CENTER for PRODUCE SAFETY

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FINAL PROJECT REPORT

Project Title

Towards a decision-support tool for identifying and mitigating on-farm risks to food safety

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Principal Investigator

Daniel S. Karp
University of California, Davis
Department of Wildlife, Fish, and Conservation Biology
One Shields Ave
Davis, CA 95616
T: 530-752-2108
E: dkarp@ucdavis.edu

Co-Principal Investigators

Kate Scow
University of California, Davis
Department of Land, Air, and Water Resources
Davis, CA 95616
T: 530-752-4632
E: kmscow@ucdavis.edu

Jeffery McGarvey
USDA ARS Foodborne Toxin and Prevention Research Unit (FTDP)
Albany, CA 94710
T: 510-559-5837
E: jeffery.mcgarvey@ars.usda.gov

Objectives

1. *To develop a grower-motivated decision-support tool that synthesizes evidence for the efficacy, feasibility, and costs of food-safety practices.*
2. *To understand the community dynamics of feces-feeding bacteria in order to harness their activity to bolster food safety.*

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FINAL REPORT

Abstract

Outbreaks of foodborne illness linked to fresh produce continue to raise questions about best practices for mitigating on-farm contamination. Scientific evidence is rapidly accumulating for how alternative practices may impact food safety, but results are often not made available to growers in a useable way. The objectives of this project were to: (i) evaluate and communicate the evidence surrounding effects of farming practices on foodborne pathogens, and (ii) specifically evaluate how biological soil amendments affect pathogen survival with focused experimentation. First, 2,489 papers were screened and 67 were selected that clearly evaluated on-farm practices for food safety. Systematic evaluation of these papers identified practices that are effective at reducing food-safety risks (*e.g.,* abandoning raw manures and installing treatment wetlands), ineffective (*e.g.,* removing non-crop vegetation), or largely unevaluated (*e.g.,* tillage and cover cropping). Second, lab experiments were coupled with a long-term soil manipulation experiment on research station field plots to trace the cascading effects of soil management on foodborne pathogen suppression in soils. Results suggested that addition of composts and cover crops increased macronutrient concentrations and shifted soil bacterial communities, influencing pathogen persistence. Specifically, composts and cover crops favored bacteria that were better able to suppress *Salmonella* and *Listeria*. However, compost effects dissipated as the growing season wore on and bacterial communities converged between treatments. Together, these results suggest that abandoning animal-based composts should be reconsidered, both because of the known benefits of composts for soil health and because it may be possible to apply amendments in such a way that food-safety risks are mitigated rather than exacerbated.

Background

Fresh produce is an important vehicle for foodborne disease outbreaks [1]. Between 2000 to 2015, three pathogens— *Listeria monocytogenes*, *Salmonella enterica* and Shiga toxin-producing *Escherichia coli* (STEC)— caused the majority of cases (>80%) of foodborne illnesses (N= 24,814) and deaths (N= 88) attributed to fresh produce consumption in the United States [2]. Attempts to lessen the food-safety risks associated with fresh produce have targeted all stages of the produce supply chain, including food production, processing, and preparation [3]. Pathogens can enter and contaminate produce farms through multiple pathways, including farm machinery, workers, irrigation water, soil amendments, and wild and domestic animals [3, 4]. As a result, scientifically informed, risk-prevention practices that address each of these possible contamination routes have been codified into law through the Food Safety Modernization Act [5].

Across the United States, growers are responding to new food-safety regulations by altering their production practices [6, 7]. Occasionally, industry buyers or the growers themselves attempt to achieve the least risky growing conditions possible, advocating for and/or implementing more extreme practices than those required by formal regulatory bodies (*e.g.,* FSMA or LGMA). Importantly, the practices adopted by formal regulatory bodies are subject to considerable scientific scrutiny [5]. But scientific literature is constantly evolving, often hidden behind paywalls, and written in inaccessible jargon. Worse, the demonstrated efficacy of particular practices may be variable across studies and/or context dependent. Practices that go beyond those adopted by FSMA or LGMA may seem intuitive, but may not be underpinned by sound science, meaning they could be ineffective, exact unintended consequences, or both.

For example, some industry buyers and growers seek to exclude all aspects of nature from the farm environment to create more sterile, hospital-like growing conditions [6, 8]. Specifically, wildlife are regularly excluded from farms via fencing, removing surrounding habitat, and/or trapping to minimize the likelihood of them defecating on vulnerable produce [9]. However, prior studies have demonstrated that the practice of removing non-crop vegetation around farm fields to minimize wildlife intrusion may both be ineffective at reducing on-farm pathogen prevalence and compromise important economic benefits to growers like pest control [6-8, 10].

Similarly, animal-based soil amendments represent a particular cause for concern for the produce industry, as studies have shown their potential to introduce foodborne pathogens to the growing environment [11]. Indeed, higher prevalence of foodborne pathogens in produce fields amended with untreated animal manures are often observed relative to fields treated with conventional fertilizers [12-15]. Spreading contaminated manures on produce fields thus carries significant food-safety risks [16, 17]. As a result, many produce farmers have stopped applying both raw manures and treated, animal-based composts in their farming operations, citing food-safety concerns [6, 7, 18]. For example, in a surveillance study, fewer than a quarter of Californian fruit and nut producers (n = 306) reported using fully composted soil amendments and less than 10% used non-composted soil manure [6].

The risk of introducing pathogens onto farms via animal-based soil amendments can be mitigated through proper composting techniques [3, 15, 19]. It is thus unclear whether refraining from applying properly composted amendments to farm fields actually improves food safety. Moreover, composts increase soil organic matter and a recent study found that organic farms with more soil organic matter hosted more diverse bacterial communities that were effective at suppressing foodborne pathogens [20]. Similarly, in an another study, soil organic matter was observed to be a significant driver of pathogen suppression [21]. Based on such evidence, growers may be able to mitigate pathogen survival on farms by increasing soil organic matter and soil microbial diversity, utilizing composts or cover crops. It is unclear, however, how long these microbial communities take to develop, how easy they are to manipulate, and which species are the most important. For example, several studies [22, 23] found that the *E. coli* survival was higher in fields amended with low-quality (artificial fertilizer/slurry) than high-quality manure (farmyard manure/compost). However, such studies did not elucidate what effect bacterial diversity might play in pathogen suppression, which bacterial species might be the most important, and how quickly communities can assemble.

More broadly, it is not always clear which of the practices that are commonly invoked to improve food safety have been thoroughly evaluated and shown to be effective versus which are likely ineffective and could have unintended consequences (*e.g.,* for environmental quality). One approach for designing farm management strategies that effectively mitigate food-safety risks associated with fresh produce would be to synthesize existing scientific literature and communicate the overarching findings via accessible formats to multiple stakeholders. In just the past few years, a plethora of new research that can directly inform on-farm management has become available. For example, studies have evaluated how pathogens are influenced by practices related to the management of wild animals [24], non-crop vegetation [25], irrigation water [26], and soil amendments [27]. From these studies and related work, existing practices can be validated and new ones can emerge for improving food-safety; for example, planting vegetated buffers [28], creating treatment wetlands [29], and minimizing irrigation/manure application at harvest [28, 30]. Increasingly, studies are also able to identify landscape scale factors that increase the risk of pathogen contamination on farms; for example, proximity to rangeland [8, 31].

The first objective of this research program was thus to synthesize existing literature pertaining to effects of on-farm management practices on foodborne pathogens. By summarizing findings and grouping results by regions, crop types, farm types, and other landscape scale factors, context-specific insights into the efficacy of existing practices may be distilled. Moreover, synthesizing existing research can also help identify practices that are widely implemented but comparatively understudied, and thus important foci for future research directions. In this vein, the second objective was to further explore the effects of soil amendments on foodborne pathogens in soils, specifically studying how properly treated animal-based composts affect soil nutrients, soil microbial communities, and foodborne pathogen survival in row crops.

Research Methods and Results

Objective 1

Evidence synthesis

The overarching goal of this objective was to identify and summarize published data concerning the effects of pre-harvest farming practices on the growth and survival of foodborne pathogens in produce fields. 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. The team was composed of: Dr. Daniel Karp (PI), project postdoc Dr. Naresh Devarajan, Dr. Daniel Weller (a food-safety expert then at Cornell University and now at the CDC), and Dr. Matthew Jones (a food-safety expert at WSU).

Next, relevant studies were identified by searching three electronic databases: PubMed, Web of Science, and PubMed Central (PMC). Searches were conducted 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: soil management, non-crop vegetation management, wildlife/animal management, and landscape context. Studies were selected for inclusion based on the following criteria:

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*, *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.

After reviewing 2,489 abstracts, a total of 163 relevant papers were finalized for in-depth reading to further filter papers according to the selection criteria (**Figure 1B**). To do so, we followed a two-hand selection procedure, where all papers (n=2489) were screened by at least two members of the science advisory team and added to the final selection list. As a team, we finalized a total of 67 papers that passed our selection criteria. Selected papers were then assigned to broad categories: soil management (n= 42), non-crop vegetation management (n=15), wildlife management (n=5), and landscape management (n=10). Next, selected management categories were sub-categorized according to the specific farming practices evaluated (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 was structured to report on: experiment type, management intervention, study period, growing region, study year, focal pathogen, farm type, sample size, and effect size. In addition, a short description of the methods and management-relevant results was reported. One team member wrote all the summaries, which were then edited by at least one other member of the scientific advisory team.

The evidence synthesis suggested that selected management practices can have significant effects on foodborne pathogen growth and survival in the produce environment. For soil management, the evidence synthesis evaluated whether soil amendments (compost, manure, biosolid/sludge), soil tillage, compost/manure storage techniques, crop types, physical covering of soil, and targeted irrigation timing reduced or increased food-safety risks. For non-crop vegetation, studies focused on treatment wetlands, hedgerows/vegetated buffers, and cover crops. For wildlife/animals, studies evaluated riparian fencing, livestock integration, and the presence/absence of wildlife/domestic animals in farm fields. Finally, the evaluated landscape factors included proximity to open/confined livestock areas, water sources, natural/semi-natural vegetation, and impermeable surfaces.

Ultimately, the evidence synthesis identified practices that are very likely to increase food-safety risks (*e.g.,* manure applications) or to reduce them (*e.g.,* treatment wetlands). It also highlighted many practices that have been quite understudied (*e.g.,* cover crops), suggesting important future research directions. Specific outcomes and conclusions regarding evaluated practices are presented below. More broadly, however, the systematic review provides an inventory of the current data on how management and landscape context affect foodborne pathogen survival/prevalence. The summaries are being compiled into an easily accessible web-based tool that can assist growers, industry stakeholders, researchers, and other stakeholders in understanding the state-of-the-science regarding on-farm, pre-harvest food-safety management.

Objective 2

Effects of composts and cover crops on soils and on pathogen persistence

We coupled field and lab experiments to evaluate the effects of soil amendments (*i.e.,* cover crops and poultry-litter compost) on soil chemistry, bacterial communities, and the survival of two foodborne pathogens in soils: *Listeria* and *Salmonella*. Our study was organized around three questions:

1) What is the impact of long-term soil management with cover cropping and compost amendments on soil physicochemical properties and bacterial communities in row crops?
2) Can soil physicochemical properties and bacterial communities alter pathogen (*i.e., Salmonella* and *Listeria*) survival in soils?
3) Do compost additions and cover cropping affect foodborne pathogen survival in soils?

The study leveraged a 27-year research station field experiment (the Century Experiment at the Russell Ranch Sustainable Agriculture Facility, University of California, Davis) to understand the cascading effects of soil management on foodborne pathogen suppression in soils. Experimental 1-acre fields (N= 72) have been continuously managed since 1993 under nine replicated cropping systems. Plots, arranged in a randomized complete block design, followed 2-year crop rotations. In this study, 12 fields managed under a corn-tomato rotation were used, all of which were cultivated with corn at the time of our experiment (2019). We compared four treatments, each triplicated and labelled as: (1) organic fields (composted poultry litter and winter cover crops), (2) conventional fields (synthetic fertilizers but no composts or cover crops), (3) cover crop only fields (winter cover crops and fertilizers but no composts), and (4) compost only fields (composts and fertilizers but no cover crops). Unlike the other treatments, compost-only fields were recently converted from a conventional wheat-tomato system in 2018.

Full details on the methods employed in this study are detailed in Devarajan et al., 2021 [32]. Briefly, however, we collected composite surface (0 to 15 cm depth) soil samples from each of the selected experimental plots on four occasions: before corn was planted, one month after planting, two months after planting, and two days before harvest. Homogenized samples were analyzed for soil physiochemical properties—including macronutrients (nitrate, ammonia, phosphorous, potassium, calcium, and magnesium), micronutrients (sodium, zinc, iron, manganese, copper, and boron), soil texture, pH, and soil organic matter—by Soiltest Farm Consultants (Moses Lake, WA).

Total soil DNA from each sample was amplicon sequenced at Integrated Microbiome Resource (Halifax, Canada). For 16S rDNA data analyses, we used Cutadapt [33] and DADA2 (version 1.12) [34] in R [35] for our bioinformatic pipeline. A total of 12,140 unique bacterial amplicon sequence variants (ASVs) were obtained. ASVs was further filtered using the package "OTUtable" [36] in R to limit ASV's to contain <0.1% and <0.01% of the total reads within a sample. Alpha diversity (*i.e.,* Shannon and Simpson diversity) was calculated at the ASV level using "phyloseq" [37]. Raw reads were archived in Sequences Read Archives (SRA) of National Center for Biotechnology Information (NCBI) with accession PRJNA656855.

Upon soil collection, samples were immediately transferred (*i.e.,* within 24 h) to a bio-safety level 2 laboratory (USDA, CA) for bioassays (*i.e.,* pathogen suppression experiments). Soil (50 g) was inoculated with either *L. monocytogenes* strain RM15994 (an isolate from the 2011 multistate cantaloupe-associated Listeria outbreak) or *S. enteric* strain RM3363NR (spontaneous nal rif-resistant mutant generated from strain RM3363, an isolate from the 2002 multistate cantaloupe-associated *Salmonella* outbreak). The average bacterial inoculum concentrations were: *L. monocytogenes* ~9.55E+07 and *S. enterica* ~ 7.67E+07 CFU per 50 grams of soil. Cups were covered and incubated at 20°C for 10 or 30 days and enumerated for the number of the pathogens remaining. Plates were incubated at 37°C for 24 h (*S. enterica*) or 48 h (*L. monocytogenes*) and the number of colonies counted. The mean population of pathogens per ml solution was converted to mean per gram population in soil (wet weight) and averaged among triplicates.

We implemented linear mixed models (LMMs) to explore relationships between management treatments, physicochemical properties, bacterial communities, and pathogen survival. All models included a random effect of 'field' to account for spatial autocorrelation. First, we used principal components analyses to derive indices of macronutrient concentrations, micronutrient concentrations, and soil texture. We then used LMMs to determine whether compost additions, cover crops, or their interactions altered soil physicochemical properties. Elapsed days since the beginning of the experiment, and its interaction with soil management treatments, were included to determine whether or not effects shifted over the growing season. Second, to examine effects on bacterial communities, we used similar models to examine soil management treatment effects on soil bacterial diversity (Shannon/Simpson diversity) and PERMANOVA to compare bacterial community composition between treatments. To determine whether bacterial communities changed over the experiment, we used a distance to centroid approach. Third, we related soil physicochemical properties to soil bacterial diversity and community composition, again with LMMs and PERMANOVA.

Finally, we related soil physicochemical properties and soil management treatments to pathogen suppression in soils. We quantified pathogen persistence as the fraction remaining after 10 and 30-day incubations. *Salmonella* and *Listeria* data were log and quarter-root transformed, respectively, to satisfy model normality assumptions. We then used LMMs to model pathogen persistence as a function of soil physicochemical properties, bacterial diversity, and bacterial community composition (using the 1^{st} and 2^{nd} axes of non-metric multidimensional scaling analyses). In separate models, we assessed soil treatment effects including compost additions, cover crop additions, elapsed time since experiment initiation, and their interactions as fixed effects. In all cases, we assessed variable significance both through model averaging (leveraging non-shrinkage variance estimates) and backwards model selection.

The outcomes of the experiment are discussed in more detail below. Overall, however, we found that soil management altered soil chemical properties, with cascading implications for bacterial communities and foodborne pathogens. Long-term compost applications and cover crops enriched soil nutrients (**Figure 2**), driving a marked shift in soil microbial communities (**Figure 3**) and an increase in some measures of soil microbial diversity. These cascading effects on soil chemical and microbial properties enhanced the ability of organic soils to suppress foodborne pathogens (**Figure 4**). Importantly, however, as the microbial communities began to converge over the course of the growing season, so too did the ability of soils to suppress foodborne pathogens (**Figure 5**). As a result, soils were largely equivalent in their ability to suppress foodborne pathogens by the end of the growing season. Additionally, we found that addition of green manure (cover crops) alone was not effective to limit the prevalence of pathogens in the selected soil samples.

Identification of suppressive soil bacteria

A series of follow-up experiments were designed to identify the mechanisms by which soil fauna regulate pathogens in selected soils. Specifically, we sought to challenge selected foodborne pathogens (*Listeria* and *Salmonella*) with multiple bacteria species to identify which bacteria are most pathogen-suppressive in soils. To do so, we plated samples from the four most suppressive soils (identified from the pathogen suppression experiment discussed above) on at least eight media (*e.g.,* pseudomonas isolation agar, tryptic soy agar, R2A agar, MRS agar, *etc.*). Soils were incubated at 30°C for 48 h in order to grow soil bacteria species, which we then isolated and cultured individually. Bacterial species were moved to 96-well plates with suitable liquid growth media and then stored at −80°C. In total, we collected ~3,500 isolates per soil sample, for a total of ~14,400 isolates. We then used a fluorescence-based assay to determine whether challenging pathogens with each of the isolates (individually) resulted in pathogen growth suppression. Unfortunately, the COVID-19 pandemic shuttered the USDA lab where we were working for long periods (*i.e.,* March 2020 to April 2021), delaying the experiments. Nevertheless, we were able to assay all isolates for *Salmonella* suppression, identifying 363 soil isolates that were able to effectively inhibit *Salmonella* growth. Efforts to repeat analyses with *Listeria* are ongoing. Once completed, we will sequence all isolates that reduce *Salmonella* or *Listeria* to identify suppressive bacteria.

Pathogen suppression on lettuce farms throughout the California Central Coast

The COVID-19 pandemic also serendipitously brought more fortunate modifications to our research plan. Originally, we had proposed to repeat our pathogen-suppression experiments at Russell Ranch for two seasons; however, the pandemic prevented our team from working at Russell Ranch in 2020. As a result, we pivoted, partnering with another project (led out of UC Berkeley and funded by the National Science Foundation) that is broadly examining the effects of farm management practices on soil health, biodiversity, and food safety on 17 lettuce farms across the California Central Coast. Through providing access to their focal farms, we sought to: (1) analyze how soil management affects pathogen suppression on 'real-world' farms rather than the experimental Russell Ranch farm, (2) focus on lettuce which is much more relevant to food safety than corn, and (3) expand our experiments to include not only *Listeria* and *Salmonella* but also pathogenic *E. coli*.

In summer 2020, we intensively sampled 17 lettuce farms using many of the same procedures as detailed above for our original pathogen suppression experiments. Briefly, we first collected surface (0–15 cm) soil samples from each farm (N=85 samples; *i.e.,* 5 composite soil samples per farm). Bioassays (pathogen suppression experiments) were then performed on soil samples within 24 h of collection in a bio-safety level 2 laboratory (USDA, CA). Experiments were performed as before, with two exceptions. First, we also assessed survival of pathogenic *E. coli* in soils. Second, we quantified the fraction of pathogens remaining only after 10 days (and not 30 days as well). A portion of each soil sample was sieved (8 mm) and then shipped to Soiltest Farm Consultants (Moses Lake, WA) to quantify macronutrients, micronutrients, soil texture, pH, and soil organic matter, as above. A separate portion (0.5 to 1 gram) of the soil sample was archived at −80°C to extract total soil DNA. Additionally, 50 grams of the soil samples were added with 50 ml of glycerol (15%) and archived at −80°C to perform possible subsequent soil bacteria identification experiments.

We found that soils on different farms varied in their abilities to suppress each of the foodborne pathogens. Interestingly, we found strong and significant correlations between soils' abilities to suppress different pathogens, with the most positive correlation between *Salmonella* and pathogenic *E. coli* suppression. That is, soils that were effective at suppressing *E. coli* survival were also quite effective at suppressing *Salmonella* survival (**Figure 6**). Unfortunately, while fieldwork and soil suppression experiments are complete, the COVID-19 pandemic delayed our ability to characterize soil microbial communities in the lab. Fortunately, personnel and funds to complete analyses of soil microbial communities, and relate them to farm management and pathogen survival, are available through the partner project. As such, experiments will be completed as soon as labs reopen.

Outcomes and Accomplishments

Objective 1

The evidence synthesis represents, to our knowledge, the first effort to (1) systemically evaluate the efficacy of pre-harvest practices in improving food safety, and then (2) translate each study into a digestible one-paragraph summary that could be easily interpreted by multiple stakeholders. Together, the science advisory team screened 2,489 papers for relevance, ultimately identifying 67 publications that satisfied all inclusion criteria and provided crucial insights into on-farm food-safety management. The science advisory team then created carefully standardized summaries of each of the 67 publications, yielding key insights into the efficacy of many on-farm practices for food safety. Specifically, practices related to soil management (6 practices evaluated), non-crop vegetation management (4 practices evaluated), wildlife/animal management (3 practices evaluated), and landscape risk factors (4 risk factors evaluated) were all summarized and evaluated. The efforts also identified many practices that are quite understudied and deserving of future study.

Identification of factors that influence the growth and/or survival of foodborne pathogens on produce fields can help assist the industry in identifying the best management practices to mitigate food-safety risks. To broadly disseminate findings, the evidence synthesis will be published in a scientific journal and hosted on a project website that allows stakeholders to easily digest the results presented from all 67 publications. Below, we detail the key insights and outcomes that arose from each of the distinct management categories.

Soil management:
Soil management was, far and away, the most studied, with nearly 2/3 of the papers evaluating a soil management practice. Our team was invited to participate in a special issue of *Philosophical Transactions of the Royal Society B* on Nature's Contribution to People by submitting a review paper entitled "Role of Soil and Soil Biota in the Regulation of Detrimental Organisms and Biological Processes." This paper has been accepted and is currently in press

[38]. Insights from the evidence synthesis were used to write this review, focused on the ability of agricultural soils to suppress (or not suppress) pathogen survival in soils.

Overall, the evidence synthesis suggested that applying raw manures carries significant food-safety risks. Seven replicated, experimental studies [14, 15, 39-43] and five observational studies [13, 17, 26, 44, 45] found higher prevalence of STEC, *Salmonella*, generic *E. coli,* and other biotic contaminants in plots amended with raw manure, compared to plots with conventional fertilizers or plots without manure. Applying manures early in the growing season, however, significantly decreased risk. For example, one replicated experimental study [46] found no major impact on food safety when plots were amended with fresh dairy manure at least 4 months before harvest. Similarly, two observational studies [13, 45] reported that aging manures for >6–12 months decreased the risk of generic *E. coli*.

In contrast, there was little evidence that applying conventional fertilizers or animal-based composts increased food-safety risks. For example, two replicated, experimental studies [47, 48] found that compost added to the soil surface had no major impact on *E. coli* O157:H7 or *Salmonella*. There was, however, variation among compost types. For example, one study in Vermont [49] and two in Georgia [16, 19] found that pathogens survived longer in plots with poultry compost, compared to plots with conventional fertilizers or dairy compost.

Many of the other evaluated practices were much less studied. A few studies [50-52] reported higher prevalence of foodborne pathogens in produce and soil samples collected from conventional farms than from organic farms. However, two other studies reported the survival of *E. coli* O157 and non-O157 to be strain, region/location, and soil specific [53, 54]. Other soil management practices such as the addition of tillage, growing low risk crops, and addition of physical covering were even less studied.

Non-crop vegetation:
Of the three practices evaluated, more research focused on treatment wetlands (N= 9 papers) than on vegetated buffers/hedgerow (N= 3) or cover crops (N= 2). In general, treatment wetlands were effective in reducing fecal indicator bacteria, fecal markers, generic *E. coli*, and pathogens (*E. coli* O157, *Listeria*, and *Salmonella*). However, one study found that generic *E. coli* concentrations increased in reservoirs, potentially due to wildlife occurrences [55]. The two studies that examined cover crops showed very context-dependent effects. In one, winter cover crops had variable effects on survival of generic *E. coli* and *L. innocua* in soil [56]. In another, the relative abundance of *E. coli* O157:H7 survival was significantly lower in soil planted with cover crops compared to unplanted controls [57]. Finally, though many growers remove non-crop vegetation around farm fields to reduce wildlife intrusion, the evidence synthesis did not suggest the practice to be effective. Removing non-crop vegetation or failing to implement a vegetated buffer either increased pathogen prevalences or did not affect pathogens at all.

Wildlife/animal management:
Few studies (N= 5) reported the effects of wildlife/animal management on foodborne pathogen prevalence and/or survival on produce fields. Management practices identified in this review included 1) riparian fencing, 2) integrated livestock grazing, and 3) studies that reported the effects of wildlife/domestic animal presence on produce farms. The two studies that examined riparian fencing suggested it was effective at preventing grazers from accessing streams and may improve microbial water quality standards; however, overland/subsurface flows can still lead to contamination of fenced waterways. In general, studies reported higher odds of detecting foodborne pathogens in the field when wild/domestic animals were observed in fields. However, one study of an integrated crop/livestock management system [58] reported that generic *E. coli* levels dropped below 1.0 $\log_{10}$ MPN/g in the soil by the 120-day standard, after cover crops were grazed by sheep.

Landscape context:
Studies were sub-categorized into four different possible landscape risk factors, including distance between produce fields and (1) open/confined livestock areas, (2) water sources/ wetlands, (3) non-grazed natural lands, and 4) urban developments. Among these, the strongest risk factor that consistently appeared was proximity to open/confined livestock areas (*e.g.,* pastures, feedlots, poultry farms). For example, one study reported that fields in proximity (≤10 miles) to poultry farms had higher generic *E. coli* prevalences than those further [26].

Objective 2

By combining analyses of soil physicochemical properties, soil bacterial communities, and bacterial survival within a long-term soil manipulation experiment, our studies demonstrated that properly composted poultry litter may not increase food-safety risks. If anything, adding properly treated composts to the soil may shift bacterial communities to favor species that are more effective at suppressing *Salmonella* and *Listeria* survival.

Our findings indicate that the introduction of cover crops and composts as soil amendments increased soil nutrients, especially macronutrients and organic matter. In turn, soil management shifted bacterial communities, with cover crop and compost additions generally increasing bacterial diversity. For example, soils with more macronutrients, more organic matter, higher initial moisture content, and fewer micronutrients tended to host more diverse bacterial communities. Likewise, changes in macronutrients, organic matter, pH, and, to a lesser extent, changes in micronutrients strongly influenced bacterial community composition. Importantly, treatment differences in bacterial diversity and composition attenuated over the growing season.

Our work also demonstrated the critical role soil physicochemical practices and bacterial communities play in regulating pathogen survival in soils. Soil with more macronutrients were more likely to suppress foodborne pathogens following both 10 and 30 days of incubation. Bacterial communities were even more important: our models predicted that, depending on the bacterial community composition, *Salmonella* would either grow by 75% or decline to 4% of its initial levels after 10 days of incubation. Subsequent analyses of ~14,400 bacterial isolates from experimental soils identified 363 isolates that were particularly effective at suppressing *Salmonella*. As mentioned, efforts to screen the ~14,400 bacterial isolates for *Listeria* are ongoing (due to COVID-19 delays), and we anticipate at least one peer-reviewed manuscript will be published detailing the most important isolates for pathogen suppression. Broadly, analyses like these may point the way towards future work developing targeted 'biocontrol' strategies for foodborne pathogens. That is, it may be possible to isolate and manufacture 'key players,' allowing growers to inoculate their soils with particularly suppressive bacteria.

In the short term, however, our studies suggest that on-farm management practices can shift bacteria communities in such a way that they become more (or less) pathogen suppressive. Both pathogens persisted better in soils with bacterial communities that were more similar to those present in the conventional treatment compared to those present in the organic treatment. As a result, adding compost tended to reduce the fraction of *Salmonella* and *Listeria* remaining in the soil. At the beginning of the growing season, models suggested that soils without composts retained 4–5x more *Salmonella* after 10 days of incubation relative to soils with composts. However, as the growing season progressed and bacterial communities converged between treatments, so too did their ability to suppress foodborne pathogens. In the absence of compost, addition of green manure (cover crops) alone was not effective in pathogen suppression. Finally, analyses of 85 soil samples across 17 lettuce farms in California's Central Coast suggest that survival of three pathogens (*Salmonella*, *Listeria,* and pathogenic *E. coli*) in soils is highly correlated. As soon as bacterial data becomes available, we anticipate writing a peer-reviewed manuscript that indicates which practices have the greatest scope for promoting suppressive soil bacteria on real-world lettuce farms.

Summary of Findings and Recommendations

Because they may introduce foodborne pathogens onto farms, growers are often reluctant to apply animal-based soil amendments to their farm fields. Indeed, surveys of growers suggest relatively low adoption of composting in California [6]. However, we found little evidence that properly treated organic soil amendments, such as compost, compromise food safety within the evidence synthesis presented here. Correspondingly, in our field study, we found that organic soils (amended with compost and cover crops) were not more conducive to pathogen survival. Instead, organic soils had more soil nutrients and more diverse soil microbial communities, both of which may have contributed to the comparatively higher rates of pathogen suppression observed early in the growing season. Even more promising, our results from lettuce farms across the Central Coast indicates that soil management affects different pathogens in remarkably similar ways (*i.e.,* suppressive capacity of *Listeria*, *Salmonella*, and *E. coli* was strongly correlated across farm fields). This suggests that actions taken to reduce the hospitability of soils for one pathogen may be effective at suppressing another.

Looking forward, it thus appears that tradeoffs between promoting soil health and food safety may not be as severe as previously considered. Organic soil amendments, including composts and cover crops, are known to improve soil health and, from our analyses, may promote bacterial communities that are particularly effective at suppressing pathogen survival. All in all, our work thus suggests that abandoning animal-based composts should be reconsidered given their benefits for soil health and potential pathogen suppression. That said, our evidence synthesis also suggests that proper composting is critical. Indeed, multiple studies demonstrated that untreated animal-based soil amendments (such as raw manure) introduce and promote foodborne pathogens on produce farms.

More generally, our work highlights the utility of evidence synthesis for on-farm food-safety management, both in identifying promising practices and ineffective ones. For example, we found multiple studies reporting on the benefits of treatment wetlands for improving microbial water quality. The evidence synthesis also pointed towards practices to avoid, namely applying raw manures close to harvest and cultivating produce near livestock. On the other hand, the evidence synthesis also suggests that some current practices may be misguided. Very little evidence suggested that removing non-crop vegetation improved food safety, for example.

Finally, a clear benefit of evidence synthesis is its ability to identify key research gaps. Many practices that may have a significant impact on food safety are severely understudied. Only a few papers published to date evaluate how foodborne pathogen persistence or prevalence change on North American produce with soil tillage, cover cropping, biosolid application, and livestock integration, among many other factors. Looking forward, we suggest that systematic evidence syntheses can serve as powerful tools for setting future research directions.

APPENDICES

Publications

1. Devarajan, N., McGarvey, J., Scow, K., Jones, M., Lee, S., Samaddar, S., Schmidt, R., Tran, T. and Karp, D. (2021), Cascading effects of composts and cover crops on soil chemistry, bacterial communities and the survival of foodborne pathogens. J Appl Microbiology (online 9 April 2021). https://doi.org/10.1111/jam.15054
2. Samaddar, S., Karp, D.S., Schmidt, R., Devarajan, N., McGarvey, J.A., Alda, P., Scow, K. (2021) Role of soil and soil biota in the regulation of detrimental organisms and biological processes. Philosophical Transactions of the Royal Society B, *In press* *manuscript accepted, available online soon.

Presentations

1. "Towards a decision-support tool for identifying and mitigating on-farm risks to food safety". D Karp, K Scow, J McGarvey, N Devarajan. Centre for Produce Safety (CPS), 2019. CPS Research Symposium Poster Session. Austin, TX.
2. "Towards a decision-support tool for identifying and mitigating on-farm risks to food safety." D Karp, K Scow, J McGarvey, N Devarajan. Centre for Produce Safety (CPS), 2020. CPS Research Symposium Webinar Series III. [Online]

News articles

1. Martin, R. 2021. Organic fertilisers found to reduce risk of foodborne pathogens on arable crops. AgriLand, 9 April [online]. Available at: https://www.agriland.co.uk/farming-news/organic-fertilisers-found-to-reduce-risk-of-foodborne-pathogens-on-arable-crops/.
2. Mayer, A. 2021. Animal compost helps reduce risk of foodborne illness. AgriPulse, 7 April [online]. Available at: https://www.agri-pulse.com/articles/15647-animal-compost-cover-crops-help-reduce-risk-of-foodborne-illness.
3. Montanari, S. 2021. Compost can help protect us from food poisoning. Popular Science, 15 April [online]. Available at: https://www.popsci.com/story/environment/compost-in-soil-fights-illness-causing-bacterias/.

Budget Summary

In total, $290,749 was allocated to this project, of which $289,348 was spent. The most significant expenses were salaries ($188,724) and associated benefits ($47,188) for the project postdoc (Naresh Devarajan, 100% time throughout the project) and a project scientist (Thao Tran, 50% time throughout the project). Devarajan led all aspects of the project, including the evidence syntheses and the soil manipulation experiments. Tran worked with Co-PI McGarvey to conduct all lab experiments, including the pathogen suppression experiments and bacterial challenge experiments. $29,832 were charged for supplies and other expenses, most notably lab consumables and costs associated with soil physicochemical analyses and DNA sequencing. $3,750 was spent on travel, both to the 2019 CPS symposium and for Devarajan to regularly travel to the USDA lab in Albany, CA, to assist in lab work. Finally, $18,947 were charged in indirect costs. The amount of money allocated was sufficient.

Figures 1–6

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

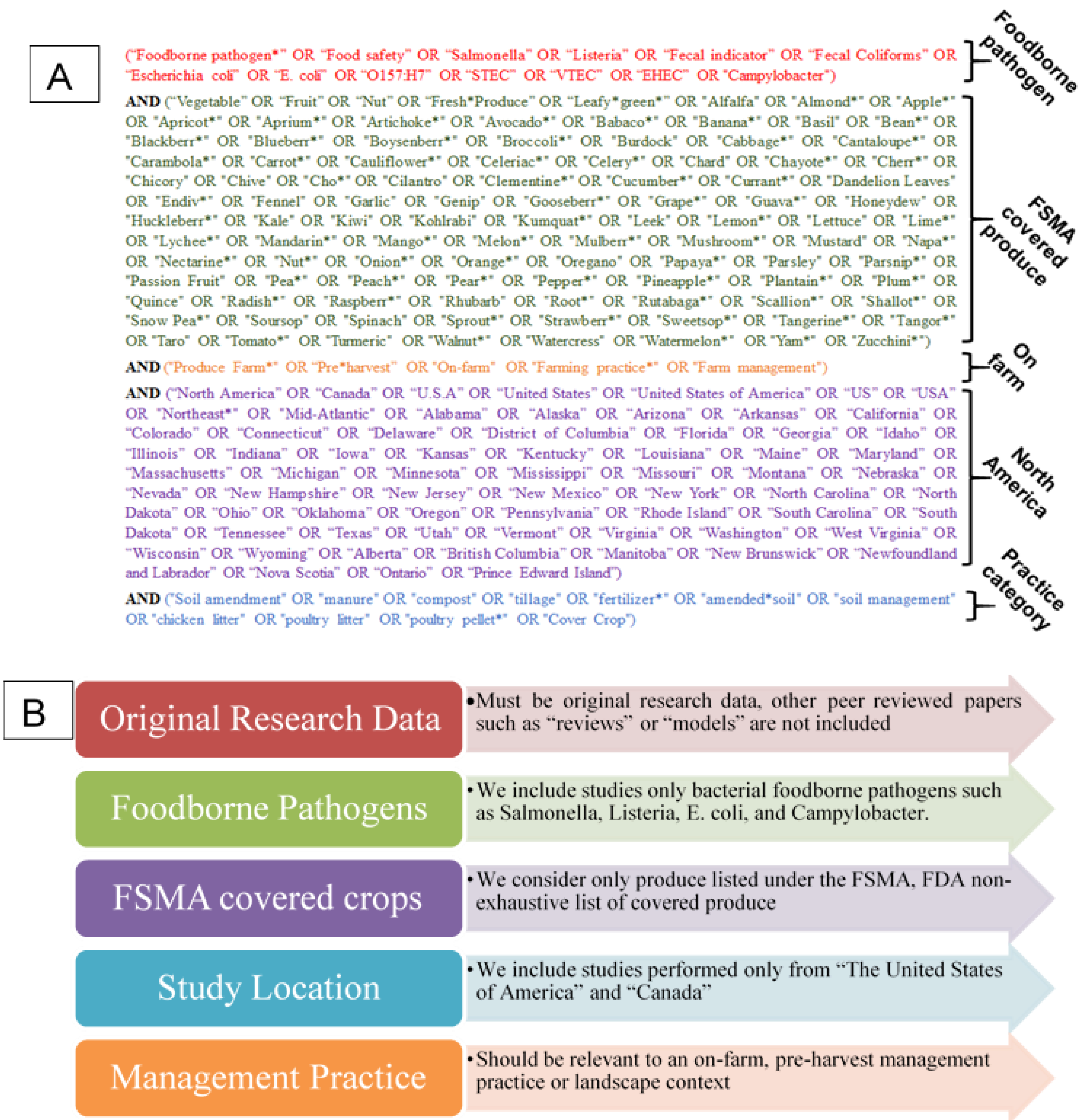


Figure 2: Long-term soil management alters soil chemical properties. Impacts of compost and cover crop treatments on macronutrients (Panel A), organic matter (Panel B), micronutrients (Panel C), initial moisture content (Panel D), pH levels (Panel E), and soil texture (Panel F). Points represent individual soil samples, colored according to each soil management treatment. For soil properties that exhibited significant changes over the growing season (Panels A/C/E), lines represent predictions from linear mixed models and shaded regions are 95% confidence intervals. For other soil properties (Panels B/D/F), large solid points and vertical lines represent predicted treatment means and 95% confidence intervals, respectively.

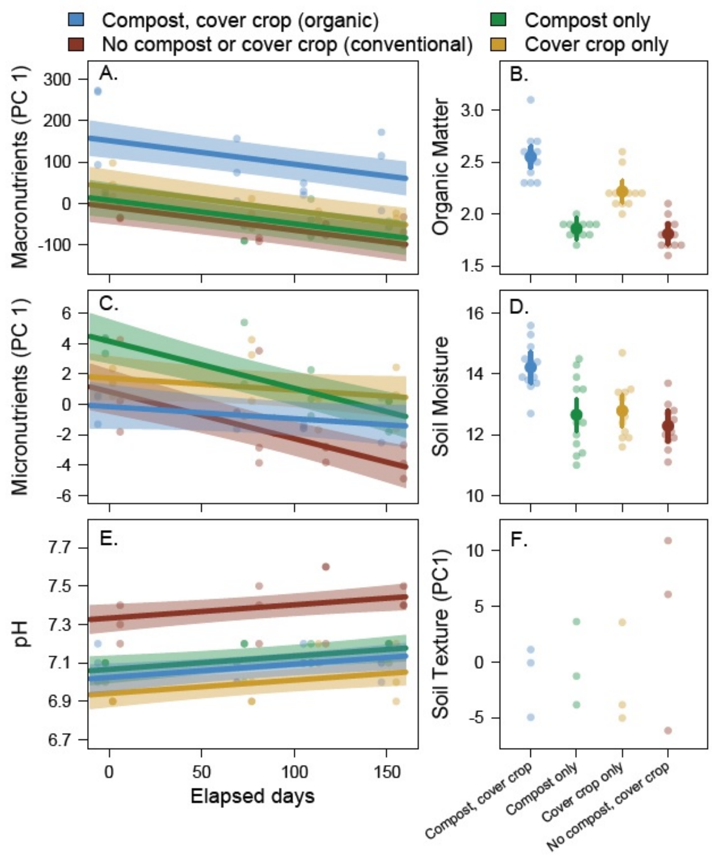


Figure 3: Bacterial communities differ between soil management treatments, especially at the beginning of the growing season. (Panel A) Effects of soil management on bacterial diversity (Simpson index) over the growing season. (Panel B) Differences in bacterial communities between treatments and (Panel C) over the growing season. (Panel D) As the growing season progressed, bacterial communities became increasingly similar between compost and non-compost treatments. Points are individual soil samples, colored according to soil management treatments (Panels A/B/D) or the elapsed days since the beginning of the experiment (Panel C). In panels A/D, lines correspond to predictions from linear mixed models; shaded regions are 95% confidence intervals. In NMDS plots (Panels B/C), distances between points correspond to differences in bacterial community composition.

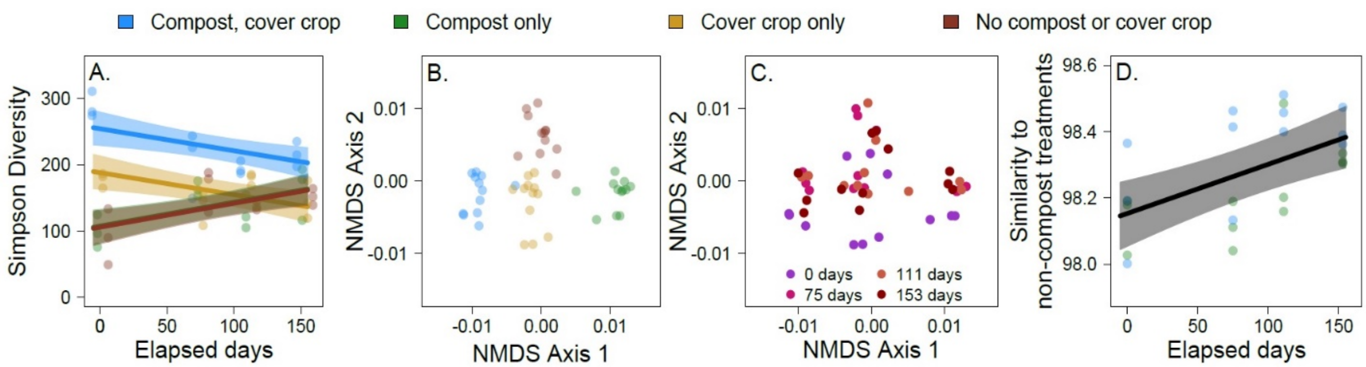


Figure 4: Bacterial communities and soil nutrient content are associated with foodborne pathogen suppression. Pathogen suppression is measured as the Mean Probable Number (MPN) of *Salmonella* or *Listeria* after 10 days of incubation, divided by the initial MPN of the inoculant (followed by log and quarter-root transformations for *Salmonella* and *Listeria*, respectively; see methods). *Salmonella* and *Listeria* concentrations exhibited steeper declines in soils with more macronutrients (Panel D/I), more micronutrients (Panel E/J), and bacterial communities (Panel B/G) more similar to those present in soils with composts and/or cover crops (*i.e.,* NMDS axis 2 in Fig. 3b). The first NMDS axis explaining variation in bacterial community composition (Panel A/F) and bacterial diversity (Simpson index; Panel C/H) did not affect foodborne pathogen suppression. Points represent soil samples, colored by management treatment. Lines represent predictions from linear mixed models; shaded regions are 95% confidence intervals.

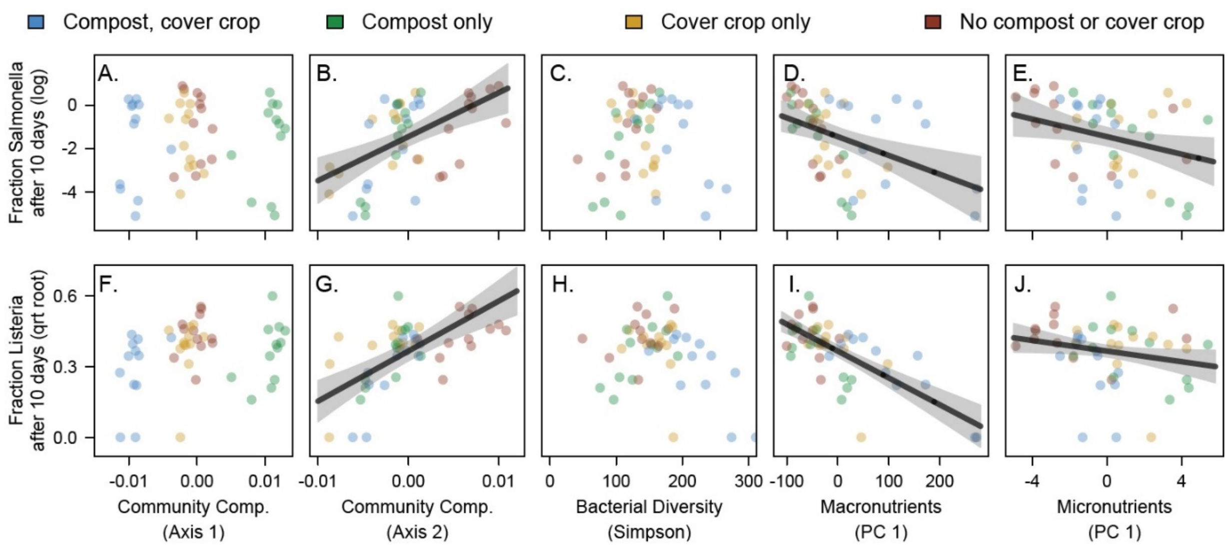


Figure 5: Composted soils are more pathogen suppressive than soils without composts, but only at the beginning of the growing season. Pathogen suppression is measured as the fraction of *Salmonella* or *Listeria* remaining in soil samples after 10 or 30 days of incubation. Points represent soil samples, colored by management treatment. Lines represent predictions from linear mixed models; shaded regions are 95% confidence intervals.

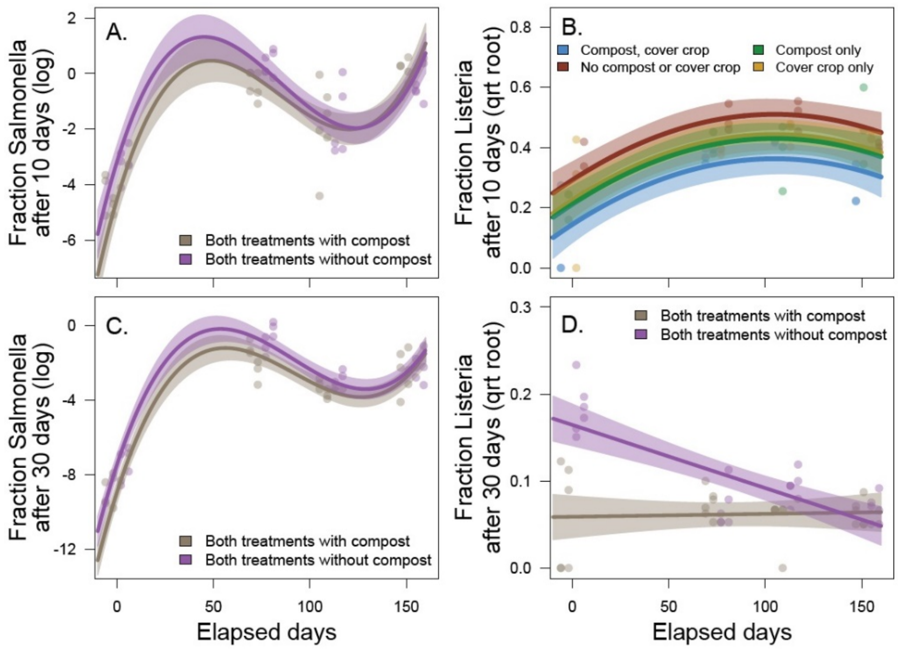
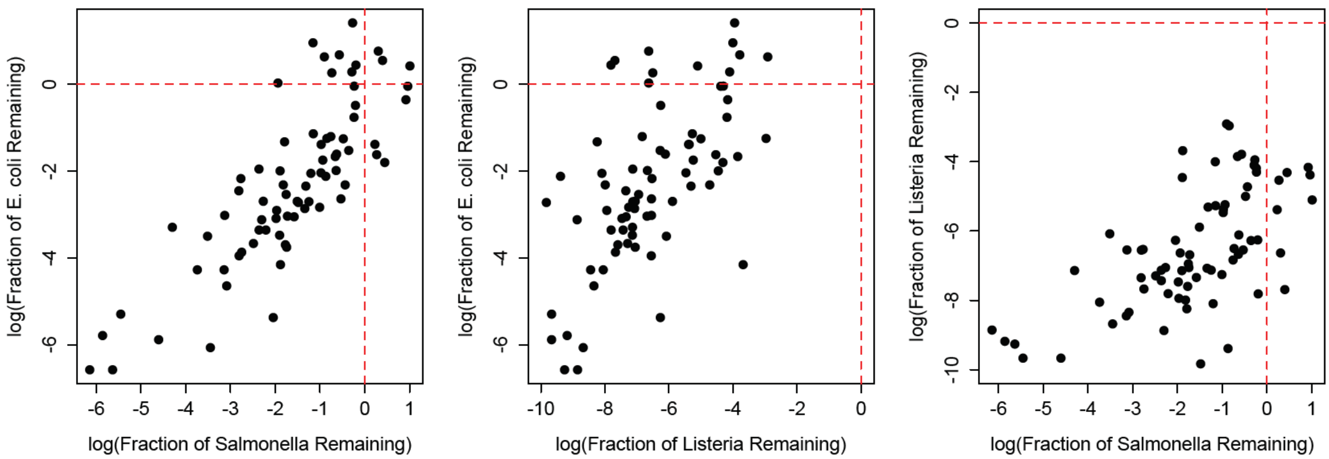


Figure 6: Correlations in soils' abilities to suppress different pathogens across 17 lettuce farms in the California Central Coast. Pathogen suppression data is presented as the (log) fraction of pathogenic *E. coli, Salmonella*, or *Listeria* remaining in soil samples after 10 days of incubation. Red lines indicate equivalent concentrations after 10 days (*i.e.,* no pathogen die-off).



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