collected from 1-6 fields per visit from each of 12 spinach farms [Colorado (N=4) and Texas (N=8)]. Five spinach samples were collected per field and generic *E. coli* levels in each sample was enumerated. At each farm visit, a questionnaire was also administered to obtain information on farm management and environmental factors. **Results:** The prevalence of generic *E. coli* was 6.6% (63/955) across all spinach samples. The odds (odds ratio = 2.4) of produce contamination with *E. coli* was higher in organic farms when compared to conventional farms. However, the difference was not statistically significant ($P=0.340$). Spinach from certified organic farms was less likely to be contaminated with generic *E. coli* than spinach from non-certified organic farms ($OR = 0.05$, $P = 0.022$).

e) ***Generic E. coli prevalence on organic versus conventional farm:*** A replicated, observational, cross-sectional study (5) conducted in June 2010 and Feb 2012, assessed the microbial safety of spinach grown by commercial farms produced by selected farms in Colorado and Texas, USA in 2010 and 2012, and found that the odds of harvesting spinach contaminated with generic *E. coli* was marginally lower in certified organic farms compared to farms that self-reported using organic practices. **Methods:** Spinach samples (N=955) collected from selected farms in Colorado (N=4) and Texas (N=8) were enumerated for *E. coli*. Information on the general farm-related management and environmental factors were obtained from the growers (questionnaire on farm management and environmental factors) and the National Resources Inventory databases (weather and

landscape factors). Variables were evaluated using a mixed-effect logistic regression model. **Results:** Generic *E. coli* was detected in 76.6% (63/955) of spinach samples. The odds of detecting generic *E. coli* was 3.7 times greater (95% CI=0.8, 16.4; P=0.89) for farms that self-reported using organic practices on the farm compared to farms that did not report using organic practices. Conversely, the odds of generic *E. coli* detection were lower for farmers that were certified organic compared to farms that weren't (Odds Ratio = 0.01; 95% CI=0.00, 0.98; P=0.049).

f) ***E. coli O157:H7 survival under organic and conventionally managed soils:*** A replicated, controlled, experimental, study (6) conducted in 2012, found that *E. coli* O157:H7 persisted significantly longer in organic compared to conventional farm soils collected in Arizona but not in soils collected in California. **Methods:** Soil was obtained from organic and conventional leafy greens farms in California [Imperial Valley (clay loam) and Salinas (sandy loam)], and Arizona (clay). In triplicate, aliquots of each soil type (500 grams) were inoculated with *E. coli* O157:H7 EDL933 cells ($5*10^6$ CFUs/gram) and sterile water (as controls). The inoculated soil was then incubated at room temperature (22±1 ℃) and the moisture content maintained at (~50%). Inoculated soils were sampled 0, 3, 6, 10, 14, 20, 27, 34, 40, and 48 days post-inoculation; inoculum levels in each sample were then enumerated. **Results:** No difference in *E. coli* O157:H7 survival was observed between organic and conventional managed soils from California: *E. coli* O157:H7 persisted for at least 30 and 20 days before reaching the detection limit (100 CFU g^{-1}) in soils from Salinas (organic 31 days, conventional 28.1 days) and Imperial Valley (organic 19.3 days, conventional 18.4 days), respectively. Contrastingly, *E. coli* O157:H7 survived significantly longer ($P < 0.05$) in organically managed soils (25.7 days) than in conventionally managed soils (15.5 days) from Yuma, Arizona.

g) ***E. coli O157 and non-O157 survival in organic versus conventionally managed soils:*** A 2014 replicated, controlled, experimental study (7) found that *E. coli* O157 and non-O157 strains persisted significantly longer in organic compared to conventional soils collected from produce fields in California (Imperial Valley, Salinas Valley) and Arizona (Yuma), USA. **Methods:** Soil samples from 16 organic and 16 conventional farms were collected from fresh produce (spinach and lettuce) growing areas in California: Imperial Valley (clay loam), Salinas (sandy loam) and Arizona: Yuma (clay). Persistence of three O157 strains (*E. coli* O157:H7 EDL933, *E. coli* O157:NM, *E. coli* O157:H7 4554), and three non-O157 strains (*E. coli* O26:H11, *E. coli* O91:H21, and *E. coli* O103:H2) were investigated in soil samples. Soil samples (500 grams) were inoculated with *E. coli* cells ($0.5 \text{ X } 10^7$ CFU/gram) and sterile water (as control). Triplicate soil samples were weighed and incubated in the dark at 20±2 ℃. Inoculated soils were sampled periodically and tested for *E. coli* via culture methods. **Results**: *E. coli* persisted for longer periods ($P<0.05$) in organic soils compared to conventional soils from Imperial Valley (3 strains of *E. coli* O157), Salinas (*E. coli* O157:NM and *E. coli* O26:H21), and Yuma (*E. coli* O26:H21 and *E. coli* O103:H2). Across all tested soils, *E. coli* O91:H21 survived the shortest (<30 days), and

E. coli O103:H2 survived the longest (60-90 days). The authors conclude that survival of *E. coli* O157 and non-O157 may be strain and soil specific.

h) ***Salmonella enterica survival in organic and conventionally managed soils:*** A 2-year replicated, controlled, experimental greenhouse study (8) conducted between 2009 and 2010, Florida, USA, found that more *Salmonella* survived on tomato plants grown in conventional than organic soil. **Methods:** During phase-1 of the experiment each greenhouse block (N=7) contained 18 tomato plants (total N=126 tomato plants), and these 18 plants were equally divided into two treatment groups, conventionally managed (N=9) and organic (N=9) soils. Organic and conventionally managed sandy loam soil was obtained from certified organic and research farms, respectively, in Florida. During both years, at each block, 6/9 conventional and 6/9 organic plants were inoculated with GFP-labelled *S. enterica* Typhimurium strains MAE110 and MAE119 while 3/9 plants were inoculated with sterile water. Plants were inoculated by dipping 3-leaflets into 15ml of *Salmonella* cocktail (10^9 CFU/ml), before fruiting stage on weeks 5 and 10 (year-1) and weeks 5, 8, 9, and 10 (year-2). Tomato leaves, fruits and seeds were sampled at specific time intervals from each plant and *Salmonella* levels were enumerated. **Results:** *Salmonella* concentrations were significantly (P = 0.0117) higher on plants grown in conventional soils (3.81 log CFU/g) than organic soils (3.27 log CFU/g). Similarly, levels of *Salmonella* inside the leaves decreased significantly (P=0.016) more in plants grown in organic soil than conventional soil. All control samples tested negative for *Salmonella*. All tomato fruit positive (1%) for *Salmonella* were from plants (3/252) grown in conventional soil.

Reference:

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1.5. Farm soil with no-till practices (5 studies)

- **Pathogens prevalence:** Four replicated experimental studies from the USA and Canada (1-4) found no difference in fecal indicator bacteria (FIB), generic *E. coli*, and *Salmonella* populations, when comparing no-till systems to system where tillage was performed in at least some of the statistical tests conducted. Of the 4 studies, one replicated, experimental study found that soil tillage in organic fields had a negative impact on *L. innocua* levels. One observational study from the USA (5) found that timing of soil tillage was a significant factor that influences the prevalence of *Salmonella* and *Listeria monocytogenes* in soil samples.

a) ***Prevalence of E. coli and Salmonella in simulated runoff from tilled versus untilled systems:*** A 2-year replicated, controlled, experimental study (1) conducted between 2009 and 2010 in Georgia, USA found no significant differences in total load of *E. coli* or *Salmonella* recovered from experimental runoff water collected from no-tillage systems and systems where tillage was performed. **Methods:** A total of 6 plots (2x3 m) were randomly assigned to conventional tillage (n=3) and no-tillage (n=3) treatments. Tillage consisted of fall disking, spring disking, and cultivator leveling. During the Fall of 2009, a cover crop mixture of rye (*Secale cereal*, 88 l ha^{-1}) and Austrian winter peas (*Lathyrus hirsutus*, 33.6 kg ha^{-1}) were planted on both the treatment areas. The cover crop was terminated with glyphosate (April, 2010) and, 2-weeks later, tillage treatment plots were plowed and conditioned. Broiler litter (4.98 Mg ha^{-1}, containing *E. coli* and *Salmonella* loads of 12.8 and 6.9 $\log_{10}$ cells ha^{-1}) obtained from a local poultry production facility was broadcasted over the fields. Litter in tillage treatment plots was incorporated (10-15 cm depth) with a field cultivator. Pearl millet (*Pennisetum glaucum*, L.R. Br.) was planted on both the treatment plots by May, 2010. To activate herbicides (Callisto) and kill the millet, fertilized plots were pre-wetted with 12.7 mm of well irrigation water. After 24 h, rainfall simulations were initiated for 70 min in the tillage (mean = 54.7mm) and no-tillage (mean = 52.8 mm) plots. Water samples from down-slope runoff were collected for every 5-min and composited for microbial analysis in time intervals 0-15, 20-25, 30-25, 40-45, 50-55, and 60-70 min. *E. coli* and *Salmonella* were enumerated. **Results:** Mean total runoff volume from no-tillage plots was approx. 150 L less than tillage plots. Broiler litter was the major source of *E. coli* (approx. 99%, based on soil density calculations) in the runoff water. Recovery of *Salmonella* was 4 $\log_{10}$ higher in the runoff water than *E. coli*. Significant differences between treatments were observed for specific time intervals (5-15 and 50-55 min sample intervals); however, no difference in the total load of *E. coli* and *Salmonella* was observed between treatments. In conclusion, tilled plots yielded more total runoff than untilled plots, but treatments did not differ in total loads of *E. coli* and *Salmonella*.

b) ***E. coli O157:H7 survival in soils with tilled versus untilled soils:*** A 2010 replicated, controlled, experimental study (2), focused on lettuce cultivated in the field in British Columbia, and Nova Scotia, Canada, found that the survival of *E. coli* O157:H7 on romaine lettuce and soil was not influenced by tillage practices. **Methods:** At each experiment station, field experiments were performed in 4 X 4 m plots (n=9). Control plots (n=3) received chemical fertilizer and treatment plots (n=6) received liquid dairy manure (60 kg N/acre). The 6 treatment plots were sub-grouped as "tilled" (n=3 and turned approx. 20cm) and "no-till" (n=3). Lettuce seedlings were transplanted by hand. After 4-weeks, plots were inoculated with *E. coli* O157:H7 isolate ATCC 700728 (10^6 CFU/ml, 100 ml/plant). Plants were irrigated via drip tape (Summerland location) and an aerial spray system (Kentville location). Lettuce heads (n=3) and composite soil (n=3) samples were later collected from each plot and *E. coli* O157:H7 isolate ATCC 700728 levels were enumerated (via a culture-based method). **Results:** *E. coli* was recovered from lettuce heads in the control plots at the Summerland (n=2) and Kentville (n=4) locations. In both experimental groups (tilled and no-till), *E. coli* in lettuce and soil declined sharply until day 7 (from 10^5 to 10^2 CFU/g on leaves and from 10^5 to 10^3 CFU/g in soil), with even lower concentrations (< log1 CFU/g) at day 21. No significant differences were observed between tilled vs. no-till soil.

c) ***Prevalence of fecal indicators and Salmonella in simulated runoff from tilled versus untilled systems:*** A 2-year replicated, controlled, experimental study (3) conducted between 2004 and 2005 in a cotton/corn field in Georgia, USA, found no difference in flow-weighed concentrations of fecal indicators when comparing levels in run-off collected from tilled versus no-tillage systems. **Methods:** Plots were cultivated under a split-plot design, each including one of the following soil treatments: conventional fertilizer with either tilled or no-till plots and poultry litter in either tilled or no-till plots. Amendments were added 2-days before planting the crops. From 1992 to 1994 fields were used to grow corn and fertilized with conventional fertilizer 168 Kg N/ha; from 1996 to 2000 (cotton) and 2000 to 2005 (corn) the fields were fertilized with 11.2 Mg/ha of poultry litter and 168 Kg N/ha of conventional fertilizer. In 2003, the fertilizer amounts were doubled (22.4 Mg/ha of poultry litter and 168 Kg N/ha of conventional fertilizers). From 1995 to 2005, a winter cover crop (rye) was added to all plots and terminated by the addition of glyphosate (2.3 l ha^{-1}). Crop residues were incorporated only in the tilled fields and not in the no-till fields, where crop residues were left on the surface. The simulations applied constant rain intensity in 2004 and variable rain intensity in 2005. The runoff and drainage water samples were collected and fecal *E. coli*, fecal enterococci, *Salmonella*, *Campylobacter*, and *C. perfringens* levels were enumerated. **Results:** Runoff volume was not different for the variable intensity rainfall simulation, but was greater in conventional tillage (176.3 l) than in no-till (35.6 l) for the constant intensity rainfall simulation. Flow weighted concentration ranges were: fecal *E. coli* (-0.3 to 3.7 $\log_{10}$ MPN 100 ml^{-1}), fecal enterococci (2.3 to 5.5 $\log_{10}$ MPN 100 ml^{-1}), and *C. perfringens* (2.7 to 3.1 CFU l^{-1}). *Salmonella* was negative in all the samples. No differences were observed between tillage treatments in flow-weighted

concentrations of fecal enterococci, *E. coli*, and *C. perfringens* for either of the rainfall simulations.

d) ***Generic E. coli and L. innocua survival in fields with tillage:*** A 3-year replicated, controlled, experimental study (4) conducted between 2013 to 2015 in a mixed vegetable field in Maryland, USA, found that in organic fields tillage negatively impacted *L. innocua* populations but found no significant differences in the persistence of *E. coli* based on samples collected before or after tillage. **Methods:** Field trials were conducted from 2013 to 2015 in a certified organic field (mixed-vegetable production, loamy sand soil, 0-5% slope, and within 40m of a woodland conservation buffer) and from 2014 to 2015 in a transitional field (corn/soybean, previously conventional, loamy sand soil, 0-2% slope, and surrounded by other fields). Field experiments (randomized complete block design) had 4 replicates in year-1 (organic only) and 5 replicates in year-2 (organic and transitional). Both fields had low organic matter (<1%) and measured 107x27 m (~0.3 ha). Selected cover crops were sown into the fields [Oct, 2013 (year-1) and Sep 2014 (Year-2)] and irrigated via overhead irrigation. After seeding, deer fencing was installed. Cover crops were tilled (moldboard plow) into the soil (flowering stage) to a depth of 15-20 cm during the month of May, 2014 and 2015. Control plots (bare ground) remained fallow throughout the fall, winter, and spring seasons. Cover crop treatments included hairy vetch (*V. villosa* Roth) at 18.14 kg/ha, crimson clover (*Trifolium incarnatum*) at 9.07 kg/ha, cereal rye (*Secale cereale* L.) at 31.75 kg/ha, a 2:3 (wt/wt) mixture of hairy vetch (9.07 kg/ha) and rye (13.60 kg/ha), and a 2:1 (wt/wt) mixture of crimson clover (9.07 kg/ha) and rye (4.54 kg/ha). A cocktail of nonpathogenic bacterial strains was sprayed onto the field 6 and 8 days after seeding in years 1 and 2, respectively. The inoculum strains included an *E. coli* isolated from a dairy farm in Clarksville, MD and *Listeria innocua* ATCC 33090. The inoculum concentrations were ~10^6 CFU/ml in year-1 and 10^4 CFU/ml (*E. coli*) and 10^3 CFU/ml (*L. innocua*) in year-2. Composite soils samples were collected [N=56 (year-1) and N=65 (year-2)] prior to field inoculation and 2 hours after inoculation. Sampling continued every 2-weeks post inoculation (until frost), monthly thereafter (until tillage) and then biweekly for 4-weeks (after tillage). *E. coli* and *L. innocua* levels in samples were enumerated. **Results:** All soil samples collected prior to tillage were negative for *E. coli* (except year-1 organic plots with mean of 0.57 log MPN/g of soil). Following tillage, *E. coli* levels significantly (P<0.001, year-2) increased in the organic treatment plots and but a very weak effect (P=0.117) was detected within the transition plots. All soil samples collected prior to tillage were positive for *L. innocua*, with mean values that ranged from 0.22 to 4.61 log MPN/g of soil. *L. innocua* populations decreased in the organic field (~1 log MPN/g) after tillage for in both the years but, and recovered to pre-tillage levels within 4 weeks post-tillage (P<0.05). In the transition plots, post-tillage *L. innocua* levels were significantly higher than pre-tillage levels (P<0.001).

e) ***Salmonella and Listeria prevalence on farms and timing of tillage:*** A 2012 replicated, observational study (5) conducted in 2012 at farms (N=21) in western, central, and eastern

New York, USA, found that timing of soil tillage was a significant factor that influences the prevalences of *Salmonella* and *Listeria monocytogenes* in soil samples. **Methods:** A questionnaire was mailed to selected farms in western (N=5), central (N=12), and eastern (N=4) New York and farm visits were performed over a 5-week period. At each sampling visit, one composite soil sample, and one drag swab were collected from each field, while irrigation water samples (N=23), and non-irrigation water samples (N=51) located within 50 m were sampled opportunistically. All samples (soil = 263 soil, drag swab = 263 drag swab, and water= 74 water samples water samples) were tested via an enrichment method for Salmonella, and Listeria monocytogenes. **Results:** *Salmonella* and *L. monocytogenes* were detected in 6.1% (16/263) and 17.5% (46/263) of fields sampled, respectively. Prevalence of *Salmonella* and *L. monocytogenes* in terrestrial samples (soil N=263 and swab samples N=263) samples were 3.4% (18/526) and 9.7% (51/526), respectively. Fields where soil was cultivated within 7 days prior to sample collection were approximately 6 times more likely (OR = 6.3; 95% CI = 1.6, 23.0) to be *Salmonella* positive than fields where soil was not cultivated for at least 30 days. Fields where soil was cultivated within 7 days prior to sample collection were approximately 8 times more likely (OR = 8.1; 95% CI = 3.3, 19.6) to be *L. monocytogenes* positive than fields where soil was not cultivated for at least 30 days.

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1.6. Grow low risk crop types (1 study): One study (1) from USA found that type of plant grown is an important factor influencing the survival of *E. coli* O157:H7 both on the produce and in the soil where the produce is grown.

a) ***E. coli O157:H7 prevalence in soil under two produce type:*** A replicated, controlled, experimental study (1) conducted in 2004 in Georgia, USA, found that *E. coli* O157:H7 survived longer in soil during the production of carrots than green onions. **Methods:** Composted cow manure (Black Gold Company, Florida) was mixed (ratio of 1:100) with 5-strains of *E. coli* O157:H7 (E0143, C7927, K262, C0083, and E0139) that carried green fluorescence protein and ampicillin resistance, to achieve an initial inoculum of approx. 10^7 CFU/g. Five parts of Tifton sandy-loam soil (the Coastal Plain Experiment Station, Tifton, GA, USA) was mixed with one part of contaminated compost and left at room temperature for 24h. Approx. 5 kg of inoculated and compost amended soil was held in twenty pots (250 x 200 mm) for growing each vegetable (baby carrot and green onions). Healthy transplants were planted into each pot (N=3/pot) and irrigated every 48 hours with municipal water. The pots were placed in a temperature-controlled chamber at 20 ℃ (with light 960 mmol/m^2/s). Samples (soil and edible root) collected on predetermined dates, in triplicate, were enumerated for *E. coli* O157:H7. **Results:** *E. coli* O157:H7 persisted on carrots at considerably high numbers (approx. 10^3 to 10^4 CFU/g) and for a longer duration (>10 weeks) than on onions (approx. 10^3 CFU/g for 1-week and 10^2 to <10 CFU/g for 7-week). Over 64 days, *E. coli* O157:H7 levels declined by 3 log CFU/g in soil from onion pots and by >2 log CFU/g on onions but only decreased over 84 days by 2.3 log CFU/g reduction in soil from carrot pots and a 1.7 log CFU/g reduction on carrots.

Reference:

1. Islam M, Morgan J, Doyle MP, & Jiang XP (2004) Fate of *Escherichia coli* O157:H7 in manure compost-amended soil and on carrots and onions grown in an environmentally controlled growth chamber. *Journal of Food Protection* 67(3):574-578.

1.7. Add mulch as a ground cover (2 studies): Two replicated, experimental studies (1, 2) from the USA found that the type of mulch used in produce farms may impact the prevalence of fecal indicator bacteria (FIB) and generic *E. coli* both on the produce and in the soil, in at least some of the statistical tests conducted. Of the 2 studies, one study reported that the prevalence of generic *E. coli* in cucumber fields was not influenced by the mulch type. The other study compared various mulch types used in lettuce production and reported that mulch type may influence the fate of enteric bacteria on lettuce.

a) ***Prevalence of E. coli in plastic mulch, straw mulch, and bare ground production systems:*** A 2-year replicated, controlled, experimental study (1) conducted between 2014 and 2015 in a cucumber field in Maryland, USA, found that *E. coli* prevalence was not different between plastic mulch, straw mulch, and bare ground systems. **Methods:** Using a randomized complete block design, 4 replicated raised beds were assigned to one of four treatments: plastic mulch, straw mulch, bare ground, or trellis (wooden frame 1.8X1.8 m) on bare ground. Each bed (1.8X0.75 m) contained 6 transplanted cucumber plants, 0.3m apart. Raw dairy manure (3.8 l/plot) was added 2 days prior to installation of plastic, straw mulch, and trellises. Plants were drip irrigated. Over 5 sample days, 4 samples each of cucumber fruit, flowers, leaves, and soil were collected and tested for *E. coli*, fecal coliforms, *Enterococcus* and *Salmonella* using culture-based methods. Presumptive *E. coli* colonies were genotyped by BOX-PCR. **Results:** All samples tested negative for *Salmonella* across all 4 treatment plots. Treatment did not appear to affect levels of *E. coli* in soil, leaves, flowers, or fruit. Overall, *E. coli* was infrequently detected in the soil (1 of 32 positive in Year 1; 12 of 32 positive in Year 2; mean of 0.7 log CFU/gram), leaves (3 of 16 samples positive on one sampling day in 2015; mean of 0.5 log CFU/g), flowers (always at lower limit of detection), and fruit (7 of 16 samples positive on one sampling day in 2015; mean of 0.6 log CFU/cucumber). Cluster analysis suggested that the source of *E. coli* may have been straw mulch rather than the soil. Production system was a factor for fecal coliforms (mean = 6.3 log CFU/cucumber) and enterococci (mean = 5.7 log CFU/cucumber) in cucumbers; specifically, cucumbers grown in bare ground had significantly lower coliform and enterococci ($P < 0.05$). However, in soils, bare ground supported significantly ($P<0.001$) higher levels of fecal coliforms (5.4 log CFU/g) than straw beds (3.8 log CFU/g). Flower samples had no significant differences in fecal coliform (6.3 log CFU/g) or enterococci (5.0 log CFU/g) levels among production systems and between sampling times. Overall, contaminated soil/mulch increased the risk of crop contamination, but crop production systems (bare ground vs mulch) seemed to play a limited role in *E. coli* transmission.

b) ***Fecal coliforms, generic E. coli, and Enterococcus spp. prevalence in lettuce that used mulches as ground cover:*** A 2-year replicated, controlled, experimental study (2) from 2013 to 2014 at farms in the Wye Research and Education Center, Maryland, USA, found that mulches used in lettuce production may impact the fate of enteric bacteria on lettuce. **Methods:** Five mulch treatment were studied: 1) bare ground (no mulch control), 2) black

polyethylene plastic mulch, 3) biodegradable (corn-based) plastic mulch, 4) paper mulch, and 5) straw mulch. Plots were arranged in a random complete block design that was drip irrigated with well water and fertilized with chicken manure pellets (3360 kg/ha). During the fall and spring season (both years) three-week-old lettuce was transplanted to the fields. Post transplantation (8-weeks later) lettuce was contaminated by overhead irrigation, with 100ml of contaminated water from a secondary lagoon receiving liquid dairy manure. Lettuce and soil samples were collected at day-0 (before and 30-min after inoculation), and thereafter at 1, 3, 5, 7, 10, and 14 days post inoculation. All samples were enumerated for generic *E. coli*, fecal coliforms and *Enterococcus* spp. **Results:** In lagoon water for generic *E. coli* levels ranged between 4.2 log to 7.7 log CFU/ml, fecal coliforms ranged between 3.4 to 7.7 log CFU/ml, and *Enterococcus* levels ranged between 1.0 to 3.2 log CFU/ml, during the course of the study. Before inoculation, 3.2% (2/63), 41% (26/63) and 1.6% (1/63) samples were positive for fecal coliforms, *Enterococcus* and *E. coli*, respectively. Bacterial counts on lettuce appeared to decrease over time and exhibited similar descending trends in all lettuce samples collected during the same season. In spring 2014, *E. coli* declined faster on bare ground-grown lettuce compared to mulch-grown lettuce ($p=0.005$), and in 2014 fall, *E. coli* levels decreased faster on paper mulch-grown lettuce compared to lettuce grown or using other mulches ($p=0.005$). Despite these findings in 2014, no significant differences in *E. coli* die-off rates were observed among mulch types, including bare-ground controls, in 2013 (spring $p=0.18$, and fall $p=0.89$).

References:

1. Micallef SA, Callahan MT, & Pagadala S (2016) Occurrence and Dispersal of Indicator Bacteria on Cucumbers Grown Horizontally or Vertically on Various Mulch Types. *Journal of Food Protection* 79(10):1663-1672.
2. Xu A, Buchanan RL, & Micallef SA (2016) Impact of mulches and growing season on indicator bacteria survival during lettuce cultivation. *Int J Food Microbiol* 224:28-39.

1.8. Grow crops using a trellis production system (1 study): A replicated experimental study (1) from the USA found that horizontal and vertical (trellis) production systems seemed to play a limited role in *E. coli* transmission to cucumbers.

a) ***Prevalence of E. coli in horizontal versus trellis production systems:*** A 2-year replicated, controlled, experimental study (1) conducted between 2014 and2015 in a cucumber field in Maryland, USA found that *E. coli* prevalence was not different between horizontal and vertical (trellis) production systems. **Methods:** Using a randomized complete block design, 4 replicated raised beds were assigned to one of four treatments: plastic mulch, straw mulch, bare ground, or trellis (wooden frame 1.8X1.8 m) on bare ground. Each bed (1.8X0.75 m) contained 6 transplanted cucumber plants, 0.3m apart. Raw dairy manure (3.8 l/plot) was added 2 days prior to installation of plastic, straw, and trellises. Plants were drip irrigated. Over5 sample days, 4 samples each of cucumber fruit, flowers, leaves, and soil were collected and tested for *E. coli*, fecal coliforms, *Enterococcus*, and *Salmonella* using culture-based detection methods. Presumptive *E. coli* colonies were genotyped by BOX-PCR. **Results:** All samples tested negative for *Salmonella* across all 4 treatment plots. Treatment did not appear to affect levels of *E. coli* in soil, leaves, flowers, or fruit. Overall, *E. coli* was infrequently detected in the soil (1 of 32 positive in Year 1; 12 of 32 positive in Year 2; mean of 0.7 log CFU/gram), leaves (3 of 16 positive on one sampling day in 2015; mean of 0.5 log CFU/g), flowers (always at lower limit of detection), and fruit (7 of 16 positive on one sampling day in 2015; mean of 0.6 log CFU/cucumber). Cluster analysis suggested that the source of *E. coli* may have been manure amended soil (49 isolates from flowers and soil shared 100% similarity). Overall, contaminated soil increased the risk of crop contamination, but the type of crop production system (horizontal versus trellised) seemed to only play a limited role in *E. coli* transmission.

Reference:

1. Micallef SA, Callahan MT, & Pagadala S (2016) Occurrence and Dispersal of Indicator Bacteria on Cucumbers Grown Horizontally or Vertically on Various Mulch Types. *Journal of Food Protection* 79(10):1663-1672.

2. Non-crop Vegetation Management:

2.1. Add treatment wetlands/Vegetative treatment areas (9 studies): Replicated, experimental studies from the USA (N=4) and Canada (N=1) (1-5) and four observational studies from the USA (4, 6-9) found that treatment wetlands/vegetative treatment areas were effective in reducing fecal indicator bacteria, fecal markers, generic *E. coli*, and pathogens (*E. coli* O157, *Listeria*, and *Salmonella*) in irrigation, runoff, and river water. Of the nine studies, one study found that generic *E. coli* concentrations increased in reservoirs, potentially due to wildlife occurrences (2) and cautioned against using reservoir as irrigation water for crops that are consumed raw.

a) ***Removal of fecal bacteria by a subsurface constructed wetland:*** A replicated, experimental, study (4) conducted between Dec 1995 and Feb 1997 in Arizona, USA found that a subsurface constructed wetland was effective at reducing bacterial load in secondary wastewater. Fecal coliform counts in the effluent exiting the wetland were below the recreational water quality standard (200 CFU/100ml) in 14 out of the 15 months the wetland was in operation. **Methods:** Serving a population of less than 11,000 people, a subsurface wetland was constructed, consisting of 3 wetland cells (each of 12.2 X 5.4 X I m). Wetland cells were lined with plastic polyvinyl chloride (0.08 cm mesh liner) and layered with gravel [14 cm fine (0.5-1.0 cm) and 28 cm coarse (7-10 cm)]. Wastewater from the Arboretum grounds was collected in a settling septic tank before being pumped into the wetlands. From Dec 1995 to Feb 1996, wastewater flowed through the first cell. From Mar-1996 to Feb-1997, wastewater flowed through cells 1-3 sequentially. To mimic natural wetlands, native emergent wetland species (N=16) were randomly planted into the gravel of each cell. Water samples were collected monthly (total months N=15) from the transfer boxes at the input to wetland and from the exit boxes of cells 1-3 (total no of sampling sites N=4). Fecal coliforms levels in water samples were enumerated. **Results:** The mean fecal coliforms count ranged from 3.24 to 6.49 in the influent but ranged from 0.60 to 4.64, 0.30 to 3.89, and 0.30 to 3.24 log CFU/100ml in output cells 1, 2 and 3, respectively. The lower levels of fecal coliforms in the effluent compared to influents suggests that the wetland was effective at treating wastewater and did significantly (P<0.0001) reduce (>99%) fecal coliform counts. The fecal coliform levels in the wetland effluent was below the recreational water quality standard (<200 CFU/100ml) in all months except July 1996.

b) ***Fecal indicator bacteria and microbial source tracking marker removal from creek water by a constructed wetland:*** A one year replicated experimental study (5) conducted in Kentucky, USA found little evidence that constructed wetlands were an effective means for removing microbial contamination in surface water. **Methods:** Creek water was pumped into a wetland (12-acre) using variable-frequency pumps. Pumps were programmed to withdraw water only when creek flow was between 1.5 and 31 ft^3/s with graduated pumping rates of 0.5 to 7 ft^3/s. Creek water was then stored in an equalization

basin (0.7 acre) prior to entering wetland treatment channels (3 acres) via gravity fed perforated pipes. The banks of the treatment channels were under vegetative cover, including native and invasive graminoids. Water from the treatment channel was gravity fed to an outlet basin (0.25 acre) before being discharged back to the creek downstream of the intake pump. Water samples were collected weekly between June and September 2017 at 5 locations (creek intake pump, equalization basin outlet, 2 locations along the treatment channel, and the outlet discharge). Levels of two fecal indicators, *E. coli* and *Enterococcus* were enumerated in the water samples. Separately, microbial source tracking markers (for human and avian fecal contamination, and fecal indicator bacteria markers for *E. coli* and Enterococci were quantified using qPCR. **Results:** *E. coli* (both PCR and culture-based) concentrations were not significantly different throughout the various stages of the wetland treatment. Specifically, 100% of samples were positive for *E. coli* according to membrane filtration with average concentrations ranging from 2.37 ± 0.61 to 2.91 ± 0.63 $\log_{10}$ CFU/100ml, while 92% of samples were *E. coli* positive according to qPCR with average concentrations ranging from 3.07 ± 0.55 to 3.53 ± 0.47 $\log_{10}$ gene copies/100 mL. Enterococci levels decreased significantly (−0.611 $\log_{10}$ CFU/100 mL, P = 0.0320, and −0.560 $\log_{10}$ gene copies/100 mL, P = 0.0284, respectively) between the creek inlet and equalization basin. However, culture-based enterococci concentrations increased significantly between the equalization basin outlet and final outlet pipe (+0.909 $\log_{10}$ CFU/100 mL, P = 0.0003). Human fecal marker (HF183) detection frequency was significantly (P value 0.0003) higher at the creek intake compared to downstream wetland sites, with HF183 only being detected at one downstream site (the equalization basin). Avian marker (GFD) levels were significantly lower at the intake (P=0.0033, with mean difference of -1.75 $\log_{10}$ gene copies/100ml) and equalization basin (P=0.0003, with mean difference of -2.08 $\log_{10}$ gene copies/1000 ml) compared to the outlet. Finally, passing water through the treatment wetland appeared ineffective at reducing levels of microbial contamination since, although there was a reduction in microbial load between inlet and equalization basin, microbial concentrations either remained level or increased between inlet and outlet. As concluded by the authors, microbial regrowth within the wetland system, and potentially additional microbial inputs from wildlife, may have contributed to the elevated microbial loads observed in later stages of wetland treatment.

c) ***Generic E. coli removal by wetlands form run-off flowing off a flood-irrigated pasture:***
A 2-year replicated experimental study (3) conducted between 2004 and 2005 in a flood-irrigated agricultural landscape in Northern California, USA, found that retention of generic *E. coli* was greater in an intact, flow-through broad-basin wetland than a degraded, channelized flow-through wetland. **Methods:** This study was conducted in two natural flow-through wetlands located on the western slope of the Sierra Nevada foothills (elevation ~350m). With a surface area of ~0.2 ha, each of the wetlands was situated at the base of s small drainage basin that collected runoff water from 6.1 ha (reference) and 9.3 ha (channelized) flood irrigated pastures. The reference wetland was composed of a broad-

basin that had no distinct down-cut or entrenched region for the length of the wetland. The channelized wetland was composed of a primary channel (1-3 m wide and 30-70 cm lower than surrounding topography) that transported most of the irrigation runoff water. Wetland water depths ranged from 0 to 60 cm. Dominant vegetation was composed of cattails and other emergent graminoid species. Irrigated pastures (upslope of wetlands) were rotationally grazed by beef cattle (3.1 to 4.5 animal unit months per ha); however, grazing was permanently excluded from both wetlands > 1 month before the first irrigation trial (May 2004). Water samples from 11 irrigation events (N=11) were collected at 15 to 30 min intervals, upstream (inflow) and downstream (outflow) from the wetlands (total samples reference wetlands N=195 and channelized wetlands N=320). Generic *E. coli* levels in water samples were enumerated. **Results:** Generic *E. coli* concentrations in runoff water from irrigated pastures (wetland inflow) were greater in the channelized wetland (median = 10,000 CFU/100 ml) compared to the reference wetland (median = 5,400CFU/100 ml). At both wetlands (reference and channelized), effluent *E. coli* concentrations were 67% and 94% lower than *E. coli* concentrations in the wetland influent, during high and low flow irrigation event, respectively. During irrigation events, reference wetlands significantly (P=0.003) decreased loads of *E. coli* by 68% whereas channelized wetland decreased *E. coli* loads by 25%. Presence of cattle grazing in pasture during one irrigation event resulted in significantly (P<0.001) greater *E. coli* in runoff water compared to irrigation events when cattle were absent.

d) ***Reduction by a constructed wetland of generic E. coli levels in run-off from tile drained produce fields:*** A 2-year replicated experimental study (2) was conducted between 2007 and 2008 in Nova Scotia, Canada, and found that a wetland-reservoir irrigation management system was effective in reducing generic *E. coli* concentrations from tile-drainage runoff. **Methods:** A 5 hectare produce field was subsurface tile drained and runoff water was allowed to flow through an outflow monitoring facility. The field was planted with soybeans in 2007 and barley in 2008. Liquid dairy manure was applied to the field at a rate of 20 t ha^{-1} (2007) and 70 t ha^{-1} (2008). Downstream from the produce fields, 2-wetland cells (identical and side-by-side) were constructed, each 512 m^2. Cattail (*Typha* spp.) were transplanted from a natural wetland and other species established naturally by Aug 2007. Tile drainage run off from the field was conveyed to and equally distributed between wetland cells. Effluent from each cell was discharged to a water level control structure and then flowed into a reservoir (0.3 ha surface area and 4.5m depth). For the study period (Nov 2007 through Dec 2008), water samples were manually collected from the sampling ports in the wetland inlets (N=231), outlets (N=301), and the reservoir outlet (N=64). Generic *E. coli* levels in the water samples were enumerated. **Results:** Generic *E. coli* concentrations ranged from 0-3200, 0-1160, and 0-2700 CFU/100ml at the wetland inlet, outlet, and reservoir outlet, respectively, with mean values of 122, 42, and 178 CFU/100ml, respectively. On average *E. coli* reductions between the wetland inlet and outlet were 63.3%. Wetland treatment performance was positively correlated (r=0.51,

$p<0.05$) with inflow rate (*i.e.*, higher flows improved the wetland treatment reductions of generic *E. coli* loads). Despite this, generic *E. coli* concentrations increased in the reservoir, potentially due to the occurrence of wildlife in the warm season (e.g., waterfowl), leading the authors to caution against using the reservoir as irrigation water for crops that are consumed raw.

e) **Removal of *fecal markers from treated wastewater by constructed wetland:*** A 2013 observational study (7) conducted in Arizona, USA found that a constructed wetland was effective in reducing fecal indicator bacteria levels in treated wastewater. **Methods:** The studied wetland, which had been in operation for 20 years, provided additional treatment for treated municipal wastewater and backwash from mixed media filters. The wetland was approx. 0.03 km^2 in size with a retention time of 7 days. Predominant vegetation included cattails, bulrushes, cottonwood, and willow. Water from the wetland was used to support turf (e.g., golf courses, parks, schools). A total of 24 water samples were collected across 3 sampling sites (inlet, intermediate, and outlet) monthly between May and December 2013. Total DNA was extracted from the samples and used to quantify molecular marker levels for *E. coli*, Enterococcus, and HF183 (a marker of human fecal contamination) using qPCR. **Results:** The overall detection rates for *E. coli*, Enterococcus, and HF183 were 100%, 62.5%, and 95.8%, respectively, with concentration ranging between 0.68 to 1.89, 0.81 to 3.88, and 1.80 to 4.22 log_{10} gene copies/100ml. The overall removal rates (when levels in the inlet and outlet samples were compared) were 6.7%, 84.0% and 66.6% for *E. coli*, Enterococci and HF183, respectively.

f) ***Removal of fecal bacteria from agricultural run-off and river water by constructed wetland systems:*** A 4-year observational study (6) was conducted between 2001 and 2004 at the Imperial and Brawley wetland systems, California, USA, using agricultural runoff water and river water, respectively. In both systems, wetlands were effective in reducing fecal coliforms and generic *E. coli* levels in runoff and river water. **Methods:** The selected wetland systems consisted of unvegetated sedimentation basins (2m deep) followed by vegetated wetland cells. The Imperial system consisted of 2-ha sediment basins in parallel, followed by four wetland cells in series (total area= 4.7 ha). Agricultural water collected in canals were controlled and diverted to the wetland system (flow=13,000–21,000m^3/day) with detention times on the order of five days in the sedimentation basins, and four days in the wetland cells. The Brawley system consisted of a 5900m^2 unvegetated sediment basin, followed by two wetland cells in series (total area= 1.8 ha). Water was pumped from the New River to the Brawley system (flow=1900–2600m^3/day) with detention times on the order of five days in the sedimentation basin, and nine days in the wetland cells. In both systems wetland cells had different bathymetric and vegetation patterns, although bulrushes were present in both systems. Water samples were collected biweekly at the system inlets and outlets. Fecal coliform and generic *E. coli* levels in water samples were enumerated. **Results:** In the Imperial system, annual mean values for the inlets were 5,157 (fecal coliforms) and 1,123 (generic *E. coli*) CFU/100ml, and for the outlets were 101 (fecal

coliforms) and 75 (generic *E. coli*) CFU/100ml. The bacterial reductions were 1.71 and 1.17 $\log_{10}$ for fecal coliforms and generic *E. coli*, respectively. In the Brawley system, annual mean values for the inlets were 88,688 (fecal coliforms) and 50,148 (generic *E. coli*) CFU/100ml, and for the outlets were 99 (fecal coliforms) and 41 (generic *E. coli*) CFU/100ml. The bacterial reductions were 2.75 and 3.13 $\log_{10}$ for fecal coliforms and generic *E. coli*, respectively. Across all 4-years, fecal coliforms and generic *E. coli* were reduced by an average of 1.5 $\log_{10}$ CFU/100ml in the Imperial system, compared to 2.7 $\log_{10}$ CFU/100ml in the Brawley system.

g) ***Generic E. coli prevalence in vegetated treatment areas receiving swine farm runoff water:*** A 4-year replicated, controlled, experimental study (1) was conducted between Jan, 2013 and Dec, 2016 at small-scale swine operations in Texas, USA. Vegetative treatment areas (VTAs) planted with perennial grasses reduced generic *E. coli* concentrations in runoff water originating from swine operations; however, the design and management of VTAs had a major impact on reductions in *E. coli* concentrations. **Methods:** Three small swine feeding operations in Bell, Brazos, and Robertson Counties, Texas were enrolled, and 3 sampling sites at each location were installed to monitor runoff water quality at the system inlet, outlet, and control site. VTAs were managed by hay removal and over-seeding with a cool-season grass (wheat or oats) in the fall. Bell County: This swine operation consisted of 0.15 ha of barn and outdoor pens, with ~30-100 swine. Runoff from barn and pens was drained via pipe to the VTA (0.34 ha coastal Bermuda grass, overseeded with oats or wheat in winter) that was isolated from surrounding fields with earthen berms. 0.48 ha of un-grazed pasture and a garden area above the pens (drained through a grassed waterway) were used as control. Brazos County: This operation consisted of 0.03 ha of barn and outdoor pens, with 20-35 sows. Runoff and drainage water from the pens entered the 0.10 ha VTA (native prairie grass, over-seeded with oats in winter). The control site was a rural residential area (1.21 ha) with a few animal pens that drained through a culvert. Robertson County: This operation consisted of a 0.03 ha outdoor walking pen and barn that contained 5-20 animals. Wash water and runoff from the swine facility were directed to the inlet of the VTA. Adjacent to the barn, an un-grazed native prairie, was sectioned into two: the first section (0.11 ha) was converted to a VTA (over-seeded with oats in the winter) and the second (0.16 ha) section as control. At all 3 locations, runoff into the VTA and outlet were routed through a H-flume. At each operation, water samples were collected at 0.5-1.5mm volumetric depth flow intervals and E. coli levels were enumerated. Over the course of 4-years: 43, 108, and 40 runoff events occurred at the Bell, Brazos, and the Robertson VTAs, respectively and water quality data were successfully collected at the inlet, outlet and control sites from: Bell (37, 28, and 41), Brazos (105, 65, and 47) and Robertson (35, 27, and 20) runoff events, respectively. **Results:** Median *E. coli* concentrations in the runoff events were: (1) Bell county: inlet (N=37, $5.0X10^6$ CFU/100ml), outlet (N=28, $3.35X10^5$ CFU/100ml), and control (N=41, $7.20X10^3$ CFU/100ml), (2) Brazos county: inlet (N=105, $2.20X10^7$ CFU/100ml), outlet (N=65,

$2.80X10^6$ CFU/100ml), and control (N=47, $6.80X10^3$ CFU/100ml), and (3) Robertson county: inlet (N= 35, $1.41X10^4$ CFU/100ml), outlet (N=27, $1.40X10^4$ CFU/100ml) and control (N=20, $3.45X10^3$ CFU/100ml). With a treatment efficiency of 94%, the Bell VTA (VTA area/source area = 2.3, VTA area /animal = 0.007) significantly ($P<0.05$) reduced median *E. coli* concentrations by $2.81X10^{12}$ CFU/ha so that reductions in *E. coli* concentrations were not significantly different from the control site. With a treatment efficiency of 29%, the Robertson VTA (VTA area/source area = 3.7, VTA area/animal = 0.014) did not significantly reduce *E. coli* concentrations ($4.1X10^9$ CFU/ha), when compared between inlet and outlet. However, the concentrations leaving the VTA were very low (1-2 times less than Bell and Brazos). One explanation for low amounts of *E. coli* entering the VTA may be that the Robertson location had enclosed pens and an alternative solid waste management procedure. Finally, although the Brazos VTA (VTA area/source area = 3.3, VTA area/animal = 0.004) had a treatment efficiency of 93%, and significantly reduced ($P<0.05$) *E. coli* concentrations to $2.59X10^{13}$ CFU/ha, the runoff leaving the VTA had much higher concentrations than the control site. The authors suggest that increased pen washing produced very *E. coli* high loads in the runoff water that exceeded the treatment capacity.

h) ***Fecal indicator removal by vegetated filter strips:*** A one-year observational study (9) conducted in Kansas, USA found that vegetative filter strips were effective in reducing concentrations of fecal coliforms, fecal *streptococci*, and generic *E. coli* in runoff from cattle feedlots during unstocked conditions. **Methods:** Four vegetative filter strips (VFS) used to control storm runoff from commercial feedlots were selected as sampling sites in the present study. Site A (350-head beef operation) was a 0.28 ha, 2-year-old strip with an average slope of 0.8%; Site-B (300-head beef operation) was a 1.25 ha, 5.5-year-old strip with an average slope of 1.25%; Site C (300-head beef operation) was a 2.7ha, 4-years old strip with an average slope of 2%; and Site D (200- head beef operation) was a 0.85 ha, 2.5-years old strip with an average slope of 0.6% and had a gated irrigation pipe to spread water evenly over the width of the VFS. All 4-sites were planted with brome (*Bromus inermis*) although, site C was planted with both fescue (*Festuca arundinacea*) and brome. In general, runoff from the feedlot was contained within the feedlot (acting as sedimentation basin) and a pipe (PVC), which delivered the runoff to the top of each VFS. Between May and November 1998, automatic samplers (samples were collected every 30 mins and removed after 24 hours) collected runoff during rain events (total N=22 feedlot runoffs) at the VFS inlets and outlets. Fecal indicator levels were enumerated in each sample using a culture-based method. **Results:** Mean fecal coliform concentration reductions at sites A, B, C, and D were 60.5%, 94.0%, 71.1%, and 96.7%, respectively. Mean *E. coli* concentration reductions at sites A, B, C, and D were 67.5%, 94.5%, 77.0%, and 96.0%, respectively. Mean fecal streptococci concentration reductions at sites A, B, C, and D were 100.0%, 100.0%, 70.8%, and 100.0%, respectively. Measured reductions (between the VFS inlet and outlet) in concentrations averaged 77%, 83%, and 83% for

fecal coliforms, generic *E. coli* and fecal streptococci, respectively. When cattle were not present, runoff from cattle feedlots, had an average concentration of one-fortieth the fecal coliform concentrations observed when cattle were present.

i) ***Generic E. coli and E. coli O157:H7 removal from dairy wastewater by constructed wetlands:*** A replicated observational study (8) conducted in California, USA found that a constructed wetland was an effective means to treat dairy wastewater. Generic *E. coli* and *E. coli* O157:H7 levels in effluents exiting the wetland showed evidence of approx. 2 log reductions in both targets when effluent was compared to influent. **Methods:** Wastewater from a dairy milking parlor was drained into a raw water lagoon. Two wetlands with parallel operation but different loading designs were used to treat the wastewater: wetland-1 had an end-loading design, and wetland-2 had a side-loading design. At both the wetlands, wastewater treatment occurred beneath the surface, which was gravel planted with reeds (*Phragmites communus*) and bulrush (*Scirpus validus*). Treated water was drained into a collection box at the end of the basin. Each month 6 samples were collected from 6 sites in: the raw wastewater, the facultative pond, each wetland inlet and each outlet. Generic *E. coli*, and *E. coli* O157 levels in each sample were enumerated. **Results:** Concentrations (CFU/ml) of generic *E. coli* ranged between $5.0X10^3$ to $3.9X10^7$ (raw waste water), $1.2X10^3$ to $5.6X10^4$ (pond), $2.4X10^3$ to $1.7X10^6$ (influents) and $3.1X10^1$ to $1.0X10^3$ (outlets). Concentrations (CFU/ml) of *E. coli* O157:H7 ranged between $1.0X10^2$ to $3.8X10^6$ (raw), $4.2X10^2$ to $8.9\ X10^2$ (pond), $1.2X10^3$ to $6.3X10^3$ (influents) and $4.8X10^1$ to $8.9X10^2$ (outlets). Overall, generic *E. coli* and *E. coli* O157:H7 in the wastewater were reduced 2 to 3 log between the raw wastewater and wetland outlet.

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2.2. Add hedgerows or vegetative buffer strips (3 studies): Three observational studies from the USA (1-3) evaluated the effects of hedgerows, adjacent non-crop vegetation, or vegetated buffer zones on likelihood of pathogens being detected in produce fields. Of the three studies, two studies reported that the presence of non-crop vegetation may improve food safety, while the third study found no difference in the prevalence of pathogens in fields managed with restored hedgerows versus conventionally managed field edges.

a) ***Generic E. coli, EHEC, and Salmonella prevalence in produce farms that removed non-crop vegetation:*** A one year observational study (1) conducted in 2015 in the California Central Coast, USA found that removal or replacing non-crop vegetation with cropland increased enterohemorrhagic *E. coli* (EHEC) prevalence over time. **Methods:** EHEC which includes *E. coli* O157:H7 and STEC and *Salmonella* data were acquired from an organic farming operation, whih collected data between 2007 and 2013. A total of 482,208 produce samples originating from 295 farms spread across USA were tested for EHEC and *Salmonella* at the organic farming operation. Data on generic *E. coli* in irrigation water that was collected monthly between 2007 and 2010, was acquired from the leafy green agricultural industry. Finally, between Oct, 2009 and Aug, 2011, fecal samples were collected from 11 wild rodent species, trapped at 9 produce farms in California, USA. Fecal samples were enriched to detect the presence/absence of *Salmonella*. Data from microbial analysis and estimates of land cover maps (data reflect changes in landcover over the study period) were used in statistical models to understand the effect of land use change on the prevalence of pathogens in the produce fields. **Results:** Across the California Central Coast, prevalence of EHEC in fresh produce increased significantly (likelihood ratio test: n = 21 region-years/482,208 tests, X^2 = 16.8, P < 0.001) from 2007 to 2013. Simultaneously (2005-2012), growers in California Central Coast replaced non-crop vegetation with bare ground buffers such that, within 50 m of produce fields, there bare ground buffers increased by (30%), riparian (9%), woodland (2%), scrub (13%), grassland (11%), and marsh/meadow (30%) cover all decreased. This study (after analyzing ~ 250,000 tests for generic *E. coli* in water and EHEC in fresh produce) found that likelihood of pathogen detection was not higher in areas surrounded by non-grazed riparian or natural vegetation compared to grazeable land. EHEC was ~100 times more likely (~0.01% vs ~1%) to be detected near grazable land (60% grazable land within 1.5 km) when compared to far from grazable land and removing riparian vegetation did not decrease EHEC prevalence over time (n = 28, X^2= 0.07, P = 0.79). Contrastingly, when farms expanded areas under crop cover by removing natural vegetation, EHEC was more likely to be detected (n = 28 farms, X^2 = 4.22, P = 0.04). Changes in *Salmonella* prevalence in either fecal or produce samples was not associated with changes in cropland or non-riparian natural vegetation.

b) ***Salmonella, E. coli O157 and Non-O157 STEC prevalence in fields with and without hedgerows:*** A 2-year observational study (2) was conducted between 2013 and 2015 in the Sacramento Valley, California, USA to monitor the prevalence of enteric pathogens in

wildlife feces in walnut orchards and tomato fields with and without field-edge wildlife habitat (i.e., fields with restored hedgerows versus conventionally managed field edges). Overall, there was no difference in the prevalence of pathogens in wildlife feces collected from restored hedgerows and fields with conventionally managed field edges. **Methods:** Four walnut orchards and 5 tomato fields (each site approx. 32 ha) in the Sacramento Valley, California, USA were selected for this study. On each site, one side of the field had a hedgerow of native shrubs and perennial grasses that was approx. 7×448 m and 10 to 20-years old. The other 3-sides of the sites were conventionally managed by discing, mowing, and/or use of herbicides to control weeds. The field edge opposite to the hedgerow (min distance 400 m) was selected as the control. Wildlife activity was monitored seasonally in both walnut orchards (summer, autumn, winter, spring; July 2013 through May 2014) and tomato fields (spring and summer; May through July 2015) with Sherman live traps and video cameras. Fecal samples collected from the traps were enriched to detect *E. coli* O157, non-O157 STEC, and *Salmonella* (total fecal samples walnut orchard N=218, tomato fields N=259). **Results:** All samples were negative for *E. coli* O157. *Salmonella* was detected in 2 of the 125 samples collected from the hedgerow side of walnut orchards; and all other samples were *Salmonella* negative. Four samples were positive for Non-O157 STEC (2/125 in walnut hedgerows and 2/93 in walnut conventional field edges). Fecal samples collected from tomato fields were all negative for all 3 pathogens.

c) ***Salmonella and Listeria prevalence in fields with and without buffer zone:*** A 2012 observational study (3) conducted in Upstate New York, USA, found a higher prevalence of *Salmonella* and *Listeria monocytogenes* in fields with no buffer compared to fields with grassy buffer zones. **Methods:** A questionnaire was mailed to selected farms in western (N=5), central (N=12) and eastern (N=4) New York to learn about farming practices. Farm visits were performed over a 5-week period (June and July, 2012). At each visit composite soil sample and a drag swab samples were collected from each field, while irrigation water samples (N=23), and non-irrigation water samples (N=51) were collected if present within 50 m of a sampled field. All samples (soil =263, drag swab =263, and 74 water samples) were tested via enrichment to determine if Salmonella or Listeria monocytogenes was resent. **Results:** *Salmonella* and *L. monocytogenes* were detected in 6.1% (16/263) and 17.5% (46/263) of fields sampled, respectively. The prevalence of *Salmonella* and *L. monocytogenes* in terrestrial samples (total n=526 i.e., soil n=263 and swab n=263) samples was 3.4% (18/526) and 9.7% (51/526), respectively. The survey defined a 'buffer zone' as a >5m grassy strip where no produce was grown. The presence of a buffer zone had a protective effect and reduced the likelihood of *Salmonella* (OR=0.1; 95% CI = 0.03, 0.6) and *L. monocytogenes* (OR = 0.5; 95% CI = 0.2, 0.9) being detected in a field compared to fields without buffer zones.

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2.3. Add winter cover crops (2 studies): Two replicated, experimental studies from the USA (1, 2) found that pathogen (*E. coli* O157:H7 and *L. innocua*) survival was significantly lower in fields with cover crops, in at least some of the statistical tests conducted. Specifically, of the two studies, one study reported that effects of cover crop on *L. innocua* and generic *E. coli* survival were context dependent. The other study reported a significant reduction in *E. coli* O157:H7 populations in fields planted with cover crops.

a) ***Generic E. coli and L. innocua survival in fields added with cover crop:*** A 3-year replicated, controlled, experimental study (1) conducted between 2013 and 2015 in a mixed vegetable field in Maryland, USA, found that the effect of cover crops on the survival of generic *E. coli* and *L. innocua* in soil were context dependent. **Methods:** Field trials were conducted from 2013 to 2015 in a certified organic field (mixed-vegetable production, loamy sand soil, 0-5% slope), and within 40m of a woodland conservation buffer and from 2014 to 2015 in a transitional (i.e., shifting from conventional to organic production) field (corn/soybean, loamy sand soil, 0-2% slope, and surrounded by other fields). Field experiments (randomized complete block design) had 4 replicates in year-1 (organic only) and 5 replicates in year-2 (organic and transitional). Both fields had low organic matter (<1%) and measured 107X27 m (~0.3 ha). Selected cover crops were sown into the fields [Oct, 2013 (year-1) and Sep, 2014 (Year-2)] and irrigated via overhead irrigation. After seeding, deer fencing was installed. Cover crops were tilled (moldboard plow) into the soil (flowering stage) to a depth of 15-20 cm in 2014 and 2015. Control plots (bare ground) remained fallow throughout the fall, winter and spring seasons. Cover crop treatments included hairy vetch (*V. villosa* Roth) at 18.14 kg/ha, crimson clover (*Trifolium incarnatum*) at 9.07 kg/ha, cereal rye (*Secale cereale* L.) at 31.75 kg/ha, a 2:3 (wt/wt) mixture of hairy vetch (9.07 kg/ha) and rye (13.60 kg/ha), and a 2:1 (wt/wt) mixture of crimson clover (9.07 kg/ha) and rye (4.54 kg/ha). A cocktail of nonpathogenic bacterial strains was sprayed onto the field 6 and 8 days after seeding in years 1 and 2, respectively (*E. coli* isolated from a Maryland dairy farm and *Listeria innocua* ATCC 33090). The inoculum concentrations were ~10^6 CFU/ml in year-1 and 10^4 CFU/ml (*E. coli*) and 10^3 CFU/ml (*L. innocua*) in year-2. Composite soils samples were collected [N=56 (year-1) and N=65 (year-2)] prior to field inoculation and 2 hours after inoculation. Sampling continued every 2-weeks post inoculation (until frost), monthly thereafter (until tillage), and then biweekly for 4-weeks (after tillage). *E. coli* and *L. innocua* levels in soils were enumerated. **Results:** Soil samples collected prior to field inoculation were negative for *L. innocua* and *E. coli* (except 2 plots with mean of 0.4 log MPN/g). Post inoculation, mean *L. innocua* levels in soil were: 6.4 log MPN/g (Year-1) and 4.7 log MPN/g (Year-2) in the organic fields and 7.5 log MPN/g (Year-2) in the transitional fields. Similarly, post inoculation, mean *E. coli* levels in soil were: 6.1 log MPN/g (Year-1) and 3.6 log MPN/g (Year-2) in the organic fields and 3.2 log MPN/g (Year-2) in the transitional fields. In all treatments, bacterial levels significantly ($P<0.001$) decreased from fall to late spring. In the organic field, both cover crop treatment ($P=0.004$) and time ($P < 0.001$) were significantly

associated with *E. coli* and *L. innocua* survival; the effect of cover crop was most significant ($P<0.001$) during the first 3 weeks of the study. Cover crop treatment plots with rye and hairy vetch/rye supported the highest mean *E. coli* populations (at ~1.8 log MPN/g of soil) which were significantly different ($P=0.007$ and $P<0.01$, respectively) from the bare-ground plots (1 log MPN/g of soil). Contrastingly, survival of *L. innocua* was higher in bare-ground plots (4.11 mean log MPN/g of soil) than in cover crop treatments. In the transitional fields, time ($P<0.001$) was the only factor significantly associated with *E. coli* and *L. innocua* levels.

b) ***E. coli O157:H7 survival in soil planted with a clover cover crop:*** A 2012 replicated, controlled, experimental greenhouse study (2) conducted in Georgia, USA found that *E. coli* O157:H7 survival was significantly lower in soil planted with clover compared to unplanted controls. **Methods:** *E. coli O157:H7* (avirulent strain B6914) was inoculated into a highly controlled soil mixture (1:3 topsoil mix and peat-based substrate) to achieve an initial concentration of 10^8 CFU/g soil. Within a controlled greenhouse environment, 4 treatments (each of 8 replicates, distributed in a 2x2 factorial design) were used to compare the effect of low and high volumetric moisture content (25 and 45% ww^{-1}, respectively) and the presence or absence of clover (*Trifolium incarnatum*, organic cover crop) on the survival of *E. coli* O157:H7 in the soil matrix. For each replicate, sterilized pots (15 cm) contained 928 grams of soil that were mixed with nano-pure water (125 ml and 350 ml for low and high moisture treatments, respectively), fertilizer (14N-P-K 5.5 g/pot), and *E. coli* O157:H7 (10^8 CFU/g soil). Clover was planted at a rate of 20 seeds/pot and the seeds were covered with an additional 1 cm of soil mixture. The pots were irrigated using an automated system to maintain the set moisture level in the soil. Control (no *E. coli* O157:H7) pots (N=3) for each treatment received only sterile nano-pure water. Soil samples were collected from each replicate pot (N= 7 weeks, N=5 samples for each treatment and N=3 for control) and *E. coli* O157:H7 was enumerated. Total DNA extracted from the soil samples were used to quantify the total bacterial load (16S rRNA gene) and *E. coli* O157:H7 (*uidA* gene) by qPCR. **Results:** *E. coli* O157:H7 concentrations in the unplanted soil with 25% and 45% moisture content at week-0, were 8.774 and 8.630 $\log_{10}$ cells/gram soil, respectively. *E. coli* O157:H7 concentrations in the clover soil with 25% and 45% moisture content at week-0 were 8.737 and 8.785 $\log_{10}$ cells/gram soil, respectively. In general, the growth of clover significantly ($P<0.01$) reduced the survival of *E. coli* O157:H7 in the 45 % soil moisture treatments, whereas no inactivation effect of clover was observed in the 25 % soil moisture treatments. However, when *E. coli* O157:H7 populations were determined relative to all bacteria (16S rRNA gene), *E. coli* O157:H7 survived at significantly lower concentrations in clover soil [at both 25% ($P<0.05$) and 45% ($P<0.001$)] when compared to unplanted soil. As canopy size increased, *E. coli* levels decreased (at 25% $y = -0.0159x + 7.657$, $r^2=0.7439$, $P=0.0315$; at 45%, $y = -0.0115x + 7.204$, $r^2=0.8604$, $P=0.0098$); the high moisture treatment resulted in larger canopies and greater *E. coli* O157:H7 reduction than the low moisture treatments.

References:

1. Reed-Jones NL, Marine SC, Everts KL, Micallef SA, & Björkroth J (2016) Effects of Cover Crop Species and Season on Population Dynamics of *Escherichia coli* and *Listeria innocua* in Soil. *Appl Environ Microb* 82(6):1767-1777.
2. Rothrock MJ, Frantz JM, & Burnett S (2012) Effect of Volumetric Water Content and Clover (*Trifolium incarnatum*) on the Survival of *Escherichia coli* O157:H7 in a Soil Matrix. *Curr Microbiol* 65(3):272-283.

2.4. Remove vegetation (weeds) in produce fields (1 study): A experimental study from the USA (1) found that surrounding vegetation (weeds) influenced the survival of Generic *E. coli* in produce surface.

a) ***Generic E. coli survival in watermelon fields with low to high non-crop vegetation:*** A replicated, controlled, experimental study (1) in 2018 at the Botanic Gardens of Louisiana State University, Louisiana, USA found generic *E. coli* exhibited lower survival on watermelon surfaces in fields with low levels of surrounding non-crop vegetation versus fields with medium and high levels of surrounding non-crop vegetation levels. **Methods:** A test field at the Botanic Gardens of Louisiana State University was divided into 3 blocks and each block had 6 plots (12X30ft^2 total N=18 plots). Plots were treated with herbicide before (24 h) transplanting watermelon seedlings and fields were overhead irrigated until flowering. After 8 days of herbicide treatment, multiple types of non-crop vegetation were observed in each plot. The non-crop vegetation was evaluated and plots were classified as low (not to small amount of non-crop vegetation), medium (~50% of plot covered in non-crop vegetation) and high (~100% of plot covered in non-crop vegetation). Generic E. coli, *Salmonella* and *E. coli* O157:H7 levels on watermelons harvested from low (N=30), medium (N=30) and high (N=20) non-crop vegetation plots were enumerated. Separately but in this same study, a cocktail of 3 generic *E. coli* strains (ATCC 23716, 25922, and 11775) with a final inoculum concentration of 10^9 CFU/ml were surface (area = 20 cm^2) coated on watermelon rind discs (N=63). Inoculated rind discs were placed randomly in plots with each level of vegetation (N=21 discs in each plot). Discs from each plot were collected at 0, 12, 36, 60, 84, and 108 hours and generic *E. coli* was enumerated. **Results:** All samples were 100% negative for *Salmonella* and *E. coli* O157:H7. The concentration of generic *E. coli* on the watermelons collected from low, medium and high vegetation levels were 1.0, 1.46, 1.23 log MPN/sample, respectively. The level of vegetation surrounding the watermelons had no significant effect (P>0.05) on the generic *E. coli* concentration. However, generic *E. coli* on watermelon rind discs reduced initially from 0-12 hours (reduction rate were 2.58, 1.14, and 0.95 log in low, medium and high, respectively), followed by a gradual increase in the generic *E. coli* concentration (i.e., after 12 h). At 108 hours, the highest reduction (3 log CFU/cm^2, average die-off rate = 0.66 log/day) was observed in low vegetation level, whereas in the medium and high vegetation level the generic *E. coli* counts recovered to initial levels (~6 log CFU/cm^2).

Reference:

1. Chhetri VS, *et al.* (2018) Effect of surrounding vegetation on microbial survival or die-off on watermelon surface in an agriculture setting. *J Food Safety* 38(6).

3. Wildlife Management:

3.1. Add riparian fencing (2 studies): One replicated, controlled, experimental study from the USA (1), and one replicated, controlled, experimental study from Canada (2) found riparian fencing was an effective measure for restricting livestock from accessing water sources (streams). Both studies found that riparian fencing may improve stream water microbial quality in at least some of the statistical tests conducted.

a) ***Generic E. coli prevalence in catchment streams with or without riparian fencing:*** A 5-year replicated, controlled, experimental study (1) conducted in Missouri, USA found that patch-burn grazing of tallgrass prairie increased generic *E. coli* concentrations relative to ungrazed controls and that riparian fencing moderately reduced this effect (but not significantly so). **Methods:** The study focused on native tallgrass prairie in Missouri, USA (first-order catchments, N=6) that was historically used for cattle grazing (from the 1900's). Management practices include: midsummer haying (every 3 years) and prescribed burning (every 3–5 years). In this study, to test if the stream was affected by patch burn-grazing, a Before-After/Control-Impact design with samples paired in time (BACI) was used. Treatments included: fire but no grazers (control, N=2), grazers present with free access to riparian areas and streams (unfenced, N=2), and grazers present with riparian fencing (fenced, N=2). Pretreatment ("Before" phase, no fire or grazing) occurred from Sep, 2009 to Mar, 2011. Post-treatment ("After" phase, with patch-burn and grazing) occurred between Apr, 2011 to Jul, 2013. Treatment and control areas were burned (i.e., 1/3 of the catchment area) on specific dates in Apr, 2011, 2012, and 2013. Riparian fencing (for 10 m, with poly-electric tape) was established on each side of the stream. In the burned treatments, yearling calves were stocked (density ~0.825 calves/ha), between May and July (for 3 years). Drinking water tanks were added in the upper catchment areas (away from the stream) to supply water for the animals. Streams were sampled monthly (only when flowing) and generic *E. coli* levels were enumerated. **Results:** During the pretreatment phase (no fire or grazing), all streams (N=6, treatment and control) exhibited similar levels of generic *E. coli* (*i.e.,* no significant differences). Post-treatment, patch burn grazing caused an increase in generic *E. coli* values (P= 0.05) when compared to control areas. Fenced riparian areas had well developed vegetation areas compared to unfenced areas, highlighting the efficacy of riparian fencing at excluding grazing activity. Fencing moderately reduced *E. coli* levels (*i.e.,* mean of 514 CFU in unfenced areas versus means of 39 CFU in fenced treatments); however, this was not significant (*P*= 0.27) likely due to variation among samples collected from a given treatment. In both grazed treatments, *E. coli* counts significantly increased by threefold to tenfold in the treatment riparian catchments when cattle were present; however, shortly after cattle were removed (within 2-months) *E. coli* counts were negligible and returned to baseline values (variable Time, *F* stat=25.43, *P*-value<0.01). Such results were consistent for all treatment years (N=3). The authors explain that even though the riparian fencing was robust at excluding cattle from

streams, shallow soil depths, over-land and subsurface flow may have impacted the study results.

b) ***Fecal indicator bacteria (FIB) prevalence in a pasture-adjacent stream with and without riparian fencing:*** A replicated, controlled, experimental study (2) in Ontario, Canada found that riparian fencing was effective at restricting pastured livestock from visiting streams and improved stream water microbial quality. **Methods:** This study was conducted in two pastures located near the outflow of a 285-ha watershed. Pasture-1 comprised approx. 1.8 ha of grazing land and had restricted cattle access (RCA). A 3-5 m wide riparian buffer (between pasture-1 and stream) was fenced with electrical wire to block cattle access to approx. 356 m of the stream channel. At pasture-1, a bridge was constructed to provide cattle with access to both sides of the stream. Situated directly downstream from pasture-1 was pasture-2 (approx. 4.8 ha of land), which included 2.2 ha of grazing land and 2.6 ha of woodland. It also included 348 m of unrestricted, stream channel access for the cattle (URCA). Livestock density in both pastures was maintained at 2.5 animals/ha. Water samples were collected twice a week at selected sampling (N=3) locations (RCA_{in}, RCAout & URCAin, and URCAout). FIB levels (fecal coliform, *E. coli* and Enterococcus) were enumerated in the samples. **Results:** Total load reduction indices at the RCA and URCA locations were: fecal coliform (N=42; RCA = $6.614X10^3$, URCA= $-6.09X10^5$), *E. coli* (N=438; RCA=$2.78X10^6$, URCA=$4.0X10^5$), and Enterococcus (N=431; RCA=$1.63X10^6$, URCA=$-2.9X10^5$). The mean percent fecal indicator bacteria reduction for the RCA were significantly greater ($p < 0.1$) than those from the URCA. In conclusion, keeping pastured livestock more than 5 m from streams may improve stream water quality.

Reference:

1. Larson DM, Dodds WK, Whiles MR, Fulgoni JN, & Thompson TR (2016) A before-and-after assessment of patch-burn grazing and riparian fencing along headwater streams. *Journal of Applied Ecology* 53(5):1543-1553.
2. Sunohara MD, *et al.* (2012) Impact of Riparian Zone Protection from Cattle on Nutrient, Bacteria, F-coliphage, *Cryptosporidium*, and *Giardia* Loading of an Intermittent Stream. *Journal of Environmental Quality* 41(4):1301-1314.

3.2. Integrated livestock in produce fields (3 studies): One observational study from the USA (1) assessed the food safety risks associated with grazing sheep within crop fields prior to planting. In all comparisons, a 120-day waiting period between grazing and harvest reduced the mean generic *E. coli* concentrations. One observational study from the USA (2) reported higher odds of generic *E. coli* in produce samples when growers reported domestic animal intrusion in the fields. Finally, one observational study from the USA (3) failed to identify significant associations between odds of detecting generic *E. coli* in a spinach sample, and the grower reporting the presence or absence of domestic animals in the spinach fields.

a) ***Generic E. coli levels in fields soils following sheep grazing:*** A replicated, observational study (1) was conducted in 2015 on an organic farm with mixed crop-livestock practices in the Central Valley of California, USA. This study found that mean generic *E. coli* levels dropped below 1.0 $\log_{10}$ MPN/g in the soil by the 120-day standard (USDA, National Organic Program) after cover crops were grazed by sheep. **Methods:** In a five-acre organic field, a cover crop mixture was planted in autumn 2014, and then grazed by a flock of sheep (n=60) in early spring 2015. The study took place in two fields (A and B), which were in turn divided into 3 paddocks. Each paddock was grazed continuously for 3-4 days, after which the sheep were removed. The fields were then prepped (composted, tilled, irrigated) and planted with onions (field A) or various melon varieties/sunflowers (field B). For each field, soil was collected from 12 random locations. Composite soil samples (day 1, 7, 14, 21, 28, 56, 84, and 112) were collected from selected 50X50 ft areas, and fecal samples on day 1 and 56. Generic *E. coli* and STEC levels were quantified. **Results:** *E. coli* levels in field A were higher (84.38% positive; mean 3.24 log10 MPN/g) compared with field B (75.00% positive; mean 2.92 log10 MPN/g). *E. coli* levels in field A decreased by 3.7 $\log_{10}$ from day 48 to day 139 days post sheep grazing, while levels in and field B decreased by 3.51 $\log_{10}$ from day 14 to day 112 days post grazing. Mean generic *E. coli* concentrations declined to below 1.0 $\log_{10}$ MPN/g by 111 days post sheep grazing (0.67 $\log_{10}$ MPN/g) in field A and 84 days post grazing (0.95 $\log_{10}$ MPN/g) in field B. Prevalence of STEC in the sheep flock during the study period was 4.17% (1/24).

b) ***Generic E. coli prevalence in spinach farms with or without domestic animals intrusion:*** A 2-year replicated, observational study (2) conducted from 2010 to 2011 in spinach fields in Western Colorado and in Southwestern Texas, USA, found that spinach contamination with generic *E. coli* was higher on farms with domestic animal presence when compared to farms where domestic animals were absent. **Methods:** A total of 955 spinach samples were collected from 1 to 6 fields per visit from each of 12 spinach farms [Colorado (n=4) and Texas (n=8)]. Each sample consisted of at least 10 spinach leaves and generic *E. coli* levels in each sample was enumerated. At each farm visit, a questionnaire was also administered to obtain information on farm management and environmental factors. The questionnaire asked, for example, whether the farm employed some strategy for excluding wildlife and/or whether domestic animals were present. **Results:** Generic *E. coli* was isolated from 6.6% (63/955) of the spinach samples. In univariate analysis, spinach was

more likely to be contaminated if the farms reported the presence of domestic animals (OR= 11.8; 95% CI=1.1, 122.9, P=0.039) compared to farms where no domestic animal were reported. However, the variable "domestic animals presence or absence" was not included in the final multivariate model presented by the authors.

c) ***Generic E. coli contamination of spinach samples was not associated with the presence of domestic or wild animals in the field***: A replicated, observational, cross-sectional study (3) conducted in June 2010 and Feb 2012, assessed the microbial safety of spinach grown by commercial farms produced by selected farms in Colorado and Texas, USA. The study found no significant association between the odds of detecting generic *E. coli* in a spinach sample and a farmer reporting the presence or absence of domestic animals. **Methods:** Spinach samples (N=955) collected from selected farms in Colorado (N=4) and Texas (N=8) were enumerated for *E. coli*. Information on the general farm-related management and environmental factors were obtained from the growers (questionnaire on farm management and environmental factors) and the NRI databases (weather and landscape factors). Variables were evaluated using a mixed-effect logistic regression model. **Results:** Generic *E. coli* was detected in 76.6% (63/955) of spinach samples. Using univariable regression analysis, the study failed to find a significant association between odds of detecting generic *E. coli* in a spinach sample, and a farmer reporting versus not reporting domestic animal intrusion into the spinach fields.

Reference:

1. Patterson L, *et al.* (2018) Persistence of *Escherichia coli* in the soil of an organic mixed crop-livestock farm that integrates sheep grazing within vegetable fields. *Zoonoses Public Health* 65(7):887-896.
2. Park S, *et al.* (2013) Generic *Escherichia coli* Contamination of Spinach at the Preharvest Stage: Effects of Farm Management and Environmental Factors. *Appl Environ Microb* 79(14):4347-4358.
3. Park S, *et al.* (2014) Farm Management, Environment, and Weather Factors Jointly Affect the Probability of Spinach Contamination by Generic *Escherichia coli* at the Preharvest Stage. *Appl Environ Microb* 80(8):2504-2515.

3.3. Decrease the presence of wildlife animals in produce fields (3 studies): One observational study from the USA (1) found that methods implemented by growers to repel wildlife did not reduce the occurrence of generic *E. coli*. In another study (2) the odds of detecting *Listeria monocytogenes* contamination in produce samples were higher when growers reported wildlife intrusion into the fields during 3 days before sample collection. Finally, a study (3) failed to identify significant association between odds of detecting generic *E. coli* in a spinach sample, and the farmer using wildlife control fences versus not using fences to keep wildlife out of the spinach fields.

a) ***Salmonella and Listeria contamination in produce fields where wildlife intrusion did or did not occur:*** A 2012 observational study (2) conducted in Upstate New York, USA, found a higher prevalence of *Salmonella* and *Listeria monocytogenes* in fields where growers reported observations of wildlife than in fields with no reported wildlife activity. **Methods:** A questionnaire was mailed to selected farms in western (N=5), central (N=12) and eastern (N=4) New York to quantify farm management approaches, and farm visits were performed over a 5-week period between (June and July, 2012). At each visit, one composite soil sample and one drag swab samples from each field, while irrigation water samples (N=23) and, non-irrigation water samples (N=51) located within 50 m from a sampled field were collected opportunistically. All samples (soil =263, drag swab =263, and 74 water samples) were enriched for separate detection of *Salmonella* and *Listeria monocytogenes.* **Results:** Prevalence of *Salmonella* and *L. monocytogenes* in terrestrial (N=526 i.e., soil n=263 and swab n=263) samples was 3.4% (18/526) and 9.7% (51/526), respectively. The prevalence of *L. monocytogenes* (30%, 22/74) was higher in water samples than *Salmonella* (11%, 8/74). Based on the final multivariable model, fields where growers reported observation of wildlife during the 3 days before sample collection had higher odds of *L. monocytogenes* (OR=6.1; 95% CI=1.3, 28.4; P=0.021) than fields where growers reported observation of wildlife 4 to 7 days prior sample collection (OR=1.0, 0.2, 4.8; P=0.978) prior to sample collection. No evidence was found between the observation of wildlife intrusion into a field and likelihood of Salmonella detection.

b) ***Generic E. coli levels in spinach farms that used and did not use wildlife deterrents:*** A 2-year replicated, observational study (1) conducted from 2010 to 2011 in spinach fields in Western Colorado and in Southwestern Texas, USA, found that farms with management practices that attempt to repel wildlife did not reduce the occurrence of generic *E. coli*. **Methods:** A total of 955 spinach samples were collected from 1 to 6 fields per visit from each of 12 spinach farms [Colorado (n=4) and Texas (n=8)]. Each sample consisted of at least 10 spinach leaves and generic *E. coli* levels in each sample were enumerated. At each farm visit, a questionnaire was also administered to obtain information on farm management and environmental factors. The questionnaire asked, for example, whether the farm employed some strategy for excluding wildlife and/or whether domestic animals were present. **Results:** Generic *E. coli* was isolated from 6.6% (63/955) of the spinach samples. Univariate analysis indicated that attempting to control wildlife increased (rather than

decreased) the odds of detecting generic *E. coli* (OR= 5.0; 95% CI= 1.9, 13.2, P= 0.001) compared to farms where no wildlife deterrent strategies were implemented. However, the presence or absence of wildlife control methods was not included in the final multivariable model presented by the authors.

c) ***Generic E. coli contamination of spinach samples was not associated with the presence of wild animals in the field***: A replicated, observational, cross-sectional study (3) conducted in June 2010 and Feb 2012, assessed the microbial safety of spinach grown by commercial farms produced by selected farms in Colorado and Texas, USA. The study found no significant association between the odds of detecting generic *E. coli* in a spinach sample and a farmer using a wildlife control fences versus not using fences. **Methods:** Spinach samples (N=955) collected from selected farms in Colorado (N=4) and Texas (N=8) were enumerated for *E. coli*. Information on the general farm-related management and environmental factors were obtained from the growers (questionnaire on farm management and environmental factors) and the NRI databases (weather and landscape factors). Variables were evaluated using a mixed-effect logistic regression model. **Results:** Generic *E. coli* was detected in 76.6% (63/955) of spinach samples. Using univariable regression analysis, the study failed to find a significant association between odds of detecting generic *E. coli* in a spinach sample, and the farmer using wildlife control fences (as opposed to not using these fences) to keep wildlife out of the spinach fields.

References:

1. Park S, *et al.* (2013) Generic *Escherichia coli* Contamination of Spinach at the Preharvest Stage: Effects of Farm Management and Environmental Factors. *Appl Environ Microb* 79(14):4347-4358.
2. Strawn LK, *et al.* (2013) Risk Factors Associated with *Salmonella* and *Listeria monocytogenes* Contamination of Produce Fields. *Appl Environ Microb* 79(24):7618-7627.
3. Park S, *et al.* (2014) Farm Management, Environment, and Weather Factors Jointly Affect the Probability of Spinach Contamination by Generic *Escherichia coli* at the Preharvest Stage. *Appl Environ Microb* 80(8):2504-2515.

4. Landscape factors

4.1. Increase distance between produce fields and open/closed livestock areas (9 studies): Six observational studies (1-7) from the USA and one experimental study from the USA (8) found produce fields located in proximity to grazable lands, rangelands, pastures, or poultry farms were at higher risk when compared to farms located farther away, in some or all comparisons. One observational study (9) failed to identify any significant associations between odds of spinach contamination by generic *E. coli* and proximity to beef or poultry operations.

a) ***Generic E. coli and EHEC prevalence in produce farms located near grazeable lands:*** A one year observational study (4) conducted in 2015 in the California Central Coast, USA found that fresh produce from farms located in the vicinity to grazable lands were at higher risk of contamination from EHEC when compared to farms located far from grazable lands. **Methods:** EHEC which includes *E. coli* O157:H7 and STEC and *Salmonella* data were acquired from an organic farming operation, which collected the data between 2007 and 2013). A total of 482,208 samples (a mix of fresh produce) originating from 295 farms (spread across USA) were tested for EHEC and *Salmonella* at the organic farming operation. Data on generic *E. coli* in irrigation water that was tested monthly between 2007-2010 was acquired from the leafy green agricultural industry. Between Oct, 2009 and Aug, 2011, fecal samples were also collected from 11 wild rodent species, trapped at 9 produce farms in California, USA. Fecal samples were enriched to detect the presence/absence of *Salmonella*. Data from microbial analyses and land cover maps (data reflect changes in landcover over the study period) were used in statistical models to understand the effect of land use change on the prevalence of pathogens in the produce fields. **Results:** Farms with more surrounding grazeable land had significantly higher EHEC prevalence in fresh produce (model averaging: n = 236,522 tests across 57 farms, $Z = 3.8$, $P < 0.001$). EHEC was ~100 times more likely to be detected near grazeable land (60% grazeable land within 1.5 km) when compared to far from grazeable land. In this study, the authors report that surrounding land cover (e.g., grazeable land) did not significantly predict the changes in the prevalence of generic *E. coli* in water, and *Salmonella* in rodents or leafy green vegetables at any spatial scale.

b) ***Generic E. coli and E. coli O157:H7 prevalence in fresh produce farms near a beef cattle feedlot:*** A 2-year replicated, experimental study (8) conducted between 2011 and 2012 in a leafy green produce field in Nebraska, USA found that spinach fields in proximity to a cattle feedlot pen were at greater risk for generic *E. coli* and *E. coli* O157:H7 contamination compared to fields far away from feedlot pens. **Methods:** Plots (N=9, 6.1X9.1 m) that were located 60, 120, and 180 m from the nearest row of feedlot pens (6,000 head capacity, beef cattle) were planted with spinach (2011, 2012), mustard greens (2012), and turnip greens (2012). Plots were planted every 2-3 weeks in a field north of the feedlot (south winds during sampling season) to provide leaves for sampling from June through September each year. Plots were enclosed with fences (2.4 m high) and poultry netted (91.4 cm high) to prevent wildlife intrusion. Plots were irrigated (overhead) with well and tank water.

Samples from the feedlot pens (N=10, 30X90 m, with 60-80 head/pen) closest to the plots were collected as source samples. Leafy greens from plots (located at 60, 120, and 180m from the source) and feedlot surface manure were collected on 6 sampling days during each study year (2011 and 2012). On each sampling day, 270 leafy green samples (i.e., 30 samples from each of 9 plots or 90 samples from each plot distance from feedlot), and 100 feedlot pen surface manure samples (10 samples/feedlot pen) were collected. Additionally, composite soil samples (N=2/plot) were collected each year before planting fresh greens. Irrigation water samples were collected periodically for each sampling year (N=3 sampling days/year). Air samples (N=15 sampling days, with 2.5h sampling period, simultaneously at 4 locations 0, 60, 120, and 180 m from feedlot, and each of the 9 plots) were collected when wind was from south, southeast, or southwest directions. Generic *E. coli* and *E. coli* O157:H7 levels were enumerated in all samples using culture-based and and immunomagnetic separation methods. **Results:** All water and soil samples were negative for generic *E. coli* and *E. coli* O157:H7 (except for one soil sample from a plot at 60m from feedlot). All air samples were negative for *E. coli* O157:H7. With average concentrations of 2.71 and 2.91 log CFU/g in 2011 and 2012, respectively, the majority of the feedlot manure samples were positive for *E. coli* O157:H7 (total positive samples = 72.5 % with 9.2% samples that were enumerable). Few leafy green samples were positive for *E. coli* O157:H7; however, the percentage of positive samples significantly ($P<0.05$) decreased as the distance from feedlot increased. Specifically, across both years, 3.5, 2.2, and 1.8% of the leafy green samples were positive for *E. coli* O157:H7 at plots located 60, 120, and 180 m away from feedlot pens, respectively. Generic *E. coli* also decreased with increasing distance from feedlot pens. Across both years, 35.5%, 29.4%, and 26.1% of the leafy green samples were positive for generic *E. coli* at plots located 60, 120, and 180 m away from feedlot pens, respectively. Average generic *E. coli* concentrations in the air samples ranged from 0 to 837.2, 0 to 16.7, 0 to 10, and 0.2 to 5.3 CFU/m^3 at plots located at 0, 60, 120, and 180 m away from feedlot pens, respectively. As reported by the authors, dry-dusty feedlot pen surfaces, environmental parameters (rain, temperature), and cattle activity had a major impact on the detection of generic *E. coli* in the air samples. Among *E. coli* O157:H7 isolates, 3 predominant subtypes represented the majority (75.2% in 2011 and 82.1% in 2012) of the feedlot pen surface isolates (total N=870, for both years). These three subtypes also were the predominant subtypes in the leafy greens isolates (representing 88.5%, total N=75 isolates for both years).

c) ***Generic E. coli prevalence in soils collected from areas under pasture cover compared to other cover types:*** A replicated, observational study (6) conducted in 2018 collected soil samples from various landscapes surrounding the Buffalo River in Minnesota, USA. The study found that land cover type was an influential driver of generic *E. coli* prevalence in surface soils, with prevalence being higher in pasture soils compared to soils in croplands, grasslands, scrublands and wetlands. **Methods:** The study area comprised 585,589 ha within a 20 km radius around the Buffalo River, MN, USA. Using a spatially explicit

landscape sampling design, the study area was stratified by surface soil pH (5.9 to 6.7, 6.7 to 7.3, and 7.3 to 8.2) for all sampling locations. The study area was further sub-divided into 5 zones (based on predicted soil pH values). At each sampling site (total N=143), 10 surface soil samples (0-4 in. depth, total samples N = 1,430) were collected. During sample collection, observations about the sampling area (land cover type, vegetation type, soil moisture, soil consolidation, and density of vegetation) were recorded. Generic E. coli levels in samples were enumerated. Following isolation, phylogroup (B1, B2, D, or E, total isolates N=3,329) was determined by single-nucleotide polymorphism qPCR assays, and whole-genome sequencing. **Results:** The landscape composition within the 20-km buffer of the Buffalo River was: 36% cropland, 29% forest, 10% open water, 6% wooded wetland, 6% pasture, 5% herbaceous wetland, 4% urban, 3% grassland, and 1% scrubland. The overall prevalence of generic *E. coli* among all soil samples was 41% (581/1428). The majority of generic *E. coli* isolates (87%, total N=3,329) belonged to the focal phylogroups (43% B1, 19% B2, 18% D, and 8% E). Regardless of phylogroup, *E. coli* prevalence was greatest in pasture (73%) and forest (73%) soils compared to wooded wetland (65%), herbaceous wetland (44%), grassland (19%), cropland (19%), and scrubland (15%) soils. However, the effect of proximity to pasture was less clear, as the prevalence of generic *E. coli* peaked at approx. 400 m from pasture, then dipped in prevalence at approx. 900 m from pasture, before peaking again at approx. 1500 m from pasture.

d) ***Listeria spp. and L. monocytogenes detection in samples collected from produce fields at different distances from pasture:*** A 2014 replicated, observational study (5) conducted in Upstate New York, USA produce fields, found that fields found that Listeria was more likely to be isolated from fields near pasture (≤ 62.5 m) compared to fields located far from pasture (> 62.5 m). **Methods:** A cross-sectional study was conducted on produce farms (total N=4) in New York State: western New York (N=2), the Hudson Valley (N=1), and the Capital District (N=1). Fields within each farm were classified by predicted prevalence of *L. monocytogenes* isolation, based on land cover factors (using models from previous studies). Selected land cover factors include: distance from water source, distance from roads, and distance from pastures. For each farm, ArcGIS shapefiles (hydrology, road, and pasture) were generated, and categorized as areas of high (e.g., areas within 62.5 m from pasture) or low (e.g., areas > 62.5 m from pasture) predicted *L. monocytogenes* prevalence, according to splits in the previously published model. Fields were then divided into 5X5 m plots, and a subset of plots was sampled randomly (i.e., one drag swab per plot from each high/low risk category). All samples (total N=1,056) were enriched to isolate *Listeria* spp. and *L. monocytogenes*. Distance from the sampling site to each land cover class (barren land, grassland, forest, scrubland, water, road, pasture, and wetlands) was calculated to facilitate identification of associations with *Listeri*a isolation. **Results:** Overall, 20% of samples (208/1056) were positive for *Listeria* spp. and 12% of samples (128/1056) were positive for *L. monocytogenes*. Prevalence of *Listeria* spp. was 24% in samples (N=12/49) collected from plots closer to pasture (i.e., ≤ 62.5 m from pasture) and 19% in samples

(11/57) collected from plots farther from pasture (i.e., > 62.5m from pasture). Prevalence of *L. monocytogenes* was 22% in samples (N=22/49) collected from plots closer to pasture (i.e., ≤ 62.5 m from pasture) and 15% in samples (9/57) collected from plots farther from pasture (i.e., > 62.5m from pasture). Based on classification tree analysis, the odds of isolating *L. monocytogenes* (OR = 2.9; 95% CI = 1.4, 6.0; P=0.005) were greater in samples collected from field areas located near as opposed to far from pasture. Similarly, based on univariable regression analysis, proximity to pasture was associated with *Listeria* positive samples (*Listeria* spp. OR=0.92, 95% CI = 0.83, 1.0; P=0.117 and *L. monocytogenes* Or=0.92, 95% CI=0.81, 1.0; P=0.148). However, the proximity to pasture was not include in the final multivariate model presented by the authors.

e) ***L. monocytogenes, Salmonella, and STEC prevalence in produce samples collected close to and far from pasture:*** A 3-year observational study (3) from 2009 to 2011 in New York State, USA, found that proximity to pasture increased the likelihood of *L. monocytogenes* detection in the fresh produce fields. **Methods:** A longitudinal field study was conducted on five produce farms in New York State: central New York (N = 1), the Finger Lakes (N = 3), and Western New York (N = 1). Selected farms were sampled between June 2009 and August 2011 (in total 9 times, once per season summer, fall, winter, and spring in each year). Within each farm, a composite surface soil samples (total N=178) and one drag swab samples (total N=175) was collected from each of four produce fields per visit. At each field, up to 5 water samples [total N=174, engineered (N=28) and surface (N=146)] and fecal samples (N=61) were collected (when available). All samples underwent 3 separate enrichment methods, to allow the isolation and detection of *L. monocytogenes*, *Salmonella*, and STEC. Classification Tree (CT) models were fit to explore associations between environmental variables, and detection of *L. monocytogenes* and *Salmonella*. **Results:** Across all samples 15.0%, 4.6%, and 2.7% of the samples were positive for *L. monocytogenes*, *Salmonella* and STEC. The prevalence of *L. monocytogenes* was higher in surface water samples (33%; 48/146) than fecal (15%; 9/61), soil (9%; 16/178), and drag swab (9%; 15/1758) samples. Similarly, prevalence of *Salmonella* was higher in surface water samples (11%; 16/146) than fecal (7%; 4/61), soil (2%; 4/178), and drag swab (2%; 3/175) samples. The prevalence of STEC was marginally higher in fecal (7%; 4/61) than drag swab (3%; 5/175), surface water (3%; 4/146), and soil (2%; 3/178) samples. Engineered water samples (N=28) were all negative for all 3 tested pathogens. The CT model determined that locations within 62.5 m of a pasture had a predicted *L. monocytogenes* prevalence of 50% (n=25/50), whereas the prevalence in soil samples collected farther than 62.5 m from pasture was 7.5% (3/40). In conclusion, being closer to pasture increased the likelihood of detecting *L. monocytogenes* in produce farm environments. The authors did not find evidence of an association between proximity to pasture and likelihood of *Salmonella* isolation.

f) ***Generic E. coli prevalence on spinach farms located at varying distances from livestock operations:*** A 2-year replicated, observational study (2) conducted from 2010 to 2011, in

spinach fields in Western Colorado and in Southwestern Texas, USA, found that generic *E. coli* was significantly more prevalent in spinach grown in fields located near (≤ 10 miles) poultry farms compared to fields farther away from poultry farms (<10 miles). The study found no evidence that generic *E. coli* was significantly more prevalent in spinach grown in fields located near (≤ 10 miles) dairy, swine and beef farms compared to fields farther away from these operations (>10 miles). **Methods:** A total of 955 spinach samples were collected from 1-6 fields per visit from each of 12 spinach farms [Colorado (n=4) and Texas (n=8)]. Each sample consisted of at least 10 spinach leaves and generic *E. coli* levels in each sample were enumerated. At each farm visit, a questionnaire was also administered to obtain information on farm management and environmental factors. In total 76 explanatory variables were considered for the univariable analysis. The variables associated with spinach contamination at P=0.20 significance level were identified and used to build a full multivariable. The full model was then reduced by backwards selection based on p-value to a final model. **Results:** The prevalence of generic *E. coli* was 6.6% (63/955) across all spinach samples. The probability of detecting generic *E. coli* was higher near (≤ 10 miles) as opposed to far (>10 miles) from poultry farms. Specifically, both univariable (OR=8.7; 95% CI = 0.9, 88.0; P=0.067) and multivariable (P<0.001; OR = 172.1; 95% CI = 21.1, 402.8) analysis showed that the odds of spinach contamination were significantly higher for fields near (within 10 miles) poultry farms compared to fields located farther away (>10 miles). Based on univariable analysis, the odds of spinach contamination were higher, but not significantly so (P=0.174) for fields in proximity (within 10-mile radius) to beef farms (odds ratio = 6.0; 95% CI = 0.5, 79.1); however, proximity to beef farms was not retained in the final multivariable model. Finally, univariable tests suggested that probability of detecting generic *E. coli* was not affected by proximity to dairy and swine farms (P> 0.2); thus, neither variable was included in the full multivariable model.

g) ***Association between generic E. coli, E. coli O157 and Salmonella spp. detection and distance to rangeland:*** A 3 year replicated, observational study (1) conducted between May 2008 and October 2010 in the Central Coast of California, USA found that generic *E. coli* was more likely to be detected in surface water on produce farms located near as opposed to far from rangelands. **Methods:** Produce farms (N=16) that grow leafy green vegetables and agricultural water sources sites (total N=241) in the Central California Coast region were repeatedly sampled during the study period. Water and sediment samples were collected from produce farms (N=16) and water sources that had public access near the farms. A total of 255 irrigation water samples and 181 sediment samples were collected from produce farms; sample types included; well water, reservoir, irrigation, furrow, ditch, pond, stream, river, and creek. A total of 389 non-irrigation water samples and 159 sediment samples were collected from water sources with public access (ditch, pond, stream, river, and creek). Generic *E. coli* levels were enumerated in each sample using a culture-based method. A subset of samples followed an enrichment method, where

E. coli O157 (N=436) and *Salmonella* (N=249) were recorded as present/absent. Models were fit to further explore associations between distance from the sample location to rangeland, riparian habitats, and roads and generic *E. coli* levels. **Results:** In total, 6.3% of the water samples (irrigation 1/60, and non-irrigation 5/36) and 4.8% of the sediment samples (irrigation 2/37, and non-irrigation 3/64) were positive for *Salmonella.* One water sample from produce farms (0.4%; total N=242) and nine water samples with public access (2.8%; total N=316) were positive for *E. coli* O157. On produce farms, 78% of the water samples (199/255; mean $7.1X10^2$ MPN/100 ml) and 36% of the sediment samples (65/81; mean $1.0X10^4$ CFU/100g) were positive for generic *E. coli*. Proximity to rangeland was significantly associated with generic *E. coli* concentrations in the water samples. For example, based on the final negative binomial regression model generic *E. coli* concentrations decreased by 3% for each 10 m increase in the distance between rangeland and the water sampling location (Coefficient = -0.003, P<0.01, 95% CI = -0.006, -0.001).

h) ***Generic E. coli contamination of spinach samples was not associated with proximity to livestock operations:*** A replicated, observational, cross-sectional study (9) conducted in June 2010 and Feb 2012, assessed the microbial safety of spinach grown by commercial farms produced by selected farms in Colorado and Texas, USA. The study did not identify any significant associations between odds of detecting generic E. coli in a spinach samples and proximity to livestock operations. **Methods:** Spinach samples (N=955) collected from selected farms in Colorado (N=4) and Texas (N=8) were enumerated for *E. coli*. Information on the general farm-related management and environmental factors were obtained from the growers (questionnaire on farm management and environmental factors) and the NRI databases (weather and landscape factors). Variables were evaluated using a mixed-effect logistic regression model. **Results:** Generic *E. coli* was detected in 76.6% (63/955) of spinach samples. Using univariable regression analysis, the study failed to find a significant association between odds of detecting generic *E. coli* in a spinach sample, and proximity to a beef or to a poultry operation.

i) ***Pathogenic E. coli, L. monocytogenes, and Salmonella prevalence in irrigation water along a proximity gradient to upstream livestock:*** A 2-year replicated, observational study (7) between 2015 and 2016 in irrigation water ditches of British Columbia, Canada, found that the proximity to and density of upstream livestock correlated with VTEC and *L. monocytogenes* detection in surface irrigation waters. **Methods:** Surface water samples (total N=223) were collected (ditch, creek, or stream) from two distinct watersheds: the Serpentine watershed and the Sumas watershed of British Columbia, Canada, once per month (between Feb and Apr, 2015) and thereafter twice per month until Aug, 2016. All water samples were enumerated for generic *E. coli*, *E. coli* (VTEC), *L. monocytogenes*, and *Salmonella*. All presumptive pathogenic colonies were serotyped by PCR. A map was created to determine the upstream water sources and presence of livestock was considered positive if there was a direct connection between the surface water and the livestock property. Correlations between pathogen occurrence and landscape factors were calculated

using point-biserial method. **Results:** Overall, *L. monocytogenes* was recovered from 10.3% of samples, followed by VTEC (4.9%) and *Salmonella* (2.7%). Neither VTEC or *Salmonella* showed any differences in occurrence between the two watersheds; however, *L. monocytogenes* occurred more in the Serpentine (18.1%) watershed compared to the Sumas (4.65%) watershed. Mean generic *E. coli* ranged from 30 to 111 CFU per 100 mL and 136 to 251 CFU per 100 mL for the Sumas and Serpentine watersheds, respectively. The likelihood of occurrence of VTEC correlated significantly with the proximity of upstream water to livestock areas, up to a distance of 2 km ($r_s = 0.812$; $n = 6$; $p = 0.050$) and 3 km ($r_s = 0.812$; $n = 6$; $p = 0.050$). The likelihood of occurrence of *L. monocytogenes* correlated significantly with livestock density within 2km upstream ($r_s = 0.841$; $n = 6$; $p = 0.036$). The authors report no significant correlations for likelihood of *Salmonell*a occurrence and livestock proximity or density.

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4.2. Increase distance between produce fields and water sources/wetlands (5 studies**):** Three observational studies (1-3) from the USA found consistent evidence that produce fields located in proximity to surface water (which also served as proxy for riparian areas) or wetlands were at higher risk of *Listeria* contamination compared to fields located farther away from water sources. One observational study (4) from the USA reported that generic *E. coli* was not significantly more prevalent in spinach grown in fields located in proximity (≤ 10 miles) to water sources compared to fields farther away (> 10 miles). Finally, one observational study (5) from the USA reported that generic *E. coli* prevalence was higher in wooded wetlands than herbaceous wetlands, grasslands, croplands, and scrubland soils.

a) ***Listeria spp. and L. monocytogenes prevalence in produce fields near versus far from water sources:*** A 2014 observational study (2) conducted in spinach fields in New York, USA found that landscape factors accurately predicted the risk of *L. monocytogenes* contamination in produce fields, with *L. monocytogenes* being more likely to be detected in fields closer to water sources (i.e., riparian areas) and roads. **Methods:** A longitudinal study was conducted in two spinach fields (0.2 ha) over a 7-week period (May – July, 2014). Field-A was categorized as high-risk, (i.e., field was ≤ 37.5 m from water source and ≤ 9.5 m from a road) and Field-B was categorized as low risk (i.e., > 37.5 m from a water source and >9.5 m from a road) for *L. monocytogenes* contamination based on models from previous studies. Both fields were divided into 21 (13X13 m) plots. Fields were prepared for planting and fertilized at a rate of 789 kg/ha (13-13-13 NPK). Fields were irrigated with creek water via overhead irrigation. Soil sample sites within each plot were randomly selected, with a new sampling site being used per plot on each visit (i.e., one sample per plot per visit). Soil samples (total N=1,092) were collected on the day of planting and 24, 48, 72, and 144-192 h after an irrigation or rain event. Water samples (total N=52) were collected from the irrigation water source, which was a creek. Fecal samples (total N=14) were collected when detected within 5m of the sampled fields or water source. Composite leaf samples (total N=334) were hand collected (beginning 36 days after planting) along the perimeter and diagonals of the plot. Samples (total N=1,492) were enriched to isolate *Listeria* spp. and *L. monocytogenes*. Isolates were identified to allelic type by partial *sigB* gene sequences. **Results:** Overall, 14% of the samples (204/1,492) were positive for *Listeria* spp.; specifically, 90% of water samples (47/52), 79% of fecal samples (11/14), 12% of soil samples (126/1,092), and 6% of leaf samples (19/334). *L. monocytogenes* was positive in 9% of the overall samples (130/1,492); specifically, 64% of fecal samples (9/14), 63% of water samples (33/52), 8% of soil samples (86/1,092) and 0.6% of leaf samples (2/334). *Listeria* were more prevalent in soil samples collected from the high-risk field, which was close to the creek and road (*Listeria* spp. 15%; 84/546, and *L. monocytogenes* 11%; 62/546) compared to the low-risk field, which was farther from creek and road (*Listeria* spp. 8%; 42/546, and *L. monocytogenes* 4%; 24/546). The odds of isolating *Listeria* spp. (OR = 2.3; 95% CI = 1.5, 3.5, P<0.01) and

L. monocytogenes (OR = 3.5; 95% CI = 2.0, 6.0, P<0.01) were 2 times and 3.5 times greater for soil samples collected from the high-risk field compared to the low-risk field.

b) Likelihood of detecting Listeria spp. and L. monocytogenes in fields close to and far from water and wetlands: A 2014 replicated, observational study (3) conducted in produce fields in New York, USA, found that fields near water and wetlands were at higher risk of *Listeria* contamination compared to fields farther away from surface water and wetlands. **Methods:** A cross-sectional study sampled 4 produce farms in Upstate New York. Using a previously published model that used land cover and soil characteristics to predict *L. monocytogenes* prevalence in farm fields, fields within each farm were stratified into different risk categories based on the predicted prevalence of *L. monocytogenes*. The land cover factors used in the model were distance from water source (e.g., riparian areas), distance from roads, and distance from pastures. With regards to proximity to water, areas <37.5 m from water were predicted to have a high *L. monocytogenes* prevalence while areas > 37.5 from of water were) predicted to have a low prevalence. Fields were divided into 5X5 m plots, and a subset of plots were randomly selected and sampled using drag swabs (i.e., one drag swab per plot). All samples (total N=1,056) were enriched to isolate *Listeria* spp. and *L. monocytogenes*. Distance from the sampling site to each land cover class (barren land, grassland, forest, scrubland, water, road, pasture, and wetlands) was calculated to facilitate identification of associations with Listeria isolation. **Results:** Overall, 20% of the samples (208/1056) were positive for *Listeria* spp. and 12% of the samples (128/1056) were positive for *L. monocytogenes*. Prevalence of *Listeria* spp. was 26% in samples (N=51/195) collected from plots close to water (i.e., ≤ 37.5 m from water) and 18% in samples (157/861) collected from plots farther away from water (i.e., > 37.5 m from water). Similarly, the prevalence of *L. monocytogenes* was 22% in samples (N=43/195) collected from plots closer to water (i.e., ≤ 37.5 m from water) and 10% in samples (85/861) collected from plots farther away from water (i.e., > 37.5 m from water). The odds of isolating *Listeria* spp. (OR=1.6, 95% CI = 1.1, 2.4; P=0.008) and *L. monocytogenes* (OR = 3.0; 95% CI = 2.0, 4.6; P<0.001) was greater in samples collected near water (and this riparian areas), compared to samples collected farther away. Proximity to water was also significantly associated with *Listeria* in univariable and multivariable regression models. According to the multivariable model, for every 100 m increase in distance from a water source, the likelihood of *Listeria* spp. decreased by 15% (OR=0.85, 95% CI=0.76, 0.95; P=0.006) and *L. monocytogenes* decreased by 24% (OR = 0.76; 95% CI = 0.65, 0.89; P<0.001). Univariable and multivariable regression analysis also showed that the likelihood of *Listeria* isolation decreased with as distance to wetlands increased. According to univariable analysis, a 100-m increase in the distance from wetlands to the sampled plot was associated with a 7% decrease in likelihood of *Listeria* spp. isolation (OR=0.93, 95% CI=0.86, 1.0; P=0.058) and an 8% decrease in likelihood of *L. monocytogenes* isolation (OR=0.92, 95%CI=0.84, 1.0; P=0.088).

c) ***Association between likelihood of L. monocytogenes, Salmonella, and STEC detection in produce farms located in proximity to water:*** A 3-year observational study (1) conducted between 2009 and 2011 in New York State, USA, found that the likelihood of *L. monocytogenes* was greater in fresh produce fields located closer to water sources as opposed to farther away. **Methods:** A longitudinal field study was conducted on five produce farms in New York. Selected farms were sampled between June 2009 and August 2011 (9 times per farms, with at least 2 sampling visits [per year] in each of the four seasons, spring, summer, winter, and fall). At each sampling visit, one composite soil sample (total N=178) and one drag swab sample (total N=175) were collected from each of four produce fields. At each field, up to 5 water samples [total N=174, engineered (N=28) and surface (N=146)] and fecal samples (N=61) were collected (when available). All samples followed 3 separate enrichment methods, to allow the isolation and detection of *L. monocytogenes*, *Salmonella*, and STEC. Classification Tree (CT) models were fit to further explore the environmental and topographic variables that were associated with the detection of *L. monocytogenes* and *Salmonella*. **Results:** Across all samples 15.0%, 4.6%, and 2.7% of the samples were positive for *L. monocytogenes*, *Salmonella*, and STEC. The prevalence of *L. monocytogenes* was higher in surface water samples (33%; 48/146) than fecal (15%; 9/61), soil (9%; 16/178), and drag swab (9%; 15/1758) samples. Similarly, prevalence of *Salmonella* was higher in surface water samples (11%; 16/146) than fecal (7%; 4/61), soil (2%; 4/178), and drag swab (2%; 3/175) samples. The prevalence of STEC was marginally higher in fecal (7%; 4/61) than drag swab (3%; 5/175), surface water (3%; 4/146), and soil (2%; 3/178) samples. All engineered water samples (N=28) were negative for all 3 pathogens. The CT model determined that locations within 37.5 m of waterways had a predicted *L. monocytogenes* prevalence of 39% (N=74) (all 74 samples within 37.5 m of mapped waterways were surface waters) compared to 12% (N=514) in locations more than 37.5 m. It is important to note that this study did identity proximity to water as a surrogate rule in the *Salmonella* CT, with samples collected near water have a substantially higher likelihood of being *Salmonella* positive compared to samples collected farther from water.

d) ***Generic E. coli prevalence in wetland soils compared to soils from areas under other cover types:*** A replicated, observational study (5) conducted in 2018 collected soil samples from various landscapes surrounding the Buffalo River, MN, USA. The study found that land cover type was an influential driver of generic *E. coli* prevalence in surface soils, with prevalence in wooded wetland soils being higher than in cropland, grassland, scrubland and herbaceous wetland soils but lower than pasture or forest soils. **Methods:** The study area comprised 585,589 ha within a 20 km radius around the Buffalo River, MN, USA. Using a spatially explicit landscape sampling design, the study area was stratified by surface soil pH (5.9 to 6.7, 6.7 to 7.3, and 7.3 to 8.2) for all sampling locations. The study area was further sub-divided into 5 zones (based on predicted soil pH values). At each sampling site (total N=143), 10 surface soil samples (0-4 in. depth, total samples N = 1,430)

were collected. During sample collection, observations about the sampling area (land cover type, vegetation type, soil moisture, soil consolidation, and density of vegetation) were recorded. Generic *E. coli* levels in samples were enumerated. Following isolation, phylogroup (B1, B2, D, or E, total isolates N=3,329) was determined by single-nucleotide polymorphism qPCR assays, and whole-genome sequencing. **Results:** Landscape composition within the 20-km buffer of the Buffalo River was: 36% cropland, 29% forest, 10% open water, 6% wooded wetland, 6% pasture, 5% herbaceous wetland, 4% urban, 3% grassland, and 1% scrubland. The overall prevalence of generic *E. coli* among all soil samples was 41% (581/1428). The majority of generic *E. coli* isolates (87%, total N=3,329) belonged to the focal phylogroups (43% B1, 19% B2, 18% D, and 8% E). Regardless of phylogroup, *E. coli* prevalence was greatest in pasture (73%) and forest (73%) soils. Following pasture and forest soils, *E. coli* prevalence was greatest in wooded wetland (65%) soils compared to herbaceous wetland (44%), grassland (19%), cropland (19%), and scrubland (15%) soils.

e) ***Generic E. coli prevalence in Spinach farms located at proximity to water resource:*** A 2-year replicated, observational study (4) conducted between 2010 and 2011, in spinach fields in Western Colorado and in Southwestern Texas, USA, found that generic *E. coli* was not significantly more prevalent in spinach fields near (≤ 10 miles) water sources compared to fields farther away (> 10 miles). **Methods:** A total of 955 spinach samples were collected from 1 to 6 fields per visit from each of 12 spinach farms [Colorado (n=4) and Texas (n=8)]. Each sample consisted of at least 10 spinach leaves and generic *E. coli* levels in each sample were enumerated. At each farm visit, a questionnaire was also administered to obtain information on farm management and environmental factors. In total, 76 explanatory variables were considered for the univariable analysis. The variables association with spinach contamination at P=0.20 significance level were identified and used to build a full multivariable. The full model was then reduced by backwards selection based on p-value to a final model. The variables associated with spinach contamination at a 20% significance level (univariate, P=0.2) were identified and included in a final multivariate model. **Results:** The prevalence of generic *E. coli* was 6.6% (63/955) across all spinach samples. Univariable regression analysis suggested that the probability of detecting generic *E. coli* was not affected by proximity to water sources (P> 0.2).

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4.3. Increase distance between produce fields and non-grazed natural lands (5 studies): Five observational studies from the USA reported the prevalence of pathogens and generic *E. coli* in produce fields near versus far from non-grazed natural lands. Three studies (1-3) found evidence that prevalence of generic *E. coli* and *Listeria* were greatest in samples near scrubland or forested areas, in some or all comparisons. Two studies (4, 5) found no significant association between the prevalence of generic *E. coli*, EHEC, and *Salmonella* in produce fields or irrigation water and distances to nearest riparian or natural vegetation area.

a) ***Generic E. coli prevalence in soils collected from areas under forest and other land cover:*** A replicated, observational study (1) conducted in 2018, collected soil samples from various landscapes surrounding the Buffalo River, Minnesota, USA. Generic *E. coli* was most prevalent in soil samples collected from forested or forest-adjacent areas compared to samples collected from cropland, grassland, scrubland, and wetland areas. **Methods:** The study area comprised a 585,589 ha within a 20 km radius around the Buffalo River, MN, USA. Using a spatially explicit landscape sampling design, the study area was stratified by surface soil pH (5.9 to 6.7, 6.7 to 7.3, and 7.3 to 8.2) for all sampling locations. The study area was further sub-divided into 5 zones (based on predicted soil pH values). At each sampling site (total N=143), 10 surface soil samples (0-4 in depth, total N = 1,428) were collected (N=1,430 samples total). During sample collection, observations about the sampling area (land cover type, vegetation type, soil moisture, soil consolidation, and density of vegetation) were recorded. Generic *E. coli* levels in samples were enumerated. Following isolation, phylogroup (B1, B2, D, or E, total isolates N=3,329) was determined by single-nucleotide polymorphism qPCR assays, and whole-genome sequencing. **Results:** The landscape composition within the 20-km buffer of the Buffalo River was: 36% cropland, 29% forest, 10% open water, 6% wooded wetland, 6% pasture, 5% herbaceous wetland, 4% urban, 3% grassland, and 1% scrubland. The overall prevalence of generic *E. coli* among all soil samples was 41% (581/1428). The majority of generic *E. coli* isolates (87%, total N=3,329) belonged to the focal phylogroups (43% B1, 19% B2, 18% D, and 8% E). Regardless of phylogroup, *E. coli* levels/prevalence was greatest in forest (73%) and pasture (73%) soils compared to wooded wetland (65%), herbaceous wetland (44%), grassland (19%), crop land (19%), and scrubland (15%) soils. Percent forest cover and percent cropland cover were strongly inversely correlated (R = -0.72, correlation tests). As identified by random forest analysis, distance to nearest forest and percent forest cover were the best predictors of *E. coli* isolation from surface soil. Similarly, the likelihood of detecting generic *E. coli* was almost 20-fold lower in cropland compared to forest areas; samples collected within 30 m from forest had a 5-fold higher risk for *E. coli* isolation compared to those sample collected farther away.

b) ***Listeria spp. and L. monocytogenes detection in samples collected from produce fields at different distances from forest and scrubland:*** A 2014 replicated, observational study (2) conducted in produce fields in New Yok, USA, found that *Listeria* was more likely to be isolated from fields near forest and scrubland compared to fields far from forest and

scrubland. **Methods:** A cross-sectional study was conducted on produce farms (total N=4) in New York State: western New York (N=2), the Hudson Valley (N=1), and the Capital District (N=1). Fields within each farm were classified by predicted prevalence of *L. monocytogenes* isolation based on land cover factors, using models from previous studies. Selected land cover factors included: distance from water source, distance from roads, and distance from pastures. Distance from the field center to land cover (barren land, grassland, forest, scrubland, water, road, pasture, and wetlands) was calculated to identify their association with *Listeria* isolation from produce fields. For each farm, ArcGIS shapefiles (hydrology, road, and pasture) were generated, and categorized as areas of high (e.g., <62.5 m from pasture) or low (e.g., > 62.5 m from pasture) predicted *L. monocytogenes* prevalence according to splits in the previously published model. Fields were then divided into 5X5 m plots, and a subset of plots was sampled randomly (i.e., one drag swab per plot from each high/low risk category). All samples (total N=1,056) were enriched to isolate *Listeria* spp. and *L. monocytogenes*. Distance from the sampling site to each land cover class (barren land, grassland, forest, scrubland, water, road, pasture, and wetlands) was calculated to facilitate identification of associations with Listeria isolation. **Results:** Overall, 20% of samples (208/1056) were positive for *Listeria* spp. and 12% of samples (128/1056) were positive for *L. monocytogenes*. Proximity to forest and scrubland were significantly associated with *Listeria* positive samples in univariable and multivariable GLMM analyses. For example, for a 100-m increase in the distance of a sampling site from forests, the likelihood of *Listeria* spp. isolation decreased by 16% (OR=0.84, 95% CI=0.74, 0.95; P=0.009) and the likelihood of *L. monocytogenes* isolation decreased by 14% (OR=0.86, 95%CI=0.74,1.0; P=0.060). In the final multivariable model, for a 100 m increase in the distance of a sampling site from forests, the likelihood of *L. monocytogenes* isolation decreased by 13% (OR=0.87, 95% CI=0.76, 0.99; P=0.031). Similarly, for the univariable analyses, for a 100-m increase in the distance of a sampling site from scrubland, the likelihood of *Listeria* spp. isolation decreased by 7% (OR=0.93, 95% CI=0.88, 0.99; P=0.044) and the likelihood of *L. monocytogenes* isolation decreased by 12% (OR=0.88, 95%CI=0.81, 0.95; P=0.002). In the final multivariable model, for a 100 m increase in the distance of a sampling site from scrubland, the likelihood of *Listeria.* spp. isolation decreased by 6% (OR=0.94, 95% CI=0.88, 1.0; P=0.042) and the likelihood of *L. monocytogenes* isolation decreased by 14% (OR=0.86, 95% CI=0.79, 0.93; P<0.001). The authors noted that the effect of proximity to natural cover may not be a function of the presence of natural cover per se but instead may be driven by the fact that the natural-agricultural border represents an ecotones (the transitional area where two ecological communities meet) and suggested growers could create buffer zones of unharvested product near the edges of fields, increase surveillance and/or decontamination of produce grown near field edges, or stage harvesting and processing so that higher-risk material (i.e., produce grown near field edges) is harvested and processed last.

c) ***Association between generic E. coli, E. coli O157 and Salmonella spp. occurrence in produce farms and proximity to vegetation and riparian areas:*** A 3 year replicated, observational study (4) conducted between May 2008 and October 2010 in the Central Coast of California, USA found no association between generic *E. coli* concentrations in produce farms and distances from fields to non-crop vegetation or riparian areas. **Methods:** Produce farms (N=16) that grow leafy green vegetables and agricultural water sources sites (total N=241) in the Central California Coast region were repeatedly sampled during the study period. Water and sediment samples were collected from produce farms (N=16) and water sources that had public access near the farms. A total of 255 irrigation water samples and 181 sediment samples were collected from produce farms; sample types included; well water, reservoir, irrigation, furrow, ditch, pond, stream, river, and creek. A total of 389 non-irrigation water samples and 159 sediment samples were collected from water sources with public access (ditch, pond, stream, river, and creek). Generic *E. coli* levels were enumerated in each sample using the culture-based method. For a subset of samples, enrichment was performed to detect *E. coli* O157 (N=436) and *Salmonella* (N=249). Models were fit to explore associations between distance from the sample location to rangeland, vegetation or riparian habitats, and roads and detection of generic *E. coli* levels. **Results:** In total, 6.3% of the water samples (irrigation 1/60, and non-irrigation 5/36) and 4.8% of the sediment samples (irrigation 2/37, and non-irrigation 3/64) were positive for *Salmonella.* One water sample from produce farms (0.4%; total N=242) and nine water samples with public access (2.8%; total N=316) were positive for *E. coli* O157. On produce farms, 78% of the water samples (199/255; mean $7.1X10^2$ MPN/100 ml) and 36% of the sediment samples (65/81; mean $1.0X10^4$ CFU/100g) were positive for generic *E. coli*. Univariable associations between generic *E. coli* concentration in irrigation water on produce farms and distances to non-crop vegetation or riparian areas were not significant (0.001 P=0.41, 95% CI = −0.001, 0.003).

d) ***Generic E. coli Salmonella and EHEC prevalence in produce farms samples collected close to as opposed to far from non-grazed riparian vegetation:*** A 2015 observational study (5) conducted in the California Central Coast, USA found no evidence that EHEC in fresh produce, generic *E. coli* in water, and *Salmonella* in rodents or leafy green vegetables were more prevalent near non-grazed riparian or semi-natural vegetation. **Methods:** EHEC, which includes *E. coli* O157:H7 and STEC, and *Salmonella* data were acquired from an organic farming operation, which collected the data between 2007 and2013. A total of 482,208 produce samples originating from 295 farms spread across USA were tested for EHEC and *Salmonella* at the organic farming operation. Data on generic *E. coli* in irrigation water that was collected monthly between 2007 and 2010, was acquired from the leafy green agricultural industry. Finally, between Oct, 2009 and Aug, 2011, fecal samples were collected from 11 wild rodent species, trapped at 9 produce farms in California, USA. Fecal samples were enriched to detect the presence/absence of *Salmonella*. Data from microbial analysis and estimates of land cover maps (data reflect

changes in land cover over the study period) were used in statistical models to understand the effect of land use change on the prevalence of pathogens in the produce fields. **Results:** The study reported that no evidence of increased prevalence of EHEC in fresh produce, generic *E. coli* in water, and *Salmonella* in rodents or leafy green vegetables near areas surrounded by non-grazed riparian or other seminatural vegetation. However, removal of riparian and other natural vegetation was significantly associated with increased *Salmonella* and EHEC prevalence, respectively.

e) ***Generic E. coli prevalence in samples collected from spinach farms close to and far from forest cover:*** A 2-year replicated, observational study (3) conducted from 2010 to 2011, in spinach fields in Western Colorado and in Southwestern Texas, USA, found that generic *E. coli* was significantly more prevalent in spinach grown in fields located near (≤ 10 miles) forests compared to fields farther away from forest (> 10 miles). **Methods:** A total of 955 spinach samples were collected from 1 to 6 fields per visit from each of 12 spinach farms [Colorado (n=4) and Texas (n=8)]. Each sample consisted of at least 10 spinach leaves and generic *E. coli* levels in each sample were enumerated. At each farm visit, a questionnaire was also administered to obtain information on farm management and environmental factors. In total 76 explanatory variables were considered for the univariable analysis. The variables associated with spinach contamination at P=0.20 significance level were identified and used to build a full multivariable. The full model was then reduced by backwards selection based on p-value to a final model. **Results:** The prevalence of generic *E. coli* was 6.6% (63/955) across all spinach samples. Based on univariable analysis, the odds of spinach contamination were significantly (P=0.002) higher for fields in proximity (within 10-mile radius) to forests (odds ratio = 0.11; 95% CI = 0.03, 0.43) compared to fields located farther away (> 10-mile radius). However, the proximity to forest was not included in the final multivariable model presented by the authors.

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4.4. Increase distance between produce fields and urban areas (5 studies): Five observational studies from the USA (1-5) reported the prevalence of generic *E. coli*, *Listeria,* and *Salmonella* in produce fields as a function of proximity to roads/impervious cover/urban areas. Two observational studies (2, 4) reported that proximity to roads increased the likelihood of *Listeria* detection in produce fields. Three observational studies (1, 3, 5) found no significant evidence that generic *E. coli* concentration in produce or irrigation water, as well as *Listeria* in produce fields, were influenced by proximity to roads, landfills, or residential areas.

a) ***Listeria spp. and L. monocytogenes detection in samples collected from produce fields at different distances from roads:*** A 2014 replicated, observational study (1) conducted in Upstate New York produce fields, USA, found no statistically significant evidence that fields closer to urban developments (*i.e.*, roads ≤ 9 m) were at higher risk of *Listeria* isolation compared to fields located farther away from urban developments (road > 9 m). **Methods:** A cross-sectional study was conducted on produce farms (total N=4) in New York State: western New York (N=2), the Hudson Valley (N=1), and the Capital District (N=1). Fields within each farm were classified by predicted prevalence of *L. monocytogenes* isolation, based on land cover factors (using models from previous studies). Selected land cover factors included: distance from water source, distance from roads, and distance from pastures. For each farm, ArcGIS shapefiles (hydrology, road, and pasture) were generated, and categorized as areas of high (e.g., areas within 62.5 m from pasture) or low (e.g., areas > 62.5 m from pasture) predicted L. *monocytogenes* prevalence, according to splits in the previously published model. Fields were then divided into 5X5 m plots, and a subset of plots was sampled randomly (i.e., one drag swab per plot from each high/low risk category). All samples (total N=1,056) were enriched to isolate *Listeria* spp. and *L. monocytogenes*. Distance from the sampling site to each land cover class (barren land, grassland, forest, scrubland, water, road, pasture, and wetlands) was calculated to facilitate identification of associations with Listeria isolation. **Results:** Overall, 20% of samples (208/1056) were positive for *Listeria* spp. and 12% of samples (128/1056) were positive for *L. monocytogenes*. Prevalence of *Listeria* spp. was 21% in samples (N=36/168) collected from plots closer to roads (i.e., ≤ 9 m from roads) and 17% in samples (121/693) collected from plots farther from roads (i.e., > 9 m from road). Prevalence of *L. monocytogenes* was 7% in samples (N=11/168) collected from plots closer to roads (i.e., ≤ 9 m from roads) and 11% in samples (74/693) collected from plots away from roads (i.e., > 9 m from road). However, the univariable and multivariable models in this study did not include proximity to roads as a food safety risk factor.

b) ***L. monocytogenes, Salmonella, and STEC prevalence in produce samples collected close to and far from urban areas:*** A 3-year observational study (2) from 2009 to 2011 in New York State, USA, found that proximity to roads or other urban developments increased the likelihood of *L. monocytogenes* detection in the fresh produce fields. **Methods:** A longitudinal field study was conducted on five produce farms in New York State: central New York (N = 1), the Finger Lakes (N = 3), and Western New York (N = 1). Selected

farms were sampled between June 2009 and August 2011 (in total 9 times, once per season summer, fall, winter, and spring in each year). At each sampling visit, a composite soil sample (total N=178) and one drag swab sample (total N=175) were collected from each of four produce fields per visit. At each field, up to 5 water samples [total N=174, engineered (N=28) and surface (N=146)] and fecal samples (N=61) were collected (when available). All samples underwent 3 separate enrichment methods, to allow the isolation and detection of *L. monocytogenes*, *Salmonella*, and STEC. Classification Tree (CT) models were fit to explore associations between environmental variables and detection of *L. monocytogenes* and *Salmonella*. **Results:** Across all samples 15.0%, 4.6%, and 2.7% of the samples were positive for *L. monocytogenes*, *Salmonella* and STEC. The prevalence of *L. monocytogenes* was higher in surface water samples (33%; 48/146) than fecal (15%; 9/61), soil (9%; 16/178), and drag swab (9%; 15/1758) samples. Similarly, prevalence of *Salmonella* was higher in surface water samples (11%; 16/146) than fecal (7%; 4/61), soil (2%; 4/178), and drag swab (2%; 3/175) samples. The prevalence of STEC was marginally higher in fecal (7%; 4/61) than drag swab (3%; 5/175), surface water (3%; 4/146), and soil (2%; 3/178) samples. Engineered water samples (N=28) were all negative for all 3 tested pathogens. The CT model determined that locations within 9.5 m of urban development (i.e., roads and other impervious substrates) had a predicted *L. monocytogenes* prevalence of 20%, when compared to 5% prevalence for locations farther than 9.5 m. In conclusion being closer to road increased the likelihood of detecting *L. monocytogenes* in produce farm environments. The authors did not find evidence of an association between proximity to road and likelihood of *Salmonella* detection.

c) ***Generic E. coli, E. coli O157 and Salmonella spp. occurrence in produce farm environmental samples collected close to and far from roads:*** A 3 year replicated, observational study (3) conducted between May 2008 and October 2010 in the Central Coast of California, USA found no association between the generic *E. coli* concentration in irrigation water on produce farms and distance to the nearest road as opposed to far from roads. **Methods:** Produce farms (N=16) that grow leafy green vegetables and agricultural water sources sites (total N=241) in the Central California Coast were repeatedly sampled during the study period. Water and sediment samples were collected from produce farms (N=16) and water sources that had public access near farms. A total of 255 irrigation water samples and 181 sediment samples were collected from produce farms; sample types included; well water, reservoir, irrigation, furrow, ditch, pond, stream, river, and creek. A total of 389 non-irrigation water samples and 159 sediment samples were collected from water sources with public access (ditch, pond, stream, river, and creek). Generic *E. coli* levels were enumerated in each sample using the culture-based method. For a subset of samples, enrichment was performed to detect *E. coli* O157 (N=436) and *Salmonella* (N=249). Models were fit to explore associations between distance from the sample location to rangeland, vegetation or riparian habitats, and roads and detection of generic *E. coli* levels. **Results:** In total, 6.3% of the water samples (irrigation 1/60, and non-irrigation

5/36) and 4.8% of the sediment samples (irrigation 2/37, and non-irrigation 3/64) were positive for *Salmonella.* One water sample from produce farms (0.4%; total N=242) and nine water samples with public access (2.8%; total N=316) were positive for *E. coli* O157. On produce farms, 78% of the water samples (199/255; mean $7.1X10^2$ MPN/100 ml) and 36% of the sediment samples (65/81; mean $1.0X10^4$ CFU/100g) were positive for generic *E. coli*. Generic *E. coli* concentration in irrigation water on produce farms was not significantly associated with the distance to the nearest road (Coefficient = -0.003, P=0.41, 95% CI = −0.01, 0.004).

d) ***Generic E. coli prevalence in spinach farms located at varying distance from roads, landfill, and residential areas:*** A 2-year replicated, observational study (5) conducted from 2010 to 2011, in spinach fields in Western Colorado and in Southwestern Texas, USA, found that generic *E. coli* was not significantly more prevalent in spinach grown in fields near (≤ 10 miles) to roads, landfills, or residential areas compared to fields farther away. **Methods:** A total of 955 spinach samples were collected from 1-6 fields per visit from each of 12 spinach farms [Colorado (n=4) and Texas (n=8)]. Each sample consisted of at least 10 spinach leaves and generic *E. coli* levels in each sample were enumerated. At each farm visit, a questionnaire was also administered to obtain information on farm management and environmental factors. In total 76 explanatory variables were considered for the univariable analysis. The variables associated with spinach contamination at P=0.20 significance level were identified and used to build a full multivariable. The full model was then reduced by backwards selection based on p-value to a final model. **Results:** The prevalence of generic *E. coli* was 6.6% (63/955) across all spinach samples. Based on univariable analysis, the odds of spinach contamination were significantly (P<0.001) higher for fields in proximity (within 10-mile radius) to roads (odds ratio = 0.07; 95% CI = 0.02, 0.28) compared to fields located farther away (> 10-mile radius) from roadways; however, the final multivariable model did not include proximity to roads. Finally, univariable tests suggested that the probability of detecting generic *E. coli* was not affected by proximity to landfills or residential areas (P> 0.2); thus, neither variable was tested in the final multivariate model.

e) ***Listeria spp. and L. monocytogenes prevalence in produce fields near versus far from roads:*** A 2014 observational study (4) conducted in spinach fields in New York, USA found that landscape factors accurately predicted the risk of *L. monocytogenes* contamination in produce fields, with *L. monocytogenes* being more likely to be detected in fields closer to roads. **Methods:** A longitudinal study was conducted in two spinach fields (0.2 ha) over a 7-week period (May – July, 2014). Field-A was categorized as high-risk, (i.e., field was ≤ 37.5 m from water source and ≤ 9.5 m from a road) and Field-B was categorized as low risk (i.e., > 37.5 m from a water source and >9.5 m from a road) for *L. monocytogenes* contamination based on models from previous studies. Both fields were divided into 21 (13X13 m) plots. Fields were prepared for planting and fertilized at a rate of 789 kg/ha (13-13-13 NPK). Fields were irrigated with creek water via overhead

irrigation. Soil sample sites within each plot were randomly selected, with a new sampling site being used per plot on each visit (i.e., on sample per plot per visit). Soil samples (total N=1,092) were collected on the day of planting and 24, 48, 72, and 144-192 h after an irrigation or rain event. Water samples (total N=52) were collected from the irrigation water source, which was a creek. Fecal samples (total N=14) were collected when detected within 5m of the sampled fields or water source. Composite leaf samples (total N=334) were hand collected (beginning 36 days after planting) along the perimeter and diagonals of the plot. Samples (total N=1,492) were enriched to isolate *Listeria* spp. and *L. monocytogenes*. Isolates were identified to allelic type by partial *sigB* gene sequences. **Results:** Overall, 14% of the samples (204/1,492) were positive for *Listeria* spp.; specifically, 90% of water samples (47/52), 79% of fecal samples (11/14), 12% of soil samples (126/1,092), and 6% of leaf samples (19/334). *L. monocytogenes* was positive in 9% of the overall samples (130/1,492); specifically, 64% of fecal samples (9/14), 63% of water samples (33/52), 8% of soil samples (86/1,092) and 0.6% of leaf samples (2/334). *Listeria* were more prevalent in soil samples collected from the high-risk field, which was close to water and roads (*Listeria* spp. 15%; 84/546, and *L. monocytogenes* 11%; 62/546) compared to the low-risk field (*Listeria* spp. 8%; 42/546, and *L. monocytogenes* 4%; 24/546), which was farther was farther from road. The odds of isolating *Listeria* spp. (OR = 2.3; 95% CI = 1.5, 3.5, P<0.01) and *L. monocytogenes* (OR = 3.5; 95% CI = 2.0, 6.0, P<0.01) were 2 times and 3.5 times greater for soil samples collected from the high-risk field compared to the low-risk field. Both univariate (P<0.01; OR=0.69; 95% CI=0.57, 0.85) and multivariate analysis OR=6.3; 95% CI = 1.6, 25; P=0.01), reported that proximity to roads was associated with an increased likelihood of isolating *L. monocytogenes* from soil samples collected in produce fields.

References:

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5. Agricultural water management

5.1. Irrigate fields with low-risk water source (12 studies**):** 8 of 12 studies (6 observational and 2 experimental) found evidence that food-safety risks varied as a function of the source of irrigation water. In general, groundwater and/or well water carried fewer food-safety risks compared to standing/surface water sources, as reported in some or all comparisons in 8 studies (1-8). Several studies also compared pathogen contamination between reclaimed/wastewater, groundwater, and roof-collected water, generally finding reclaimed/wastewater to carry the highest risks (2, 9). That said, other studies showed that bacterial loads can exceed FSMA irrigation water standards in roof-collected water (10) but that bacterial loads may depend on the type of the roofing material used (11). Finally, one study (12) reported very low incidence of pathogen detection in produce and soil and due to this low prevalence could not link pathogen detection to the type of irrigation water used.

a) ***Salmonella prevalence in pond versus well irrigation water:*** A 4-year replicated, controlled, observational study (1) conducted in tomato fields in Delmarva VA, USA, found higher prevalence of *Salmonella* in pond irrigation water compared to well irrigation water. **Methods:** Four field experiments were performed between 2012 and 2015 using a split-pot design. The plots used in the split-pot were: fresh poultry litter, poultry litter ash, and conventional fertilizer. Amendments were added 1 week before transplanting 7-week-old tomatoes into the field, to achieve a standard rate of P_2O_5 (100 kg / hectare); specifically, 25 Kg of fresh poultry litter, 4 kg of poultry litter ash, or 2 Kg of conventional fertilizer (Triple Super Phosphate) were applied. Each plot was 12.5 m x 0.6 m, and contained ~30 tomato plants. Plants were drip irrigated daily (~6L water/sub plot). Pond and well irrigation water were sampled once monthly: Aug 2012 and Dec 2013 (17 months), 2014 (12 months), and 2015 (12 months). *Salmonella* concentration in water samples were enumerated by a culture-based method. **Results:** *Salmonell*a was consistently isolated from the pond water each year. The prevalence of *Salmonell*a in pond water was 64.7% (2012 to 2013; average 0.77±0.31 MPN/L), 11.8% (2014; average 4.06±1.86 MPN/L), and 12.2% (2015; 2.30±1.0 MPN/L). All samples were negative for *Salmonella* from irrigation well water. The authors report no significant relationships between environmental factors (rain and temperature) and *Salmonella* prevalence in irrigation pond water. In conclusion, use of untreated pond water may increase the likelihood of *Salmonella* detection in produce.

b) ***Salmonella enterica, STEC, and generic E. coli prevalence in ground and surface irrigation water:*** A replicated, observational study (8) from July to September 2012, at tomato farms in Maryland, Delaware and New Jersey, USA, found that generic *E. coli* counts were significantly lower in ground water samples when compared to surface water samples. **Methods:** Based on a grower survey, tomato farms (N=24) in Maryland,

Delaware, and New Jersey, USA were classified as organic certified/non-certified (N=12) or conventional (N=12). During the harvest season, tomato fruit, soil, compost, and water samples were collected from the fields and levels of STEC, *Salmonella enterica*, and indicator bacteria (generic *E. coli* and total coliforms) were enumerated. *Salmonella* (*invA*) and STEC (*stx1* and *stx2*) markers were detected using PCR amplification; presumptive PCR-positive samples were culture confirmed by enrichment. A total of 422 samples were collected from 24 farms: 259 tomato samples (130 conventional and 129 organic), 45 soil samples, 9 pond sediment samples, 7 compost samples, and 102 water samples (N=40 wells, N=17 ponds, N=4 creeks and streams, and N=41 end of irrigation system lines). 23 farms used drip/trickle irrigation, and 1 farm used overhead sprinkler irrigation. **Results:** Few samples were positive for *stx* genes (3/422 samples, 0.7%): 2 from surface irrigation water samples (*stx*2) and 1 from tomato fruit (*stx*1 and *stx*2). Similarly, 5/422 (1.18%) of the samples were positive for *Salmonella inv*A: 2 tomato samples, 2 water samples, and 1 soil sample. All samples were negative for STEC and *Salmonella* following enrichment. Prevalence of generic *E. coli* was 36.1% (26/72) ground water samples and 70% (21/30) in surface water samples, a statistically significant difference ($P< 0.0001$). End of the line water samples (both ground $p=0.351$, and surface $p=0.451$) were not significantly different from the source surface water samples. In conclusion, the source of irrigation water was a significant factor for generic *E. coli*, where groundwater had lower prevalence when compared to surface water samples.

c) ***Salmonella, Listeria, and E. coli (pathogenic and generic) prevalence in ground versus secondary-treated sewage and roof-harvested water:*** A replicated experimental study (2) between July and October, 2017, at spinach farms in PA, USA, examined the suitability of 3 different irrigation water sources for spinach irrigation. The study found no pathogens (*Salmonella*, *L. monocytogenes*, and *E. coli* O157:H7) in irrigation water collected from ground, secondary-treated wastewater, and roof-harvested rain water (or in produce samples). However, ground water may contain higher generic *E. coli* populations when compared to secondary-treated wastewater and roof harvested rain water. **Methods:** Two field experiments were performed in summer (July, 2017) and fall (October, 2017). The study included 3 different irrigation water sources: ground water, secondary treated wastewater, and roof-harvested rain water. Each field consisted of 6 plots, two plots per water type, and each of which was 5 X 2 m. Up to six weeks, the spinach plants in all 6 plots were spray irrigated once a week with ground water. After 6 weeks, spinach plants were spray irrigated once a week with the corresponding water treatment (N=3, 16 liters per plot). Spinach and surface soil samples were collected pre-irrigation (N=1) and post-irrigation (N=8, for 2-weeks at 0, 1, 2, and 4 days post-irrigation). All samples (spinach N=240, water N=12, and soil N=240) were enumerated for generic *E. coli* and selected pathogens (*Salmonella*, *L. monocytogenes*, and *E. coli* O157:H7) via enrichment methods. Before irrigation, water samples (N=12) from all 3 treatments were enumerated for generic *E. coli*, Enterococci, and the presence/absence of pathogens. **Results:** All samples (water,

soil, and spinach) were negative for *Salmonella, L. monocytogenes*, and *E. coli* O157:H7. All water samples were positive for generic *E. coli* (range from 0.6 to 3.99 CFU per 100 ml) and Enterococci (range from 0.3 to 3.9 CFU per 100 ml), except one wastewater sample. Secondary treated wastewater had less generic *E. coli* (range from 0 to 0.85 CFU per 100 ml) and Enterococci (range from 0 to 1.76 CFU per 100 ml) when compared to groundwater (*E. coli* range from 0.6 to 3.9; Enterococci range from 0.8 to 3.9 CFU per 100 ml) and roof harvested rainwater (*E. coli* range from 0.3 to 2.7; Enterococci range from 2.3 to 2.9 CFU per 100 ml). The authors report that the source of the irrigation water was a significant factor for generic *E. coli* populations ($P<0.0001$, degree of freedom=2, F value = 12.38) but not a significant factor for *Enterococcus* populations.

d) ***Generic E. coli prevalence in spinach from farms irrigated with various water sources:*** A 2-year replicated, observational study (3) conducted from 2010 to 2011 in spinach fields in Western Colorado and the Southwestern Texas, USA, found that the odds of spinach contamination with generic *E. coli* was significantly higher in fields irrigated with pond water compared to other water sources (well, reservoir, municipal, and river). **Methods:** A total of 955 spinach samples were collected from 1 to 6 fields per visit from each of 12 spinach farms [Colorado (n=4) and Texas (n=8)]. Each sample consisted of at least 10 spinach leaves and generic *E. coli* levels in each sample were enumerated. At each farm visit, a questionnaire was also administered to obtain information on farm management and environmental factors. The questionnaire asked, for example, what is the source of irrigation water used in each farm (pond/well/municipal/reservoir/river). In total, 76 explanatory variables were considered for the univariate analysis. The variables associated with spinach contamination at P=0.20 significance level was identified and used to build a full multivariate model. The full model was then reduced by backwards selection based on p-value to a final model. **Results:** The prevalence of generic *E. coli* was 6.6% (63/955) across all spinach samples. Based on univariate analysis, spinach from fields irrigated with well (OR= 0.30: 95% CI=0.06, 1.51, P=0.144) and reservoir (OR= 0.08; 95% CI= 0.01, 0.40; P= 0.002) water were less likely to be contaminated with generic *E. coli* compared to spinach from fields irrigated with pond (OR= 24.4; 95% CI = 2.1, 280.1; P=0.010) water. The other sources of irrigation water (municipal, river) were not significant risk factors ($P>0.2$). Based on final multivariate model, the odds of spinach contamination with generic *E. coli* was higher in pond water (OR=64.4; 95% CI=4.9, 855.3; P=0.002) compared to all other water sources. However, it is important to note that only 1 out of 12 farms used pond water for spinach irrigation.

e) ***Listeria and Campylobacter prevalence in lettuce fields irrigated with manure-contaminated water:*** A 3-year replicated, experimental study (12) between 2010 and 2012 from Quebec, Canada, found no evidence that irrigating with manure-contaminated water increased pathogen incidence on produce or in soil. **Methods:** Field experiments were conducted in summer of 2010 to 2012 on muck soil, Quebec, Canada. The experimental plots were arranged in a split-plot factorial design. Specifically, plots were sub-divided into

two irrigation water sources (manure contaminated vs aerated pond water) as the main factor and 3 pre-harvest time intervals (21, 7, and 3 days) as the sub-plot factor, resulting in 6 production conditions (2 water sources x 3 time intervals), with each replicated 4 times. Each plot contained 104 lettuce plants (with 13 rows in each plot). Water from an aerated pond (recharged by ground water, minimal surface runoff, and no history of manure application in the surrounding landscape) was pumped into 2 (10,000 L) reservoirs. One of the reservoirs was contaminated with manure (mix of hog and bovine slurry, 2.33 L of manure in 10,000 L of water). Lettuce fields were then spray irrigated with either contaminated or aerated pond water. Samples of manure, soil, water, and lettuce were tested for the presence/absence of *Listeria monocytogenes* and *Campylobacter* spp. All presumptive colonies were confirmed via PCR. **Results:** All samples were negative for *C. jejuni*. All aerated pond water samples were negative for *C. coli* and *L. monocytogenes*. Overall, 3 out of 18 manure samples and 3 out of 54 irrigation water samples were positive for *C. coli*. All manure samples (N=18) and irrigation water samples (except one, 53/54) were negative for *L. monocytogenes*. All soil samples (N=288) and lettuce samples (N=288) were negative for *C. coli* and *L. monocytogenes* (except one sample positive for *L. monocytogenes* in lettuce). Overall, this study had low incidence of pathogen detection in soil and lettuce samples; hence, the authors could not link pathogen incidence in produce and soil with the type of irrigation water used.

f) ***Generic E. coli and Enterococci prevalence in spinach irrigated with creek water, roof-harvested rainwater, and reclaimed water:*** A one-year replicated, experimental study (9) in 2018 in MD, USA, found higher levels of generic *E. coli* and Enterococci on spinach irrigated with reclaimed water when compared to spinach irrigated with creek or roof-harvested rain water. **Methods:** Spinach seeds were planted into container (N=50, 8.25X1.25 cm) that contained sandy soil. Planted containers were maintained in a growth chamber at 18-20 ℃. Following this, 6 trays were randomly assigned to each irrigation water treatment: reclaimed water (from a local wastewater facility), roof-harvested rain water, and creek water. Spinach plants were irrigated weekly using freshly collected treatment water (1L/tray). At each week, at 0 and 24h post-irrigation, spinach samples were collected aseptically. Spinach collected after week-2 had repeated exposure to the treatment water. At each week, water and spinach samples were enumerated for generic *E. coli* and enterococci. All samples followed an enrichment method to report the presence/absence of pathogens (*Salmonella*, *L. monocytogenes*, *Clostridium perfringens* and *E. coli* O157:H7). **Results:** All samples were negative for *Salmonella*, *L. monocytogenes*, and *E. coli* O157:H7. Generic *E. coli* in creek and roof-harvested rain water were within the limits of FSMA standards for irrigation water (< 2.1 log CFU/100 mL) and ranged from 0.0 to 1.45 log CFU/100 mL. Reclaimed water often exceeded the FSMA standards for irrigation water, with generic *E. coli* populations ranging from 2.3 to 3.36 log CFU/100 mL. Enterococci was negative in all samples (except one reclaimed water and one creek water sample). At 0 h post-irrigation, higher generic *E. coli* recovery

was observed from spinach irrigated with reclaimed water (25-100%) when compared to spinach irrigated with creek water (0-25%) or roof-harvested rainwater (0-25%). Similarly, at 24 h post-irrigation, higher generic *E. coli* recovery was observed from spinach irrigated with reclaimed water (0-75%) compared to spinach irrigated with creek water (0-25%) or roof-harvested rainwater (0%). With repeated irrigation treatments (week 2-4), the recovery of generic *E. coli* increased from 25 to 100% in spinach irrigated with reclaimed water compared to 0-25% in spinach irrigated with creek or roof-harvested rainwater. Likewise, the recovery of enterococci was higher in spinach irrigated with reclaimed water (0-66%) when compared to creek (0-17%) or roof-harvested rainwater (0%). The authors conclude that reclaimed water may not be a good alternative to irrigate fresh produce compared to creek or roof-harvested rainwater.

g) ***Prevalence of fecal E. coli, Enterococci, and other bacteria in roof-harvested rain water:*** A 3-year replicated, observational study (10) from 2014 to 2016 in PA, USA, found that roof-harvested rain water often posed a risk to food safety as fecal *E. coli* and Entercocci levels in these water samples (>10^3 per 100 mL) often (60%) exceeded the FSMA standards for irrigation water. **Methods:** Participants in this study were asked to fill out an online survey regarding the rain water collection barrel characteristics. Between 2014 to 2016, 38 water samples (1L) were collected from 34 roof-harvested rain water barrels (polyethylene, <1 to 10 years old) in PA, USA. Water samples were enumerated for Fecal *E. coli* and Enteroccoci. Total DNA extracted from water samples were used to quantify selected targets (*Acanthamoeba* spp., *Naegleria fowleri*, *Legionella* spp., *L. pneumophila*, *M. avium*, *M. intracellulare*, *Pseudomonas aeruginosa* and *C. jejuni*) via qPCR. The roofing material was often made of asphalt shingle (26 roofs, 50%), though others included plastic sheeting (3 sites, 12%), tar (1 site, 4%), slate (1 site, 4%), and rubber (2 roofs, 8%). Gutters were mostly made of aluminum (24 sites, 92%), but other materials included steel (1 site, 4%) and plastic (1 site, 4%). Water samples were collected from the barrel spigot (96%), except one barrel, where water samples were collected from the top of the barrel. Participants reported signs of wildlife at 39% of the study sites, and trees at 46% of the study sites. At all study sites, participants never treated the roof-harvested rain water and 15/38 sampling events occurred on wet days. **Results:** Overall, 62% and 77% of the water samples were positive for *E. coli* (mean=290 CFU/100mL) and Enterococci (mean=155 CFU/100mL), respectively. Prevalence of opportunistic pathogens included: *Legionella* spp. (57.9%; mean = 2.47 X 10^4 copies per L), *M. intracellulare* (44.7%; mean = 2.59 X 10^3 copies per L), *M. avium* (21.1%; mean = 1.30 X 10^3 copies per L), *Acanthamoeba* spp. (18.4%; mean = 7.48 X 10^4 copies per L), *P. aeruginosa* (5.30%; mean = 4.57 X 10^7 copies per L), and *C. jejuni* (2.60%; mean = 2.82 X 10^2 copies per L). All samples were negative for *N. fowleri* and *L. pneumophila*. In conclusion, roof-harvested rainwater often exceeded the FSMA standard for irrigation water.

h) ***Generic E. coli and Salmonella spp. levels in standing water compared to other farmland water sources:*** A 3 year replicated, observational study (4) conducted between May 2008

and October 2010 in the Central Coast of California, USA reported higher generic *E. coli* levels in standing water (furrow, ditch) when compared to other water sources (river, pond, and well) but conducted no statistical tests to confirm this as a significant difference. **Methods**: Produce farms (N=16) that grow leafy green vegetables and agricultural water sources sites (total N=241) in the Central California Coast region were repeatedly sampled during the study period. Water and sediment samples were collected from produce farms (N=16) and water sources that had public access near the farms. A total of 255 irrigation water samples and 181 sediment samples were collected from produce farms; sample types included: well water, reservoir, irrigation, furrow, ditch, pond, stream, river, and creek. A total of 389 non-irrigation water samples and 159 sediment samples were collected from water sources with public access (ditch, pond, stream, river, and creek). Generic *E. coli* levels were enumerated in each sample using a culture-based method. A subset of samples followed an enrichment method, where *E. coli* O157 (N=436) and *Salmonella* (N=249) were recorded as present/absent. **Results:** In total, 6.3% of the water samples (irrigation 1/60, and non-irrigation 5/36) and 4.8% of the sediment samples (irrigation 2/37, and non-irrigation 3/64) were positive for *Salmonella*. One water sample from produce farms (0.4%; total N=242) and nine water samples with public access (2.8%; total N=316) were positive for *E. coli* O157. On produce farms, 78% of the water samples (199/255; mean 7.1X102 MPN/100 ml) and 36% of the sediment samples (65/81; mean 1.0X104 CFU/100g) were positive for generic *E. coli*. The levels of generic *E. coli* in water samples were: well (N=6, all samples were negative), reservoir (N= 22, mean=82 CFU/100mL), pond (N=19, mean=230 CFU/100mL), standing water (i.e., furrow, ditch N=38, mean=2,500 CFU/mL), and running water (i.e., river, creek, stream N=35, mean=1,000 CFU/100mL.

i) ***E. coli, enterococci and Salmonella prevalence in roof-harvested rain water:*** A replicated, observational study (11) between March and September, 2017 in Utah, USA, found that rain water collected from wood shake and asphalt shingle had poor microbial water quality (*E. coli* and Enterococci) when compared to synthetic slate roofing materials. A quantitative microbial risk assessment (QMRA) reported that the health risk associated with produce irrigated with roof harvested rain water varied by roofing material, irrigation water with-holding period, and exposure frequency. **Methods:** At the University of Utah campus, 6 buildings which had either asphalt shingle, wood shake, or synthetic slate roofs were selected. An map (tree canopy density, within 1 km from the building) was used to approximate the presence of bird feces on the roofs. Samples (roof top rain water, total N=30) were collected from the downspouts of selected buildings. Water samples were enumerated for *E. coli* and Enterococci. Concentrations of *E. coli*, Enterococci and *Salmonella* were quantified by qPCR. A QMRA model was evaluated to estimate the probability of illness risks associated with ingestion of lettuce that has been irrigated using roof harvested rain water. **Results:** Overall, 12 out of 30 samples were positive for *Salmonella* (values were below the detection limit i.e., 20 gene copies per 100 mL). Bacterial abundances in runoff were not correlated ($P > 0.05$) with tree canopy area. Based

on the QMRA response model: 1) the probability of illness is greatest from exposure to enterococci in runoff water from asphalt when compared to synthetic slate and wood shake roofing materials, 2) the probability of illness is greatest from exposure to *E. coli* in runoff water from wood shake when compared to asphalt and synthetic roofing materials, 3) the probability of illness was greatest when consuming lettuce immediately after irrigation (however withholding irrigation water for one day significantly ($P<0.05$) reduced *E. coli* by 2 log orders of magnitude).

j) ***Salmonella, STEC, and generic E. coli prevalence in produce fields irrigated with ground versus surface water sources:*** A 2-year replicated, observational study (5) was conducted from 2012 to 2013 on farms (N=32) in Maryland, Delaware, and New Jersey, USA. The study found that ground water sources had lower generic *E. coli* counts than surface water samples. **Methods:** Field samples were collected from 32 farms (15 conventional and 17 organic) in fall 2012 and spring 2013. Sample types included: foliage of leafy greens (N=369 leaves, some of which touched the ground or were soiled), irrigation water (N=124 from well, pond, ground or river water), sediment (N=13 from pond or river), field soil (N=60), and compost (N=11). All samples were enumerated for generic *E. coli*; presence of *Salmonella* (*invA*) and STEC (*stx1* and *stx2*) were determined using qPCR amplification, followed by culture-based isolation for PCR positive samples. **Results:** STEC gene prevalence (qPCR) was 0.3% (2/577) across all samples. *Salmonella invA* prevalence was 4.2% (24/577) across all samples (leafy greens N=15, water N=6, compost N=1, and soil/sediment N=2). *Salmonella* presence was culture confirmed in 9 of 24 samples (leafy greens N=8 and pond sediment N=1). Overall, 10% of samples were positive for generic *E. coli*: leafy greens (6%), irrigation water (18%), soil (10%), sediment (38%), and compost (27%) samples. All ground water samples were negative and 78.6% of the surface samples were positive for generic *E. coli*. In this study, there was no association between generic *E. coli* levels in irrigation water and leafy green produce samples.

k) ***Generic E. coli, fecal coliforms and Enterobacteriaceae prevalence in tomatoes irrigated with open surface ponds versus ground water:*** A two year replicated, experimental study (6) between 2009 and 2010 in Maryland, USA, found that levels of fecal indicators varied significantly among irrigation water sources (ponds vs ground water). However, when tomato plants were spray irrigated (2 pond water types vs ground water), the levels of fecal indicators were not significantly different in tomato surface wash samples. **Methods:** At the Wye Research and Education Center, plots (Nassawango silt loam soil) were arranged in a randomized complete block design (5 block per plot, spaced at 9.1 m apart) that contained 3 experimental units. During 2009, the units were composed of paired rows of tomatoes ("juliet" variety) and only one row of tomato in 2010. Tomato plants were transplanted from a greenhouse and placed under 7 to 14 day spray irrigation schedules. Irrigation water sources included: a ground water well, a surface pond, and a pond treated with copper-sulfate. Water from each source was mixed separately with chemicals

(fertilizers, pesticides) and applied to fields (6 applications in 2019 and 5 applications in 2010) in a separate boom sprayer. Water samples were collected (bi-weekly or weekly for both years) from the source and at the sprayer (4 sampling dates only in 2010). Tomato samples were collected weekly: 7 and 6 fruit sampling days in 2009 and 2010, respectively. All samples were enumerated for fecal coliforms, generic *E. coli*, and *Enterobacteriaceae*. To detect the presence/absence of *Salmonella* all samples followed an enrichment method. **Results:** All samples tested negative for *Salmonella*. All ground water samples were below the limit of detection for fecal coliforms, generic *E. coli*, and *Enterobacteriaceae*. Levels of fecal coliforms (range from 2.0 to 5.16 log CFU/100 ml), generic *E. coli* (range from 0 to 4.1 log CFU/100mL), and *Enterobacteriaceae* (range from 3.04 to 6.3 log CFU/100 ml) in water samples collected from the pond sources (surface pond and pond treated with copper-sulfate) varied based on the sampling period/season. Levels of fecal coliforms were not significantly different from the source to the sprayer, except for ground water sample (where the mean level of fecal coliforms at source and sprayer were 0 and 1.37 log CFU/100 mL, respectively). Ground water and surface pond water samples were negative for generic *E. coli* at the source and sprayer. Generic *E. coli* levels were 1.50 and 1.78 log CFU/100 mL at the source and sprayer for copper-sulfate treated pond water. In 2009, tomatoes irrigated with surface pond water (4.01 log CFU/100mL) had significantly ($P<0.05$) higher levels of fecal coliforms when compared to tomatoes irrigated with ground (2.66 log CFU/100 mL) and copper-sulfate treated pond water (2.45 CFU/100 mL). No significant differences were reported in the levels of fecal coliforms (for 2010 tomato samples) and *Enterobacteriaceae* (for 2009 tomato samples). In 2010, tomatoes irrigated with ground water (4.32 log CFU/100 ml) had significantly ($P<0.05$) lower levels of *Enterobacteriaceae* than tomatoes irrigated with copper-sulfate treated pond water (5.28 log CFU/100 ml), however no significant difference was reported for the levels of *Enterobacteriaceae* between tomatoes irrigated with ground and surface pond water. The authors report that the association between the levels of fecal indicators present in the irrigation water sources and on the tomato fruits is not straight forward and may be difficult to develop reliable metrics for testing agricultural water quality.

l) ***Generic E. coli levels in canal and surface reservoir irrigation waters:*** A one year replicated, observational study (7) in 2010 from Ohio, USA found that generic *E. coli* levels were higher in canal water when compared to surface reservoir water. **Methods:** Two irrigation canals and four surface reservoirs that provided irrigation water to farmlands were monitored for generic *E. coli* over a growing season (2010). Early in the season, samples were collected twice a month (between April and May) and weekly thereafter (June to October). In total, 227 water samples were collected. All samples were enumerated for generic *E. coli*. Precipitation data and the changes in generic *E. coli* levels after precipitation occurred were monitored. **Results:** In general, 97% (219/226) samples tested positive for generic *E. coli*. The median levels of generic *E. coli* in irrigation water sourced from canals ($N=79$, 2.5 $\log_{10}$ MPN/100mL) were significantly ($P<0.05$) higher when

compared to surface reservoirs (N=148, 1.5 $\log_{10}$ MPN/100mL. Generic *E. coli* levels in canal waters (47%) exceeded the LGMA standard (≤235 MPN/100 mL) for irrigation waters more frequently when compared to surface reservoirs (12%). Following rainfall (20 mm or more), generic *E. coli* levels were significantly higher ($P<0.05$) in canal water whereas *E. coli* levels were not significantly associated with precipitation in reservoirs.

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5.2. Choose low-risk irrigation types (10 studies): Six experimental studies found evidence that food safety risks associated with produce contamination under pre-harvest conditions can be mitigated by choosing low risk irrigation types. In 4 out of the 6 studies, produce irrigated under furrow/surface or drip irrigation systems carried lower food-safety risks when compared to sprinkler/spray/overhead/foliar irrigation systems (1-4). One study (5) reported no significant difference in food safety risks when produce fields used sprinkler versus drip irrigation systems and one study (6) reported that drip irrigation delivered more pathogen to plant roots when compared to furrow irrigation. In addition, four studies (7-10) found evidence that sprinkler irrigations systems (and/or rainfall events) can spread foodborne pathogens from contaminated feces to fresh produce.

a) ***Escherichia coli prevalence in lettuce irrigated with sprinkler, furrow, and drip irrigation systems:*** A two year replicated experimental study (1) between 2007 and 2008 in Yuma, Arizona, USA found that *E. coli* levels were higher in fields irrigated with sprinkler systems when compared to furrow and drip irrigation systems. However, *E. coli* survived longer in soil collected from furrow irrigated fields when compared to drip and sprinkler irrigation treatments. **Methods:** A pilot (control) study in 2007, assessed the prevalence of generic *E. coli* in lettuce and soil samples irrigated with canal water. All pilot study samples were negative for generic *E. coli*. Following this, an experimental study was conducted between Jan and Apr, 2008. Experimental plots (35X15 m) were arranged within a random block design and included 2 lettuce types (romaine, iceberg) that were grown on Indio silty clay loam in 2 bed sizes (1 and 2 m wide). Three irrigation types were tested: 1) solid-set overhead sprinkler, 2) drip irrigation (14 cm underground), and 3) furrow irrigation (surface applied). Under drip and sprinkler irrigation systems, a cocktail (two non-pathogenic *E. coli* K-12 strains: LMM1010 and ATCC 25253) of cultures that contained approx. 10^8-10^9 cells/mL were injected into the irrigation water stream (total 2L of culture in a 20 min run). For furrow irrigation, a controlled plastic container delivered 75 mL of the cocktail culture/min. The approx. amount of *E. coli* contaminated water delivered to the fields were: 55, 40, and 37 L via furrow, sprinkler and drip irrigation systems. Irrigation water was sourced from a canal recharged by the Colorado River. Soil and lettuce samples were collected in triplicate on 1 (3 h post irrigation), 3, 7, 10, and 14 days post-irrigation. Irrigation canal water samples were collected weekly between 2006 and 2009. All samples were enumerated for *E. coli*. **Results:** Lettuce and soil samples collected from the pilot study (control) were below the limit of detection for generic *E. coli* (<10 CFU/g). Generic *E. coli* in canal irrigation water ranged from <10 to 75 $\log_{10}$ CFU/Kg. In the experimental study, on day-1 (3 h post-irrigation) and day-3, tracer *E. coli* was lowest in lettuce cultivated under drip and furrow irrigation systems (all samples <10 CFU/g, except one furrow treatment sample that was 820 CFU/g) when compared to sprinkler irrigation system (range from $1.7X10^2$ to $2X10^6$ CFU/g). Tracer *E. coli* in irrigated lettuce (all 3 treatments) were below the limit of detection after day 7 [except two samples at day-10: furrow (50 CFU/g) and drip (40 CFU/g)]. The tracer *E. coli* was higher

and survived longer (up to 15 days) in soil collected from the furrow irrigated plots (range from <10 to 3700 CFU/g) when compared to sprinkler (range from <10 to 1293 CFU/g, up to 3 days) and drip irrigated plots (range from <10 to 470 CFU/g, up to 3 days).

b) ***Salmonella transfer to melons in furrow versus drip irrigated fields:*** A 3- year, replicated, experimental study (6) between 2009 and 2011 from California, USA, found that drip irrigation systems delivered more *Salmonella* to the plant roots when compared to furrow irrigation systems. However, all produce samples (from both treatments) were negative when assayed for the internalization potential of *Salmonella* in melon fruits. **Methods:** In a greenhouse, 3 melon cultivars (Cantaloupe Oro Rico, Top Mark, and Honeydew "Summer Dew" HMX 4593) were planted in UC mix (33% peat, 25% sand, 42% fir bark) and irrigated daily. In total, 66 plants were established and fertilized with 50% Hoagland's solution. At flowering stage, 400 mL of *S. enterica* (Typhimurium strain *a*PTVS150, log 7 CFU/mL) was added to the root ball of the plants. Post-inoculation (day 15 and 49) plants were harvested and treated separately (soil, vine, and fruit) to detect the presence/absence of *Salmonella* in soil and internalization potential of *Salmonella* in melon vines and fruits. During summer (2009, 2010, and 2011) melon seedlings (3 cultivars as above) were transplanted to the field (UC Research Farm, Davis, CA, USA, soil type: Yolo silt clay loam). *Furrow irrigation:* In a field, 9 beds were raised with 18 corresponding furrows. In total, each bed contained 36 seedlings (spacing 77 cm: 18 cantaloupe and 18 honey dew). At flowering stage, each furrow was inoculated with *Salmonella* (via porous infusion sachets, N=4 per furrow, spaced approx. 7.5 m apart, final conc. log 7.5 CFU/50 g of sand) and fields were irrigated to a uniform furrow depth. Care was taken to avoid direct contact between melon and irrigation water. A second inoculation event was repeated as above, after 24-days from the first inoculation. At each irrigation event, soil and water samples were collected from each furrow. *Sub-surface drip irrigation:* In an adjacent field, 5 beds were prepared and melons were transplanted (total N=36, each bed split in half N=18 cantaloupe and N=18 honey dew) and irrigated via a sub-surface drip. At flowering stage, *Salmonella* culture (500 mL of log 9 CFU/mL) was added to a pressurized tank (1000 L) that supported the sub-surface drip lines. The irrigation lasted 2h and post-irrigation water samples were collected from each bed and the end of the drip line. Post-inoculation, melons were harvested (in total N=491) from the furrow and drip irrigated treatment plots on day-30 and day-43, respectively. Samples were treated separately to detect the presence/absence of *Salmonella* on the melon surface and internalization potential of *Salmonella* in melon fruits. Post-irrigation soil samples (on day-5, 21 and 40) were collected from each treatment plot. *Soil contamination:* 20 melon plants (N=10 cantaloupe and N=10 honey dew) at the flowering stage were inoculated with *Salmonella* (500 mL of log 8 CFU/mL), within the root zone area (14 cm radius). Plants were either drip (N=10) or furrow (N=10) irrigated. Post-inoculation (48 h) plant vines, soil in contact with root, and root balls were sampled. Samples were enumerated to detect the presence/absence of *Salmonella* or the internalization potential of *Salmonella*. **Results:** Post-inoculation (until

day-49), all greenhouse soil samples were positive for *Salmonella* (N=9 out of 9). In plant samples, *Salmonella* was detected only in Oro Rico cultivar (vine sample N=2/2) at day-15 (post-inoculation) and all other plant samples (vine N=20/22 and melon N=14/14) were negative for *Salmonella.* *Field experiments:* Post-inoculation (0 h), *Salmonella* levels were lower in water samples collected from plots irrigated under furrow treatment (range from 2 to 3 log CFU/mL) when compared to drip treatment (range from 4 to 6 log CFU/mL). *Salmonella* survived in field water (until day-21 post-inoculation) under furrow irrigated treatment plots (range from 2.3 to 2.4 log CFU/mL) whereas water samples under drip irrigated plots were not assessed for *Salmonella*. *Salmonella* levels were lower (5-day post-inoculation) in soil collected from furrow treatment plots (3.9 log CFU/g) when compared to drip treatments (4.10 CFU/g). In both treatments, *Salmonella* populations tended to decline, but the population were still quantifiable until day-21 (2.7 and 2.6 log CFU/mL) and day-37 (1.6 and 1.9 CFU/mL) under furrow and drip irrigation treatment groups, respectively. On day-41, *Salmonella* populations were not quantifiable but all soil samples were still positive under the furrow treatment (6/6), whereas drip treatment soil samples were not assessed for *Salmonella*. Soil samples collected around the furrow bed edge and furrow bed center were all negative for *Salmonella* (except 2 samples; total N=90). All melon samples were negative for *Salmonella* and no internalization (fruit total N=401) was detected under both the treatment groups. *Salmonella* was detected on the melon rind surface (day-35; cantaloupe 3/14, honey dew 2/15, day-41: cantaloupe 8/8, and day-43: cantaloupe 15/21) under the furrow treatment, whereas drip treatment melon rind samples were not assessed for *Salmonella*. In conclusion, contaminated water resulted in soil contamination, however the lateral movement of *Salmonella* across the soil profile was not detected in the furrow treatment (soil from bed center and bed edge were negative) whereas the drip treatment delivered *Salmonella* directly to the plant root base. However, the risk of *Salmonella* contamination was higher on melon surfaces that were in contact with contaminated sources (soil/water), specifically in the furrow irrigation treatment.

c) ***E. coli O157:H7 transfer to lettuce in drip and sprinkler irrigated fields:*** A 3-year, replicated, experimental study (3) between 2007 and 2009 in California, USA, found no significant difference in *E. coli* population levels in inoculated lettuce plants irrigated by drip versus sprinkler irrigation systems. However, later in the growing season, inoculated lettuce from sprinkler irrigation treatments reported significantly more positive samples when compared to drip irrigation treatments. **Methods:** Field trials (N=4) were conducted using a split-plot design to evaluate the treatment effects of drip and overhead sprinkler irrigation systems. Each treatment contained 3 blocks (44 m long) with 9 beds (1m). All beds were seeded with two rows of lettuce (*Lactuca sativa*) spaced 30 cm apart. Prior to the first irrigation, pesticide was added to all beds (Kerb 50 W, Dow Agrosciences, 2.24Kg/ha). Unfarmed beds (N=10) were raised between the two treatment groups to avoid cross-contamination. Fields were irrigated 2 times weekly; sub-surface drip 2 to 4 h per irrigation, and sprinkler 1.5 to 2.5 h per irrigation; water applied to the field ranged from

28 to 46 cm. After plant thinning (30 days post-seeding), plots were fertilized with 80 kg nitrogen/ha. A culture of attenuated *E. coli* O157:H7 ATCC 700728 (inoculum conc: low=10^5 or high=10^7 CFU/mL) was inoculated onto the lettuce plants (2 or 4-week-old) via spray bottle and on the soil surface (5-days after planting) via backpack sprayer. Post-inoculation lettuce samples were collected on day-0 (0h and 2h after inoculation), day-2, and weekly thereafter (on days 7, 14, 21, 28, and 35). In total 2,586 lettuce heads were harvested during the 3-year study period. Prior to field inoculation and post-inoculation (day-0, 2, 7, 15, 21, 28 or 35), 5 surface soil samples per bed (N=18) were collected from all plots. All samples were enumerated for *E. coli* O157:H7. **Results:** All soil samples (in all 4 trials) collected before field inoculation and by the end of the field study (at day-21 or 35) were negative for *E. coli* O157:H7. All control samples were negative for *E. coli* O157:H7. In general, the authors report no significant difference in the *E. coli* population levels between inoculated plants irrigated by drip or sprinkler treatment methods. Hence, the results of *E. coli* levels from both treatment groups were pooled together and presented. After inoculation *E. coli* O157:H7 levels declined over the growing season. For example, when 4-week-old lettuce plants were inoculated, *E. coli* populations ranged from 3.6 log to 4.2 log CFU/plant at day-0 (0 h post-inoculation). Within 1h (post-inoculation) the *E. coli* populations declined by 1 log CFU/plant and ranged from 2.3 to 3.4 log CFU/plant. After 48 h post-inoculation, all plant samples were below the limit of quantification (2.3 log to 2.6 log CFU/plant), and all samples needed to be enriched to report the presence/absence of *E. coli* O157:H7. Similarly, *E. coli* populations decreased in soil with time and all samples were negative by day-15 (*E. coli* populations in soil: at 0h average=4.7 log CFU/g; at 2h average=4.2 log CFU/g; at day-7 = 2 out of 6 soils beds were positive). Similar results were reported when 2-week-old lettuce plants were inoculated with *E. coli*. A rapid decrease in *E. coli* populations occurred between 0-h (5 log CFU/plant) and 2-h (2.5 log CFU/plant); following this, 50%, 47% and 0.8% of the plants were positive at day-7, day-14, and day-21, respectively. Later in the growing season, however, the irrigation method influenced the percent positive samples for *E. coli*. For example, at selected sampling days, the number of positive samples were significantly ($P<0.05$) greater among inoculated plants irrigated via sprinkler systems compared to drip irrigation systems (day-14: N=60, drip=23% and sprinkler=45%, day-35, N=75: drip=17% and sprinkler=38% positive samples). There was one exception (trail-3, day-7, N=60; drip=82% and sprinkler=62%), where the drip system had significantly ($P<0.05$) more positive samples when compared to sprinkler irrigation systems.

d) ***E. coli O157:H7 prevalence in lettuce irrigated with sprinkler and surface irrigation methods:*** A one year replicated, controlled, experimental study (2) from New Jersey, USA, found that lettuce plants were more often positive for *E. coli* under sprinkler irrigation when compared to surface irrigation treatments. **Methods:** Pots (5 in) were filled with a 1:1 mixture of Canadian peat moss and agricultural grade vermiculite. Seeds of green ice lettuce (3 sets of 48) were planted at 10-day intervals. Plants were irrigated daily, and

fertilized with a Pete's General Purpose 15-15-15 fertilizer. On the day of inoculation, plants were moved to a lab and grouped by age (20, 30, and 40 days of age). Each set of plants from each age group were split into two treatment groups (N=24 each): surface and spray irrigation. Under the surface irrigation treatment, each pot soil was covered by plastic (13 cm) to avoid contact between the soil and plant. Plants were surface irrigated once with 200 ml of *E. coli* O157:H7 (GFP) contaminated water. Under the spray irrigation treatment, plants were irrigated once with approx. 100 ml of *E. coli* O157:H7 contaminated water via. a spray bottle. Control plants (N=2) were surface and spray irrigated with potable water. On the day of harvest (40 days of age), plants from each treatment were split into two lots (N=12): one lot received 100 mL of water and the other plants were immersed in chlorine (200 ppm) for 1 min. All samples were enumerated for *E. coli* O157:H7. **Results:** In general, spray irrigated plants were significantly ($P<0.05$) more often positive for *E. coli* O157:H7 when compared to surface irrigated treatment plants: 40-day old plants (spray=11/11, surface=5/12), 30-day old plants (spray =9/10, surface =0/10), and 20-day old plants (spray =9/11, surface =1/10). Similarly, when plants were chlorine treated, spray irrigated plants were significantly ($P<0.05$) more positive for *E. coli* O157:H7 when compared to the surface irrigated treatment: 40-day old plants (spray=11/11, surface=1/10), 30-day old plants (spray =8/9, surface =1/11), and 20-day old plants (spray =3/10, surface =0/11).

e) ***E. coli O157:H7 prevalence in lettuce irrigated with sprinkler and drip irrigation methods:*** A 2-year, replicated, experimental study (5) between 2009 and 2010 in the Salinas Valley, California, USA, found that the persistence of *E. coli* O157:H7 on lettuce plants was not significantly different when irrigated under sprinkler versus drip irrigation systems. **Methods:** Lettuce plants (*Lactuca sativa*) were grown following standard commercial practices in a split block design. In total, 4 trials were conducted between 2009 and 2010: 2-trials in June (early season) and 2-trails in Aug-Oct (late season). In each trial, plants were irrigated with sprinkler (N=3 blocks) and drip (N=3 blocks) irrigation systems. Plants (4-weeks-old) were contaminated with *E. coli* O157:H7 ATCC700728 (10^7 CFU/plant) using spray bottles. In all trials, control plants (from the same field) were not contaminated. Post-inoculation, lettuce plants (N=48 per sampling day, i.e., N=12 per treatment and control) were sampled at 0h, 2h, 1, 7, 14, 21, and 28 days. Complete plants were enumerated for *E. coli* O157:H7 until day-14; at day 21 and 28 plants were further split into inner and outer leaves and samples were enumerated for *E. coli* O157:H7. Total DNA extracted from the plant leaf samples were used to quantify (qPCR) the total bacterial abundance in the lettuce phyllosphere and 16S rRNA gene sequence analysis (Pyrosequencing). **Results:** All control samples were negative for *E. coli* O157:H7. Post-inoculation, the average number of generic total bacteria ranged between log 5.1 to log 7.7 cells/g of leaf. Generic total bacteria increased 3 to 12-fold between day-2 and day-28 in inoculated plants. Plants under sprinkler treatments had more generic bacteria when compared to the drip treatment. In general, post-inoculation *E. coli* O157:H7 levels

declined rapidly, and by day-7 (post inoculation) all samples were below the limit of detection. Samples were enriched to report the presence/absence of *E. coli* O157:H7. In all trials, *E. coli* O157:H7 percent positive samples in inoculated plants ranged between: day-2 (N=96, 58% to 92%), day-7 (N=72, 54% to 63%), day-14 (N=96, 13% to 38%), and day-21 (N=96, 8% to 25%). Based on 16S rRNA sequence analysis, *E. coli* was detected sporadically in inoculated plants and, when found, the relative abundance was less than 0.001%. Persistence of *E. coli* O157:H7 on lettuce plants were not significantly different based on irrigation treatments. However, the bacterial communities were significantly ($P \leq 0.05$) different in 8/12 sampling days between the irrigation treatments.

f) ***E. coli O157:H7 and avian pathogen survival on produce irrigated under spray or drip treatments:*** A one year replicated, experimental study (4) from Maryland, USA, found that pathogenic *E. coli* levels were significantly (P=0.03) higher in pot mix and persisted longer on spray treated basil plants when compared to drip treatment. **Methods:** For basil plants, a mix of two avian pathogenic *E. coli* (APEC) strains (APEC*stx*+, APEC*stx*-), *E. coli* O157:H7, and *E. coli* O104:H4 were diluted 10-fold into a sterile dairy manure slurry (10^6 CFU/mL). For lettuce and spinach plants, a mix of 4 APEC (*stx*-) strains and *E. coli* O157:H7 were diluted into a sterile poultry litter extract (10^6 CFU/mL). Disinfected basil seeds were planted under sterile BSL-2 lab conditions into a container (4X3.5X3.5 cm) with pot mix (85% Canadian sphagnum peat moss). Both spinach and lettuce were grown under lab conditions in a container with sterile fine sandy loam soil. Plants were irrigated once a week (20 mL per container) and fertilized with 1.32 g/L of Jack's Classic All Purpose 20-20-20 fertilizer. Fourteen-day old basil plants were contaminated (3 mL) with each strain individually, via drip or spray irrigation. Post-inoculation, plants and pot-mix samples were collected on days 0, 1, 4, 7 and 10. Lettuce (30 day old) and spinach (28 day old) plants were contaminated directly by placing 100 uL of the inoculum on foliar surface. After 30 mins, leaves were harvested. All samples were enumerated for pathogenic *E. coli*. **Results:** *E. coli* persisted for significantly ($P<0.0001$) longer duration, and at higher populations, in pot mix than on plants. On lettuce and spinach, both (APEC and *E. coli* O157:H7) pathogens were positive until day-17. For spray and drip treatments, *E. coli* was positive at day-10 from pot mix samples at 3.31 and 2.64 log CFU/g, respectively. When basil plants were spray and drip irrigated, *E. coli* was not detected after day 4 and day 1, respectively. In conclusion, *E. coli* persisted at significantly (P=0.03, day-10) higher levels on spray treated plants when compared to drip treated plants.

g) ***E. coli transfer from fecal pellet to lettuce irrigated with sprinkler systems:*** A one year replicated, controlled, experimental study (10) from New York, USA, found that sprinkler irrigation systems supported the transfer of *E. coli* from fecal sources to lettuce plants. **Methods:** Experimental fields consisted of 3 cells (8.5X59.5 m) that were divided into seven plots (8.5X8.5 m). Bare ground buffers bordered both the field cells (3.1 m) and between the cells (8.5X59.5 m). Each cell contained 5 longitudinal beds (1.2 m wide) that were separated by furrows (0.6 m). Each bed was planted with lettuce seeds (0.4 m apart)

and plants were thinned after 4 weeks. Plants were irrigated as needed via an overhead sprinkler irrigation system. Irrigation was ceased a week before the harvest. Plots (N=20) were randomly assigned to one of the four treatments: fecal placement at 72, 48, 24 or 2.5 h before irrigation. A control plot (N=1) did not receive fecal pellets. Rabbit feces pellets (5 gram) were spiked with a cocktail of non-pathogenic *E. coli* (TVS 353, TVS 354 and TVS 355 average concentration of $3.65X10^8$ CFU/5g). Fecal pellets were randomly placed on the soil surface in a furrow between lettuce rows (minimum of 7m between each pellet). Following this, fields were irrigated via a sprinkler (2.5 h approx. 25mm of water). Post irrigation, six lettuce heads (total N=140, including intact, inner and outer leaf samples) close to the fecal pellet, surface soil underneath the fecal pellet, and the fecal pellets were sampled. Distance between the fecal pellet and lettuce head, distance to nearest sprinkler, and post-irrigation field water pools were recorded. All samples were enumerated for *E. coli*. **Results:** All control samples were negative. In general, 89% of the intact lettuce heads (range 1.0 to 3.0 $X10^6$ MPN/head), 75% of the inner leaf samples (range 1.0 to 3.0 $X10^3$ MPN/head), and 80% of the outer leaf samples (range 1.0 to $2.8X10^5$) were positive for *E. coli*. The average percent of *E. coli* that transferred from the closest fecal pellet to the lettuce head, inner, and outer leaf was 0.028%, 0.0001%, and 0.003%, respectively. The percent of *E. coli* that transferred from the source (fecal pellet) to the nearest lettuce head was significantly greater for lettuce heads irrigated 2.5 h after fecal sample placement compared to heads 24 h (-1.5; 95% CI=-2.14, -0.84; P<0.001), 48 h (-2.6, 95% CI=-3.24, -1.89; P<0.001) and 72 h (-1.02; 95%CI=-1.7, -0.3; P=0.004) after placement. The percent of *E. coli* that transferred from the source to the lettuce head was significantly greater for outer leaves (1.29; 95%=0.45, 2.14; P=0.003) and whole head (1.86; 95%CI=1.19, 2.52; P <0.001), when compared to inner leaves. There was a significant decrease in the percent of *E. coli* that transferred from the source to the lettuce head for: i) each 10 cm increase in the distance between the source and lettuce (-0.34; 95% CI=-0.44, -0.24; P<0.001), and ii) for each 1 m increase in the distance between the lettuce heads and sprinkler (-0.08, 95% CI=-0.15, -0.02; P=0.008). The authors report a similar result when fecal age, distance between source and lettuce head, lettuce sample type, and the distance between sprinkler and lettuce head were included in a multivariable model for *E. coli* levels on lettuce heads at harvest.

h) <u>Salmonella and generic E. coli transfer from inoculated soil to produce during splash events:</u> A four year replicated experimental study (9) between 2013 and 2016 in Georgia, USA, found that splash water (caused by sprinkler or rain) supported the transfer of generic *E. coli* and *Salmonella* from contaminated soil to produce. **Methods:** Four experiments were performed in this study: one microcosm experiment and 3 field experiments. <u>Microcosm experiment:</u> To access the survival of *Salmonella* in soil, each microcosm (wet N=4, 200 mL of sterile water added; dry N=4, no water added) contained 1.5 kg of Tifton loamy soil in a 3.1 L plastic container that were placed in a bio-safety cabinet. The surfaces of the microcosm soils (N=3) were contaminated with 100 mL of *Salmonella* (3 log CFU/mL). One microcosm under each treatment was not inoculated (control). Post-

inoculation composite soil samples (surface, 15 g each) were collected on days 0, 2, 4, 6, and 8. *Field experiment-1* (on a commercial produce farm, Georgia, USA) assessed the effect of splash events caused by overhead irrigation (pond water) and natural rain on the prevalence of naturally occurring *Salmonella* and *E. coli* in produce fields (broccoli, mustard greens, and cantaloupe). At the time of crop harvest, 6 overhead irrigation and 3 rainfall events were studied. Five sampling locations were selected based on the density of the crop. At each location, five aluminum (30.5X30.5X10 cm) pans were installed at heights of 5, 10, 20, 40, and 80 cm to capture the irrigation water and splash water. Representative produce samples (N=14) were collected from each sampling location. Before each irrigation event a water sample at the source was collected. All samples were enumerated for *E. coli* and *Salmonella*. *Field experiment-2* (on a horticulture farm, Georgia, USA) assessed whether contaminated (*Salmonella* GFP labelled) soil could transfer the pathogen to produce via splash water during a simulated rainfall event. GFP-labelled *Salmonella* (7 log CFU/mL, 300 mL) was inoculated (via tank sprayer) to fields (soil and produce concentrations of 5 log CFU/cm^2) of harvestable cabbage (total N=8 plots, 0.5X6 m). Four plots were assigned as wet treatments (irrigated every 2-days before rain event) and four plots as dry treatments (soil covered by plastic sheet during irrigation). Irrigation water was sourced from a well, and irrigation events lasted for approx. 20-30 min (0.8 cm). Soil samples were collected before inoculation (control). After inoculation, soil (N=3) samples and produce samples (N=3) were collected from each plot (days 0, 2, 4, 6, and 8). At day-9, aluminum pans were installed (30 cm away from crop, outside plot area of 3X3 m) to collect splash at varying heights (5, 10, 20, 40, and 80 cm). Following this well water was used to stimulate a rainfall event (30 min, 110mm/h) which covered a plot area of 3x3 m. All samples were enumerated for *Salmonella*. *Field experiment-3*: *Salmonella*-GFP was sprayed on the produce and soil in field experiment-2. In field experiment-3, *Salmonella*-GFP was sprayed only to the soil and care was taken to avoid contact with produce (kale, collard greens). Experiments were conducted on one dry and wet plot (0.6X6.7 m each). Before a natural rain event, a culture of *Salmonella*-GFP (7 log CFU/mL) was applied to field soil (between rows) in each plot. Post-inoculation produce samples (kale N=6 and green collards N=6) and soil samples (N=3) were collected from each plot. Aluminum pans (N=10) were installed: 5 cm (N=3), 10 cm (N=3), 20 cm (N=2) and 40 cm (N=2) above ground. The natural rain lasted 2 h (approx. 7.5 mm). Post-rain, soil and produce samples (at each sampling event; soil N=3, kale N=6, collard greens N=6) were collected 0-h, 1-h, 5 and 10 days after the rain event. Parallel to these experimental studies, one observational study collected surface soil samples (N=120) across 8 farms in southern Georgia. All samples were enumerated for *Salmonella*. **Results:** All samples were negative for *Salmonella* by day-8 (field) and day-10 (microcosm). By day-8 and 10, *Salmonella* had been reduced by 3 and 4 log CFU/g, respectively. *Salmonella* survival was not significantly different between dry (die-off rate constant/day range from 0.05 to 0.78) and wet soil (die-off rate constant/day range from 0.23 to 0.96). All field experiments

provided evidence for the transfer of generic *E. coli* or *Salmonella* via splash water. Generic *E. coli* was detected in splash water up to 10 cm (aluminum pan height) and *Salmonella* was not detected in any splash sample collected from uncontaminated fields. All soil samples (N=120) were negative for *Salmonella* from the surveillance study (N=120, across 8 farms in southern GA). Splash water samples were positive for *Salmonella* (up to 40 cm pan height), only when fields were artificially contaminated with *Salmonella*. At each pan height, *Salmonella* detected in splash water was higher (but not significant P=0.07) in wet plots (for example at 5cm, mean range from 1.67 to 7.43 CFU/100mL) when compared to dry plots (at 5 cm, mean range from 0.38 to 2.78 CFU/100mL). Contamination levels were significantly (P=0.04) greater in produce harvested from wet plots (mean = 0.24 CFU/g) when compared to dry plots (mean = 0.04 CFU/g). Post-irrigation, *Salmonella* levels decreased over time and all produce samples were negative by day-8. Finally, *Salmonella* was detected in splash water collected from pans placed 30 cm away (horizontally) from the contaminated site.

i) ***E. coli transfer from feces to lettuce irrigated with sprinkler system:*** A replicated, controlled, experimental study (8) from California, USA, found that sprinkler irrigation systems supported the transfer of *E. coli* from fecal slurries to lettuce heads. **Methods:** Experimental fields contained 10 beds (0.6 m wide) with furrows (0.4 m wide). Two rows of romaine lettuce seeds were planted (approx. 0.3m apart) in each bed. Fields were irrigated (between 5 and 7 days, for 2 h) with ground water via overhead sprinklers (N=12, 30X30 ft), weeded, and fertilized as needed. Irrigation was suspended 5 days before the experiment. 12 h prior to the start of the experiment, a fecal slurry (5g, source: chicken and rabbit, negative for *E. coli* O157:H7) was mixed with *E. coli* (TVS 354) culture to achieve a final concentration that ranged from 10^7 to 10^8 CFU/g feces. In the field, eight lettuce heads were randomly grouped to form a cluster (total N=23 clusters). Inoculated, fecal slurries were placed on the soil surface in middle of the lettuce cluster on days 4, 2, 1, and 0 (1h) before irrigation. One cluster of lettuce (N=8) did not receive a fecal slurry (control). Following this, fields were irrigated via sprinkler for 1h. In field, the following factors were recorded: distance (cm) between the four closest sprinklers to the fecal deposit, distance (cm) between each lettuce head to the fecal deposit, lettuce orientation relative to wind direction and fecal deposit (upwind or downwind), age of inoculated feces, and source of feces (chicken or rabbit). Post-irrigation, lettuce (total N=180 heads) and fecal samples were harvested from each cluster. All samples were enumerated for *E. coli* (TSV 354). **Results:** After irrigation events (days 4, 2, 1, and 0), *E. coli* levels were higher in the fecal slurry, when compared to pre-irrigation levels, but not significantly P>0.05. For example, pre-irrigation *E. coli* levels were lower in chicken fecal slurry (range from 7.7 $X10^7$ to $2.0X10^8$ CFU/g) when compared to post-irrigation *E. coli* levels (range from $2.6X10^9$ to $4.6X10^{11}$). 96.7% of the lettuce heads from the treatment groups were positive for *E. coli* (mean = $6.5X10^4$ MPN/head) and all lettuce heads from the control group were negative for *E. coli*. Additional lettuce samples (N=2 per cluster) were collected outside the 152.4

cm no-harvest buffer zone (LGMA). All samples tested positive for *E. coli* and ranged from 1.5 to $4.5X10^2$ MPN/head. Age of the fecal slurry and the source of the fecal slurry were not significantly associated with *E. coli* transfer from feces to lettuce. For each additional cm in the mean distance between the fecal slurry and sprinkler (N=4), *E. coli* concentrations on lettuce heads decreased approx. 1.4% (0.0137; 95% CI=0.0048, 0.022; P=0.002). For each additional cm of distance from feces to lettuce head, *E. coli* concentrations decreased by approx. 1.1% (-0.0107; 95% CI= -0.021, -0.0007; P=0.037). Lastly, for each additional cm of irrigation water applied to the fields, the concentration of *E. coli* in lettuce heads increased by 297% (1.0895; 95% CI=0.068, 2.11; P=0.037).

j) <u>E. coli O157:H7 transfer from animal feces to lettuce irrigated with sprinkler systems:</u>

A one year replicated experimental study (7) from California, USA, found that sprinkler irrigation systems supported the transfer of *E. coli* O157:H7 to lettuce heads. **Methods:** Each field contained 12 beds (61 cm wide) that were separated by furrows (46 cm). Two rows of lettuce (romaine) seeds were planted in each bed and plants were thinned after 4-weeks. Plants were irrigated weekly via over-head sprinkler irrigation systems (as needed every 5-7 days). Six lettuce heads were randomly grouped to form a cluster. Rabbit feces (5 g) spiked with *E. coli* O157:H7 ($1.29X10^8$ CFU/5g) was placed on the soil surface in the middle of the lettuce cluster on days 3, 2, 1, and 0 (immediately before irrigation). When each scat was placed, the distance to the nearest sprinkler, and the distance to each lettuce heads were recorded. Following this, fields were irrigated via sprinkler (for 2.5h, approx. 1.25 to 3.85 mm water per plot). After irrigation, selected lettuce samples were harvested from each cluster (total N=192). Throughout the study, irrigation water, rodent feces, and air samples were monitored for *E. coli* O157:H7. Negative control samples were included from adjacent plots (without feces). All samples were enumerated for *E. coli* O157:H7. **Results:** All control, air, rodent, and water samples tested negative for *E. coli* O157:H7. In general, 38% of the lettuce heads were positive for *E. coli* O157:H7 (range from 1.3 to $2.3X10^5$ MPN/gram or $7.3X10^3$ MPN per lettuce head). None of the inner lettuce sections were positive for *E. coli* O157:H7, as all positive samples represented the outer lettuce sections. Age of feces (-0.134, 95% CI= -0.18, -0.08, P<0.001), distance between feces and lettuce (-0.305, 95% CI= -0.38, -0.23, P<0.001), and distance between sprinkler and lettuce (-0.133, 95% CI=-0.17, -0.09, P<0.001) were significantly associated with the transfer of *E. coli* O157:H7 from feces to lettuce. Based on the regression model, for each additional increase in distance (approx. 8 cm) from feces to lettuce, there was an approx. 0.10-fold reduction of *E. coli* per lettuce head. Moreover, for each 24h between fecal deposition and irrigation event, there was an 0.04-fold reduction of *E. coli* per lettuce head. In conclusion, *E. coli* are readily transferred from feces to lettuce head via sprinkler irrigation.

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