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Agrochemicals indirectly increase survival of *E. coli* O157:H7 and indicator bacteria by reducing ecosystem services

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Abstract. Storm water and agricultural runoff frequently contain agrochemicals, fecal indicator bacteria (FIB), and zoonotic pathogens. Entry of such contaminants into aquatic ecosystems may affect ecology and human health. This study tested the hypothesis that the herbicide atrazine and the fungicide chlorothalonil indirectly affect the survival of FIB (*Escherichia coli* and *Enterococcus faecalis*) and a pathogen (*E. coli* O157:H7) by altering densities of protozoan predators or by altering competition from autochthonous bacteria. Streptomycin-resistant *E. coli*, *En. faecalis*, and *E. coli* O157:H7 were added to microcosms composed of Florida river water containing natural protozoan and bacterial populations. FIB, pathogen, and protozoan densities were monitored over six days. Known metabolic inhibitors, cycloheximide and streptomycin, were used to inhibit autochthonous protozoa or bacteria, respectively. The inhibitors made it possible to isolate the effects of predation or competition on survival of allochthonous bacteria, and each treatment increased the survival of FIB and pathogens. Chlorothalonil's effect was similar to that of cycloheximide, significantly reducing protozoan densities and elevating densities of FIB and pathogens relative to the control. Atrazine treatment did not affect protozoan densities, but, through an effect on competition, resulted in significantly greater densities of *En. faecalis* and *E. coli* O157:H7. Hence, by reducing predaceous protozoa and bacterial competitors that facilitate purifying water bodies of FIBs and human pathogens, chlorothalonil and atrazine indirectly diminished an ecosystem service of fresh water.

Key words: agrochemicals; atrazine; bacterivorous protozoa; chlorothalonil; Enterococcus faecalis; Escherichia coli; Escherichia coli *O157:H7; fecal indicator bacteria; zoonotic pathogens.*

Introduction

Wide-scale application of agrochemicals has become increasingly scrutinized in recent years for possible contributions to human health risks, including potentially carcinogenic effects and prolonging the survival of agriculturally associated zoonotic pathogens (Carmichael et al. 1997, Kudva et al. 1998, Fratamico et al. 2004, Semenov et al. 2009, Lebbad et al. 2010, Ziemer et al. 2010). Agrochemicals have been shown to alter population densities, community composition, predator–prey relationships, and ecosystem dynamics of freshwater systems (DeLorenzo et al. 2001, Downing et al. 2008, Verro et al. 2009*a*, *b*, Debenest et al. 2010). The mechanisms by which agrochemicals alter ecosystem function can be either direct or indirect and either beneficial (i.e., resulting in or increasing ecosystem services) or harmful (i.e., increasing risks to aquatic species, communities, and human health; decreasing ecosystems services [McMahon et al. 2012]). Previous research has shown that neither the herbicide atrazine nor the fungicide chlorothalonil directly affect the survival of selected fecal indicator bacteria (FIB) and zoonotic pathogens (Breazeale and Camper 1972, Staley et al. 2012); however, atrazine has been found to elevate *Escherichia coli* and enterococci densities in sediments via indirect effects (Staley et al. 2010). Specifically, atrazine treatments had an algal-mediated, indirect effect on FIB survival, wherein a significant inverse correlation was observed between phytoplankton densities (which decreased upon herbicide exposure) and *E. coli* densities in the sediment (Staley et al. 2011). Other indirect effects of atrazine and other agrochemicals may significantly alter ecosystem services and functions (Rohr and McCoy 2010). For example, the fungicide chlorothalonil is directly toxic to species at multiple trophic levels (i.e., amphibians, gastropods, and zooplankton) which results in indirect effects, such as decreasing decomposition, increasing dissolved oxygen and net primary productivity, and facilitating algal blooms and altering ecosystem functions (McMahon et al. 2012). Due to the myriad potential indirect effects and how significant they may be, further investigation of multiple trophic levels is necessary to understand how agrochemical application can affect ecosystem functions.

The effects of agrochemicals on organisms could be trait-mediated, altering behavior, immunity, physiology, or morphology, and have been observed in multicellular organisms, particularly amphibians (Rohr et al. 2003,

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2006, 2008*b*, Rohr and Crumrine 2005, Rohr and Palmer 2005), as well as microbes such as protozoa (DeLorenzo et al. 1999, 2001, De Laender et al. 2010, Debenest et al. 2010). Alternatively, indirect effects of agrochemicals may be density-mediated, altering the abundance of a target species' food resources, parasites, competitors, or predators. These effects have been seen in multicellular organisms (Rohr et al. 2006, 2008*a*, *b*, 2009, Raffel et al. 2008) as well as protozoa and bacteria (Dive et al. 1980, Ekelund 1999, Sumpono et al. 2003, Foit et al. 2010, Staley et al. 2011). As both agriculturally derived zoonotic pathogens and agrochemical residues are likely to be present in agricultural runoff, understanding the mechanism (direct or indirect) and direction (beneficial or adverse) of agrochemical effects in impacted water bodies is essential to utilizing agricultural practices that best safeguard human and ecosystem health.

Predation by bacterivorous protozoa and competition with native bacteria are important controls on allochthonous bacteria in aquatic environments (Wanjugi and Harwood 2012, Korajkic et al. 2013*a*, *b*). Previous studies have shown that protozoan densities are reduced when exposed to cycloheximide, a chemical that inhibits protein synthesis in eukaryotic organisms, and, as a consequence, the populations of their bacterial prey increase (Marino and Gannon 1991, Davies et al. 1995). Streptomycin and kanamycin, antibiotics that inhibit bacterial protein synthesis via binding to 16S rRNA, have been shown to reduce competition and increase the densities of bacteria resistant to these antibiotics (Wanjugi and Harwood 2012). Agrochemicals that are toxic to protozoan predators may exhibit similar effects as cycloheximide, reducing protozoan populations and increasing bacterial densities. Agrochemicals may also affect bacterial populations differently, potentially conferring a competitive advantage on unaffected or beneficially affected species.

Agriculturally associated pathogens, such as enteropathogenic *Es. coli* strains, *Salmonella enterica*, *Cryptosporidium* spp., *Giardia* spp., and zoonotic influenza viruses, are endemic or emerging human pathogens in many parts of the world (U.S. Environmental Protection Agency 2005, 2009*a*, *b*, Lebbad et al. 2010). Many of these agriculturally derived pathogens are waterborne and can enter water bodies via storm water and agricultural runoff containing fecal material from livestock, such as cattle, swine, and poultry (Fratamico et al. 2004, Berry et al. 2007, Brooks et al. 2009). In addition to fecal contamination, runoff frequently introduces agrochemicals into water bodies, which could potentially affect the survival of zoonotic pathogens and fecal indicator bacteria (FIB), as well as autochthonous microbes.

Presently, regulatory standards for microbial water quality only consider densities of non-pathogenic FIB, such as *E. coli* and *Enterococcus* spp. (U.S. Environmental Protection Agency 1983, 2002*a*). However, not all pathogens are as susceptible to environmental stressors as FIB. Even closely related, agriculturally derived pathogens have physiological differences from FIB and are also subjected to different rates of predation from bacterivorous protozoan predators (Hayashi et al. 2001, Jenkins et al. 2011). A recent study has found that *E. coli* O157:H7 persists longer than non-pathogenic *E. coli* and enterococci in the presence of protozoan predation and ultraviolet (UV) light stress (Jenkins et al. 2011), while another demonstrated better survival of *E. coli* O157:H7 compared to FIB, particularly in sediments (Wanjugi and Harwood 2012). Given the differences in physiology, predation, and stress-response, it is reasonable to believe that FIB and pathogens may exhibit different responses to agrochemicals.

To investigate the effects of agrochemicals on predation and competition with autochthonous bacteria, simplified microcosms were established using river water and sterilized sediment. Streptomycin-resistant FIB and pathogens (*E. coli*, *En. faecalis*, and *E. coli* O157:H7) were inoculated in order to allow the use of streptomycin to reduce competition from autochthonous bacteria. The study was limited to these bacteria as the indirect effects on multiple bacterial species are unknown and may differently affect the overall bacterial community. These bacteria were chosen as they represent FIB, which are presently utilized by regulatory agencies, and a pathogen, for which the FIB are used as surrogates, and are, therefore, most relevant for regulatory policies and management decisions. Cycloheximide was used to isolate the effect of predation on the bacteria by inhibiting protozoan activity. Microcosms were dosed with atrazine or chlorothalonil to examine effects of agrochemicals on bacterivorous predation by a natural protozoan population and on competition with natural bacterial populations. Control microcosms were maintained under unamended (no addition of agrochemical or antimicrobial agent) conditions. Atrazine and chlorothalonil were the selected agrochemicals because, in the United States, they are both within the top two agrochemicals in usage within their agrochemical class (Kiely et al. 2004).

Experimental Design

Competition and predation from natural microbial populations

Microcosms were established in the University of South Florida Botanical Gardens (Tampa, Florida, USA) in an outdoor greenhouse 18–24 September 2012, with a mean water temperature of 25.7°C, and standard deviation of 3.5°C, in each microcosm. Microcosms consisted of 0.95-L glass mason jars containing 600 mL of water collected from the Hillsborough River (Tampa, Florida, USA) containing autochthonous bacteria and protozoan species and 100 mL of sediment, also collected from the Hillsborough River, which was sterilized via baking in an oven

(Despatch Oven, Minneapolis, Minnesota, USA) for 24 hours at 177°C. One-third of microcosms were dosed with cycloheximide (200 μg/mL) to reduce protozoan predation, one-third were dosed with streptomycin (100 μg/mL) to diminish autochthonous competition, and the remaining one-third received neither streptomycin nor cycloheximide. Cycloheximide and streptomycin will hereafter be collectively referred to as inhibitors. All microcosms were covered with plastic wrap to allow for light penetration, but prevent contaminants from entering the system.

Prior to inoculation, *E. coli* MG6155, *En. faecalis* 19 433 (American Type Culture Collection, Manassas, Virginia, USA), and *E. coli* O157:H7 EDL933 were passaged on Luria-Britani agar (Thermo Fisher Scientific, Waltham, Massachusetts, USA), supplemented with streptomycin (100 μg/mL) in order to select for spontaneous streptomycin-resistant mutants. Streptomycin-resistant strains were prepared for inoculation by streaking for isolation on trypticase soy agar and incubating overnight at 37°C. Isolated colonies were inoculated into brain–heart infusion broth and incubated at 37°C overnight. Each individual culture was then centrifuged at 105 912 m/s^2 for five minutes (IEC Multi, Thermo Fisher Scientific, Waltham, Massachusetts, USA) and the pellet was resuspended in sterile buffered water (0.0425 g/L KH_2PO_4 and 0.4055 g/L $MgCl_2$) twice (American Public Health Association 1999). Each suspension was then diluted 1:10 in sterile buffered water, and 0.7 mL of each diluted bacterial suspension was aseptically inoculated into every microcosm ($\sim 10^7$ CFU/100 mL for all bacterial species).

One hour following inoculation, one of three treatments was randomly applied to each microcosm, a solvent control (DI water amended with 0.002% acetone, used as a solvent for all agrochemicals), the herbicide atrazine, or the fungicide chlorothalonil at the expected environmental concentration (EEC; 102 μg/L for atrazine, 170 μg/L for chlorothalonil [U.S. Environmental Protection Agency 2001]).Within each different inhibitor condition (diminished predation, diminished competition, or neither), each agrochemical treatment was represented and replicated thrice in separate spatial blocks for a total of 27 separate microcosms divided among three spatial blocks.

Water samples were collected from each microcosm one hour following bacterial inoculation, but immediately prior to agrochemical application (time 0, $T0$). Water samples were collected from every microcosm again after one day ($T1$), two days ($T2$), three days ($T3$), and six days ($T6$).

Sample collection, filtration, and bacterial and protozoan enumeration

Water samples were collected by pipetting 10-mL volumes into sterile centrifuge tubes. Samples were placed on ice for transport to the laboratory and processed within 30 minutes of collection. Samples were filtered through a nitrocellulose membrane filter (0.45-μm pore size, 47 mm diameter; Thermo Fisher Scientific, Waltham, Massachusetts, USA) and bacteria were enumerated via standard membrane filtration methods (American Public Health Association 1999). *E. coli* colonies were enumerated on mTEC agar at 35°C for two hours, followed by incubation at 41°C for 22 hours (U.S. Environmental Protection Agency 2002*c*); enterococci were enumerated on mEI agar at 41°C for 24 hours (U.S. Environmental Protection Agency 2002*b*); and *E. coli* O157:H7 was enumerated on sorbitol MacConkey agar at 35°C for 24 hours (Novicki et al. 2000). Protozoa were quantified by haemocytometer as per manufacturer's instructions (HyClone, Pittsburg, Pennsylvania, USA) using unfiltered water samples. If no protozoa were visible under the haemocytometer, one-half of the limit of detection (LOD) was recorded (the LOD was 2.5×10^3 cells/mL, 1.25×10^3 cells/mL was the value used for statistical analysis).

Statistical analysis

All data were analyzed using Statistica v.12 software (Statsoft, Tulsa, Oklahoma, USA). All response variables (bacterial and protozoan densities) were log-transformed and blocking effect was included in all analyses. The residuals were always carefully scrutinized to ensure that the assumptions of the analysis were met. To assess agrochemical effects on bacterial densities with natural predators, a repeated-measures, regression-based, multivariate analysis of variance (MANOVA) was conducted, where the repeated-measures factor was the bacterial density on the four sampling intervals ($T1$, $T2$, $T3$, and $T6$). The $T0$ densities for both bacterial and protozoan densities were used as continuous covariates to control for stochastic variation upon inoculation. In these analyses, interactions between among- and within-tank (repeated-measures) factors were always included. This allowed us to examine treatment-by-time interactions. In these analyses, the response variables were the densities of *E. coli*, *En. faecalis*, and *E. coli* O157:H7 on each sampling date. The full model included the main effects of inhibitors (no inhibitor, diminished predation, or diminished competition), the three agrochemical treatments (no agrochemical, atrazine, or chlorothalonil), two-way interactions, and repeated-measures main effects and interactions. ANOVAs were also conducted on each individual bacterial species to ensure no univariate effects were missed.

Protozoa were analyzed separately using ANOVA. The response variable was the log-transformed protozoan densities, including a repeated-measures factor consisting of the protozoan densities on each sampling interval ($T1$, $T2$, $T3$, and $T6$). The full model included the main effects of inhibitors (no inhibitor, diminished predation, or diminished competition), the three agrochemical treatments (no agrochemical, atrazine, or chlorothalonil), two-way interactions, and repeated-measures main effects and interactions.

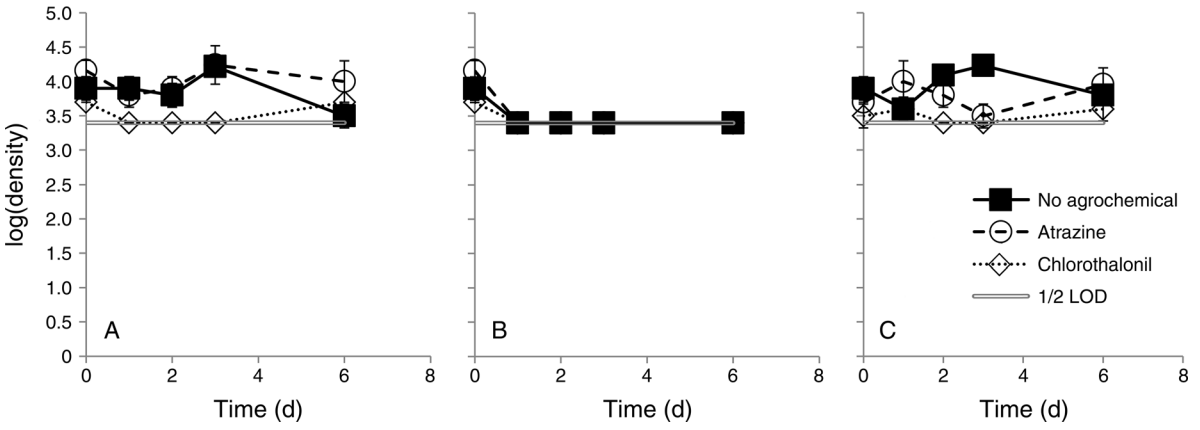

FIG. 1. Densities (measured as cells/mL; mean ± SD; $n = 3$) of naturally occurring protozoa in microcosms. (A) no inhibitors, including the completely unamended control; (B) cycloheximide addition; and (C) streptomycin addition. The level of one-half of the limit of detection (LOD) is shown.

RESULTS

The inhibitor cycloheximide and the agrochemical chlorothalonil had significant main effects on protozoan densities ([$F_{2,17} = 16.60$, $P < 0.001$] and [$F_{2,17} = 16.47$, $P < 0.001$], respectively), as protozoan densities were significantly reduced (by $\sim 10^{0.5}$ cells/mL) in cycloheximide and chlorothalonil treatments (Fig. 1). In non-inhibited (no streptomycin or cycloheximide) chlorothalonil treatments, protozoan densities showed signs of recovery by the end of the study, in that final densities were similar to inoculation densities (Fig. 1A). A significant interaction was found between the agrochemical and inhibitor treatments ($F_{4,17} = 5.93$, $P = 0.004$), which was driven by the reduction of protozoan densities in the presence of chlorothalonil only, but not in cycloheximide- or streptomycin-amended treatments with chlorothalonil. It should be noted that both chlorothalonil and cycloheximide reduced protozoan densities below the limit of detection, so detection of an additive effect of chlorothalonil and cycloheximide would not have been possible. Neither streptomycin nor atrazine treatment significantly affected protozoan densities, which remained at $\sim 10^4$ cells/mL throughout the experiment (Fig. 1A and C).

Bacterial densities were significantly increased, compared to the control, by the inhibitors streptomycin and cycloheximide ($F_{6,22} = 27.96$, $P < 0.005$; Figs. 2 and 3), as expected. Post-hoc tests revealed significantly higher densities of *En. faecalis* and *E. coli* O157:H7 in cycloheximide-amended microcosms than in streptomycin-amended microcosms ($P = 0.011$ and 0.005, respectively). Bacterial densities in cycloheximide treatments declined little throughout the experiment (holding at $\sim 10^6$–10^7 colony forming units [CFU]/100mL), with the exception of enterococci densities, which declined by two orders of magnitude by the sixth day (Fig. 2 D–F). In the streptomycin-amended treatment, all bacteria exhibited a steady decline with *E. coli* and enterococci densities decreasing approximately five orders of magnitude over six days and *E. coli* O157:H7 densities decreasing by approximately two orders of magnitude in all agrochemical treatments (Fig. 2 G–I). *E. coli* O157:H7 had greater persistence than either FIB in all microcosms over the course of the experiment (Fig. 2). A significant main effect of agrochemical treatment was also noted ($F_{6,22} = 9.89$, $P < 0.005$), as bacterial densities in chlorothalonil treatments remained significantly greater relative to control microcosms (Fig. 3). Atrazine also resulted in significantly greater *En. faecalis* and *E. coli* O157:H7 densities relative to the control ($P = 0.015$ and 0.001, respectively).

A significant interaction between agrochemical and inhibitor treatments was observed ($F_{12,29.39} = 7.08$, $P = 0.030$). Bacterial densities in the non-inhibited condition were significantly elevated (by approximately three to four orders of magnitude) in the presence of chlorothalonil; however, increased bacterial densities were not observed when cycloheximide- or streptomycin-amended treatments were dosed with chlorothalonil (Fig. 2A–C and Fig. 3).

DISCUSSION

The environmental stress of predation and competition on bacterial survival has been well established (Barcina et al. 1997, Byappanahalli et al. 2012, Wanjugi and Harwood 2012, Korajkic et al. 2013*a*); however the effect agrochemicals have on these stressors, and the ensuing result on bacterial survival, is poorly understood. Agrochemicals could prolong the survival of FIB and bacterial pathogens by lessening predatory or competitive stress, increasing the perceived or real risks to human health (Ekelund 1999). Conversely, pesticide residues could provide an ecosystem service by increasing predation or competition, having detrimental effects on bacterial persistence, effectively "killing two birds with one stone."

Effects which indirectly influence bacterial survival, either through density- or trait-mediated effects on

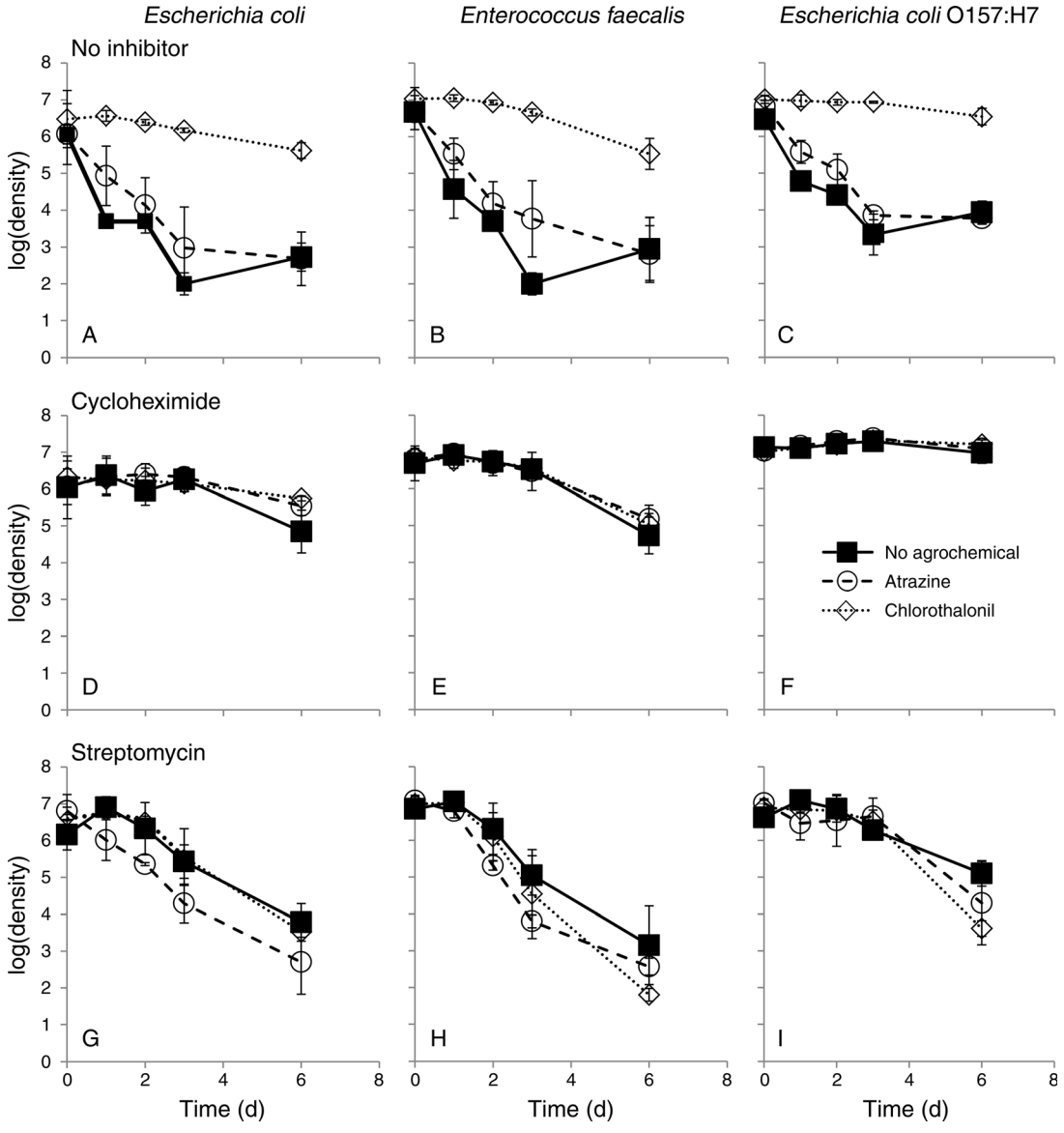

FIG. 2. (A, D, and G) *Escherichia coli*, (B, E, and H) *Enterococcus faecalis*, and (C, F, and I) *Escherichia coli* O157:H7 densities (measured as [colony-forming units (CFU)]/100 mL; mean ± SD; $n = 3$ replicates) as a function of time in microcosms with or without agrochemicals and panels (A–C) no inhibitors added (unamended), panels (D–F) cycloheximide addition, and panels (G–I) streptomycin addition.

predators, competitors, or nutrient resources, can be more common and complex than direct effects (Rohr et al. 2006, Relyea 2009). For example, while previous research has indicated that there are no direct effects of atrazine or chlorothalonil on the bacterial targets investigated in this experiment (Staley et al. 2012), an indirect effect of agrochemicals, mediated by altering algal populations, has been shown to significantly affect FIB densities (Staley et al. 2011). Therefore, understanding the indirect effects of agrochemicals on the survival of FIB and pathogens, mediated via effects on predation or competition, is important for a better understanding of how pesticides can influence ecosystem services and risks to human health.

The top-down pressure of predation as well as the pressure of competition can have significant negative impacts on bacterial survival (Wanjugi and Harwood 2012, Korajkic et al. 2013*a*). In this study, bacterial densities were significantly elevated, relative to unamended conditions, when either competition (autochthonous bacteria) or predation (protozoa) was decreased by inhibitors. Protozoan and bacterial densities were affected similarly by cycloheximide and the fungicide chlorothalonil. Further, predation was found to be a more prominent stressor on bacterial popula-

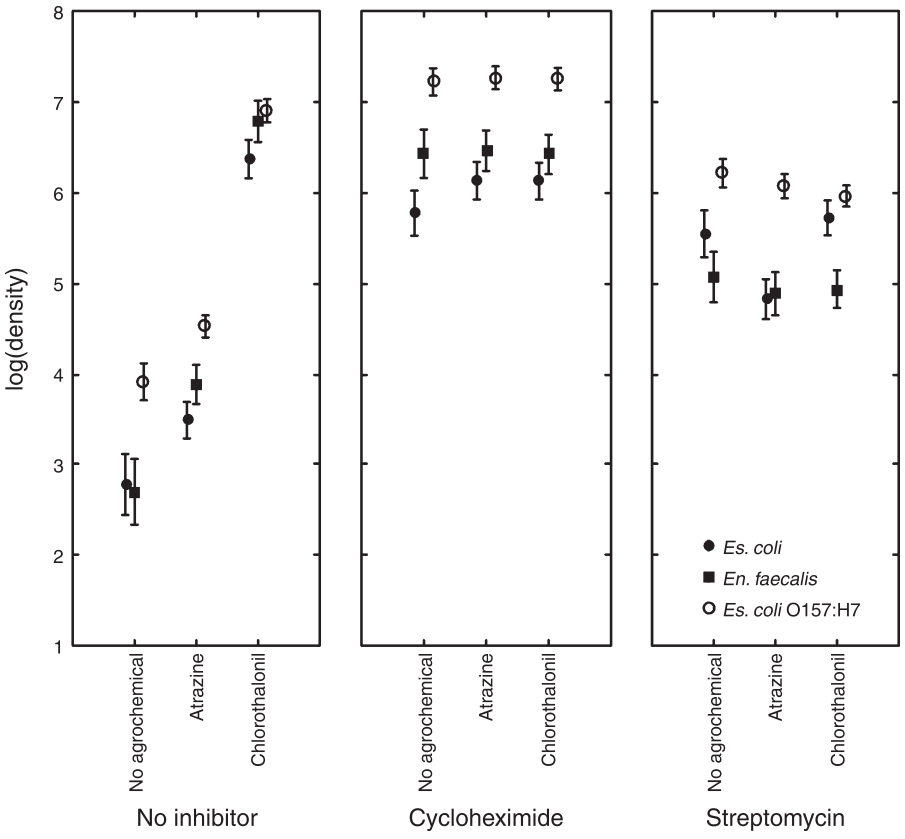

FIG. 3. The effect of inhibitors and agrochemical treatments on mean fecal indicator bacteria (FIB) and pathogen densities in microcosms (measured as CFU/100 mL; mean ± SE; $n = 3$ replicates) over the entire six-day period assessed by repeated ANOVA measures.

tions than competition, as bacterial densities were highest when protozoan predation was diminished in cycloheximide-amended treatments (Fig. 3). Previous studies support these results, as bacterivorous predation has been found to be a more significant stressor than competition, accounting for upwards of 90% of bacterial decay in lake systems (Anderson et al. 1986, Gurijala and Alexander 1990, Byappanahalli et al. 2012).

The fungicide chlorothalonil significantly influenced both bacterial and protozoan densities. Elevated bacterial densities in non-inhibited microcosms dosed with chlorothalonil were similar to those observed in the cycloheximide-amended treatments, where protozoa were undetectable after 24 hours. Previous research has also shown that chlorothalonil reduced levels of heterotrophic protist species (Downing et al. 2004) and may be toxic to a variety of protozoa (Bending et al. 2007). These results suggest that chlorothalonil had a density-mediated, indirect effect on FIB and pathogens, similar to cycloheximide, wherein bacterial densities remain elevated as a consequence of decreased protozoan predation. However, protozoan densities in microcosms treated only with chlorothalonil conditions (no cycloheximide) recovered over the six-day sampling period, possibly because protozoa developed resistance to chlorothalonil or because the fungicide had decayed over the duration of the study. The half-life of chlorothalonil can range from several hours to over a month, depending on the ambient conditions (i.e., sediment or water location, movement speed of water, pH, amount of humic substances, aeration); therefore, degradation of the fungicide may have occurred over the course of this study (Davies 1988, U.S. Environmental Protection Agency 1999).

The failure of chlorothalonil to reduce protozoan densities in the presence of streptomycin was unexpected. Our original hypothesis was that streptomycin and chlorothalonil would have an additive, positive effect on bacterial survival; however, no significant difference was observed in either bacterial or protozoan densities in the streptomycin/chlorothalonil treatment compared to the streptomycin-only treatment. Furthermore, the chlorothalonil treatment produced significantly greater bacterial survival than the streptomycin/chlorothalonil treatment. These results suggest an interaction between streptomycin and chlorothalonil that inhibits the toxicity of the fungicide toward protozoa. Previous research has suggested this interaction, as the antifungal effects of chlorothalonil on *Rhziobium* spp. were shown to be

diminished in the presence of streptomycin (Habte 1985).

Atrazine treatment did not significantly affect protozoan densities or the effect of protozoa on bacterial populations, which was unexpected. Previous research has demonstrated that atrazine can increase heterotrophic activity, abundance, and induce an increase in predation behavior in facultative heterotrophic protozoan species (DeLorenzo et al. 2001, Downing et al. 2004, Debenest et al. 2009, 2010). It should be noted that we did not attempt to characterize the composition of the protozoan population in this study, so the fraction of facultative heterotrophic protozoa in the microcosms is unknown. Facultative heterotrophic algal and protozoan species have generally been found to comprise 3–5% of the total algal and protozoan populations in freshwater environments, with facultative heterotrophic algal species making up as much as 25–50% of the total algal populations in surface waters (Arenovski 1994, Carrias et al. 1996). Additionally, facultative heterotrophic protozoa can significantly impact community structures, outcompeting obligate heterotrophs and autotrophs (Tittle et al. 2003).

While atrazine did not cause a significant decrease in bacterial densities via increased predation, atrazine treatment resulted in significantly greater densities of *En. faecalis* and *E. coli* O157:H7, but not the nonpathogenic *E. coli* compared to the control. However, the increase in bacterial densities attributed to atrazine treatment was not observed in microcosms amended by both atrazine and streptomycin, i.e., bacterial concentrations were very similar in microcosms amended only with streptomycin, compared to those that received both the inhibitor and atrazine. We hypothesize that the effect of atrazine was not seen in the streptomycin-amended microcosms because the inhibitor alone caused a pronounced increase in bacterial survival that masked the relatively smaller effect of atrazine. Increased bacterial abundance in unamended atrazine treatments may have been via a lethal effect on phytoplankton, whose die-off could provide more nutrients for allochthonous bacteria, or a negative effect on the autochthonous competitors. The experiments were not designed to discriminate between these possibilities.

The indirect effects of agrochemicals on bacterivorous predation have been little explored and are very poorly understood. These experiments revealed that agrochemicals have a significant effect on bacterivorous predation, resulting in an indirect, density-mediated effect on the survival of allochthonous FIB and pathogens. Most notably, chlorothalonil, when present in freshwater systems, reduces heterotrophic protozoan densities, thereby decreasing predation, which results in elevated bacterial densities and persistence. Therefore, chlorothalonil diminishes an ecosystem service by reducing the ability of protozoa to aid in purifying water bodies of introduced allochthonous pathogens (i.e., from sewage spills or storm water or agricultural runoff).

Increased bacterial survival as a result of chlorothalonil and/or atrazine application could facilitate the persistence and possibly growth of zoonotic pathogens such as *E. coli* O157:H7 in impacted water bodies, increasing risks to human health. Further, densities of pathogenic *E. coli* O157:H7 remained significantly higher than densities of FIB in all microcosms, not only those treated with chlorothalonil. Extended persistence of *E. coli* O157:H7 relative to FIB, even in the presence of competition and predation, has been reported previously (Jenkins et al. 2011, Staley et al. 2011, 2012), suggesting that the present regulatory standards, which rely on FIB densities, may underestimate the risk posed by *E. coli* O157:H7.

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