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

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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 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.

Keywords

Biological control | biotic resistance | pathogenic *E. coli* | food safety | ecosystem services | coprophage

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; Karp et al., 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: Scarabeidae) 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 et al., 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., 2014, Jones et al., 2019) and flies (Loy, 1972). Three traps were 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; Cartwright, 1974; Gordon and Cartwright, 1980; Arnett et al., 2002; Gordon and Skelley, 2007). Flies were identified to the family level (*per* McAlpine et al., 1987). Only those taxa with known dung associations were used for analysis (*per* Encyclopedia of Life [http:// http://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-liter 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 hrs. 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 hrs at 37° C. After incubation, up to 4 colonies (of the 8) were tested using a Latex 0157 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 heteroskadastic 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 'lsmeans', 'ggplot2', 'dunn.test', 'plyr', and 'vegan' packages (Dinno, 2017; Lenth, 2016; Oksanen et al., 2018; R core team, 2017; Wickham, 2009; Wickham, 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 2,688 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, Table1B).

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

4. Discussion

Coprophagous insects provide key ecosystem services to agriculture (Losey and Vaughan, 2006; Nichols, 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 et al., 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. vomitora*. 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, H., 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. vomitora* 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.

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Appendix A. Supplementary data.

Supplementary data associated with this article can be found, in the online version, at http://xx

References

Ackers, M.L., Mahon, B.E., Leahy, E., Goode, B., Damrow, T., Hayes, P.S., Slutsker, L., 1998. An outbreak of *Escherichia coli* O157:H7 infections associated with leaf lettuce consumption. Journal of Infectious Diseases 177, 1588–1593.

Arnett, R.H., Thomas, M.C., Skelley, P.E. (eds.) 2002. American Beetles, Volume II: Polyphaga: Scarabaeoidea through Curculionoidea. Vol. 2. CRC press.

Bach, S.J., McAllister, T.A., Veira, D.M., Gannon, V.P.J., Holley, R.A., 2002. Transmission and control of *Escherichia coli* O157: H7—a review. Canadian Journal of Animal Science 82, 475-490.

Bang, H.S., Lee, J.H., Kwon, O.S., Na, Y.E., Jang, Y.S., Kim, W.H., 2005. Effects of paracoprid dung beetles (Coleoptera: Scarabaeidae) on the growth of pasture herbage and on the underlying soil. Applied Soil Ecology 29, 165-171.

Barrios-Garcia, M.N., Ballari, S.A., 2012. Impact of wild boar (*Sus scrofa*) in its introduced and native range: a review. Biological Invasions 14, 2283-2300.

Bell, L.W., Moore, A.D., Kirkegaard, J.A., 2014. Evolution in crop–livestock integration systems that improve farm productivity and environmental performance in Australia. European Journal of Agronomy 57, 10-20.

Beretti, M., Stuart, D., 2008. Food safety and environmental quality impose conflicting demands on Central Coast growers. California Agriculture 62, 68-73.

Bertone, M., Watson, W., Stringham, M., Green, J., Washburn, S., Poore, M., Hucks, M., 2005. Dung beetles of central and eastern North Carolina cattle pastures. North Carolina Cooperative Extension, North Carolina State University, Raleigh, NC.

Bishop, A.L., McKenzie, H.J., Spohr, L.J., Barchia, I.M., 2005. Interactions between dung beetles (Coleoptera: Scarabaeidae) and the arbovirus vector *Culicoides brevitarsis* Kieffer (Diptera: Ceratopogonidae). Australian Journal of Entomology 44, 89-96.

Borg I., Groenen P., 1997. Modern Multidimensional Scaling: Theory and Applications. Springer-Verlag, New York, 471 pp.

Bugguide.net. available from https://bugguide.net/node/view/15740 (accessed December 2017).

Cartwright, O.L., 1948. The American species of *Pleurophorus* (Coleoptera: Scarabaeidae). Transactions of the American Entomological Society 74, 131-145.

Cartwright, O.L., 1974. *Ataenius*, *Aphotaenius*, and *Pseudataenius* of the United States and Canada. Smithsonian Contributions to Zoology 154, 1-106.

Centers for Disease Control and Prevention Outbreak Surveillance Data, 2010. Available from https://www.cdc.gov/fdoss/index.html (Accessed 6 June 2018).

Clarke, K.R., 1993. Non-parametric multivariate analyses of changes in community structure. Australian Journal of Ecology 18, 117–143.

Crowder, D.W., Northfield, T.D., Strand, M.R., Snyder, W.E., 2010. Organic agriculture promotes evenness and natural pest control. Nature 466, 109-112.

Dinno, A., 2017. dunn.test: Dunn's Test of Multiple Comparisons Using Rank Sums. R package version 1.3.5. available from https://CRAN.R-project.org/package=dunn.test

Encyclopedia of Life. Available from http://www.eol.org. Accessed December 2017.

Floate, K.D., 2011. Arthropods in Cattle Dung on Canada's Grasslands. Arthropods of Canadian Grasslands (Volume 2): Inhabitants of a Changing Landscape 2, 71–88.

Gable, J., Northfield T.D., Crowder, D.W., Steffan, S.A., Snyder W.E., 2012. Niche engineering reveals complementary resource use. Ecology 93, 1994-2000.

Garibaldi, L.A., Carvalheiro, L.G., Vaissiere, B.E., Gemmill-Herren, B., Hipolito, J., Freitas, B.M., ... Zhang, H., 2016. Mutually beneficial pollinator diversity and crop yield outcomes in small and large farms. Science 351, 388–391

Gautam, R., Bani-Yaghoub, M., Neill, W. H., Döpfer, D., Kaspar, C., Ivanek, R., 2011. Modeling the effect of seasonal variation in ambient temperature on the transmission dynamics of a pathogen with a free-living stage: example of *Escherichia coli* O157: H7 in a dairy herd. Preventive Veterinary Medicine 102, 10-21.

Gordon, R.D., Cartwright, O.L., 1980. The Western Hemisphere species of *Rhyssemus* and *Trichiorhyssemus* (Coleoptera: Scarabaeidae). Smithsonian Contributions to Zoology 317, 1-29.

Gordon, R.D., Skelley. P.E., 2007. A monograph of the Aphodiini inhabiting the United States and Canada (Coleoptera: Scarabaeidae: Aphodiinae). Memoirs of the American Entomological Institute 79, 1-580.

Graczyk, T.K., Knight, R., Tamang, L., 2005. Mechanical transmission of human protozoan parasites by insects. Clinical microbiology reviews, 18, 128-132.

Habeeb, M.A., Mahdi, M.A., 2012. Mechanical transmission of bacteria via animal agents truefly species. Advanced Studies in Biology 4, 583-591.

Hancock, D.D., Besser, T.E., Rice, D.H., Ebel, E.D., Herriott, D.E., Carpenter, L.V., 1998. Multiple sources of *Escherichia coli* O157 in feedlots and dairy farms in the northwestern USA. Preventive Veterinary Medicine 35, 11–9.

Herrero, M., Thornton, P. K., Notenbaert, A. M., Wood, S., Msangi, S., Freeman, H. A., Bossio, D., Dixon, J., Peters, M., van de Steeg, J., Lynam, J., Parthasarathy Rao, P., Macmillan, S., Gerard, B., McDermott, J., Seré, C., Rosegrant, M., 2010. Smart investments in sustainable food production: revisiting mixed crop-livestock systems. Science 327, 822-825.

Holzschuh, A., Steffan-Dewenter, I., Tscharntke, T., 2008. Agricultural landscapes with organic crops support higher pollinator diversity. Oikos 117, 354-361.

Hutton, S.A., Giller, P.S., 2003. The effects of the intensification of agriculture on northern temperate dung beetle communities. Journal of Applied Ecology 40, 994–1007.

Hwang, J.S., Kim, Y.J., Bang, H.S., Yun, E.Y., Kim, Y.T., Kim, S.R., Jeon, J.P., 2008. Isolation of antibacterial response genes from the dung beetle *Copris tripartitus* (Coleoptera : Scarabaeidae) immunized with *Escherichia coli*. European Journal of Entomology 105, 355–359.

Iwasa, M., Makino, S.I., Asakura, H., Kobori, H., Morimoto, Y., 1999. Detection of Escherichia coli O157: H7 from Musca domestica (Diptera: Muscidae) at a cattle farm in Japan. Journal of Medical Entomology, 36, 108-112.

Jay, M.T., Cooley, M., Carychao, D., Wiscomb, G.W., Sweitzer, R.A., Crawford-Miksza, L., Asmundson, R.V., 2007. *Escherichia coli* O157:H7 in feral swine near spinach fields and cattle, Central California Coast. Emerging Infectious Diseases 13, 1908–1911.

Jones, M.S., Tadepalli, S., Bridges, D.F., Wu, V.C.H., Drummond, F.A., 2015. Suppression of *Escherichia coli* O157:H7 by dung beetles (Coleoptera: Scarabaeidae) using the lowbush blueberry agroecosystem as a model system. PLoS ONE 10, e0120904

Jones, M. S., Fu, Z., Reganold, J. P., Karp, D. S., Besser, T. E., Tylianakis, J. M., & Snyder, W. E. 2019. Organic farming promotes biotic resistance to foodborne human pathogens. Journal of Applied Ecology 56, 1117-1127.

Junqueira, A. C. M., Ratan, A., Acerbi, E., Drautz-Moses, D. I., Premkrishnan, B. N., Costea, P. I., ... & Subramanian, P., 2017. The microbiomes of blowflies and houseflies as bacterial transmission reservoirs. Scientific Reports *7*, 16324.

Kaartinen, R., Hardwick, B., Roslin, T., 2013. Using citizen scientists to measure an ecosystem service nationwide. Ecology 94, 2645-2652.

Karp, D.S., Baur, P., Atwill, E.R., De Master, K., Gennet, S., Iles, A., Kremen, C., 2015. The unintended ecological and social impacts of food safety regulations in California's Central Coast Region. BioScience 65, 1173-1183.

Karp, D.S., Gennet, S., Kilonzo, C., Partyka, M., Chaumont, N., Atwill, E.R., Kremen, C., 2015. Comanaging fresh produce for nature conservation and food safety. Proceedings of the National Academy of Sciences 112, 11126–11131.

Karp, D.S., Moses, R., Gennet, S., Jones, M.S., Joseph, S., M'Gonigle, L.K., Ponisio, L.C., Snyder, W.E., Kremen, C., 2016. Agricultural practices for food safety threaten pest control services for fresh produce. Journal of Applied Ecology 53, 1402-1412.

Kennedy, C.M., Marra, P.P., Fagan, W.F., Neel, M.C., 2010. Landscape matrix and species traits mediate responses of Neotropical resident birds to forest fragmentation in Jamaica. Ecological Monographs 80, 651–669.

Larsen, T.H., Forsyth, A., 2005. Trap spacing and transect design for dung beetle biodiversity studies. Biotropica 37, 322–325.

Lenth R.V., 2016. Least-Squares Means: The R Package lsmeans. Journal of Statistical Software 69, 1-33. doi:10.18637/jss.v069.i01

Letourneau, D.K., Jedlicka, J.A., Bothwell, S.G., Moreno, C.R., 2009. Effects of natural enemy biodiversity on the suppression of arthropod herbivores in terrestrial ecosystems. Annual Review of Ecology, Evolution, and Systematics 40, 573-592.

Letourneau, D.K., Bothwell, S.G., Robert, A., Michael, R. K., Sharkey, M.J., Stireman, J.O., 2015. Habitat eradication and cropland intensification may reduce parasitoid diversity and natural pest control services in annual crop fields. Elementa: Science of the Anthropocene 3, 1–13.

LGMA, 2013. Commodity specific food safety guidelines for the production and harvest of lettuce and leafy greens. Sacramento, CA. Retrieved from http://www.caleafygreens.ca.gov/sites/default/files/California LGMA metrics 08 26 13 Final.pdf

Lichtenberg, E.M., Kennedy, C.M., Kremen, C., Batáry, P., Berendse, F., Bommarco, R., ... Winfree, R., 2017. A global synthesis of the effects of diversified farming systems on arthropod diversity within fields and across agricultural landscapes. Global Change Biology 23, 4946-4957.

Liu, Q., Tomberlin, J. K., Brady, J. A., Sanford, M. R., Yu, Z., 2008. Black soldier fly (Diptera: Stratiomyidae) larvae reduce *Escherichia coli* in dairy manure. Environmental Entomology 37, 1525-1530

Losey, J. E., Vaughan, M., 2006. The economic value of ecological services provided by insects. BioScience 56, 311-323.

Lowell, K., Langholz, J., Stuart, D., 2010. Safe and sustainable: Co-managing for food safety and ecological health in California's Central Coast region. Available at ucfoodsafety. ucdavis.edu/files/198568.pdf. Accessed June 6, 2015.

Loy, V.A., 1972. Environmental Factors and Their Effects on House Fly (Musca Domestica L.) Ecology and Suppression. Retrieved from University of Arizona Libraries campus repository.

Manning, P., Slade, E.M., Beynon, S.A., Lewis, O.T., 2016. Functionally rich dung beetle assemblages are required to provide multiple ecosystem services. Agriculture, Ecosystems & Environment 218, 87-94.

Marsh, C. J., Louzada, J., Beiroz, W., Ewers, R.M., 2013. Optimising bait for pitfall trapping of Amazonian dung beetles (Coleoptera: Scarabaeinae). PLoS ONE 8, e73147.

McAlpine, J.F., 1987. Morphology and terminology – adults, p. 675-1332. In: J.F. McAlpine; B.V. Peterson; G. E. Shewell; H.J. Teskey; J. R Vockeroth, D.M. Wood (eds.). Manual of Nearctic Diptera. Vol. 2. Monograph 28. Research Branch Agriculture Canada.

Newell, D.G., Koopmans, M., Verhoef, L., Duizer, E., Aidara-Kane, A., Sprong, H., … Kruse, H., 2010. Food-borne diseases - the challenges of 20 years ago still persist while new ones continue to emerge. International Journal of Food Microbiology 139, S3-15.

Nichols, E., Spector, S., Louzada, J., Larsen, T., Amezquita, S., Favila, M.E., 2008. Ecological functions and ecosystem services provided by Scarabaeinae dung beetles. Biological Conservation 141, 1461-1474.

Nichols, E., Alarcón, V., Forgie, S., Gomez-Puerta, L. A., Jones, M.S., 2017. Coprophagous insects and the ecology of infectious diseases of wildlife. ILAR Journal 58, 336-342.

Northfield, T.D., Snyder, G.B., Ives, A.R., Snyder, W.E., 2010. Niche saturation reveals resource partitioning among consumers. Ecology Letters 13, 338-348.

O'Hea, N.M., Kirwan, L., Finn, J.A., 2010. Experimental mixtures of dung fauna affect dung decomposition through complex effects of species interactions. Oikos 119, 1081–1088.

Oksanen J., Guillaume Blanchet, F., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Henry, M., Stevens, H., Szoecs, E., Wagner, H., 2018. vegan: Community Ecology Package. R package version 2.4-6. https://CRAN.R-project.org/package=vegan

Olsen, A.R., 1998. Regulatory action criteria for filth and other extraneous materials: III. Review of flies and foodborne enteric disease. Regulatory Toxicology and Pharmacology 28, 199-211.

Olsen, A.R., Hammack, T.S. 2000. Isolation of *Salmonella* spp. from the housefly, *Musca domestica* L., and the dump fly, *Hydrotaea aenescens* (Wiedemann) (Diptera: Muscidae), at caged-layer houses. Journal of Food Protection 63, 958-960.

Painter, J.A., Hoekstra, R.M., Ayers, T., Tauxe, R.V., Braden, C.R., Angulo, F.J., Griffin, P.M., 2013. Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008. Emerging Infectious Diseases 19, 407-415.

Parker, J.E., Crowder, D.W., Eigenbrode, S.D., Snyder, W.E., 2016. Trap crop diversity enhances crop yield. Agriculture, Ecosystems & Environment 232, 254-262.

Pennington, H., 2010. *Escherichia coli* O157. The Lancet 376, 1428-1435.

Quinn, G. P., Keough, M. J. (2002). *Experimental design and data analysis for biologists*. Cambridge University Press.

R Core Team, 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Reisen, W.K., Fang, Y., Martinez, V.M., 2005. Avian host and mosquito (Diptera: Culicidae) vector competence determine the efficiency of West Nile and St. Louis encephalitis virus transmission. Journal of Medical Entomology 42, 367-375.

Reganold, J. P., Wachter, J. M., 2016. Organic agriculture in the twenty-first century. Nature Plants 2, 15221.

Salton, J.C., Mercante, F.M., Tomazi, M., Zanatta, J.A., Concenco, G., Silva, W.M., Retore, M., 2014. Integrated crop-livestock system in tropical Brazil: Toward a sustainable production system. Agriculture, Ecosystems & Environment 190, 70-79.

Scott, J.G., Warren, W.C., Beukeboom, L.W., Bopp, D., Clark, A.G., Giers, S.D., Liu, N., 2014. Genome of the house fly, *Musca domestica* L., a global vector of diseases with adaptations to a septic environment. Genome Biology 15, 466. http://doi.org/10.1186/s13059-014-0466-3

Snyder, W.E., Ives, A.R., 2001. Generalist predators disrupt biological control by a specialist parasitoid. Ecology 82, 705-716.

Snyder, W.E., Tonkyn, D.W., Kluepfel, D.A., 1998. Insect mediated dispersal of the rhizobacterium *Pseudomonas chlororaphis*. Phytopathology 88, 1248-1254.

Snyder, W.E., Snyder, G.B., Finke, D.L., Straub, C.S., 2006. Predator biodiversity strengthens herbivore suppression. Ecology Letters 9, 789-796.

Talley, J.L., Wayadande, A.C., Wasala, L.P., Gerry, A.C., Fletcher, J., DeSilva, U., Gilliland, S.E., 2009. Association of *Escherichia coli* O157: H7 with filth flies (Muscidae and Calliphoridae) captured in leafy greens fields and experimental transmission of *E. coli* O157:H7 to spinach leaves by house flies (Diptera: Muscidae). Journal of Food Protection 72, 1547-1552.

Tanada, Y.O., Fuxa, J.R., 1987. The pathogen population. Epizootiology of Insect Diseases 113-157.

USDA, 2017. Guidance & Instructions for Accredited Certifying Agents & Certified Operations. https://www.ams.usda.gov/rules-regulations/organic/handbook. (Accessed 18 September 2017).

Wales, A.D., Carrique-Mas, J.J., Rankin, M., Bell, B., Thind, B.B., Davies, R. H., 2010. Review of the carriage of zoonotic bacteria by arthropods, with special reference to *Salmonella* in mites, flies and litter beetles. Zoonoses and Public Health 57, 299–314.

Wickham, H., 2009. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, 2009.

Wickham, H., 2011. The split-apply-combine strategy for data analysis. Journal of Statistical Software 40, 1-29.

Zar J., 1999. Biostatistical Analysis. Fourth edition. Prentice Hall. Upper Saddle River, NJ.

Figure/table captions

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

Fig 2. Non-metric multidimensional scaling (NMSD) 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).

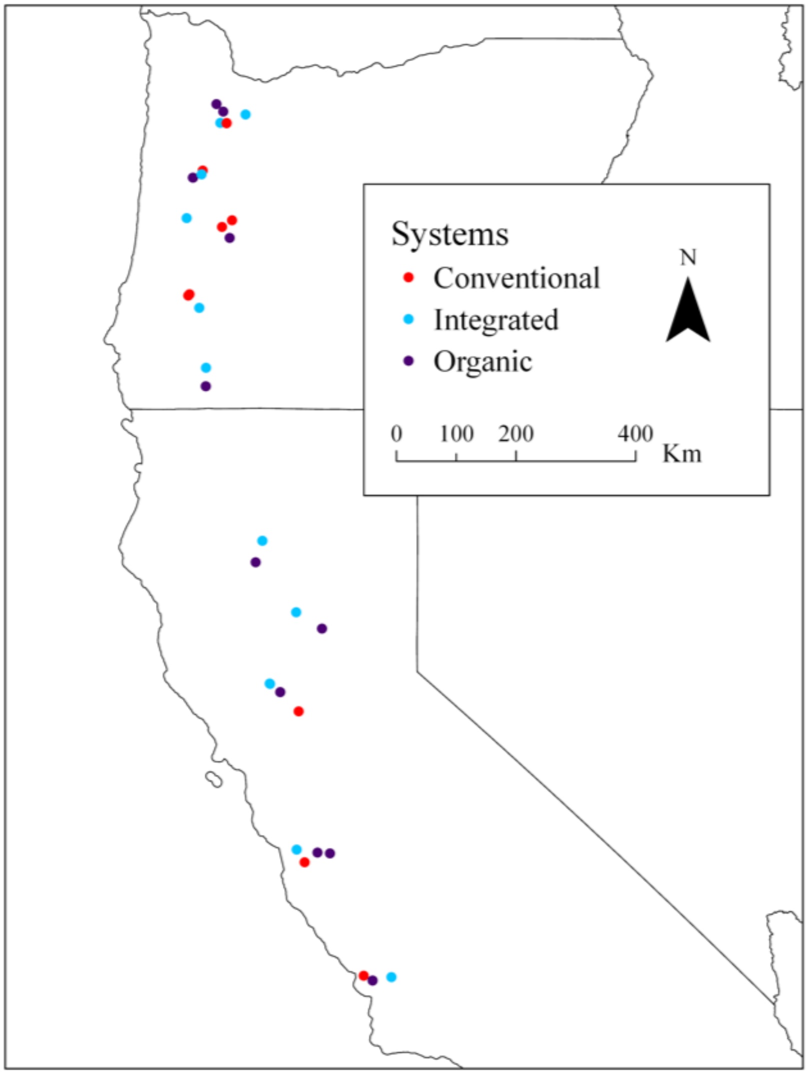
Fig. 3. Fig. 3. Number of *E. coli* O157:H7 from (A) soil samples and (B) broccoli leaf surfaces. Treatment groups are: no coprophagous insect control (C), *C. vomitoria* flies only (F), *O. taurus*

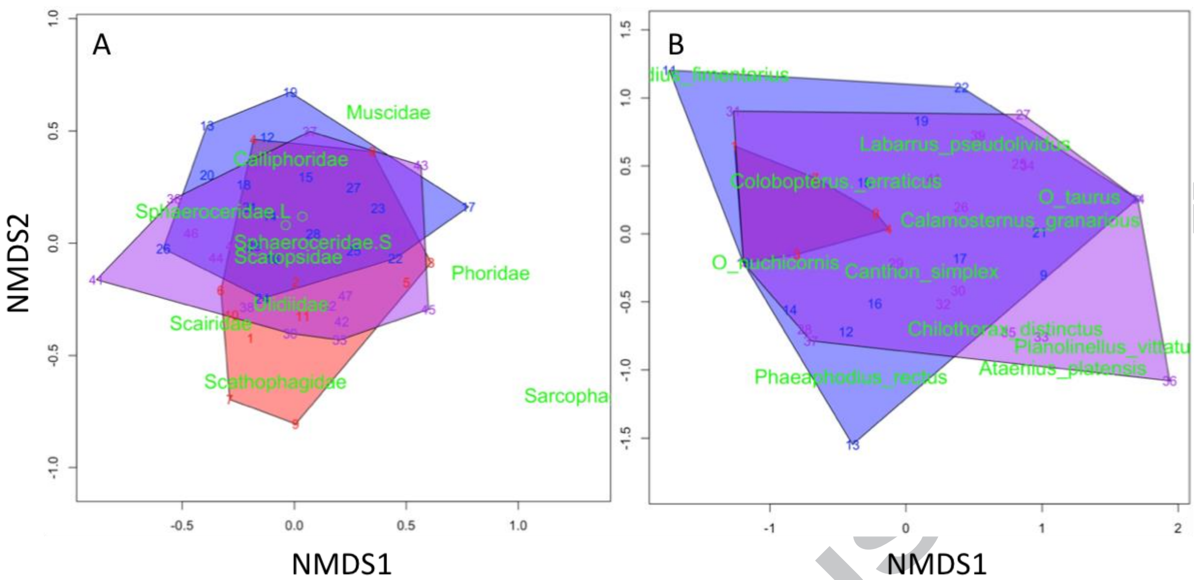
beetles only (B), and both flies and beetles (F+B). Data are means ± SE of log-transformed colony forming units (CFU).

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

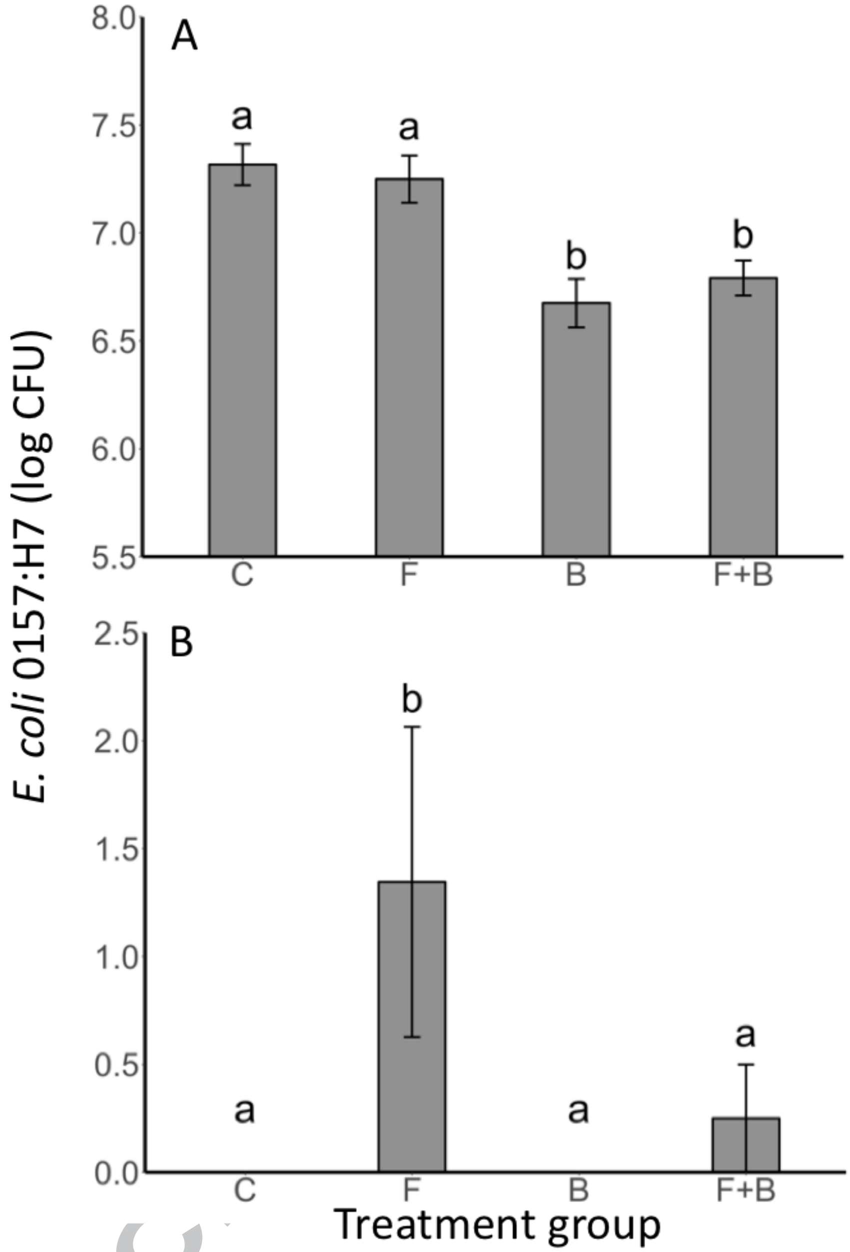
Fig. SI 1. Experimental Microcosm setup.

Table 1. Pairwise comparisons of pathogen levels found in experimental soil (A), on broccoli leaves (B), and on flies (C).

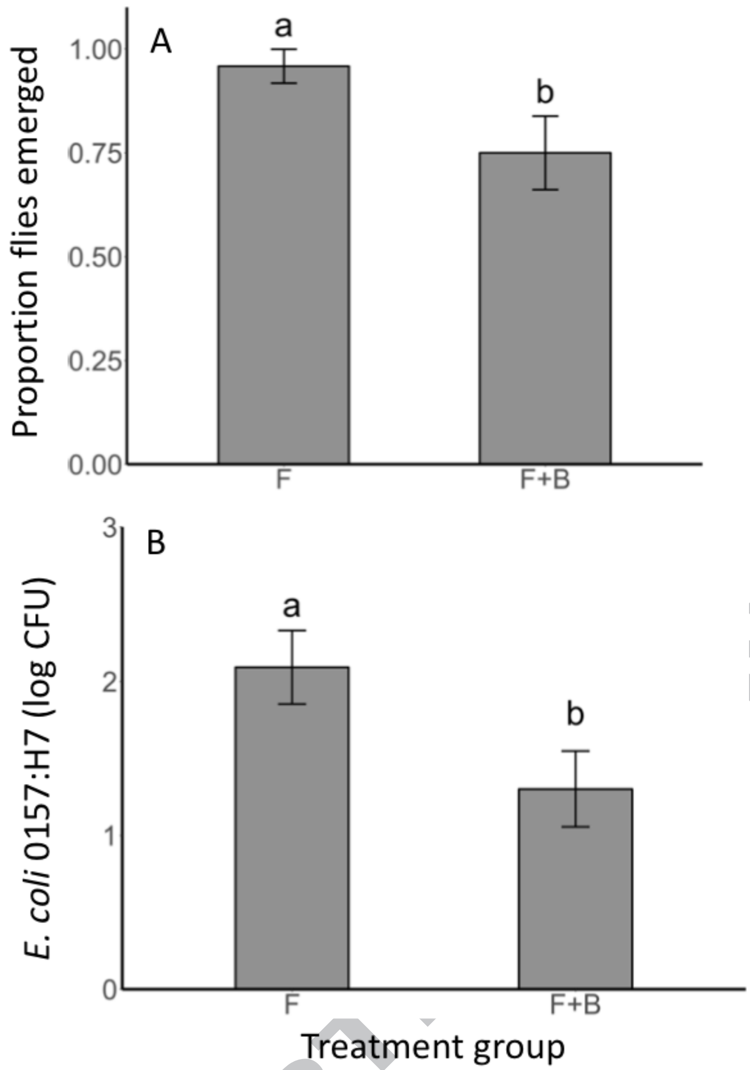

Systems
Conventional
Integrated
Organic
N
0
100
200
400
Km



A
B
NMDS1
NMDS2
Muscidae
Calliphoridae
Sphaeroceridae.L
Sphaeroceridae.S
Scatopsidae
Phoridae
Ulidiidae
Sciaridae
Scathophagidae
Sarcopha
Labarrus_pseudolividus
Colobopterus_erraticus
O_taurus
Calamosternus_granarius
O_nuchicornis
Canthon_simplex
Chilothorax_distinctus
Planolinellus_vittatu
Ataenius_platensis
Phaeaphodius_rectus



A
B
E. coli 0157:H7 (log CFU)
8.0
7.5
7.0
6.5
6.0
5.5
a
a
b
b
C
F
B
F+B
2.5
2.0
1.5
1.0
0.5
0.0
b
a
a
a
Treatment group


A
a
b
Proportion flies emerged
1.00
0.75
0.50
0.25
0.00
F
F+B
B
a
b
E. coli 0157:H7 (log CFU)
3
2
1
0
F
F+B
Treatment group

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

Highlights

- Produce contamination with human pathogens might be suppressed or enhanced by coprophagous insects.
- We examined how farming practices impact dung beetle and coprophagous fly communities and possible broccoli contamination by *E. coli* O157:H7.
- The dung beetle *Onthophagus taurus* directly suppressed *E. coli* persistence in feces and reduced their spread by the dung-feeding fly *Calliphora vomitora*.
- The beneficial beetle commonly occurred only on organic farms, suggesting possible links between food safety and organic farming worthy of further investigation.

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