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REVIEW ARTICLE

A synthesis of the effects of pesticides on microbial persistence in aquatic ecosystems

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Abstract

Pesticides have a pervasive presence in aquatic ecosystems throughout the world. While pesticides are intended to control fungi, insects, and other pests, their mechanisms of action are often not specific enough to prevent unintended effects, such as on non-target microbial populations. Microorganisms, including algae and cyanobacteria, protozoa, aquatic fungi, and bacteria, form the basis of many food webs and are responsible for crucial aspects of biogeochemical cycling; therefore, the potential for pesticides to alter microbial community structures must be understood to preserve ecosystem services. This review examines studies that focused on direct population-level effects and indirect community-level effects of pesticides on microorganisms. Generally, insecticides, herbicides, and fungicides were found to have adverse direct effects on algal and fungal species. Insecticides and fungicides also had deleterious direct effects in the majority of studies examining protozoa species, although herbicides were found to have inconsistent direct effects on protozoans. Our synthesis revealed mixed or no direct effects on bacterial species among all pesticide categories, with results highly dependent on the target species, chemical, and concentration used in the study. Examination of community-level, indirect effects revealed that all pesticide categories had a tendency to reduce higher trophic levels, thereby diminishing top-down pressures and favoring lower trophic levels. Often, indirect effects exerted greater influence than direct effects. However, few studies have been conducted to specifically address community-level effects of pesticides on microorganisms, and further research is necessary to better understand and predict the net effects of pesticides on ecosystem health.

Keywords

algae, bacteria, fungi, fungicide, herbicide, insecticide, protozoa

History

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Introduction

Global pesticide usage is increasing, and the presence of pesticides, which include herbicides, fungicides, and insecticides, has become pervasive in freshwater and marine ecosystems. Between 2000 and 2007, global pesticide use increased from 2.27 billion to 2.36 billion kg (U. S. Environmental Protection Agency 2012). In 2001, over five billion kilograms of chemicals were used in the United States alone (Kiely et al. 2004), and pesticides were detected in over 90% of developed watersheds (defined as any watershed dominated by agricultural, urban, or mixed land use) and in over 50% of groundwater wells (Gilliom 2007).

To safeguard against pesticide contamination, the United States Environmental Protection Agency (USEPA) benchmarks have been established at the "no-effect" level for pesticides, wherein a pesticide concentration below the benchmark would not be expected to have any adverse effects, while a concentration above the benchmark might adversely affect the target organism(s) (Table 1) (Gilliom 2007; Gilliom et al. 2006;

Table 1. Partial listing of USEPA pesticide benchmarks for human and aquatic health.

Pesticide group	Pesticide	Soil half-life (days)	Human (μg L^{-1})	Fish (μg L^{-1})*	Invertebrates (μg L^{-1})*	Nonvascular plants (μg L^{-1})*	Vascular plants
Insecticides	carbofuran	50		44	1.115	0.75	
	chlorpyrifos	30	100	0.9	0.05	140	
	diazinon	40		45	0.105	3700	
	dimethoate	7	130	3100	21.5	84	
	endosulfan	50	150	0.05	0.3	428	
	fenitrothion	6		860	1.15		
	lindane	450	3960	0.85	0.5		
	malathion	1		16.5	0.295	2400	>9630
	methoxychlor	120		7.5	0.7		
	permethrine	40		0.395	0.0106	68	
Herbicides	2,4-D	10			12500		
	atrazine	60		2650	360	<1	0.001
	bentazone	20		>50000	>50000	4500	5350
	diuron	90		200	80	2.4	15
	glyphosate	47		21500	26600	12100	11900
	linuron	60		1500	60	13.7	2.5
	paraquat	1000		6000	600	0.396	71
	simazine	60		3200	500	2.24	140
	tebuthkron	360		53000	148500	50	135
	tri-allate	82	1650	600	45.5	120	2400
Fungicides	captan	3	3300	13.1	4200	320	>12700
	chlorothalonil	30		5.25	1.8	6.8	630
	dicloran	140	16500				
	fenpropimorph	4	4950				
	propiconazole	30	3000	425	650	21	4828
	thiophanate methyl	7		4150	2700	930	>4700
	thiram	15	462	21	105	140	1600

*Values are based on USEPA acute toxicity standards.

U. S. Environmental Protection Agency 2012). Based upon these USEPA benchmarks, pesticides exceeded standards for human health in ~10% of agricultural streams, ~7% of urban streams, and ~1% of groundwater tested, while benchmarks for aquatic health were exceeded in 57% of agricultural and 83% of urban streams (Gilliom 2007; U. S. Environmental Protection Agency 2004; U. S. Environmental Protection Agency 2012). Similarly, according to the European Union (EU) directives, ~43% of median pesticide levels in surface waters of northeastern Greece (Vryzas et al. 2009) and 12% of groundwater tested in northern Spain exceeded regulatory standards (Hildebrandt et al. 2008).

In addition to understanding how parent pesticide compounds affect watersheds, it is also important to recognize that pesticide intermediates can produce significant effects on the health of aquatic ecosystems. Pesticide intermediates are the breakdown products produced via photolytic, hydrolytic, or microbially induced decay of the parent pesticide compound. Many of these intermediate compounds are not biologically inert and can have magnified or altogether different effects (positive or negative) than the parent compound (Kralj et al. 2007; Yanze-kontchou and Gschwind 1994; Zeinat et al. 2008).

While most pesticides are designed to target a specific pest or particular pest group, the use of pesticides and their ensuing intermediates can have additional effects on non-target species (Rohr et al. 2006a). For instance, pesticide exposure has resulted in decreased biodiversity, toxicity to certain algae and diatoms resulting in harmful algal blooms, and alterations in ecosystem food webs, such as increases in heterotrophic activity (Beketov et al. 2013; Benton et al. 2003; Debenest et al. 2010; Malaj et al. 2014; Robinson and Sutherland 2002). Projections suggest that continued extension of current agricultural practices could produce further harmful effects (Tilman 1999). Multiple studies have investigated the effects of pesticides on amphibian, arthropod, and fish species (Desneux et al. 2007; McMahon et al. 2011; Relyea 2009; Rohr et al. 2003; Rohr and McCoy 2010; Rohr and Palmer 2005; Rohr et al. 2006b; Rohr et al. 2008a,b; Stark and Banks 2003); however, relatively little research has addressed the effects of pesticides on microorganisms.

Microorganisms, including algal, bacterial, protozoan, and fungal species, are fundamental components of aquatic ecosystems, providing crucial services such as primary production, decomposition, and nutrient cycling. Aquatic microbial communities potentially include a host of waterborne bacterial, protozoan, and fungal pathogens. These pathogens may be natural (autochthonous) inhabitants of aquatic ecosystems, for example, *Vibrio* species, or they may be allochthonous intruders contributed by human or animal waste. Therefore, any pesticide impact resulting in alteration of microbial communities could result in dramatic changes and damage to an impacted water body, as well as posing a risk to human health. Of the studies on interactions between pesticides and microorganisms, most have examined the role of both naturally occurring and laboratory-derived microorganisms in the biodegradation of pesticides (Anderson et al. 2002; Cycoń et al. 2009; De Souza et al. 1998; Levanon 1993; Seffernick et al. 2007; Wackett et al. 2002; Zeinat et al. 2008). As many microorganisms can breakdown pesticides, using them as a resource, and pesticides can be directly toxic to microorganisms, the effects of pesticides on microorganisms are difficult to predict *a priori*.

The difficulty in assessing many of the studies investigating the effects of pesticides on microorganisms is that, often, different systems (i.e., lab cultures, microcosms, and field

studies) are employed along with different target organisms and pesticide concentrations. Of the studies reviewed, some used pesticide concentrations that are ecologically relevant, that is, levels that could be expected to be found in water bodies as a consequence of stormwater or agricultural runoff from neighboring watersheds. Others use concentrations consistent with direct pesticide applications. Finally, other studies test pesticide concentrations that are not ecologically relevant, and thus are above the expected environmental concentrations based on application instructions. Throughout this review, reference will be made to whether pesticide concentrations used were environmentally relevant, meaning that the concentration was at or below what would be expected in a natural water body (based on reports from agencies such as the World Health Organization, USEPA, and the US Department of Health and Human Services Agency for Toxic Substances and Disease Registry). In addition to variation in concentrations, studies vary in their endpoints, with some examining direct, population-level effects of a pesticide and others considering direct and indirect effects on microbial communities. As a consequence of a lack of standardization among concentrations, pesticides, organisms, and study complexity, a review of the existing literature seemed more practical than a quantitative meta-analysis.

This review considers studies that have examined the effects of pesticides on populations or communities of microorganisms, including microscopic phototrophs (i.e., algae and cyanobacteria), heterotrophic bacteria, protozoans, and aquatic fungi. Studies were limited to freshwater, estuarine, and marine watersheds, including the underlying sediments and laboratory culture studies, as these often investigate the effects of pesticides on waterborne microbes. Pesticide effects in unsaturated soils were not reviewed. Trends in pesticide effects for pesticide categories and classes on specific microbial groups are explained, where possible. Where studies have found conflicting results, this review will address potential reasons for these conflicts. Additionally, by examining studies that have focused on direct and indirect effects (see below), this review will summarize the ecological mechanism(s) by which pesticides act on microbial communities. Ultimately, this review provides a detailed summary of the net pesticide effects observed on microorganisms so that the effects of pesticides on impacted aquatic ecosystems can be better anticipated, as well as highlights the need for continued research, particularly at the community level.

Type and direction of pesticide effects

The effects of pesticides on microbes may be direct (examining only a single species at the population level) or indirect (examining multiple different species and/or trophic levels at the community level) and either beneficial or adverse (Clements and Rohr 2009; Verro et al. 2009). Pesticide exposure may prove directly toxic to microbes (an adverse effect) or pesticides may be utilized by microbes, particularly bacterial species, as a nutrient source, facilitating growth and/or survival (a beneficial effect). Additionally, direct effects may be masked by indirect pesticide effects. For example, an initial decrease in a population as a result of a directly toxic pesticide may be ameliorated by an accompanying decrease in predation or competition, masking the initial adverse effect over time (Rohr et al. 2006b).

While many direct effects might be easily predicted (i.e., the direct toxicity of an herbicide on an algal species), indirect effects are more common and complex than direct effects and are often more difficult to predict (Relyea 2009; Rohr and Crumrine 2005; Rohr et al. 2006a). Relatively few studies have investigated how indirect effects of pesticides influence microbial survival, despite the frequent observation that such effects are important drivers of the microbial community structure (Jousset 2012; Korajkic et al. 2013b; Wanjugi and Harwood 2012). Indirect effects might be either density- or trait-mediated (Rohr et al. 2006a). Density-mediated indirect effects would be those that result in either the increase or decrease in abundance of a predator, competitor, parasite, or food resource, which subsequently affects the focal organism (Raffel et al. 2008; Rohr et al. 2008a,b; Rohr et al. 2009). For instance, pesticide application might reduce the abundance of a bacterivorous protozoan species, facilitating the survival of bacterial prey (a beneficial effect for the bacteria). Pesticides might also act to reduce certain microbial populations while leaving others unscathed, which would decrease competition for resources shared by the affected and unaffected populations. In addition to density-mediated indirect effects, pesticides have the capacity to alter traits of organisms, such as behavior, immunity, physiology, and morphology, resulting in trait-mediated indirect effects (Rohr and Crumrine 2005; Rohr et al. 2003; Rohr et al. 2006a; Rohr and Palmer 2005; Rohr et al. 2008a,b; Rohr et al. 2009). For example, upon exposure to an herbicide that affects photosynthesis, a facultatively heterotrophic protozoan (capable of photosynthetic or heterotrophic metabolism) might switch from an autotrophic to a heterotrophic mode, causing increased predation on the microbial prey population (an adverse effect) (Debenest et al. 2010; Staley et al. 2014). Pesticides might also stimulate an increase or decrease in antiparasite or antipredator behaviors, affecting overall survival (Rohr et al. 2009). Understanding both direct and indirect effects is therefore essential to being able to accurately predict the net impact of pesticide residues on aquatic ecosystems.

Study search criteria

Studies examining the effects of pesticides on microorganisms were selected using the National Institutes of Health PubMed (http://www.ncbi.nlm.nih.gov/pubmed) and Web of Science databases. Boolean searches were performed consisting of a pesticide classification and microbial taxa. Pesticide classifications ranged from the general term "pesticide" to more specific pesticide categories (herbicide, insecticide, and fungicide), pesticide groups within categories (i.e., organophosphates, carbamates, s-triazines, aromatics, etc.), and, at the most specific, pesticide names (i.e., atrazine, malathion, diazinon, etc.). Terms used for microbial taxa ranged from the general term "microorganism" or "microbe" to more specific microbial taxa (including algae, cyanobacteria, bacteria, protozoa, and fungi). The term "heterotrophic" was also included with bacteria and protozoa, as well as limiting search terms to "aquatic fungi," to further specify searches. No particular microbial species (i.e., specifically *Escherichia coli* or *Tetrahymena pyriformis*)

were searched for. Additionally, terms such as "microbial persistence" or "microbial survival" (also repeated with more specific taxa names replacing "microbial") were also used to remove studies focusing on biodegradation rather than microbial fate from the search results. Also, as there are thousands of pesticides, it was necessary to limit the scope of this review to only those pesticides that were more commonly used, to allow for synthesis of results and avoid an encyclopedic listing of study results. Where only one or two studies could be found utilizing a particular pesticide, those studies were not included unless they represented the sole instance of an experiment utilizing the particular pesticide group.

Statistics

To determine whether types of pesticides generally have beneficial, neutral, or adverse effects on microbes and whether their effects depended upon the microbial taxon, each species in our database received a -1, 0, or 1 if a focal pesticide had a significant adverse effect, no significant effect, or a significant beneficial effect on its survival, respectively. Treating the species as the replicate, we then used a generalized linear model with an ordinal multinomial error distribution to test whether microbial density was influenced significantly by pesticide type (insecticide, herbicide, or fungicide), microbial taxon (algae, bacteria, fungi, or protozoa), and their interaction. Note that an ordinal multinomial distribution assumes an ordered categorical response variable ($-1 < 0 < 1$), but it does not assume that distances between categories are known or constant. This is appropriate in this case because the distance between a significant adverse effect and a non-significant effect is not the same for every pair of species. We chose not to conduct a traditional meta-analysis based on effect sizes because there were many variations among the methods and effects in these studies. For each taxon by chemical class combination, we also evaluated whether the observed frequency of significant adverse effects, null effects, or significant beneficial effects was greater than expected by chance (with the conservative assumption of an equal probability of falling in any of the three categories) using an observed versus expected Chi-square test. All statistics were conducted in Statistica version 6.0 (Statsoft Inc., Tulsa, OK, USA) with an α of 0.05.

Specific effects of pesticides

Insecticides

The general mode of action of most commercial insecticides is nervous system disruption (Table 2). Organophosphate insecticides (i.e., parathion, diazinon, malathion, chlorpyrifos, etc.) and carbamate insecticides (i.e., carbaryl and carbofuran) hydrolyze acetylcholine and inhibit acetylcholinesterase, resulting in a buildup of the neurotransmitter acetylcholine and eventual death of the organism (Fukuto 1990). Pyrethroid insecticides and organochlorine insecticides (i.e., dichlorodiphenyltrichloroethane [DDT], aldrin, dieldrin, and endrin) act by binding to γ-aminobutyric acid or GABA receptors, preventing chloride anions from entering nerve cells (Coats 1990).

Overall, insecticides caused generally negative effects on algal, bacterial, protozoan, and fungal species (Tables 3 and 4, Figure 1). When considering effects by pesticide (rather than

Table 2. Pesticides and mechanism of action.

Pesticide category	Groups included	General toxic effect	Specific site of action
Herbicide	Glyphosate	Amino Acid Synthesis	EPSP Synthase
	Sulfonyl Ureas, Imidazolinones	Amino Acid Synthesis	Acetolactate Synthase
	Glufosinate	Amino Acid Synthesis	Glutamine Synthetase
	Triazines, Anilides, Phenyl Carbamates, Ureas, Biscarbamates, Benzimidazoles, Uracils, Quinones, Hydroxynitriles	Photosynthesis	Hill Reaction of Photosystem II
	Bipyridiniums, Heteropentalenes	Photosynthesis (Bleaching)	Photosystem I
	Diphenyl ethers, Oxadiazoles, iV-phenly imides	Photosynthesis (Bleaching)	Protoporphyrinogen Oxidase
	Aryoxyphenoxy propionates, Cyclohexanediones, Chloroacetamide	Lipid Biosynthesis	Acetyl-CoA Carboxylase
	Pyridazinones, Fluridone, w-Phenoxybenzamides, 4-Hydroxypyridines	Carotenoid Biosynthesis	Phytoene Desaturase
	Aminotriazole	Carotenoid Biosynthesis	Lycopene Cyclase
	Dichlormate	Carotenoid Biosynthesis	ζ-Carotene Desaturase
	Iosxazolidinones	Carotenoid Biosynthesis	IPP Isomerase and/or Prenyl Transferase
	Dinitroanilines, Phosphoric amides, Chiorthaldimethyl, Propyzamide, Cholchicine, Terbutol	Mcrotubule Biosynthesis	β-Tubulin
	Dichlobenil	Cellulose Biosynthesis	Cellulose Synthase
	Asulam	Folate Biosynthesis	Dihydropteroate Synthase
Insecticides			
Organophosphates	Carbamates	Nervous System Inhibition	Acetylcholinesterase
Organochlorines	Boranes, Chlorinated Acylics, Cyclodienes, Cyclohexanes	Nervous System Inhibition	GABA Receptor
Pyrethroids		Nervous System Inhibition	GABA Receptor
Fungicides	Aromatic hydrocarbons, Triazoles	Membrane Biosynthesis	Lipid Biosynthesis
	Cinnamic acid amide, Morpholine, Triazoles	Membrane Biosynthesis	Sterol Biosynthesis
	Hydrochloride	Membrane Biosynthesis	Intracellular Membrane Components
	Glucopyranosyl antibiotics, Tetracycline antibiotics	Amino Acid Synthesis	
	Phenylpyrroles, Dicarboximides	Signal Transduction	
	Pyrimidinamines	Respiration	NADH oxido-reductase Inhibitors
	Pyridine carboxamides, Benzamides, Gxathiin carboxamides	Respiration	Succinate-dehydrogenase Inhibitors
	2,6-dinitroanilines, Dinitrophenyl crotonate	Respiration	Oxidative Phosphorylation Uncouplers

Table 3. Summary of studies finding direct pesticide effects that were positive, neutral, or negative.

Taxa	Pesticide category	Direction of effect			Mixed	Total studies
		Positive	Neutral	Negative		
Algae	Insecticides	0	1	16	1	18
	carbamate	–	–	4	–	4
	organochlorine	–	1	6	1	8
	organophosphate	–	–	6	–	6
	Herbicides	0	0	10	2	12
	Fungicides	0	0	3	0	3
Bacteria	Insecticides	1	6	5	1	13
	carbamate	–	–	3	–	3
	organochlorine	–	1	–	–	1
	organophosphate	0	5	2	1	8
	pyrethroid	1	–	–	–	1
	Herbicides	1	3	3	1	8
	Fungicides	0	1	3	1	5
Fungi	Insecticides	0	0	3	0	3
	carbamate	–	–	1	–	1
	organophosphate	–	–	2	–	2
	Herbicides	0	0	4	0	4
	Fungicides	0	0	4	0	4
Protozoa	Insecticides	0	0	11	0	11
	carbamate	–	–	3	–	3
	organochlorine	–	–	3	–	3
	organophosphate	–	–	5	–	5
	Herbicides	0	1	2	0	3
	Fungicides	0	0	2	0	2

by study as in Table 3, as several studies used more than one pesticide), 92% (of 25 insecticides), 50% (of 20 insecticides), 100% (of 17 insecticides), and 100% (of three insecticides) of insecticides caused direct negative effects on algae (including cyanobacteria), bacteria, protozoa, and aquatic fungi, respectively (Table 4). Eight percent and 35% of insecticides had neutral effects on algae and bacteria, respectively, while 15% of insecticides had direct positive effects on bacterial species (Table 4). While insecticides tend to produce generally negative effects for most microbes, there are some differences in direct effects produced among pesticide groups.

Organophosphates

Organophosphates, as a class, have adverse direct effects on algal growth; however, of the six studies resulting in direct negative effects, only one was conducted at insecticide concentrations that were environmentally relevant (Table 5). Mala-

Table 4. Summary of pesticides with reported direct effects that were positive, neutral, or negative.

Taxa	Pesticide category	Direction of effect			Total chemicals	X^{2a}	P^a
		Positive	Neutral	Negative			
Algae	Insecticides	0	2	23	25	38.96	<0.001
	carbamate	–	–	6	6	12.00	0.002
	organochlorine	–	2	11	13	15.86	<0.001
	organophosphate	–	–	6	6	12.00	0.002
	Herbicides	0	2	33	35	58.69	<0.001
	Fungicides	0	0	4	4	8.00	0.018
Bacteria	Insecticides	3	7	10	20	3.70	0.157
	carbamate	–	–	4	4	8.00	0.018
	organochlorine	–	1		1	–	–
	organophosphate	2	6	6	14	2.29	0.319
	pyrethroid	1	–		1	–	–
	Herbicides	2	6	7	15	2.80	0.247
	Fungicides	1	1	7	9	8.00	0.018
Fungi	Insecticides	0	0	3	3	6.00	0.050
	carbamate	–	–	1	1	–	–
	organophosphate	–	–	2	2	–	–
	Herbicides	0	0	4	4	8.00	0.018
	Fungicides	0	0	5	5	10.00	0.007
Protozoa	Insecticides	0	0	17	17	34.00	<0.001
	carbamate	–	–	3	3	6.00	0.050
	organochlorine	–	–	6	6	12.00	0.002
	organophosphate	–	–	8	8	16.00	<0.001
	Herbicides	0	2	2	4	2.00	0.368
	Fungicides	0	0	3	3	6.00	0.050

[a]Results of an observed versus expected Chi- square test assuming an equal expectation for each of the three categories.

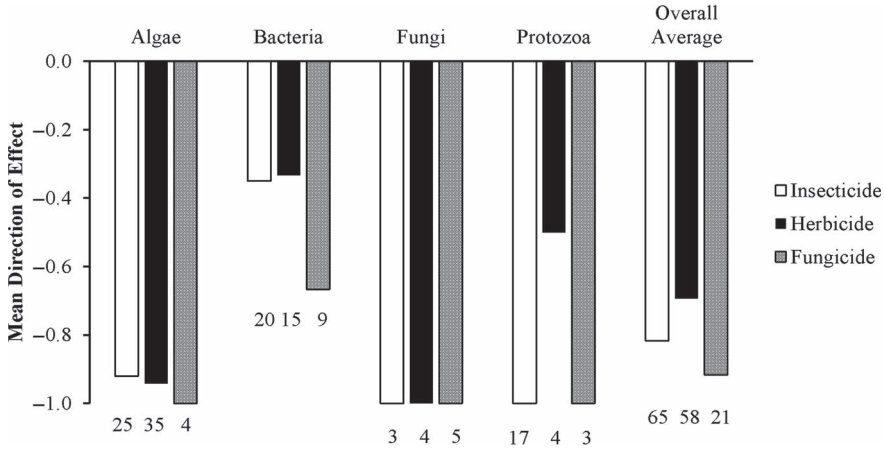

Figure 1. Mean direction of pesticide "direct" effects (+ 1 for significant positive effects, 0 for null effects, and − 1 for significant negative effects) for each taxon. Sample sizes appear below each bar.

thion inhibited chlorophyll production and resulted in death of cyanobacteria at concentrations between 1 and 1000 μg L^{-1}, overlapping with ecologically relevant concentrations of malathion (DeLorenzo et al. 2001; Torres and O'Flaherty 1976). The organophosphate diazinon inhibited growth of cyanobacteria at concentrations of 400 000 and 500 000 μg L^{-1} (Singh 1973). Although also at concentrations greater than would be expected in the environment, diazinon was toxic to planktonic algae at 10 000 μg L^{-1} (Butler et al. 1975). Parathion inhibited reproduction in *Chlorella fusca* at concentrations of 7860 μg L^{-1} (Faust et al. 1994). Fenitrothion inhibited growth of both phytoplankton and cyanobacteria at concentrations between 800 and 24 400 μg L^{-1} (Kent and Currie 1995). Chlorpyrifos inhibited growth of the marine diatom *Minutocellus polymorphus* at a concentration of 240 μg L^{-1} (Walsh et al. 1988).

Organophosphate insecticides also produced direct negative effects on protozoa (Table 6), and were frequently toxic to protozoa at concentrations lower than that which were toxic to algae; however, only one of the five studies reviewed used pesticide concentrations not exceeding environmentally relevant concentrations. Fenitrothion and chlorpyrifos inhibited the growth of *Tetrahymena pyriformis* (at 500–2500 μg L^{-1} and 1000–10 0000 μg L^{-1}, respectively) and at higher concentrations of fenitrothion (5000–10 000 μg L^{-1}), cell death also resulted (Lal et al. 1987). Organophosphates were generally toxic to the ciliate *Colpidium campylum* at much higher concentrations than would be expected in the environment, exceeding 10 000 μg L^{-1} (Dive et al. 1980). Parathion was found to inhibit survival of *Euglena gracilis* at a concentration of 1200 μg L^{-1} (Moore 1970). Malathion was lethal to ciliates (at concentrations between 1000 and 30 000 μg L^{-1}) and inhibited growth of *E. gracilis* (at a concentration of 7250 μg L^{-1}) (Moore 1970; Weber et al. 1982). Another study found that malathion significantly reduced swimming velocity of *E. gracilis* at concentrations ranging from 6000 to 50 000 μg L^{-1} (Azizullah et al. 2011).

Organophosphate insecticides have also induced negative effects on aquatic fungi (Table 7). Diazinon, at concentrations ranging between 0.05 and 50 μg L^{-1}, promoted sporulation of *Heliscella stellata* (Flores et al. 2014). Although above environmentally relevant concentrations, dimethoate (at 2500 μg L^{-1}) increased fungal mycelia density, zoosporangia concentration, and the number of sexual and asexual organs in the species *Achlya racemosa, Dictyuchus monosporus, Saprolegnia ferax, Thraustotheca clavata,* and *Allomyces arbuscula* (Khallil and Omar 1993). However, in the same study, dimethoate concentrations ranging from 5000 to 75 000 μg L^{-1} completely inhibited mycelial growth of all five species.

The direct effects of organophosphate insecticides on bacteria have been less consistent than direct effects on other taxa (Table 3), although neutral effects were most commonly observed at environmentally relevant concentrations (Table 8). For example, diazinon was not found to effect the abundance of rumen bacteria in laboratory cultures at concentrations ranging from 0 to 500 000 μg L^{-1} (Williams et al. 1963). Similarly, diazinon, at concentrations as high as 0.005 μg L^{-1}, did not inhibit the growth of *E. coli* or *Klebsiella pneumoniae* (Higgins and Hohn 2008). Malathion was also not directly toxic to *E. coli* or *Bacillus subtilis* in *in vitro* tests (Kerszman 1993), and no effects on growth rate or survival of *E. coli*, *Enterococcus faecalis*, *E. coli* O157:H7, or *Salmonella enterica* Typhimurium were observed in simplified *in vitro* microcosms using environmentally relevant concentrations of 101 and 202 μg L^{-1} (Staley et al. 2012).

However, when environmentally realistic concentrations of organophosphates were exceeded, negative and positive effects were observed (Tables 3 and 8), although in most cases this was likely due to other confounding factors or marked differences in toxicity assessment. At concentrations of 3 300 000–9 900 000 μg L^{-1}, well above environmentally relevant concentrations, chlorpyrifos increased abundance of *Listeria monocytogenes, Salmonella* sp., *Shigella* sp., and *E. coli* O157:H7 100–1000-fold over a 96-h period in laboratory cultures (Guan et al. 2001). However, in the same study, diazinon (at 14 600 000 μg L^{-1}) had toxic effects on *L. monocytogenes, Salmonella* sp., *Shigella* sp., and *E. coli* O157:H7 after 24 h in laboratory cultures, decreasing concentrations from approximately 10^2 CFU ml^{-1} to undetectable levels. However, the addition of diazinon also greatly reduced the solution pH to 3.70, which would have contributed to decreases in bacterial densities (Guan et al. 2001). Additionally, malathion inhibited bacterial growth in sludge at concentrations of 500 μg L^{-1}, although this concentration is also higher than would be expected in the environment (Pai et al. 2009). One study of

Table 5. Studies examining direct effects of pesticides on algae and cyanobacteria.

Pesticide Category	Pesticide Group	Pesticides Used	Pesticide Concentration ($\mu g\ L^{-1}$)	Ecologically Relevant	Matrix	Focal Microbes	Direction of Effect	Reference
Insecticide	carbamate	carbaryl	25000	N	Lab Culture	planktonic algae	–	(Butler, Deason, et al. 1975)
	carbamate	carbaryl	5000–14000	N	Lab Culture	*Anabaena flos-aquae, Microcystis flos-aquae, M. aeruginosa, Selenastrum capricornutum, Scenedesmus quadricauda, Sc. obliquus, Chlorella vulgaris, C. pyrenoidosa*	–	(Ma, Lu, et al. 2006)
	carbamate	carbaryl	3667	N	Lab Culture	*Scenedesmus quadricuauda, Selenastrum cupricornutum, Nitzchia* sp., *Cyclotella meneghiana, Microystis aeruginosa, Oscillatoria* sp., *Pseudoanabaena* sp., *Anabaena inaequalis, Aphanizomenon flos-aquae*	–	(Peterson, Boutin et al. 1994)
	carbamate	carbaryl	100–100000	Y	Lab Culture	*Monochrysis lutheri, Dunaliella euchlora, Chlorella* sp., *Protococcus* sp., *Phaeodactylum tricornutum*	–	(Ukeles 1962)
	carbamate	carbofuran	13000–281000	N	Lab Culture	*Anabaena flos-aquae, Microcystis flos-aquae, M. aeruginosa, Selenastrum capricornutum, Scenedesmus quadricauda, Sc. obliquus, Chlorella vulgaris, C. pyrenoidosa*	–	(Ma, Lu, et al. 2006)
	carbamate	carbofuran	667	N	Lab Culture	*Scenedesmus quadricuauda, Selenastrum cupricornutum, Nitzchia* sp., *Cyclotella meneghiana, Microystis aeruginosa, Oscillatoria* sp., *Pseudoanabaena* sp., *Anabaena inaequalis, Aphanizomenon flos-aquae*	–	(Peterson, Boutin et al. 1994)
	organochlorine	aldrin	100000	N	Lab Culture	freshwater algae	–	(Clegg and Koevenig 1974)
	organochlorine	benzene hexachloride	>30000	N	Lab Culture	*Cylindrospermum* sp., *Aulosira fertilissima*, *Plectonema boryanum*	–	(Singh 1973)
	organochlorine	DDT	3.6–36	Y	Unknown	*Selenastrum capricornutum*	–	(Lee, Fang et al. 1976)
	organochlorine	DDT	200–600	N	Lab Culture	*Monochrysis lutheri, Dunaliella euchlora, Chlorella* sp., *Protococcus* sp., *Phaeodactylum tricornutum*	–	(Ukeles 1962)
	organochlorine	dieldrin	100000	N	Unknown	freshwater algae	–	(Clegg and Koevenig 1974)
	organochlorine	endosulfan	41500 and 28500	N	Lab Culture	*Chlorella vulgaris, Anabaena doliolum*	–	(Mohapatra and Mohanty 1992)
	organochlorine	endrin	100000	N	Lab Culture	freshwater algae	–	(Clegg and Koevenig 1974)
	organochlorine	endrin	500000	N	Lab Culture	*Cylindrospermum* sp., *Aulosira fertilissima*, *Plectonema boryanum*	Neutral	(Singh 1973)
	organochlorine	lindane	4220	N	Lab Culture	*Chlorella fusca*	–	(Faust, Altenburger et al. 1994)
	organochlorine	lindane	>100000	N	Lab Culture	*Cylindrospermum* sp., *Aulosira fertilissima*, *Plectonema boryanum*	–	(Singh 1973)
	organochlorine	lindane	1000–9000	N	Lab Culture	*Monochrysis lutheri, Dunaliella euchlora, Chlorella* sp., *Protococcus* sp., *Phaeodactylum tricornutum*	–	(Ukeles 1962)

(*Continued*)

Table 5. (Continued)

Pesticide Category	Pesticide Group	Pesticides Used	Pesticide Concentration (μg L^{-1})	Ecologically Relevant	Matrix	Focal Microbes	Direction of Effect	Reference
	organochlorine	methoxychlor	100	Y	Lab Culture	*Chlorella pyrenoidosa*	–	(Kricher, Urey et al. 1975)
	organochlorine	methoxychlor	10	Y	Lab Culture	planktonic algae	Neutral	(Butler, Deason, et al. 1975)
	organochlorine	mirex	100	Y	Lab Culture	*Chlorella pyrenoidosa*	–	(Kricher, Urey et al. 1975)
	organophosphate	chlorpyrifos	240	N	Lab Culture	*Minutocellus polymorphus*	–	(Walsh, McLaughlin et al. 1988)
	organophosphate	diazinon	400000–500000	N	Lab Culture	*Cylindrospermum* sp., *Aulosira fertilissima*, *Plectonema boryanum*	–	(Singh 1973)
	organophosphate	diazinon	10000	N	Lab Culture	planktonic algae	–	(Butler, Deason, et al. 1975)
	organophosphate	fenitrothion	800–24400	N	Lab Culture	freshwater phytoplnakton	–	(Kent and Currie 1995)
	organophosphate	malathion	1–1000	Y	Lab Culture	*Chlorella vulgaris, Chlorococcum hypnosporum, Oscillatoria lutea, Tribonema, Stigeoclonium tenue, Vaucheria geminata*	–	(Torres and O'Flaherty 1976)
	organophosphate	parathion	7860	N	Lab Culture	*Chlorella fusca*	–	(Faust, Altenburger et al. 1994)
Herbicide	s-triazine	atrazine	0.12–5.8	Y	Marine Mesocosms	*Thalassiosira puntigera, T. rotula, Nitzschia pungens, Skeletonema costatum, Phaeocystis globosa*	–	(Bester, Huhnerfuss et al. 1995)
	phenoxy	2,4-D	4000	N	Lab Culture	planktonic algae	–	(Butler, Deason, et al. 1975)
	s-triazine	atrazine	1000	N	Lab Culture	planktonic algae	–	(Butler, Deason, et al. 1975)
	bipyrimidine	diquat	19–395	Y	Lab Culture	*Anabaena flos-aquae, Microcystis aeruginosa, Selenastrum capricornutum, Chlorella vulgaris*	–	(Campbell, Bartell et al. 2000)
	s-triazine	atrazine	90–3000	Y	Lab Culture	*Selenastrum capricornutum, Chlorella vulgaris, Chlamydomonas reinhardi, Scenedesmus quadricauda, Microcystis* sp., *Anabaena flosque*	–	(Fairchild, Ruessler, et al. 2009)
	chloroacetanilides	metazachlor	58	Y	Lab Culture	*Chlorella fusca*	–	(Faust, Altenburger et al. 1994)
	glyphosate	glyphosate	377000	N	Lab Culture	*Chlorella fusca*	–	(Faust, Altenburger et al. 1994)
	phenoxy	2,4-D	88900	N	Lab Culture	*Chlorella fusca*	–	(Faust, Altenburger et al. 1994)
	s-triazine	simazine	73	Y	Lab Culture	*Chlorella fusca*	–	(Faust, Altenburger et al. 1994)
	subtituted urea	bentazon	42500	N	Lab Culture	*Chlorella fusca*	–	(Faust, Altenburger et al. 1994)
	subtituted urea	chlorotoluron	23	Y	Lab Culture	*Chlorella fusca*	–	(Faust, Altenburger et al. 1994)
	subtituted urea	methabenzthiazuron	44	Y	Lab Culture	*Chlorella fusca*	–	(Faust, Altenburger et al. 1994)
	thiocarbamates	tri-allate	3870	N	Lab Culture	*Chlorella fusca*	–	(Faust, Altenburger et al. 1994)
	glyphosate	glyphosate	8900–89000	N	Freshwater Mesocosms	periphyton	–	(Goldsborough and Brown 1988)

(*Continued*)

Table 5. (Continued)

Pesticide Category	Pesticide Group	Pesticides Used	Pesticide Concentration (μg L^{-1})	Ecologically Relevant	Matrix	Focal Microbes	Direction of Effect	Reference
	bypyrimidine	paraquat	10000	N	Freshwater Mesocosms	freshwater cyanobacteria, chlorophytes, chrysophytes	–	(Kosinski 1984)
	s-triazine	atrazine	10000	N	Freshwater Mesocosms	freshwater cyanobacteria, chlorophytes, chrysophytes	–	(Kosinski 1984)
	s-triazine	atrazine	130–620 nM	Y	Lab Culture	*Navicula* sp. and *Nephroselmis pyriformis*	–	(Magnusson, Heimann et al. 2008)
	substituted urea	diuron	16–33 nM	Y	Lab Culture	*Navicula* sp. and *Nephroselmis pyriformis*	–	(Magnusson, Heimann et al. 2008)
	aldehyde	acrolein	1000	N	Lab Culture	*Scenedesmus quadricuauda, Selenastrum cupricornutum, Nitzchia* sp., *Cyclotella meneghiana, Microystis aeruginosa, Oscillatoria* sp., *Pseudoanabaena* sp., *Anabaena inaequalis, Aphanizomenon flos-aquae*	–	(Peterson, Boutin et al. 1994)
	bipyrimidine	diquat	733	N	Lab Culture	*Scenedesmus quadricuauda, Selenastrum cupricornutum, Nitzchia* sp., *Cyclotella meneghiana, Microystis aeruginosa, Oscillatoria* sp., *Pseudoanabaena* sp., *Anabaena inaequalis, Aphanizomenon flos-aquae*	–	(Peterson, Boutin et al. 1994)
	glyphosate	glyphosate	2848	N	Lab Culture	*Scenedesmus quadricuauda, Selenastrum cupricornutum, Nitzchia* sp., *Cyclotella meneghiana, Microystis aeruginosa, Oscillatoria* sp., *Pseudoanabaena* sp., *Anabaena inaequalis, Aphanizomenon flos-aquae*	–	(Peterson, Boutin et al. 1994)
	phenoxy	2,4-D	2917	N	Lab Culture	*Scenedesmus quadricuauda, Selenastrum cupricornutum, Nitzchia* sp., *Cyclotella meneghiana, Microystis aeruginosa, Oscillatoria* sp., *Pseudoanabaena* sp., *Anabaena inaequalis, Aphanizomenon flos-aquae*	Neutral	(Peterson, Boutin et al. 1994)
	pyridine	picloram	1760	N	Lab Culture	*Scenedesmus quadricuauda, Selenastrum cupricornutum, Nitzchia* sp., *Cyclotella meneghiana, Microystis aeruginosa, Oscillatoria* sp., *Pseudoanabaena* sp., *Anabaena inaequalis, Aphanizomenon flos-aquae*	Neutral	(Peterson, Boutin et al. 1994)
	s-triazine	atrazine	2667	N	Lab Culture	*Scenedesmus quadricuauda, Selenastrum cupricornutum, Nitzchia* sp., *Cyclotella meneghiana, Microystis aeruginosa, Oscillatoria* sp., *Pseudoanabaena* sp., *Anabaena inaequalis, Aphanizomenon flos-aquae*	–	(Peterson, Boutin et al. 1994)
	s-triazine	simazine	2667	N	Lab Culture	*Scenedesmus quadricuauda, Selenastrum cupricornutum, Nitzchia* sp., *Cyclotella meneghiana, Microystis aeruginosa, Oscillatoria* sp., *Pseudoanabaena* sp., *Anabaena inaequalis, Aphanizomenon flos-aquae*	–	(Peterson, Boutin et al. 1994)

(*Continued*)

Table 5. (Continued)

Pesticide Category	Pesticide Group	Pesticides Used	Pesticide Concentration (μg L^{-1})	Ecologically Relevant	Matrix	Focal Microbes	Direction of Effect	Reference
	substituted urea	tebuthiuron	5900	N	Lab Culture	*Scenedesmus quadricuauda, Selenastrum cupricornutum, Nitzchia* sp., *Cyclotella meneghiana, Microystis aeruginosa, Oscillatoria* sp., *Pseudoanabaena* sp., *Anabaena inaequalis, Aphanizomenon flos-aquae*	–	(Peterson, Boutin et al. 1994)
	phenoxy	2,4-D	1–100000	Y	Lab Culture	*Chlorella vulgaris, Chlorococcum hypnosporum, Oscillatoria lutea, Tribonema, Stigeoclonium tenue, Vaucheria geminata*	Neutral	(Torres and O'Flaherty 1976)
	s-triazine	atrazine	1–1000	Y	Lab Culture	*Chlorella vulgaris, Chlorococcum hypnosporum, Oscillatoria lutea, Tribonema, Stigeoclonium tenue, Vaucheria geminata*	–	(Torres and O'Flaherty 1976)
	s-triazine	simazine	1–1000	Y	Lab Culture	*Chlorella vulgaris, Chlorococcum hypnosporum, Oscillatoria lutea, Tribonema, Stigeoclonium tenue, Vaucheria geminata*	–	(Torres and O'Flaherty 1976)
	substituted urea	diuron	0.02–400	Y	Lab Culture	*Monochrysis lutheri, Dunaliella euchlora, Chlorella* sp., *Protococcus* sp., *Phaeodactylum tricornutum*	–	(Ukeles 1962)
	substituted urea	fenuron	0.02–400	Y	Lab Culture	*Monochrysis lutheri, Dunaliella euchlora, Chlorella* sp., *Protococcus* sp., *Phaeodactylum tricornutum*	–	(Ukeles 1962)
	substituted urea	monuron	0.02–400	Y	Lab Culture	*Monochrysis lutheri, Dunaliella euchlora, Chlorella* sp., *Protococcus* sp., *Phaeodactylum tricornutum*	–	(Ukeles 1962)
	substituted urea	neburon	0.02–400	Y	Lab Culture	*Monochrysis lutheri, Dunaliella euchlora, Chlorella* sp., *Protococcus* sp., *Phaeodactylum tricornutum*	–	(Ukeles 1962)
	s-triazine	atrazine	20	Y	Lab Culture	*Minutocellus polymorphus*	–	(Walsh, McLaughlin et al. 1988)
Fungicide	imidazole	prochloraz	24	Y	Lab Culture	*Chlorella fusca*	–	(Faust, Altenburger et al. 1994)
	organomercury		1	Y	Lab Culture	marine diatoms and phytoplankton	–	(Harriss, White et al. 1970)
	triazine	anilazine	1390	Y	Lab Culture	*Chlorella fusca*	–	(Faust, Altenburger et al. 1994)
	triazole	propiconazole	83	Y	Lab Culture	*Scenedesmus quadricuauda, Selenastrum cupricornutum, Nitzchia* sp., *Cyclotella meneghiana, Microystis aeruginosa, Oscillatoria* sp., *Pseudoanabaena* sp., *Anabaena inaequalis, Aphanizomenon flos-aquae*	–	(Peterson, Boutin et al. 1994)

note also found toxic effects of chlorpyrifos (46 300 μg L^{-1}), diazinon (10 300 μg L^{-1}), and parathion (8500 μg L^{-1}) on *Vibrio phosphoreum* (Somasundaram et al. 1990); however, in addition to noting that these concentrations were all above environmental expectation, the method of assessing toxicity, based upon bioluminescence, was also markedly different from the other study reviewed here and may have be the result of a sublethal effect.

Overall, the direct effects of organophosphate insecticides are negative for algal, protozoan, and fungal species, both at

Table 6. Studies examining direct effects of pesticides on protozoa.

Pesticide category	Pesticide group	Pesticides used	Pesticide concentration ($\mu g\ L^{-1}$)	Ecologically relevant	Matrix	Focal microbes	Direction of effect	Reference
Insecticide	carbamate	carbaryl	>10000	N	Lab Culture	*Colpidium campulum*	–	(Dive et al. 1980)
	carbamate	carbaryl	1000–100000	Y	Estuarine Mesocosms	ciliate protozoa	–	(Weber et al. 1982)
	carbamate	carbomran	9000–50000	N	Lab Culture	*Euglena gracilis*	–	(Azizullah et al. 2011)
	organochlorine	aldrin	>10000	N	Lab Culture	*Colpidium campulum*	–	(Dive et al. 1980)
	organochlorine	DDT	10000–100000	N	Lab Culture	*Tetrahymena pyriformis*	–	(Lal et al. 1987)
	organochlorine	dieldrin	>10000	N	Lab Culture	*Colpidium campulum*	–	(Dive et al. 1980)
	organochlorine	lindane	>10000	N	Lab Culture	*Colpidium campulum*	–	(Dive et al. 1980)
	organochlorine	methoxychlor	>10000	N	Lab Culture	*Colpidium campulum*	–	(Dive et al. 1980)
	organochlorine	mirex	0.9	Y	Lab Culture	*Tetrahymena pyriformis*	–	(Cooley et al. 2007)
	organophosphate	chlorpyrifos	100–2500	Y	Lab Culture	*Tetrahymena pyriformis*	–	(Lal et al. 1987)
	organophosphate	dimethoate	>10000	N	Lab Culture	*Colpidium campulum*	–	(Dive et al. 1980)
	organophosphate	fenitrothion	500–10000	Y	Lab Culture	*Tetrahymena pyriformis*	–	(Lal et al. 1987)
	organophosphate	malathion	6000–50000	N	Lab Culture	*Euglena gracilis*	–	(Azizullah et al. 2011)
	organophosphate	malathion	>10000	N	Lab Culture	*Colpidium campulum*	–	(Dive et al. 1980)
	organophosphate	malathion	7250	N	Lab Culture	*Euglena gracilis*	–	(Moore 1970)
	organophosphate	malathion	1000–30000	N	Estuarine Mesocosms	ciliate protozoa	–	(Weber et al. 1982)
	organophosphate	parathion	1200	N	Lab Culture	*Euglena gracilis*	–	(Moore 1970)
Herbicide	bipyrimidine	diquat	2940	N	Lab Culture	*Euglena gracilis, Ochromonas danica, Navicula pelliculosa, Cryptomonas ovata*	–	(Campbell et al. 2000)
	phenoxy	2,4-D	>10000	N	Lab Culture	*Colpidium campulum*	Neutral	(Dive et al. 1980)
	substituted urea	chlorotoluron	298500	N	Lab Culture	*Tetrahymena pyriformis*	–	(Nelieu et al. 2010
	substituted urea	fenuron	>10000	N	Lab Culture	*Colpidium campulum*	Neutral	(Dive et al. 1980)
Fungicide	carbamate	nab am	50–10000	N	Lab Culture	*Euglena gracilis*	–	(Moore 1970)
	carbamate	thiram	300	N	Lab Culture	*Colpidium campulum*	–	(Dive et al. 1980)
	carbamate	vapam	1000–10000	N	Lab Culture	*Euglena gracilis*	–	(Moore 1970)

and above environmentally relevant concentrations; however, at concentrations that would be expected within the environment, organophosphate insecticides have not produced any direct effects on bacterial species. At elevated organophosphate concentrations, direct effects are influenced by the specific chemical being utilized, the concentration applied, and the target bacteria, as both negative and positive effects have been reported (Table 8). In several studies, confounding variables (i.e., low pH) and differences in toxicity assessment might better explain non-neutral effects.

When more complex systems are explored and indirect effects are possible, that is, when diminished predation by protozoa increases bacterial survival, organophosphate insecticides seemed to be generally beneficial for lower trophic levels. The application of chlorpyrifos to a freshwater pond system was associated with cyanobacterial blooms, which was hypothesized to be a possible result of the removal of top-down pressures associated with predation and/or a bottom–up effect by increasing phosphorous concentrations (Butcher et al. 1977). Chlorpyrifos removed predation by herbivorous crustaceans, resulting in increased phytoplankton, cyanobacteria, and periphyton growth (Hurlbert et al. 1972). In mesocosms designed to simulate a tidal creek, chlorpyrifos applied at 10 $\mu g\ L^{-1}$ increased heterotrophic bacterial abundance, while decreasing heterotrophic ciliate and flagellate (protozoan) abundance (DeLorenzo et al. 1999b). Similarly, parathion at 50 000 $\mu g\ L^{-1}$ tended to increase abundance of heterotrophic bacteria in water samples collected from a natural lake (Lopez et al. 2006). However, another study revealed no significant effects of the pesticide on natural protozoan, bacterial, or fungal species in microcosms, even when concentrations exceeded water quality criteria threefold (~12 000 $\mu g\ L^{-1}$) (Pratt et al. 1993). Similarly, malathion at 101 and 202 $\mu g\ L^{-1}$ did not have any significant effect on *E. coli* or *Enterococcus* spp. in the sediment of complex freshwater mesocosms (Staley et al. 2010). While none of these studies were designed to specifically isolate indirect effects of organophosphates, the algal and bacterial populations tended to increase in community settings. Generally, organophosphates seem to remove top–down pressures, allowing algal blooms or increased bacterial abundance, suggesting that indirect effects may be more pronounced than the negative direct effects on many populations of lower trophic levels.

Carbamates

Carbamate insecticides have similarly been found to have adverse direct effects on algae, protozoan, and fungal species, both at and above environmentally relevant concentrations (Tables 5–7). At environmentally relevant concentrations, carbaryl had an EC_{50} ranging from 5000 to 14 000 $\mu g\ L^{-1}$ for green algae and cyanobacteria (Ma et al. 2006). Carbaryl inhibited carbon uptake in diatoms and green algae at concentrations ranging from 100 to 100 000 $\mu g\ L^{-1}$ (Ukeles 1962), and at concentrations exceeding environmental relevance (25 000 $\mu g\ L^{-1}$) it was toxic to planktonic algae (Butler et al. 1975). Carbaryl and carbofuran exhibited direct toxicity to phytoplankton species at 3667 and 667 $\mu g\ L^{-1}$, respectively (Peterson et al. 1994), and carbofuran had an environmentally relevant EC_{50} ranging from 13 000 to 281 000 $\mu g\ L^{-1}$ for cer-

Table 7. Studies examining direct effects of pesticides on aquatic fungi.

Pesticide category	Pesticide group	Pesticides used	Pesticide concentration (μg L^{-1})	Ecologically relevant	Matrix	Focal microbes	Direction of effect	Reference
Insecticide	carbamate	carbaryl	2500	Y	Lab Culture	*Batrachochytrium dendrobatidis*	–	(Hanlon and Paris 2012)
	organophosphate	diazinon	0.05–50	Y	Lab Culture	*Heliscella stellata, Lunaspora curvula,* Sigmoid type-2 aquatic fungi	_	(Flores et al. 2014)
	organophosphate	dimethoate	2500–75000	N	Lab Culture	*Achyla racemosa, Dictyuchus monosporus, Saprolegnia ferax, Thraustotheca clavata,* and *Allomyces arbuscula*	_	(Khallil and Omar 1993)
Herbicide	bipyrimidine	diquat	2–10	Y	Lab Culture	*Helicoon elegans, Pseudoaergerita matsushimae, Hormiactus ontariensis, Beverwykella pulmonaria,* and Sigmoid type-2 fungi	_ _	(Fronda and Kendrick 1995)
	glyphosate	glyphosate	50000–500000	N	Lab Culture	*Annulatascus velatisporus, Camposporium antennatum, Helicosporium griseum,* and *Massarina sp.*	_	(Tsui et al. 2001)
	glyphosate	glyphosate	500	Y	Lab Culture	*Batrachochytrium dendrobatidis*	_	(Hanlon and Paris 2012)
	s-triazine	atrazine	0.0106–106	Y	Lab Culture	*Batrachochytrium dendrobatidis*	_	(McMahon et al. 2013)
Fungicide	aromatic	chlorothalonil	0.0176–1.76 and 32–176	Y	Lab Culture	*Batrachochytrium dendrobatidis Cryptococcusflavescens, Trichoderma*	_	(McMahon et al. 2013)
	aromatic	chlorothalonil	>260	Y	Lab Culture	*hamatum, Fusarium sporotrichioides, Mucor hiemalas, Pythium* spp., *Helicoon richonis, Helicodendran tubulosum*	_ _ _	(Dijksterhuis et al. 2011
	imidazole	imazalil	0.1–100	Y	Lab Culture	*Heliscella stellata, Lunaspora curvula,* Sigmoid type-2 aquatic fungi *Cryptococcus flavescens, Trichoderma*	_	(Flores et al. 2014)
	imidazole	imazalil	10000–210000	N	Lab Culture	*hamatum, Fusarium sporotrichioides, Mucor hiemalas, Pythium* spp., *Helicoon richonis, Helicodendran tubulosum*	_ _ _	(Dijksterhuis et al. 2011)
	phosphanoglycine	Thiophanate methyl	1500	N	Lab Culture	*Batrachochytrium dendrobatidis*	_	(Hanlon and Paris 2012)

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Table 8. Studies examining direct effects of pesticides on bacteria.

Pesticide category	Pesticide group	Pesticides used	Pesticide concentration ($\mu g\ L^{-1}$)	Ecologically relevant	Matrix	Focal microbes	Direction of effect	Reference
Insecticide	carbamate	carbaryl	9400000	N	Lab Culture	*E. coli* 0157:H7, *Salmonella typhimurium, Sal. enteritidis, Shigella sonnei, S. flexneri, Listeria moncytogenes*	–	(Guan et al. 2001)
	carbamate	carbaryl	5000	N	Lab Culture	*Vibrio phosphoreum*	–	(Somasundaram et al. 1990)
	carbamate	carbaryl	100000	N	Estuarine Mesocosms	sediment bacteria	–	(Weber and Rosenberg 1984)
	carbamate	carbofuran	20500	Y	Lab Culture	*Vibrio phosphoreum*	–	(Somasundaram et al. 1990)
	organochlorine	lindane	500–500000	N	Rumen Fluid Microcosms	rumen bacteria *E. coli* 0157:H7, *Salmonella*	Neutral	(Williams et al. 1963)
	organophosphate	chlorpyrifos	6600000	N	Lab Culture	*typhimurium, Sal. enteritidis, Shigella sonnei, S. flexneri, Listeria moncytogenes*	+	(Guan et al. 2001)
	organophosphate	chlorpyrifos	46300	N	Lab Culture	*Vibrio phosphoreum E. coli 0157:H7, Salmonella*	–	(Somasundaram et al. 1990)
	organophosphate	diazinon	14600000	N	Lab Culture	*typhimurium, Sal. enteritidis, Shigella sonnei, S. flexneri, Listeria moncytogenes*	–	(Guan et al. 2001)
	organophosphate	diazinon	0.005	Y	Lab Culture	*Eshcerichia coli, Klebsiella pneumoniae*	Neutral	(Higgins and Hohn 2008)
	organophosphate	diazinon	10300	N	Lab Culture	*Vibrio phosphoreum*	–	(Somasundaram, Coats 1990)
	organophosphate	diazinon	500–500000	Y	Rumen Fluid Microcosms	rumen bacteria *E. coli* 0157:H7, *Salmonella*	Neutral	(Williams et al. 1963)
	organophosphate	dimethoate	14800000	N	Lab Culture	*typhimurium, Sal. enteritidis, Shigella sonnei, S. flexneri, Listeria moncytogenes*	–	(Guan et al. 2001)
	organophosphate	dimethoate	500–500000	N	Rumen Fluid Microcosms	rumen bacteria	Neutral	(Williams et al. 1963)
	organophosphate	malathion	Unknown		Unknown Sludge	*Escherichia coli, Bacillus subtilis*	Neutral	(Kerszman 1993)
	organophosphate	malathion	500	N	Chamber Microcosms	heterotrophic bacteria	~	(Pai et al. 2009)
	organophosphate	malathion	101, 202	Y	Freshwater Mesocosms	*Escherichia coli, Enterococcus faecalis Eschericia coli, E. coli* 0157:H7,	Neutral	(Staley et al. 2010)
	organophosphate	malathion	101, 202	Y	Freshwater Microcosms	*Salmonella typhimurium, Enterococcus faecalis*	Neutral	(Staley et al. 2012)
	organophosphate	parathion	8500	N	Lab Culture	*Vibrio phosphoreum E. coli* 0157:H7, *Salmonella*	–	(Somasundaram et al. 1990)
	pyrethroid	permethrine	1000000	N	Lab Culture	*typhimurium, Sal. enteritidis, Shigella sonnei, S. flexneri, Listeria moncytogenes*	+	(Guan et al. 2001)
Herbicide	bipyrimidine	diquat	25000–50000	N	Lab Culture	*Erwinia carotovora, Pseudomonas fluorescens, Bacillus* sp.	Neutral	(Breazeale and Camper 1972)
	bipyrimidine	paraquat	25000–50000	N	Lab Culture	*Erwinia carotovora, Pseudomonas fluorescens, Bacillus* sp. *E. coli* 0157:H7, *Salmonella*	Neutral	(Breazeale and Camper 1972)
	bipyrimidine	paraquat	20000000	N	Lab Culture	*typhimurium, Sal. enteritidis, Shigella sonnei, S. flexneri, Listeria moncytogenes E. coli* 0157:H7, *Salmonella*	‘	(Guan et al. 2001)

(*Continued*)

Table 8. (Continued)

Pesticide category	Pesticide group	Pesticides used	Pesticide concentration (μg L^{-1})	Ecologically relevant	Matrix	Focal microbes	Direction of effect	Reference
	glyphosate	glyphosate	13400000	N	Lab Culture Sludge	*typhimurium, Sal. enteritidis, Shigella sonnei, S. flexneri, Listeria moncytogenes*		(Guan et al. 2001)
	glyphosate	glyphosate	500	Y	Chamber Microcosms	heterotrophic bacteria *E. coli* 0157:H7, *Salmonella*		(Pai et al. 2009)
	phenoxy	2,4-D	31400000	N	Lab Culture	*typhimurium, Sal. enteritidis, Shigella sonnei, S. flexneri, Listeria moncytogenes*	–	(Guan et al. 2001)
	phenoxy	2,4-D	5 and lOmM		Lab Culture	*Escherichia coli, Klebsiella pneumoniae*	–	(Higgins and Hohn 2008)
	phenoxy	2,4-D	100700	N	Lab Culture	*Vibrio phosphoreum*	–	(Somasundaram, Coats 1990)
	s-triazine	atrazine	25000–50000	N	Lab Culture	*Erwinia carotovora, Pseudomonas fluorescens, Bacillus* sp.	Neutral	(Breazeale and Camper 1972)
	s-triazine	atrazine	0.25–22.5	Y	Lab Culture	*Escherichia coli, Klebsiella pneumoniae*	Neutral	(Higgins and Hohn 2008)
	s-triazine	atrazine	1–100	Y	Lab Culture Freshwater Microcosms	*Escherichia coli, Enterococcus faecalis Eschericia coli, E. coli* 0157:H7,	+	(Koutsotoli et al. 2005)
	s-triazine	atrazine	102, 204	Y		*Salmonella typhimurium, En terococcus faecalis*	Neutral	(Staley et al. 2012)
	s-triazine	simazine	0.000025–0.0025	Y	Lab Culture	*Escherichia coli, Klebsiella pneumoniae*	Neutral	(Higgins and Hohn 2008)
	substituted urea	chlorotoluron	68200	N	Lab Culture	*Vibrio fisherii E. coli* 0157:H7, *Salmonella*	–	(Nelieu et al. 2010)
	substituted urea	linuron	22600000	N	Lab Culture	*typhimurium, Sal. enteritidis, Shigella sonnei, S. flexneri, Listeria moncytogenes E. coli* 0157:H7, *Salmonella*	+	(Guan et al. 2001)
Fungicide	aromatic	chlorothalonil	20000000	N	Lab Culture Freshwater Microcosms	*typhimurium, Sal. enteritidis, Shigella sonnei, S. flexneri, Listeria moncytogenes Eschericia coli, E. coli* 0157:H7,	+	(Guan et al. 2001)
	aromatic	chlorothalonil	169, 338	Y		*Salmonella typhimurium, Enterococcus faecalis*	Neutral	(Staley et al. 2012)
	aromatic	dicloran	0.14	Y	Unknown	*Salmonella* YG1041 *E. coli* 0157:H7, *Salmonella*	–	(de Oliviera et al. 2009)
	carbamate	thiram	13400000	N	Lab Culture	*typhimurium, Sal. enteritidis, Shigella sonnei, S. flexneri, Listeria moncytogenes*	–	(Guan et al. 2001)
	carbamate	thiram	3000–70000	N	Freshwater Mesocosms	sediment bacteria	–	(Milenkovski et al. 2010)
	dicarboximide	cap tan	3000–70000	N	Freshwater Mesocosms	sediment bacteria	–	(Milenkovski et al. 2010)
	hydrochloride	acriflavine	1000	N	Lab Culture	*Staphylococcus aureus*	–	(Kawai and Yamagishi 2009)
	morpholine	fenpropimorph	3000–70000	N	Freshwater Mesocosms	sediment bacteria	–	(Milenkovski et al. 2010)
	triazole	propiconazole	3000–70000	N	Freshwater Mesocosms	sediment bacteria	–	(Milenkovski et al. 2010)

tain species of green algae and cyanobacteria (Ma et al. 2006). Protozoan species were also adversely affected, with carbofuran application reducing cell growth of *E. gracilis* after 72 h of exposure, at a concentration of 50 000 μg L^{-1}, although this concentration is higher than would be expected in the environment (Azizullah et al. 2011). Toxic effects of carbaryl on protozoa when applied at a concentration of up to 10 000 μg L^{-1} have also been observed (Weber et al. 1982). At concentrations exceeding 10 000 μg L^{-1}, carbaryl was also toxic to protozoan *C. campylum*, although these concentrations are not environmentally relevant. The aquatic fungal species, *Batrachochytrium dendrobatidis*, a pathogen of amphibians, had significantly decreased zoosporangia densities when exposed to 2500 μg L^{-1} of carbaryl (Hanlon and Parris 2012).

Bacterial studies examining the direct effects of carbamate insecticides also produced adverse effects, regardless of whether or not insecticide concentrations exceeded environmental expectations (Table 8). Carbaryl had a half maximal effective concentration (EC_{50}) of 5000 μg L^{-1} on *V. phosphoreum,* reducing optical density by 50%, while metabolites of carbaryl had an EC_{50} of 3700 μg $L^{-1}$and carbofuran had an EC_{50} of 20 500 μg L^{-1} (Somasundaram et al. 1990). Carbaryl concentrations at 9 400 000 μg L^{-1} were also toxic to *L. monocytogenes, Salmonella* sp., *Shigella* sp., and *E. coli* O157:H7 after 24 h in laboratory cultures (Guan et al. 2001). Weber and Rosenberg (1984) found that bacterial diversity was diminished at a carbaryl concentration of 100 000 μg L^{-1} in model estuarine systems.

When the experimental design allowed observation of indirect effects of carbamate on target species by the chemicals' effects on factors such as competition or predation, carbamates have resulted in positive effects for some species and trophic levels. In mixed cultures, growth of *E. faecalis* was inhibited by a carbaryl concentration of 5000 μg L^{-1}, while *Staphylococcus aureus* and *S. enterica* experienced increased growth rates at the same concentration (Guthrie et al. 1981). In aquatic mesocosms designed to simulate pond communities, carbaryl application resulted in a decrease in zooplankton abundance when applied over a range of 100–20 000 μg L^{-1}, while microbial and phytoplankton concentrations increased (Downing et al. 2008). Affected ecosystems in this study showed signs of recovery after 40 days, but changes in zooplankton population diversity were noted (Downing et al. 2008).

Organochlorines

Direct effects of organochlorine insecticides have been mostly negative, although, where neutral effects were observed, this was likely the result of reduced chemical concentrations or parameters (i.e., ATP production or survival) that were not measured in all studies investigating the same chemical (Table 5). DDT, at concentrations both in the environmentally relevant range, 3.6–36 μg L^{-1}, and above 200–600 μg L^{-1}, inhibited photosynthesis in marine algae (Lee et al. 1976; Ukeles 1962). Mirex and methoxychlor, at environmentally relevant concentrations of 100 μg L^{-1}, also inhibited growth of *C. pyrenoidosa* (Kricher et al. 1975), although methoxychlor at 10 μg L^{-1} had no effect on certain species of green algae and cyanobacteria, likely as a result of the reduced concentration (Butler et al. 1975). All other studies reviewed utilized organochlorine concentrations exceeding environmentally relevant concentrations. Benzene hexachloride was toxic to cyanobacteria at concentrations exceeding 30 000 μg L^{-1} (Singh 1973). Similarly, lindane had adverse effects on algal species with concentrations ranging from 1000 to 9000 μg L^{-1} (Faust et al. 1994; Ukeles 1962). Endosulfan was toxic to cyanobacteria (*Anabaena doliolum*) and green algae (*C. vulgaris*) at concentrations $>$ 28 500 and 41 500 μg L^{-1}, respectively (Mohapatra and Mohanty 1992). Aldrin, dieldrin, and endrin, at concentrations of 100 000 μg L^{-1}, lowered ATP production, but did not result in reduced algal abundance (Clegg and Koevenig 1974). Endrin at 500 000 μg L^{-1} had no effect on cyanobacterial species (Singh 1973), although this study measured only survival and not ATP production.

Organochlorine insecticides also have adverse direct effects on protozoan species at and above environmentally relevant concentrations (Table 6). Mirex (0.9 μg L^{-1}) and DDT (10 000–100 000 μg L^{-1}) produced detrimental effects on *T. pyriformis* cell growth (Cooley et al. 2007; Lal et al. 1987). Endosulfan, dieldrin, endrin, aldrin, lindane, and methoxychlor were toxic to *C. campylum* at concentrations $>$ 10 000 μg L^{-1} (Dive et al. 1980). Limited research has been conducted on the direct effects of organochlorine insecticides on bacteria. A single study of the effects of organochlorines on bacteria found no direct effect of lindane at concentrations ranging from 500 to 500 000 μg L^{-1} on rumen bacteria (Table 8) (Williams et al. 1963).

Studies on organochlorines where indirect effects were possible are limited. In a study utilizing mesocosms to simulate a tidal creek, endosulfan, at concentrations of 1 and 10 μg L^{-1} resulted in decreased heterotrophic bacterial abundance and increased phototroph abundance, although many cyanobacterial species became undetectable (DeLorenzo et al. 1999b). In another study of community level effects, endosulfan (at 1 μg L^{-1}) increased phototrophic carbon assimilation, although diatom, dinoflagellate, and algal species were significantly reduced (Downing et al. 2004). Aldin and lindane, at concentrations of 50 000 μg L^{-1}, resulted in increased heterotrophic bacterial abundance in samples taken from a natural lake (Lopez et al. 2006). In samples isolated from a tropical estuary system, bacterial abundance was reduced by endosulfan, DDT, and lindane at concentrations ranging from 2 to 2000 μg L^{-1}, with bacteria in the water column experiencing greater detrimental effects than those in the sediment (Rajendran et al. 1990). In streams treated with 124 μg L^{-1} of methoxychlor, conidia production of aquatic hyphomycetes increased by up to 3–5 times (Suberkropp and Wallace 1992). Given the differences in observed effects, it is difficult to draw conclusions regarding the community-level effects of organochlorines. Unlike carbamate and organophosphate insecticides, these studies have reported decreases in both heterotrophic and phototrophic populations, although which populations are affected may be dependent upon the chemical and concentration used, as well as the system being studied (i.e., lake, creek, or estuary).

Pyrethroids

Pyrethroid insecticides have received little attention in comparison to organophosphate, carbamate, and organochlorine

insecticides (Tables 3 and 4). Guan et al. (2001) found that a commercially available solution of pyrethroid insecticides caused a 100–1000-fold increase in the abundance of *L. monocytogenes, Salmonella* spp., *Shigella* spp., and *E. coli* O157:H7 after 96 h. However, the concentrations they applied, 500 000–1 500 000 μg L^{-1}, far exceed what would be expected in the environment. At the community level, pyrethroids indirectly facilitated bacterial survival by reducing predation by *Daphnia magna* at environmentally relevant concentrations ranging from 0 to 3 μg L^{-1} (Foit et al. 2010).

Benzamides

Currently, there is limited research on the effect of benzamides on microorganisms. In watersheds treated with the insecticide dimlin at 0.03 kg ha^{-1}, the biodiversity of aquatic fungi increased with no observed direct effects on conidia production or fungal densities (Dubey et al. 1995).

Insecticide summary

With the exception of pyrethroid and benzamide insecticides, for which research is limited, all other insecticide groups tend to have direct negative effects on algal, protozoan, and fungal species. A χ^2 test revealed significantly more negative direct effects of insecticides for both algal and protozoan taxa ($P < 0.001$ for both), while the quantity of direct negative effects for fungal taxa was nearly significant, but should be considered with caution because of a low sample size for fungi ($P = 0.05$, Table 4). Direct effects of insecticides on bacteria have generally been negative, with the exception of the organophosphate group, where several studies have shown no effect of organophosphates at environmentally relevant concentrations. Whether organophosphate insecticides will produce a negative or no effect on bacteria seems to be dependent upon the target bacterial species, organic content in the culture media, and the specific chemical in use. A χ^2 test revealed that there was no significant tendency toward positive, neutral, or negative direct effects of insecticides on bacterial species ($P = 0.157$, Table 4). At the community level, however, insecticides tend to have favorable impacts on the lower trophic levels, generally algal and bacterial taxa, by reducing top-down pressures from protozoan or zooplankton predation. Additionally, algal and bacterial species have shorter generation times than taxa at higher trophic levels and thus probably recover sooner from any direct effects.

Bioconcentration of insecticides

In addition to studies reporting direct and indirect effects (positive or negative) of insecticides on microorganisms, several studies have revealed that various algal, protozoan, and bacterial species can bioconcentrate certain organophosphate and organochlorine insecticides, with no beneficial or adverse effects to those species. For example, 1000 μg L^{-1} of parathion was concentrated 100-fold over a seven-day period by the green algae *Scenedesmus obliquus*, the cyanobacterium *Anacystis nidulans,* and protozoan species *E. gracilis, Paramecium bursaria,* and *P. multimicronucleatum* (Gregory et al. 1969). Additionally, parathion was found to be concentrated 1000-fold by *T. pyriformis* (Shalesh and Anil 2007). Similar effects have been observed for organochlorine insecticides. DDT was bioconcentrated 1000-fold by *S. obliquus*, *A nidulans*, *T. pyriformis, E. gracilis, P. bursaria,* and *P. multimicronucleatum* (Gregory et al. 1969). Endosulfan was concentrated over 1,000-fold in cyanobacteria (Rao and Lal 1987), and mirex was concentrated by 1000-fold in marine algae (Hollister et al. 1975). Aldrin, dieldrin, and endrin were also concentrated by algal species (Vance and Drummond 1969), and dieldrin was concentrated 1000-fold by *T. pyriformis* (Bhatnagar et al. 1988).

While none of these studies reported any positive or negative direct effects on the microbes, the potential for community-level effects in environmental settings as a consequence of bioconcentration should be considered. Several studies (listed above) observed community-level effects of insecticides, generally concluding that the effect of reducing higher predatory trophic levels indirectly benefits algal and bacterial populations. These results, at least in part, may be attributed to bioconcentration and resulting in biomagnification of insecticides by organisms belonging to predatory guilds. Additionally, while microorganisms are the subject of this review, it should be noted that pesticides can also have toxic effects on higher-order aquatic organisms, such as amphibians, arthropods, and fish (Rico et al. 2011; Rohr et al. 2008a,b; Yu et al. 2013). Therefore, while there may be no observed direct effects of pesticides on microorganisms, the potential for concentration and biomagnification must be taken into account when considering how pesticide applications affect aquatic ecosystems, and ultimately human health.

Herbicides

Herbicides comprise a vast array of differing biochemical groups and, as such, encompass a variety of modes of action (Table 2). Herbicides can act by blocking amino acid synthesis through inhibition of 5-enolpyruvl-shikimate-3-phosphate synthase or EPSP synthase, acetolactate synthase, or glutamine synthetase (Duke 1990). Carotenoid, lipid, microtubule, cellulose, or folate synthase may also be inhibited (Duke 1990). The most prominent mode of action of herbicides is via inhibition of photosynthesis. One mechanism by which photosynthesis may be compromised is through bleaching, where pesticides act in concert with photosystem I to produce free radicals (Duke 1990). The majority of herbicides, including triazines, anilides, ureas, phenyl, and carbamates (Table 2) act by blocking electron transport through disruption of the Hill reaction in photosystem II (Duke 1990).

As might be expected, herbicides have almost exclusively negative direct effects on algal species, as well as protozoa and fungi (Table 3, Figure 1). However, direct effects of herbicides on bacteria tend to vary depending on the group of herbicide used (Table 3, Figure 1). When considering effects by pesticide rather than by study (as many studies used more than one pesticide), 94% of herbicides (of 35 herbicides) had negative direct effects, while 6% produced no effects on algal and cyanobacterial species (Table 4). Fifty percent (of four herbicides) had negative effects on protozoa, while the other 50% had no direct effects (Table 4). One hundred percent of herbicides (of four herbicides) had direct negative effects on aquatic fungi (Table 4). Positive direct effects of herbicides on bacterial species were reported for 13% of herbicides (of

15 reviewed here), negative effects were reported for 47% of herbicides, and no effects were reported for 40% (Table 4).

Direct effects of herbicides on algal species have generally been negative, both at and about environmentally relevant concentrations (Table 5). At environmentally relevant concentrations, the bipyridinium herbicide diquat was generally more toxic to cyanobacteria and golden algae (EC_{50} of 22–46 μg L^{-1}) than green algae (EC_{50} of 19–395 μg L^{-1}) (Campbell et al. 2000). Inhibition of carbon uptake by 99–100% was also observed in diatom and cyanobacteria species when exposed to diquat at 700 μg L^{-1} (Peterson et al. 1994). The aldehyde herbicide acrolein, as well as the substituted urea herbicide tebuthiuron (at 1000 μg L^{-1} and 5900 μg L^{-1}, respectively), also inhibited carbon uptake by nine algal species, although these concentrations exceeded environmental relevance (Peterson et al. 1994). In excess of what would be expected in the environment (10 000 μg L^{-1}), paraquat significantly reduced cyanobacterial respiration (Kosinski 1984).

Phenylurea herbicides also have toxic effects on algal species (Table 5), with diuron being the most toxic of the group, followed by monuron, neburon, and fenuron (Ukeles 1962). Diuron produced lethal effects on marine algae at a concentration of 4 μg L^{-1} (Ukeles 1962), and *C. fusca* reproduction was inhibited by chlortoluron at 23 μg L^{-1} (Faust et al. 1994). Diuron also had an EC_{50} between 16 and 33 nM for estuarine microalgae (Magnusson et al. 2008). Peterson et al. (1994) found that the glycine derivative glyphosate had an EC_{50} of 2848 μg L^{-1} (exceeding environmentally expected concentrations) for cyanobacterial and green algal species. Glyphosate also had an EC_{50} ranging from 8900 to 89 000 μg L^{-1} (also exceeding environmental relevance) for different freshwater periphyton communities and significantly reduced carbon uptake in diatoms and cyanobacteria (Goldsborough and Brown 1988). The acetanilide herbicide metachlor also inhibited carbon uptake in green algae, and both metachlor (at 3000 μg L^{-1}) and the imidazolinone herbicide imazethapyr (at 67 μg L^{-1}) reduced carbon uptake in cyanobacteria (Peterson et al. 1994).

Multiple triazine herbicides were tested at and above expected environmental concentrations and they resulted in decreased carbon uptake in green algae, diatoms, and cyanobacteria (Peterson et al. 1994). Simazine disrupted reproduction of *C. fusca* at a concentration of 73 μg L^{-1}, respectively (Ukeles 1962). Low concentrations of atrazine, that is, 0.12–5.8 μg L^{-1}, resulted in increased amino acid production, lower pH, and lower chlorophyll production in marine phytoplankton (Bester et al. 1995). Additionally, and within the range of environmental relevance, atrazine had an EC_{50} ranging from 90 to 3000 μg L^{-1} for certain species of algae (Fairchild et al. 2009). Atrazine also had an EC_{50} ranging from 120 to 630 nM for two species of estuarine microalgae (Magnusson et al. 2008). Atrazine was also toxic to green algae and cyanobacteria at 1000 μg L^{-1} (Butler et al. 1975).

Although most herbicides produce negative direct effects on algal species, the effects of specific chemicals, and the concentrations required to achieve toxicity can be particularly species dependent. For example, Butler et al. (1975) found that 2,4-D (at 4000 μg L^{-1}) was toxic to planktonic algae, but that no inhibitory effect was observed at lower concentrations. Similarly, in a study of nine algal species, while a majority of herbicides examined did have adverse direct effects (as stated above), no inhibition of algal growth was observed with exposure to 2,4-D at a lower concentration of 2917 μg L^{-1} (Peterson et al. 1994). Additionally, no inhibition of chlorophyll production was noted with 2,4-D at concentrations ranging from 1 to 100 000 μg L^{-1} (Torres and O'Flaherty 1976), although it should be noted that the measurement of chlorophyll production measured in this study differed from measurements of growth in the previous two studies. Lastly, picloram (1760 μg L^{-1}), while found to inhibit growth of most cyanobacterial species, actually promoted growth of four algal species tested (Peterson et al. 1994); while this supports the idea of species-specific differences regarding herbicide effects, it should also be noted that this is the only study reviewed here to test this chemical.

In contrast to algal species, fewer studies have examined the direct effects of herbicides on heterotrophic bacterial and protozoan species, although direct effects were negative for all but triazine herbicide, both at and above environmentally relevant concentrations(Tables 6 and 8). At environmentally relevant concentrations, coliform growth was reduced when exposed to 2,4,-D at concentrations of 5–10 mM (Higgins and Hohn 2008). Additionally, although exceeding environmentally relevant concentrations, another study found a significant reduction in bacterial growth rates when 25 000–50 000 μg L^{-1} of diquat or paraquat was present (Breazeale and Camper 1972). Glyphosate, at a concentration of 500 μg L^{-1}, reduced the growth of heterotrophic bacteria (Pai et al. 2009). Although potentially a result of indirect effects, the bipyridinium herbicide diquat, at concentrations ranging from 300 to 30 000 μg L^{-1}, decreased the abundance of freshwater algal and bacterial species and protozoan species richness (Melendez et al. 1993). At concentrations exceeding environmental relevance, phenoxyalkane herbicides 2,4-D and 2,4,5-T had EC_{50} values for *V. fisheri* of 100 700 and 51 700 μg L^{-1}, respectively; while their metabolites had much lower EC_{50} values of 5000 and 1800 μg L^{-1}, respectively. These results suggest that metabolites of these herbicides are more toxic than their parent compounds (Somasundaram et al. 1990). Diquat had an EC_{50} value of 2940 μg L^{-1} for *E. gracilis* (Campbell et al. 2000). Phenlyurea herbicides diuron and chlortoluron also had EC_{50} values of 68 200 and 298 500 μg L^{-1}, respectively, for *V. fisherii* and 10 700 and 8200 μg L^{-1}, respectively for *T. pyriformis* (Nelieu et al. 2010). Linuron exposures of 11 300 000 and 33 900 000 μg L^{-1} for 96 h caused a 100–1000-fold increase in the abundance of *E. coli* O157:H7, *L. monocytogenes, Salmonella* sp., and *Shigella* sp.; however, glyphosate and paraquat resulted in significant decreases of the same bacterial species after one hour (Guan et al. 2001).

In contrast to most other herbicide groups, triazine herbicides have tended to produce neutral effects on bacterial and protozoan species (Tables 6 and 8). However, Higgins found no effect of atrazine (0.25–22.5 μg L^{-1}) or simazine (0.000025–0.0025 μg L^{-1}) on coliform growth (Higgins and Hohn 2008). Similarly, Breazeale and Camper (1972) also found no significant effect of atrazine on bacterial growth at 25 000 and 50 000 μg L^{-1}. At concentrations of 102 and 204 μg L^{-1}, atrazine had no effect on *E. coli*, *Ent. faecalis*, *S. enterica* Typhimurium, or *E. coli* O157:H7 growth or sur-

vival in simplified microcosms (Staley et al. 2012). The lack of effects of both atrazine and its metabolite deethylatrazine on both bacterial and protozoan abundance have also been reported (DeLorenzo et al. 1999b). A singular study found atrazine concentrations ranging from 0.1 to 100 μg L^{-1} allowed for the growth *E. coli* and *Ent. faecalis* populations; however, in this study, no pesticide-free control was used for comparison (Koutsotoli et al. 2005).

Herbicides have also had negative effects on aquatic fungi at and above environmentally relevant concentrations (Table 7). Glyphosate at 500 μg L^{-1} significantly decreased zoosporangia concentration and zoospore production of the pathogenic fungi *B. dendrobatidis* (Hanlon and Parris 2012). Atrazine also reduced growth of *B. dendrobatidis* (0.0106–106 μg L^{-1}) both in culture and on infected tadpoles (McMahon et al. 2013; Rohr et al. 2013). The bipyridylium herbicide diquat (at concentrations between 0.2 and 10 μg L^{-1}) inhibited the growth of *Helicoon elegans*, *Pseudoaergerita matsushimae*, *Hormiactus ontariensis*, and *Beverwykella pulmonaria* to varying degrees among the species (Fronda and Kendrick 1985). Although exceeding environmentally relevant concentrations, glyphosate (at 50 000–500 000 μg L^{-1}) inhibited biomass production in *Annulatascus velatisporus, Composporium antennatum, Helicosporium griseum,* and *Massarina* sp., though the degree of inhibition varied among these species (Tsui et al. 2001).

Community-level effects of herbicides

Atrazine is one of the most widely used pesticides in studies examining community-level effects. Multiple studies have been conducted detailing the effects of atrazine exposure on phytoplankton, revealing that negative effects are dependent on atrazine concentration, length of exposure, and species of phytoplankton (Huber 1993; Solomon et al. 1996). In contrast, several studies have shown that periphyton growth can be indirectly stimulated with ecologically relevant concentrations of atrazine (Brock et al. 2000; Herman et al. 1986; Pratt et al. 1988; Rohr et al. 2012; Rohr et al. 2008a,b; Staley et al. 2011). Atrazine toxicity has also been demonstrated toward macrophytes, benthic invertebrates, zooplankton, fish, and amphibian species (Rohr and McCoy 2010; Solomon et al. 1996). Atrazine application can therefore result in profound shifts across multiple trophic levels, resulting in a myriad of potential indirect effects on algae, bacteria, and protozoa, depending on the complexity of the system.

In several studies, atrazine exposure has had an indirect effect mediated via a reduction in competition among phototrophs. Atrazine affected experimental pond communities at concentrations as low as 1–5 μg L^{-1} and as high as 500 μg L^{-1}, reducing green algae and flagellate abundance while increasing cryptomonads and golden algae (de Noyelles et al. 1982). Another study by Hamala and Kolliq (1985) found that a concentration of 100 μg L^{-1} of atrazine reduced cryptomonads and diatoms but increased cyanobacteria (Hamala and Kolliq 1985). Additionally, studies have observed algal-mediated indirect effects of atrazine, often resulting in the increase of bacterial and protozoan abundance. In the sediments of complex freshwater mesocosms, the addition of atrazine at 102 or 204 μg L^{-1} increased abundance of *E. coli* and *Enterococcus* spp. (Staley et al. 2010) compared with controls. Further, in simplified microcosms where algal populations were allowed to establish, atrazine application at 102 μg L^{-1} resulted in decreases in both phytoplankton and *E. coli* abundance in the water column, but increases in *E. coli* abundance in the sediments (Staley et al. 2011). A reduction in chlorophyll coupled with increases in cyanobacteria and bacterial abundance was reported with atrazine concentrations ranging from 40 to 160 μg L^{-1}, although bacterial abundance decreased over 45 h (DeLorenzo et al. 1999a). In the same study, atrazine had no significant effect on the abundance of small ciliates, although large ciliates and small flagellates increased, while large flagellate abundance declined (DeLorenzo et al. 1999a). At concentrations of 20 and 200 μg L^{-1}, green and golden algae decreased, while increasing the relative abundance of diatoms and heterotrophic protozoa (Downing et al. 2004).

Further, atrazine tends to stimulate heterotrophic microbial activity in community settings. In water samples collected from a natural lake, an increase in heterotrophic bacterial abundance with an atrazine concentration of 50 000 μg L^{-1} was observed (Lopez et al. 2006). Similarly, Hamala and Kolliq (1985) found that a concentration of 100 μg L^{-1} increased heterotrophic activity (Hamala and Kolliq 1985). Stimulation of heterotrophic bacteria was also observed for atrazine concentrations ranging from 3 to 100 μg L^{-1}, although at concentrations ranging from 100 to 300 μg L^{-1}, primary production was practically eliminated in laboratory experiments (Pratt et al. 1997). In a study utilizing freshwater microcosms to assess the impact of atrazine (at 102 μg L^{-1}) on competition and predation, no effect was observed on autochthonous protozoan predators; however, inoculated strains of *Ent. faecalis* and *E. coli* O157:H7 remained significantly elevated, likely as a result of atrazine reducing autochthonous (naturally present) competitors (Staley et al. 2014).

Community-level effects on biofilm algae and bacteria have also been studied using the substituted urea herbicide diuron. At environmentally relevant concentrations ranging from 0.07 to 7 μg L^{-1}, diuron reduced diatom abundance and photosynthetic efficiency (Ricart et al. 2009). Unlike many of the community-level studies using atrazine, however, diuron also reduced bacterial abundance. By contrast, another study using slightly higher concentrations of diuron (10 μg L^{-1}) did find an increase in bacterial abundance, productivity, and diversity; the mechanism for this was hypothesized as being due to phototrophic lysis and an increase in the amount of organic matter released as a result of diuron exposure (Pesce et al. 2006).

Herbicide summary

The studies reviewed here have demonstrated population-level direct effects of herbicides on algal and fungal taxa that were significantly negative ($P < 0.001$ and $P = 0.018$, respectively; Table 4). Again, we encourage interpreting the fungal results with caution because of low sample sizes in this group. However, the community-level, indirect effects of herbicides, particularly atrazine, make predictions of the effects of herbicides difficult in natural environments. While direct effects of herbicides on individual algal species are almost exclusively nega-

tive, the indirect effects, mediated via a reduction of competition, tend to favor certain phototrophic taxa at the community level (i.e., decreasing diatoms, but increasing cyanobacteria). In contrast to algal and fungal taxa, we found no significant tendency toward positive, neutral, or negative direct effects of herbicides on bacterial or protozoal species ($P = 0.247$ and 0.368, respectively; Table 4). Although the majority of studies assessing direct effects of herbicides on bacterial and protozoan populations have found negative effects (with the exception of triazine herbicides), at the community level, a decline in phototrophs has generally been associated with an increase in heterotrophic bacterial and protozoan populations (Pesce et al. 2006; Pratt et al. 1997). As is the case with insecticides, these studies suggest that indirect positive effects of herbicides, particularly on heterotrophic taxa, may outweigh direct negative effects. Given the relative paucity of community-level studies compared with those examining direct effects, further research involving multiple trophic levels is needed to be able to make accurate predictions of how herbicides will affect impacted aquatic environments.

Fungicides

Fungicides, like herbicides, have a variety of different potential modes of action (Table 2), with many tending to function as general biocides (Maltby et al. 2009). Aromatic hydrocarbon fungicides, triazoles, and hydrochlorines are generally deleterious to membrane synthesis, acting to block either lipid or sterol biosynthesis or inhibiting the biosynthesis of intracellular membrane components (Yang et al. 2011). Fungicides (e.g., pyrimidinamines, pyridine carboxamides, and benzamides; Table 2) may also act by diminishing cellular respiration, either by disrupting oxidative phosphorylation or specifically targeting NADH oxidoreductase or succinate dehydrogenase (Yang et al. 2011). Further, phenylpyrrole and dicarboximide fungicides act by blocking signal transduction (Yang et al. 2011).

Relative to herbicides and insecticides, few studies have investigated the direct effects of fungicides on algal species; however, at environmentally relevant concentrations, the direct effects have been consistently negative (Table 5, Figure 1). A triazole derivative of propiconazole (83 μg L^{-1}) inhibited carbon uptake by as much as 31% in the green algae *S. quadricauda* and cyanobacterium *Microcystis* (Peterson et al. 1994). Harris, White et al. (1970) found that organomercury fungicides at concentrations of less than 1 μg L^{-1} reduced growth and photosynthesis in phytoplankton and diatom species. The triazine fungicide anilazine (1390 μg L^{-1}) and the imidazole fungicide prochloraz (24 μg L^{-1}) were also toxic to *C. fusca* (Faust et al. 1994).

Direct negative effects of fungicides on heterotrophic protozoan species have also been consistently negative (Figure 1), although these studies utilized fungicide concentrations exceeding environmentally relevant concentrations (Table 6). Fungicides have been found to be harmful to ciliate protozoa, specifically *C. campylum* (Dive et al. 1980). At concentrations ranging from 50 to 10 000 μg L^{-1}, thiocarbamate fungicides nabam and vapam were found to inhibit growth of the flagellate protozoan *E. gracilis* (Moore 1970). While all the studies listed provide evidence of direct negative effects of fungicides on algal and protozoan species, it should be noted that these studies represent only a small variety of fungicides on a limited sampling of algal and protozoan species.

Relatively few studies have investigated the effects of fungicides on aquatic fungal species; however, those studies which have been conducted have, unsurprisingly, found negative effects (Table 7, Figure 1). The imidazole fungicide, imazalil, reduced the sporulation of *Heliscella stellata, Lunulospora curvula,* and other sigmoid type-2 aquatic fungi at concentrations ranging between 0.1 and 100 μg L^{-1} (Flores et al. 2014). Imazalil also had a EC_{100} on aquatic fungi at concentrations ranging from 10 000 to 210 000 μg L^{-1} (Dijksterhuis et al. 2011). The fungicide chlorothalonil (at 0.0176–1.76 and 32–176 μg L^{-1}) reduced the growth of *B. dendrobatidis* in culture and on infected tadpoles (McMahon et al. 2013). Chlorothalonil was also toxic to aquatic fungi with an EC_{100} at concentrations exceeding 260 μg L^{-1} (Dijksterhuis et al. 2011). Also, although exceeding environmentally relevant concentrations, the phosphanoglycine fungicide, thiophanate methyl, significantly decreased the zoosporangia number of *B. dendrobatidis* at 1500 μg L^{-1} (Hanlon and Parris 2012).

Similar to herbicides, the direct effects of fungicides on bacterial species tend to be negative, but can be highly variable among specific pesticides and dependent on the concentration used (Table 8). Acriflavine had dose-dependent bactericidal effects on *S. aureus* with concentrations as low as 1000 μg L^{-1} (Kawai and Yamagishi 2009). At concentrations that exceed environmentally relevant concentrations, captan, thiram, fenpropimorph, and propiconazole, with EC_{50} values ranging from 3000 to 70 000 μg L^{-1}, produced toxic effects, measured via a decline in leucine incorporation and denitrification of a bacterial community in a constructed wetland (Milenkovski et al. 2010). Thiram (13 400 000 μg L^{-1} in lab cultures) also significantly reduced *Salmonella* spp., *Shigella* spp., *L. monocytogenes,* and *E. coli* O157:H7 densities relative to controls, although this result was likely due to the accompanying shift in pH to 9.21 (Guan et al. 2001). In the same study, the abundance of *E. coli* O157:H7, *Salmonella* sp., *Shigella* sp., and *L. monocytogenes* increased by 100–1000-fold when exposed to commercially available chlorothalonil at concentrations of 10 000 and 30 000 μg/L over 96 h (Guan et al. 2001). However, at environmentally relevant concentrations, chlorothalonil at 170 or 340 μg L^{-1} had no effect on abundance or growth rate of *E. coli, Ent. faecalis, E. coli* O157:H7, or *Sal. enterica* Typhimurium in simplified microcosms (Staley et al. 2012). That these studies found conflicting effects of chlorothalonil is likely because of the use of different matrices (laboratory cultures vs. microcosms) and highly different concentrations of chlorothalonil. Lastly, dichloran, at a concentration of 0.14 μg L^{-1}, had almost no effects on *Sal. enterica* Typhimurium, contributing less than 0.1% to mutagenic activity (de Oliviera et al. 2009). While the direct effects of fungicides on bacterial species tend to be negative, these results seem to be highly dependent on the specific fungicide and concentration used; further, the relative lack of studies makes any generalization of the effects of fungicides on bacterial species cautionable.

Community-level effects of fungicides

Community-level effects of fungicides have not been extensively investigated. In the sediments of complex freshwater mesocosms, chlorothalonil had no significant effect on sediment *E. coli* or *Enterococcus* spp. levels at concentrations of 170 or 340 μg L^{-1} (Staley et al. 2010). However, in a study by Downing, DeLorenzo et al. 20 μg L^{-1} of chlorothalonil was found to stimulate heterotrophic bacterial activity, increase diatoms and chlorophytes, and reduce golden algae and heterotrophic protozoa (Downing et al. 2004). Similarly, captan, applied at 50 000 μg L^{-1}, increased heterotrophic bacterial abundance in a lake ecosystem (Lopez et al. 2006). In freshwater microcosms, 170 μg L^{-1} chlorothalonil reduced autochthonous protozoan densities as effectively as cycloheximide (a known inhibitor of protein synthesis in eukaryotes), providing an indirect positive effect on bacterial densities (Staley et al. 2014). Utilizing complex (i.e., containing multiple trophic levels) freshwater mesocosms, chlorothalonil (at concentrations of 164 and 328 μg L^{-1}) was found to have ecosystem-level effects, increasing mortality to zooplankton and algal species (as well as amphibians and gastropods), while decreasing decomposition and water clarity and elevating dissolved oxygen and primary productivity (McMahon et al. 2012). Also utilizing complex freshwater mesocosms, chlorothalonil (at 164 μg L^{-1}) increased chlorophyll *a* production in phytoplankton and periphyton, and decreased leaf litter decomposition and the abundance of herbivorous species (Halstead et al. 2014). Based upon these studies in community settings, fungicides have a more pronounced effect on higher trophic levels, thereby mediating increases in algal and bacterial abundance, which is similar to the effects of many insecticides. An additional possibility is that certain bacterial strains are capable of metabolizing fungicides or utilizing organic material released from dying fungal species. While no studies reviewed here have specifically examined that possibility, additional nutrients from either source could facilitate bacterial proliferation.

Fungicide summary

While the number of studies considering the effects of fungicides on microorganisms has been relatively few, most fungicides seem to exhibit generally biocidal effects. A χ^2 test revealed a significantly greater occurrence of negative direct effects for algal, bacterial, and fungal taxa ($P = 0.018$, 0.018, and 0.007, respectively) and a nearly significant number for protozoal taxa ($P = 0.05$; although 100% of studies reviewed here found negative direct effects). Similar to other pesticide categories, however, the indirect, community-level effects of fungicides seem to target higher trophic levels, resulting in increases of lower trophic levels, such as algal and bacterial species.

Pesticide Summary

Based upon the studies reviewed, the direct effects of all pesticide types produced, on average, negative effects for algal, protozoal, fungal, and bacterial species (Figures 1 and 2; all averages are negative). Importantly, there was a significant pesticide type-by-taxon interaction ($\chi^2_1 = 7.11$, $P = 0.007$), indicating that the effect of a given pesticide type was dependent on the taxon examined (Figure 2). This is not surprising given that pesticides are designed to target particular taxa, such as herbicides being designed to target photosynthetic organisms. This interaction seemed to be most strongly driven by the fact that all three pesticide types seemed to have similar adverse effects on algae and fungi, but protozoans were much more strongly affected by insecticides and fungicides than herbicides (Figures 1 and 2). Interestingly, there was also a significant main effect of pesticide type ($\chi^2_1 = 6.04$, $P = 0.048$) because, on average, fungicides seemed to be most deadly to microbes and herbicides least deadly to microbes (Figures 1 and 2). There was also a significant main effect of taxon ($\chi^2_1 = 16.31$, $P < 0.001$) because, on average, fungi and algae seemed to be most sensitive to pesticides, and bacteria were least sensitive to pesticides (Figures 1 and 2).

While, on average, direct effects of all pesticide categories on all microbial taxa are negative, these direct negative effects tend to be offset when studies investigated community-level, indirect effects. In community settings, both insecticides and fungicides had a tendency to reduce heterotrophic predators, resulting in increases in the abundance of lower trophic levels, particularly algal and bacterial species. Community-level effects of herbicides have been mostly limited to the specific herbicide atrazine (although with several exceptions) making generalization of the indirect effects of herbicides more difficult. However, the studies available do reveal a tendency for the reduction of phototrophs, increasing the abundance of heterotrophic taxa, mostly bacterial and protozoan species. Additionally, increases in bacterial abundance in the presence of any pesticide might also result from increased nutrient sources, either from direct metabolism of pesticides or from released organic material from species experiencing lethal or lytic effects.

Ecological relevance and areas for further research

Based upon the studies presented in this review and the ubiquity of pesticides in water bodies in the United States, EU, and throughout the world, it is likely that most aquatic ecosystems are in some way affected by the presence of pesticides. Understanding these effects is important to safeguard both aquatic

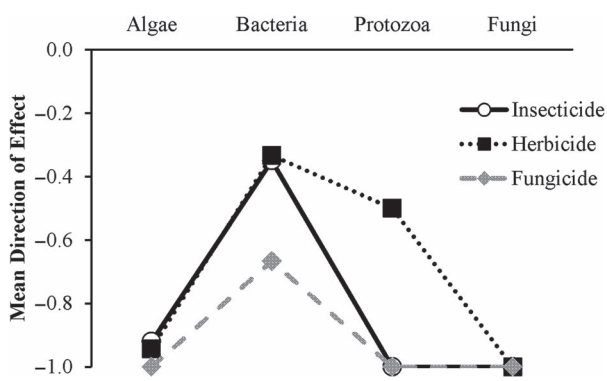

Figure 2. Interaction between pesticide type (insecticide, herbicide, or fungicide) and taxon (algae, bacteria, protozoa, or fungi) on microbial densities (+1 for significant increases in densities, 0 for null effects, and-1 for significant decreases in densities).

and human health. Multiple studies have revealed the capacity of microbes to concentrate pesticides, serving as vectors for pesticide delivery to higher trophic levels (Cooley et al. 2007; Gregory et al. 1969; Jo et al. 2011; Lal et al. 1987). Furthermore, studies have revealed that direct effects of pesticides on one trophic level may indirectly affect the abundance of species at higher or lower trophic levels (Foit et al. 2010; Relyea 2009; Staley et al. 2011).

Understanding the ecological effects of pesticides and potentially different responses among bacterial and protozoan species may also have implications for microbial regulatory criteria and human health. Presently, microbial water quality is assessed by quantifying the abundance of fecal indicator bacteria (FIB; i.e., *E. coli* and enterococci) in contaminated water bodies (U. S. Environmental Protection Agency 2002). Epidemiological studies have shown that elevated concentrations of FIB have been correlated with increased risk of contracting gastrointestinal disease (Colford et al. 2007; Wade et al. 2008; Wade et al. 2003). However, different survival patterns among FIB (*E. coli* and enterococci) have been observed (Korajkic et al. 2013a). Further, not all microbial, protozoan, or viral pathogens exhibit the same persistence in secondary environments as do FIB (Jenkins et al. 2011; Korajkic et al. 2013b; Staley et al. 2014; Wanjugi and Harwood 2012). As pesticides might have different effects on different bacterial and protozoan species, it is increasingly important to understand whether FIB are affected identically to the pathogens they are used to predict or whether the ubiquity of pesticides in contaminated water bodies is confounding regulatory measures.

Of the studies reviewed, predicting pesticide effects on bacterial species seems to be the most problematic. The general trend of direct negative effects on algae, protozoa, and aquatic fungi has emerged for many pesticides. However, the direct effects of pesticides on bacterial species are less easily generalized. For any given pesticide, studies have tended to show contrasting or null effects depending on the bacterial species and concentration and specific pesticide examined, the duration of exposure, the presence of sediments and humic materials, and environmental factors (e.g., pH and DO). Given the broad diversity of physiological strategies utilized by bacteria, it is perhaps not surprising that we were unable to draw any general conclusions regarding how different classes of pesticides affect bacteria.

However, understanding direct effects of pesticides on any microorganism may be less important than understanding the indirect effects that occur when microbial populations interact in an ecosystem. Collectively, the studies reviewed here show that a particular direct effect of a given pesticide observed in a simplified study may not manifest in a more complex system that includes various taxa and trophic levels. In many cases, a direct negative effect on a target species has been found to be offset at the community level, suggesting that indirect effects have a strong influence on the net effect (direct + indirect effects) of a pesticide. However, despite the importance of indirect effects, few studies have examined indirect and/or net effects of pesticides at the community level (DeLorenzo et al. 1999a; DeLorenzo et al. 1999b; Downing et al. 2008; Downing et al. 2004; Lopez et al. 2006; Staley et al. 2010; Staley et al. 2011; Staley et al. 2014). Nevertheless, the handful of studies that have examined the effects of pesticides on microbial communities suggest that there is trophic downgrading, a pattern that seems to be occurring globally as a result of various stressors (Estes et al. 2011). The more rapid recovery of lower trophic levels coupled with the greater sensitivity of higher trophic levels to many pesticides, such as insecticides and fungicides, seems to be driving this trophic downgrading.

As pesticide use intensifies, the impact of pesticide residues on aquatic ecosystems is likely to increase. Given the importance of microbial activities in these environments, the ability to accurately predict the effects of pesticides is essential to safeguarding ecosystems. Further, understanding how pesticide residues influence the fate of both pathogenic and regulatory associated microbes, particularly bacteria, is important for the protection of human health. Despite the urgent need to understand how pesticides influence different trophic levels and impact the ecosystem as a whole, most studies have focused on direct effects, utilizing *in vitro* studies. To better understand and more accurately predict the impact of pesticides in real-world aquatic environments, more systematic research is needed at the community-level impacts with a focus on determining the net effects (direct and indirect effects) of pesticides on microorganisms.

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