



Organic farms conserve a dung beetle species capable of disrupting fly vectors of foodborne pathogens

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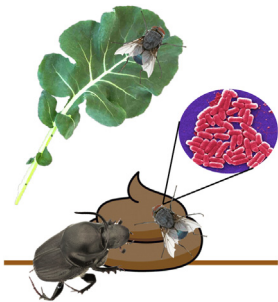
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GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:

Biological control
Biotic resistance
Pathogenic *E. coli*
Food safety
Ecosystem services
Coprotophage

ABSTRACT

Produce can be contaminated with enteric bacteria when livestock or wildlife feces are deposited in vegetable fields. Coprophagous beetles and flies might mitigate this threat as they feed but could also transmit pathogens if they contact plants. Improved food safety will result only from farming practices that enhance coprophage benefits and limit harms. On 49 mixed-vegetable farm fields across the western US states of Oregon and California, we found differences in coprophagous fly community composition under organic versus conventional management practices. While dung beetle (Coleoptera: Scarabaeidae) community assemblages did not differ significantly based on farm management system, organic farms fostered populations of *Onthophagus taurus*, a dung beetle species that is a known antagonist of human-pathogenic *Escherichia coli*. We examined the possible implications for food safety of interactions between *O. taurus* and a common fly species on the farms, *Calliphora vomitora*, in microcosms containing pig (*Sus scrofa*) feces inoculated with human pathogenic *E. coli* O157:H7 and placed near broccoli (*Brassica oleracea*) plants. In the absence of dung beetles, *Calliphora vomitora* readily acquired the bacteria and transmitted them to broccoli foliage. In the presence of the dung beetle *O. taurus*, however, *E. coli* in the soil and fly survivorship were reduced, and the pathogen was rarely recovered from foliage. Altogether, our results suggest the potential for *O. taurus* to both directly suppress enteric pathogens in vertebrate feces and to indirectly reduce the spread of these bacteria by co-occurring flies. The beneficial beetle *O. taurus* was common only on organic farms, suggesting these benefits of beetle-fly interference for food safety

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<https://doi.org/10.1016/j.biocontrol.2019.104020>

Received 11 December 2018; Received in revised form 23 May 2019; Accepted 2 July 2019

Available online 03 July 2019

1049-9644/ © 2019 Published by Elsevier Inc.

could be more likely under this farming regime. Future research that investigates interactions between the many other common dung beetle and fly species on these farms would help fully delineate any net benefit of these species-rich coprophage communities, and the farming systems that shape them, for food safety.

1. Introduction

Consumption of fresh produce contaminated with enteric pathogenic bacteria and viruses continues to be a leading threat to human health (CDC, 2010). It is estimated that 9 million foodborne illnesses occur each year in the United States alone (Painter et al., 2013). Produce can be contaminated in the field when livestock or wildlife feces are deposited on produce as the animals pass through or fly over fields (Ackers et al., 1998; Jay et al., 2007; Newell et al., 2010; Pennington, 2010; Newell et al., 2010). Currently, in the United States, these risks are mitigated by a series of rules and regulations generated by food processors and government agencies (e.g., LGMA, 2013). These often lead growers to modify their farms by installing fencing that blocks entry by ambulatory wildlife, maintaining bare ground buffer zones around fields, and avoiding harvesting any produce near observed livestock or wildlife feces, among other practices (Beretti and Stuart, 2008; Lowell et al., 2010). These moves toward farm-habitat simplification likely harm beneficial wildlife, including pollinating insects that improve fruit set and predatory birds and arthropods that contribute to biological pest control (Letourneau et al., 2015; Beretti and Stuart, 2008; Karp et al., 2015a, 2016), but could be justified if they improve food safety.

Unfortunately, efforts to exclude wildlife associated with food safety rules and regulations appear to instead increase food safety risk, rather than reduce it (Karp et al., 2015a). Indeed, Karp et al. (2015b) found that human-enteric-pathogen contamination of fresh produce was more frequently detected in simplified landscapes modified by habitat removal. Jones et al. (2019) suggested a possible explanation for this observation: working on mixed-vegetable farms spanning the US west coast, they found that both landscape simplification and agrochemical-intensive farming practices led to degraded biodiversity among feces-feeding (“coprophagous”) dung beetles (Coleoptera: Scarabaeidae) and soil bacteria (Jones et al., 2019); in turn, reduced coprophage biodiversity correlated with slower rates of feces removal and extended survival of human-pathogenic *Escherichia coli* O157:H7. Interestingly, diverse communities of dung beetles and soil bacteria were maintained on farms using organic methods, with the potential to restore the ecosystem services these beneficial coprophages provide (Jones et al., 2019). Altogether, work to date suggests (1) that landscape simplification leads to reduced coprophage biodiversity that endangers food safety (Karp et al., 2015a,b; Jones et al., 2019) and (2) that this harm may be reversed through ecologically friendly farming techniques that benefit coprophages (Jones et al., 2019). Indeed, ensuring food safety may be an important, if underappreciated, benefit of on-farm biodiversity.

While dung beetles can rapidly remove feces from agricultural lands (Losey and Vaughan, 2006) and suppress pathogens (Jones et al., 2015, 2019), coprophagous flies (Diptera) fill a complicated role in food safety. On the one hand, flies of many species consume vertebrate feces (Floate, 2011) and so may, like dung beetles, reduce the persistence of foodborne pathogens (Liu et al., 2008). On the other hand, coprophagous flies are known to acquire and transmit human-pathogenic enteric bacteria while feeding (Hancock et al., 1998; Olsen and Hammack, 2000; Wales et al., 2010; Scott et al., 2014). Once flies become internally or externally contaminated with these bacteria, they can transport the pathogens onto produce (Talley et al., 2009). This mix of pathogen suppression and transmission by flies suggests the potential to simultaneously contribute to and detract from food safety. Coprophagous flies also compete with dung beetles for fecal resources (O’Hea et al., 2010; Floate, 2011), although whether this weakens the

consistent food-safety benefits of the beetles is unknown. Altogether, we suggest that more work is needed to put flies into a broader community context that evaluates their net contribution to ecosystem services/disservices related to food safety.

Here, we first report results from a two-year field survey of dung beetle and fly communities on highly diverse, mixed vegetable farms across the U.S. states of Oregon and California [see Jones et al. (2019) for a detailed farm description]. These farms were managed using one of three farming systems: conventional vegetable, organic assorted vegetable, and organic assorted vegetable alongside livestock production (hereafter called an “integrated system”). The livestock on integrated farms might create food-safety risks (Newell et al., 2010), but also might support particularly robust coprophage communities (Bertone et al., 2005; Jones et al., 2019). We coupled this on-farm survey with a microcosm experiment in a biosafety facility where we could safely expose differing fly and dung beetle communities to pig (*Sus scrofa*) feces contaminated with pathogenic *E. coli* O157:H7 and track any resulting movement of the bacteria from feces to nearby broccoli (*Brassica oleracea*) plants. Our project sought to determine (1) how farming practices impact dung beetle and coprophagous fly communities, (2) how farming practices influenced numbers of the dung beetle *Onthophagus taurus* that previous work has demonstrated to be particularly effective at removing pathogenic *E. coli* from feces (Jones et al., 2019), and (3) how interactions between *O. taurus* and the common coprophagous fly species *Calliphora vomitora*, a known vector of foodborne pathogens (Olsen, 1998), might impact the persistence of enteric pathogens and their transmission to produce.

2. Materials and methods

2.1. Field survey

Dung beetles and flies were collected during two years across 49 vegetable farm fields in California and Oregon, USA, with 23 fields in 2014 and 26 fields in 2015 (Fig. 1). These fields were attributed to farming system as follows: 4 and 7 “conventional” fields, 9 and 9 “organic” fields, and 10 and 10 “integrated” fields in 2014 and 2015, respectively (Fig. 1). Conventional farms relied on synthetic agrochemical inputs; organic farms were either certified organic (USDA, 2017) or uncertified but still relied on natural means of fertilization and pest suppression without using synthetic agrochemicals; and integrated farms followed organic principles for vegetable production, with or without being certified, and also raised livestock and/or poultry as part of their production system. All farms that we visited produced broccoli, with this crop chosen because of its (1) long growing season across our entire study region, and (2) low-growing habit and frequent raw consumption by humans, both of which might increase risk of contamination by enteric pathogens that leads to human illness (Jones et al., 2019). In both years, sampling started in the southern part of the study range (central California) in mid-March and continued northward concurrent with farmers growing broccoli, ending in northern Oregon in late June.

On each farm, coprophagous arthropods were surveyed using pitfall traps baited with 20 g of frozen organic pig feces (modified from Larsen and Forsyth (2005); see Jones et al. (2019)). Pig feces were used to bait traps because these animals are often reared on integrated livestock farms and are common reservoirs for human pathogens as feral wildlife (e.g., Jay et al., 2007; Barrios-Garcia and Ballari, 2012). Pig feces also are known to be broadly attractive to coprophagous beetles (Marsh et al., 2013; Jones et al., 2019) and flies (Loy, 1972). Three traps were

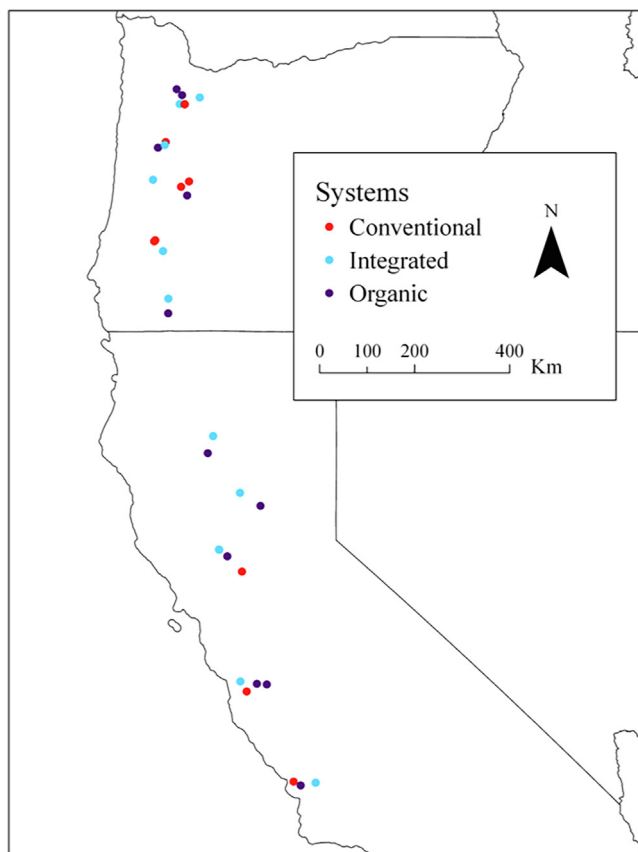


Fig. 1. Map of field collection sites in California and Oregon, USA.

placed into each vegetable field, 25 m apart from each other and 25 m from the field edge to minimize edge effects. Traps were left open in the field for 3 days before that set of traps, and insects within them, were collected. A second set of traps were set following these same methods, such that each farm was sampled twice during the same week (Jones et al., 2019). Dung beetles were identified to the species level (per Cartwright, 1948, 1974; Gordon and Cartwright, 1980; Arnett et al., 2002; Gordon and Skelley, 2007). Flies were identified to the family level (per McAlpine, 1987). Only those taxa with known dung associations were used for analysis (per Encyclopedia of Life [<http://www.eol.org>] and/or BugGuide [<https://bugguide.net/node/view/15740>]).

2.2. Lab experiment

We conducted a lab experiment to examine how the individual and combined impacts of dung beetles and/or coprophagous flies impacted (1) persistence of enteric pathogenic bacteria in feces, (2) movement of pathogens from feces to the foliage of nearby broccoli plants, and (3) performance of the insects themselves. We chose to use the dung beetle *Onthophagus taurus* (Schreber) and the fly *Calliphora vomitoria* (Linnaeus) for this experiment as these two species are commonly found together on farms we sampled, and were easily attainable. Adult *O. taurus* were field collected immediately prior to the experiment. Pupal *C. vomitoria* were purchased (Tri-State Outfitters, Moscow, ID) and reared in a growth chamber (maintained at 26 °C with a 16:8 light:dark cycle) for a single generation to obtain early 3rd instar larvae for use in the experiment. We chose to use *E. coli* O157:H7 in the experiment as our model pathogen because these bacteria are known contaminants of fresh produce (e.g., Jay et al., 2007) that are suppressed by *O. taurus* (Jones et al., 2019) and acquired and moved by *C. vomitoria* (Habeeb and Mahdi, 2012). We again used pig feces for the reasons described

previously. Because we were working with human pathogens, this work was conducted at the Biosafety Level 2 Field Disease Investigation Unit Laboratory at Washington State University, Pullman, WA.

Experimental units were 1-L plastic “deli dishes” (Harvest-Pack brand, Commerce, CA) with fine nylon mesh lids, with the addition of a water tube containing a single broccoli (*B. oleracea*) leaf from the first true leaf stage (SI Fig. 1). Field soil collected from the Washington State University Tukey Research Farm (Pullman, WA) was added to each arena at a depth of ~5 cm. Into each microcosm, we placed on the soil surface 20 g of fresh pig feces previously inoculated with 4 strains of human pathogenic *E. coli* O157:H7 (see SI Materials and Methods for details including media prep, experimental set-up, and specific pathogen strains used). Insect treatment groups were (1) **Control**, to which we added no beetles and no flies, (2) **Flies**, to which we added 6 early third-instar *C. vomitoria*, (3) **Beetles**, to which we added 6 field-collected *O. taurus*, and (4) **Flies + Beetles**, to which we added 6 fly larvae and 6 beetles. We established 4 replicates of each treatment within a fully randomized design. After 11 days, flies had emerged and the proportion of flies emerged was calculated. Collectively, insects fed on feces for 13 days before the experiment was terminated.

Soils, flies, and leaves were processed for pathogen enumeration. Separately, 50 g of soil, the entire plant sprout, or pooled groups of flies (from each replicate), were added to buffered peptone water representing a 10^{-1} dilution. Serial dilutions (of $10^{-2.5}$ – 10^{-4}) were made using sterile saline and plated in triplicate on SMAC_{CT,NAL30}-Plates were incubated at 37 °C for 24 h. After incubation we counted each plate that had approximately 30–300 sorbitol negative colonies only for each sample. Eight well-isolated colonies were selected from each sample, plated to blood agar plates, and incubated for 18–24 h at 37 °C. After incubation, up to 4 colonies (of the 8) were tested using a Latex O157 kit (see SI Materials and Methods for details including media prep, experimental set-up, and specific pathogen strains used).

2.3. Data analyses

2.3.1. Field survey

We used non-metric multi-dimensional scaling (NMDS) to separately describe the variation in the composition of dung beetle and fly communities in conventional, organic, and integrated farming systems (per Kennedy et al., 2010). NMDS is a nonparametric ordination technique effective for graphically depicting multivariate relationships in ecological data, via maximizing the rank correlation between calculated distances in an original matrix and distances in reduced ordination space (Clarke, 1993). The NMDS was performed in the “vegan” package of program R v 3.4.2 (R Core Team, 2017; Oksanen et al., 2018) using a Bray-Curtis dissimilarity matrix (Borg and Groenen, 1997) derived from taxon relative abundances at the farm level. Overall, statistical significance was determined using Analysis of Similarities (ANOSIM).

2.3.2. Lab experiment

All analyses were performed on log-transformed counts of colony forming units (CFUs). Pathogen levels in microcosm soils were analyzed using an analysis of variance (ANOVA) followed by a post-hoc Tukey HSD test to assess pairwise comparisons. These data adhered to the assumptions of normality and homoscedasticity. Because the pathogen levels on both the broccoli leaf surfaces and emerged flies were non-normal with highly heteroskedastic variance, these pathogen levels were analyzed using a Kruskal-Wallis rank test; leaf surface levels were followed by a post-hoc Dunn’s test to assess pairwise comparisons (Zar, 1999). Fly emergence was analyzed using a single factor logistic regression, followed by a contrast analysis to understand differences in emergence with ‘beetles present’ vs. ‘no beetle present’ (Quinn and Keough, 2002). Analyses were completed using R (version 3.4.2), including the ‘lsmmeans’, ‘ggplot2’, ‘dunn.test’, ‘plyr’, and ‘vegan’ packages (Dinno, 2017; Lenth, 2016; Oksanen et al., 2018; R Core Team, 2017;

Wickham, 2009, 2011). R code is available from the authors by request.

3. Results

3.1. Field survey

In total on our farms, we collected 27,357 flies, with the potential vector of concern (family Calliphoridae) comprising 6.7% of all specimens (SI data [Flies]). We collected 2688 dung beetles, with our focal species (*Onthophagus taurus*) comprising 22.8% of all specimens (SI data [Beetles]).

Analyses of community similarity confirmed farm management types had unique fly communities (Fig. 2A; $R = 0.0966, p = 0.009$), but not unique beetle communities (Fig. 2B; $R = 0.0610, p = 0.105$). Visually, the NMDS indicated that the known pathogen-vectors of interest (Calliphorid flies) were embedded within all farm management types, while our most effective known pathogen suppressor (*O. taurus*; Jones et al., 2019) was associated most strongly with organic and integrated farms (Fig. 2).

3.2. Lab experiment

Levels of *E. coli* O157:H7 in the soil were significantly lower in treatments that contained the dung beetle *O. taurus* than in treatments where these beetles were not present (Fig. 3A, Table 1A, $F_{(3,15)} = 10.29, p = 0.0012$). Specifically, pathogen levels were lower in “Beetles” relative to both “Control” and “Flies”, and “Flies + Beetles” relative to both “Control” and “Flies”. Conversely, pathogen levels were not significantly different between “Flies + Beetles” relative to “Beetles”, or “Flies” relative to “Control” (Fig. 3A, Table 1A).

Escherichia coli levels on the broccoli leaves were significantly higher in the “Flies” treatment relative to all other treatments (Fig. 3B; Table 1B, $p = 0.0525$). Neither “Flies + Beetle” relative to “Beetle”, “Flies + Beetle” relative to “Control”, nor “Beetle” relative to the “Control” were significantly different from one another (Fig. 3b, Table 1B).

Fly emergence (proportion of flies emerged) was (marginally) lower in the treatments including beetles (Fig. 4A, $p = 0.0702$). On the emerged flies themselves, pathogen levels were significantly higher on the flies that emerged from the “Flies” treatment than from the

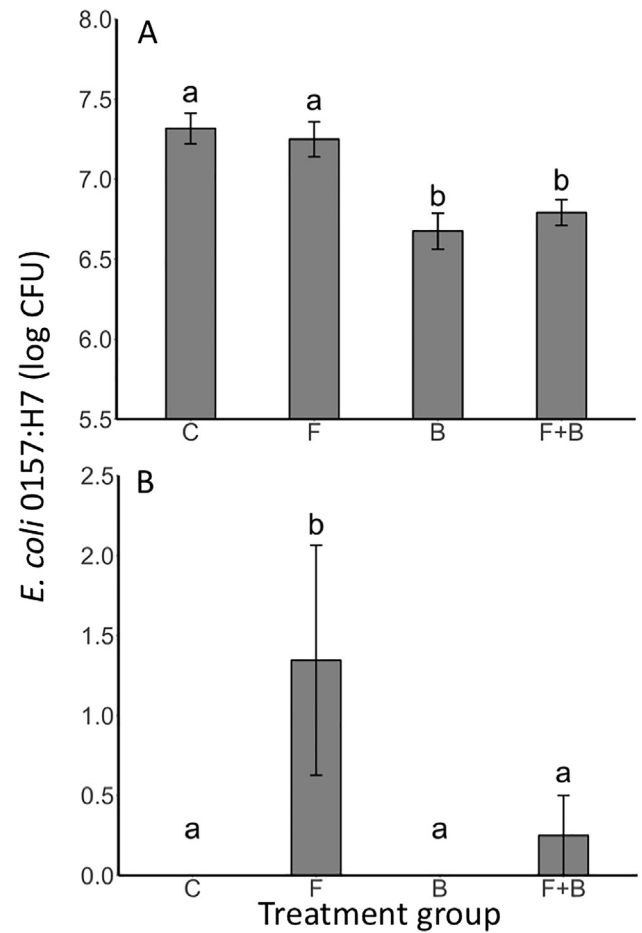


Fig. 3. Number of *E. coli* O157:H7 from (A) soil samples and (B) broccoli leaf surfaces. Treatment groups are: no coprophagous insect control (C), *C. vomitoria* flies only (F), *O. taurus* beetles only (B), and both flies and beetles (F + B). Data are means \pm SE of log-transformed colony forming units (CFU).

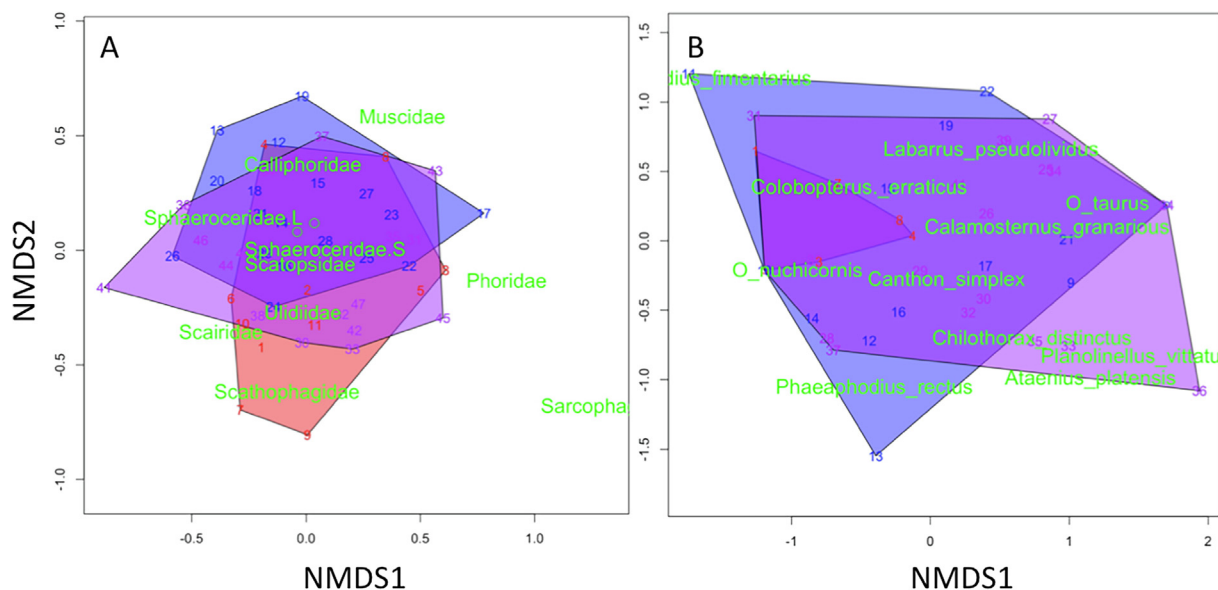


Fig 2. Non-metric multidimensional scaling (NMDS) plots of (A) fly and (B) dung beetle communities from farms managed using either conventional methods (red), organic methods (blue), or organic methods with integrated livestock (purple). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1

Pairwise comparisons of pathogen levels found in experimental soil (A), on broccoli leaves (B), and on flies (C).

A. In Soil	
Treatments	P-value
Beetles:Control	0.0033
Beetles:Flies	0.0076
Flies + Beetles:Control	0.0139
Flies + Beetles:Flies	0.032
Flies + Beetles:Beetles	0.8447
Flies:Control	0.9629
B. On Leaf	
Treatments	P-value
Fly:Control	0.0081
Fly:Beetles	0.0081
Fly: Flies + Beetles	0.0478
Flies + Beetles:Beetles	0.231
Flies + Beetles:Control	0.231
Beetles:Control	0.5
C. On Flies	
Treatments	P-value
Flies + Beetles:Flies	0.0433

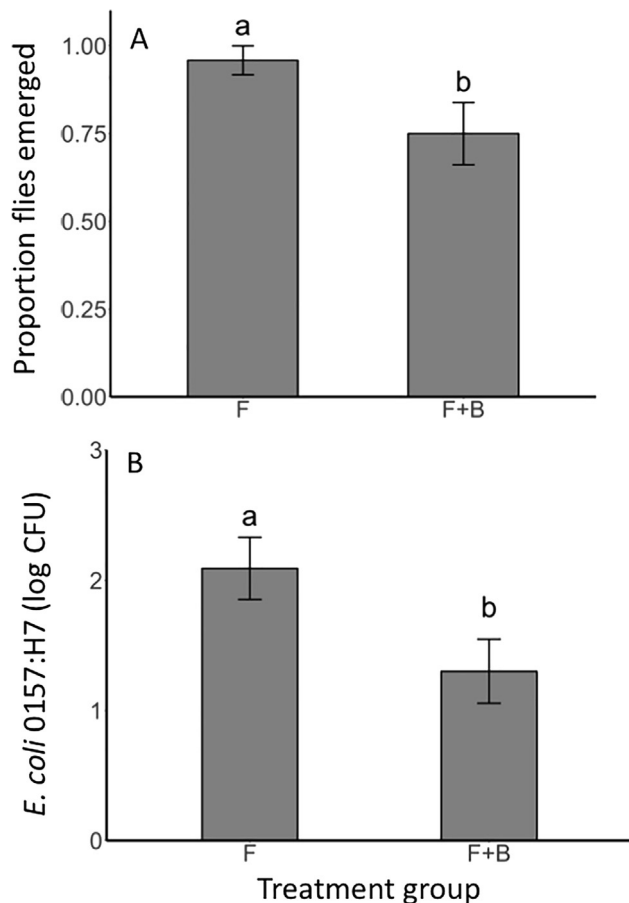


Fig. 4. (A) Fly emergence and (B) per capita numbers of pathogenic *E. coli* on flies. Data are means \pm SE.

“Flies + Beetles” treatment (Fig. 4B, Table 1C, $p = 0.0433$).

4. Discussion

Coprothagous insects provide key ecosystem services to agriculture (Losey and Vaughan, 2006; Nichols et al., 2008) by reducing pasture fouling (= feces removal) (Bertone et al., 2005; Kaartinen et al., 2013) and, as part of this work, facilitating nutrient cycling (Bang et al., 2005; Manning et al., 2016). However, perhaps less appreciated is the contribution of coprophages to ensuring food safety (Nichols et al., 2017) as they consume feces contaminated with human enteric pathogenic bacteria (e.g., Jones et al., 2015, 2019). From our field survey of the fly and dung beetle communities across CA and OR (USA), the R values (ANOSIM statistics) are relatively low, suggesting the variation/separation in the communities can be partially attributed to the farm management system. Calliphorid flies, which are previously known to transmit pathogenic *E. coli* to leafy greens (Talley et al., 2009) were one of the most abundant flies collected throughout our study. Importantly, we found this group of flies to occur in all farm management systems. Olsen (1998) provides a comprehensive review of this group of “filth” flies and indicates that they are widespread across agroecosystems and well known to transmit enteric pathogens. Interestingly, the dung beetle species *O. taurus*, previously found to be highly suppressive of pathogenic *E. coli* (Jones et al., 2019), occurred most commonly in the integrated and organic farm management systems, as opposed to conventional fields. This suggests that, while a fly species likely to vector *E. coli* occurs in all systems, the dung beetle species most likely to reduce the pathogen occurs most often in the two organic systems.

Our field survey revealed differing communities of flies and dung beetles across farms and, in some cases, across farming systems (Fig. 2, SI data beetles and SI data flies). We next used a microcosm experiment to examine whether these insect-community differences might impact food safety. Jones et al. (2015, 2019) previously reported that dung beetles, including the species *O. taurus* used in our experiment, were able to suppress levels of pathogenic *E. coli* O157:H7 in vertebrate feces. We again found evidence for this, as bacterial densities were reduced ca. 92% in the presence versus absence of these dung beetles. This reduction could be due to lethal digestion of the bacteria during beetle feeding (e.g., Snyder et al., 1998), although an antimicrobial effect of contact with dung beetle cuticles has also been suggested in the literature (Hwang et al., 2008). Feeding by *C. vomitoria* flies yielded no reduction in *E. coli* numbers; we could not find any literature with which to compare this finding. Because the flies moved bacteria to leaf surfaces, as has been reported elsewhere for related fly species (e.g., Talley et al., 2009), the individual impact of the flies was to allow normal persistence of the pathogen while facilitating pathogen movement to the foliage where they could eventually lead to foodborne illness in humans (e.g., Bach et al., 2002). Therefore, the individual effect of these beetles was largely beneficial, and of these flies largely harmful, from the standpoint of food safety.

Ecosystem services delivered by communities of insects can reflect a summing of both positive and negative impacts of individual species (Straub and Snyder, 2008). An example of this comes from the community of predatory insects and parasitoid wasps that attack aphids on *B. oleracea* plants (Snyder et al., 2006; Gable et al., 2012). The predators feed on parasitoids developing within the aphids (Snyder et al., 2006), a form of intraguild predation that can disrupt overall aphid suppression (e.g., Snyder and Ives, 2001). However, the predators also complement one another by foraging on different parts of leaves, such that only a diverse community of predators occupying these spatially distinct feeding niches can provide aphid control everywhere the pests occur on plants (Snyder et al., 2006; Gable et al., 2012). Indeed, the net effect of diverse predator and parasitoid communities on these *B. oleracea* plants is to improve aphid suppression by providing beneficial spatial-niche complementarity that counterbalances any harmful intraguild predation (Snyder et al., 2006; Gable et al., 2012).

Something roughly analogous appeared to be at work when we paired dung beetles with flies in our experiment. In the presence of dung beetles, persistence of *E. coli* in soil was reduced providing fewer opportunities for flies to become infested with the bacteria and then transport them to plant foliage (Fig. 3B). Dung beetles also reduced fly survivorship (Fig. 4A), so that fewer flies were present in any case. This means that dung beetles continued to benefit pathogen suppression while also lessening the risk that *C. vomitoria* flies might otherwise pose to food safety. While we did not examine specifically how dung beetles are lowering fly survivorship, we suspect that either dung beetles directly harm fly larvae as the beetles feed or that the beetles outcompete the fly larvae for food (e.g., Bishop et al., 2005). Clearly, more work is needed to clarify this point.

Our work suggests that organic farming might be a management approach that allows growers to better harness the benefits of one species of coprophagous insect capable of benefitting safe food production, the dung beetle *O. taurus*. Similarly, Jones et al. (2019) found that organic vegetable farms fostered diverse communities of dung beetles and antagonistic soil bacteria that reduce persistence of human pathogenic *E. coli*. Here, we expand these findings to suggest that organic farms that house robust numbers of *O. taurus* also have the potential to limit harm to food safety that otherwise might be posed by the coprophagous fly *C. vomitoria*. These possible contributions to food safety join a long list of ecosystem services thought to be improved on organic farms, including biological control (Crowder et al., 2010), pollination (Holzschuh et al., 2008), and enhanced soil health (Reganold and Wachter, 2016).

At the same time, however, organic management appeared to reshape communities of coprophagous flies, specifically with the house/stable flies (family Muscidae) being more common in organic fields and the dung flies (family Scathophagidae) being more common in conventional fields. Both of these groups are also known to transmit enteric pathogens (Iwasa et al., 1999; Graczyk et al., 2005; Junqueira et al., 2017). It is not clear why farming system had such relatively strong impacts on fly compared to dung beetle communities, although differences in fertility management and pesticide applications that appear to impact dung beetles (Jones et al., 2019) could be possible explanations. Future work is needed to determine how *O. taurus* interacts with the many other fly species found on these farms, and how the flies interact with other dung beetle species. This information will be needed before we can fully assess whether organic farming attracts enough beneficial dung beetles to counteract any harmful effects of the fly species that the farms also harbor.

In addition to improving chemical and biological soil attributes, contributing to higher quality pasture and reducing greenhouse gas emissions (Salton et al., 2014), bringing livestock onto farms can diversify farmers' production which in turn attracts a wider customer base and provides income stability (Herrero et al., 2010; Bell et al., 2014). An obvious risk to this farming approach, however, is the possibility that feces produced by the livestock will contaminate produce with enteric pathogens (e.g., Newell et al., 2010; Pennington, 2010). However, the work reported here provides further evidence (see also Jones et al. (2019)) that integrated livestock farms also can attract beneficial coprophages like *O. taurus* with the potential to, at least partly, offset any enhanced risks to food safety. In general, more work is needed to identify farming practices that enhance beneficial coprophage biodiversity and increase their ability to biotically resist the persistence and spread of enteric pathogens, while discouraging populations of potentially harmful coprophages like *C. vomitoria* with the potential to worsen food safety risks. Also, work is needed to determine whether a relatively subtle reshaping of fly community structure on integrated livestock farms is introducing new risks to food safety not seen for the flies on vegetable-only farms managed using either organic or conventional practices.

We now know that insect biodiversity on farms can improve the delivery of the key ecosystem services of pollination (Kremen et al.,

2002; Garibaldi et al., 2016) and natural pest control (Letourneau et al., 2009; Crowder et al., 2010; Northfield et al., 2010). These benefits can be enhanced by diverse crop and non-crop plantings that provide more resources to beneficial insects (Parker et al., 2016; Lichtenberg et al., 2017). Ensuring food safety, once seen as a unique exception (e.g., Beretti and Stuart, 2008; LGMA, 2013) to this broader pattern, now seems instead to be another example of insect biodiversity leading to enhanced ecosystem services. The work presented here suggests the possibility that diversified farming systems that attract particularly beneficial species of dung beetle have the potential to mediate risks that coprophagous flies might pose to food safety. However, several important gaps remain to be filled. First, it is unclear whether the beneficial dung beetle-fly interactions seen in our microcosms reflect interactions likely to occur in the larger, more complex environments that real farms provide. It would be valuable, if logistically challenging, to examine enteric pathogen levels in/on flies on farms with simple versus complex dung beetle communities. Second, it is not entirely clear what relative risk coprophagous flies pose as vectors of enteric pathogens in a field context where bacteria and viruses may come into contact with produce through many different routes (Newell et al., 2010). However, it is perhaps reassuring that some of the same mechanisms leading to positive versus negative diversity effects among other insects – complementarity and interference – might also be at work within coprophage communities. This suggests that studies in other systems may provide a roadmap for gaining a better understanding of biodiversity-food safety relationships.

CRedit authorship contribution statement

Matthew S. Jones: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Resources, Project administration, Funding acquisition, Supervision, Writing - original draft, Writing - review & editing. **Stephanie A. Wright:** Methodology, Investigation, Data curation, Writing - original draft, Writing - review & editing. **Olivia M. Smith:** Formal analysis, Visualization, Writing - original draft, Writing - review & editing. **Thomas E. Besser:** Conceptualization, Methodology, Supervision, Writing - original draft, Writing - review & editing. **David H. Headrick:** Conceptualization, Investigation, Data curation, Resources, Supervision, Writing - original draft, Writing - review & editing. **John P. Reganold:** Conceptualization, Supervision, Writing - original draft, Writing - review & editing. **David W. Crowder:** Formal analysis, Supervision, Writing - review & editing. **William E. Snyder:** Conceptualization, Resources, Project administration, Funding acquisition, Supervision, Writing - original draft, Writing - review & editing.

Acknowledgements

The project was supported by USDA NIFA Organic Transitions program grant 2014-03354. MSJ was supported by USDA NIFA predoctoral fellowship 2016-04538. We thank C. Crawford, A. Urango-MacDonald, C. Freeman, A. Watts, L. Mae, and R. McIntosh for field and lab assistance. We thank R. McPeak for taxonomic assistance and K. Jones for GIS/map-making assistance.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biocontrol.2019.104020>.

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