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Pesticides alter ecosystem respiration via phytoplankton abundance and community structure: Effects on the carbon cycle?

Samantha L. Rumschlag[1] | Dale A. Casamatta[2] | Michael B. Mahon[1] | Jason T. Hoverman[3] | Thomas R. Raffel[4] | Hunter J. Carrick[5] | Peter J. Hudson[6] | Jason R. Rohr[1]

[1]Department of Biological Sciences, Eck Institute for Global Health, Environmental Change Initiative, University of Notre Dame, Notre Dame, Indiana, USA

[2]Department of Biology, University of North Florida, Jacksonville, Florida, USA

[3]Department of Forestry and Natural Resources, Purdue University, West Lafayette, Indiana, USA

[4]Department of Biological Sciences, Oakland University, Rochester, Michigan, USA

[5]Department of Biology, Institute for Great Lakes Research, Central Michigan University, Mount Pleasant, Michigan, USA

[6]Department of Biology, Pennsylvania State University, State College, Pennsylvania, USA

Correspondence

Samantha L. Rumschlag, Department of Biological Sciences, Eck Institute for Global Health, Environmental Change Initiative, University of Notre Dame, Notre Dame, IN, USA.
Email: srumschl@nd.edu

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Abstract

Freshwater systems are critical to life on earth, yet they are threatened by the increasing rate of synthetic chemical pollution. Current predictions of the effects of synthetic chemicals on freshwater ecosystems are hampered by the sheer number of chemical contaminants entering aquatic systems, the diversity of organisms inhabiting these systems, the myriad possible direct and indirect effects resulting from these combinations, and uncertainties concerning how contaminants might alter ecosystem metabolism via changes in biodiversity. To address these knowledge gaps, we conducted a mesocosm experiment that elucidated the responses of ponds composed of phytoplankton and zooplankton to standardized concentrations of 12 pesticides, nested within four pesticide classes, and two pesticide types. We show that the effects of the pesticides on algae were consistent within herbicides and insecticides and that responses of over 70 phytoplankton species and genera were consistent within broad taxonomic groups. Insecticides generated top-down effects on phytoplankton community composition and abundance, which were associated with persistent increases in ecosystem respiration. Insecticides had direct toxic effects on cladocerans, which led to competitive release of copepods. These changes in the zooplankton community led to a decrease in green algae and a modest increase in diatoms. Herbicides did not change phytoplankton composition but reduced total phytoplankton abundance. This reduction in phytoplankton led to short-term decreases in ecosystem respiration. Given that ponds release atmospheric carbon and that worldwide pesticide pollution continues to increase exponentially, scientists and policy makers should pay more attention to the ways pesticides alter the carbon cycle in ponds via changes in communities, as demonstrated by our results. Our results show that these predictions can be simplified by grouping pesticides into types and species into functional groups. Adopting this approach provides an opportunity to improve the efficiency of risk assessment and mitigation responses to global change.

KEYWORDS

aquatic ecology, carbon cycle, disturbance, ecosystem respiration, pesticides, phytoplankton, synthetic chemicals, zooplankton

1 | INTRODUCTION

The importance of aquatic systems to life on earth cannot be overstated. Humanity relies on freshwater as a precious natural resource for drinking, food production, and carbon capture (Meybeck, 2003; Vörösmarty et al., 2005; World Water Assessment Programme, 2009). Freshwater systems are hotspots for biodiversity (Balian et al., 2008; Dudgeon et al., 2006), and they play a crucial role in the capture, storage, and release of atmospheric carbon (Holgerson & Raymond, 2016). Yet, the benefits of economic productivity, spurred by access to water, have been accompanied by impairments that threaten aquatic systems (Vörösmarty et al., 2010). In particular, the increasing rate and toxicity of synthetic chemical pollution, which outpaces many other global change drivers, presents a major threat to freshwater systems globally (Bernhardt et al., 2017; Schulz et al., 2021). In the United States alone, more than 500 million pounds of pesticide active ingredients are applied every year (Atwood & Paisley-Jones, 2017). These applications have led to well-documented and widespread contamination of freshwater (Dudgeon et al., 2006; Gilliom et al., 2006; Stone et al., 2014) and are implicated as a contributing factor to biodiversity declines in these systems (Malaj et al., 2014; Stehle & Schulz, 2015).

Despite the magnitude of the threat that pesticide pollution imposes on freshwater systems, funding agencies have largely ignored the intricacies that these disturbances can yield on communities and ecosystems (Bernhardt et al., 2017; Burton et al., 2017). Pesticides have the potential to alter carbon cycling in freshwater systems via changes in the abundance or composition of community members (Bundschuh et al., 2021; Halstead et al., 2014; McMahon et al., 2012; Rumschlag et al., 2020). Yet, risk assessment approaches used by many federal agencies are often removed from studying ecosystem-level effects. For instance, classic toxicological, single-species test of model organisms fail to predict complex suite of effects that occur when pesticides enter freshwater systems (Bernhardt et al., 2017; Gessner & Tlili, 2016; Rohr et al., 2016), which can occur through indirect effects of pesticides mediated through species interactions and food web structures (Kidd et al., 2014; Rumschlag et al., 2019). As an alternative, mesocosm studies, which incorporate naturally complex communities (EFSA Panel on Plant Protection Products & their Residues, 2013) allows for the evaluation of whether pesticide exposure can directly or indirectly alter ecosystem functions, including ecosystem metabolism (Bernhardt et al., 2010; Gessner & Tlili, 2016; Halstead et al., 2014).

Predicting the cumulative effects of pesticides on freshwater systems is enormously challenging because of the diverse array of pesticides to which ecosystems are exposed, combined with the diversity of organisms that exist in freshwater systems. In the United States and Europe, tens of thousands of synthetic chemicals are registered for use, and in the United States alone more than 350 pesticides are applied annually in agriculture (Baker & Stone, 2015). Furthermore, freshwater systems are home to about 125,000 described species, even though they only occupy 0.8% of earth's surface (Balian et al., 2008; Dudgeon et al., 2006). Predicting these effects could be simplified if the effects of pesticides are similar within pesticide types (e.g. insecticides and herbicides that are designed to target arthropod and primary producers, respectively) or pesticide classes (i.e. chemical classes of pesticides that share similar chemical structures and molecular targets within a pest). Furthermore, predicting the effects of pesticides could be simplified if organisms that are taxonomically related or share similar functional roles within an ecosystem have similarities in their responses to pesticides, a trend that has been shown in previous toxicological research (Hua & Relyea, 2014; Ippolito et al., 2012; van den Berg et al., 2021). For example, our previous research has shown consistency in the effects of pesticides by class and type on parasite transmission, ecosystem functions, and macroinvertebrate and amphibian communities (Rumschlag et al., 2019, 2020). In addition, organisms that share functional roles in a community have been shown to respond similarly to types and classes of pesticides (Rumschlag et al., 2020). But to date, no study has attempted to evaluate the consistency of responses of phytoplankton community members to pesticide classes and types, which could be important for predicting the effects of pesticides on freshwater systems' ability to process carbon, given the fundamental role algae play in aquatic metabolism. While herbicides might have consistent effects on algae through direct toxicity (Brock et al., 2000), insecticides could also alter the algal community indirectly, through top-down effects on zooplankton (Brock et al., 2000; Wijngaarden et al., 2005).

In the current study, we conducted an outdoor, replicated mesocosm experiment focused on exploring the effects of a diverse array of pesticides on phytoplankton and zooplankton communities and their contributions to ecosystem respiration. Phytoplankton and zooplankton were chosen as focal communities because: both groups have relatively short generation times (e.g., upwards of a day for phytoplankton, Laws, 2013 and weeks for zooplankton, Kalff, 2002) allowing them to establish population dynamics within the duration of this experiment, both groups are taxonomically diverse allowing for the assembly of diverse communities in this experiment, and finally both groups are important contributors to ecosystem metabolism (Kalff, 2002). Algae and macrophytes convert sunlight into biomass via photosynthesis, and zooplankton are the primary consumers of algae, driving population dynamics of algae through top-down control mechanisms. Our objectives were to: (1) determine the consistency in the effects of pesticides by type, class, and individual pesticide on freshwater phytoplankton communities; (2) gauge the consistency of responses of phytoplankton community members to pesticides within five broad taxonomic groups (green algae, diatoms, cryptophytes, cyanobacteria, and euglenoids); (3) evaluate how herbicides and insecticides alter phytoplankton abundance and community composition via direct toxicity and indirect, top-down effects mediated by the zooplankton community; and (4) examine how respiration of aquatic systems is altered by pesticide-induced perturbations to aquatic communities over time.

We proposed four hypotheses. First, we hypothesized that the effects of pesticides would be consistent within pesticide types and classes; pesticides with similar taxonomic targets or similar chemical structures would have similar directional effects on phytoplankton communities. This hypothesis is motivated by the nestedness of the biologic activity of pesticides by class and by type. Pesticides of similar types (e.g., insecticides and herbicides) have similar targets in the environment (e.g. arthropod and primary producers, respectively), so pesticides within types likely have similar directional effects on non-target taxa (e.g. zooplankton and algae, respectively). Classes of pesticides share modes of action, meaning that they target the same biochemical and molecular pathways (e.g., triazine herbicides bind to the QB protein in the photosystem II reaction center blocking photosynthesis), which would drive similarity of observed directional effects within classes on phytoplankton communities. Second, we hypothesized that the responses of phytoplankton to pesticide exposures would be similar within broad taxonomic groups; a trend shown in other studies (Hua & Relyea, 2014; Ippolito et al., 2012; van den Berg et al., 2021). Third, we hypothesized that herbicides would cause direct reductions in abundance of phytoplankton across broad taxonomic groups, while insecticides would increase phytoplankton abundance and alter community composition through top-down effects on the zooplankton community. And finally, we hypothesized that pesticides would induce changes in ecosystem respiration; the duration of these changes would be explained by the environmental persistence of the pesticides and the generation time of the organism to which the pesticide is directly toxic. For instance, we predicted that respiration in communities exposed to herbicides might recover quickly because photosynthetic phytoplankton have short generation times and thus might rebound rapidly from the direct toxicity of herbicides. In contrast, respiration in communities exposed to insecticides might recover more slowly because direct toxicity occurs to longer-lived zooplankton that might have top-down effects on algae. In addition, we predicted that pesticides that persist for short durations of time in the environment would cause short-term disruptions to respiration because as the pesticide degrades, the community would recover more quickly from the initial perturbation relative to more persistent pesticides.

2 | METHODS

2.1 | Aquatic communities and experimental design

A randomized-block experiment was performed at the Russell E. Larsen Agricultural Research Center (Pennsylvania Furnace, PA, USA) using replicated mesocosm ponds. Mesocosms were 1100 L cattle tanks covered with 60% shade cloth lids. The spatial block was distance from a tree line in the mesocosm field. We filled mesocosms with 800 L of water and 300 g mixed hardwood leaves. Mesocosms were inoculated with zooplankton, phytoplankton, and periphyton that were homogenized from four local ponds. Three weeks later, after these additions, pesticides were applied. To mimic the complex food web structure of natural ponds, we also added two snail, three larval anuran, one larval dragonfly, one water bug, one water beetle, one larval salamander, and one backswimmer species to each mesocosm on the day of pesticide applications, just prior to application. More specifically, each mesocosm received 11 *Helisoma* (*Planorbella*) *trivolvis*, 10 *Physa gyrina*, 20 *Hyla versicolor*, 20 *Lithobates palustris*, 20 *Lithobates clamitans*, two *Anax junius*, two *Belostoma flumineum*, five *Hydrochara* sp., three *Ambystoma maculatum*, and six *Nototeca undulata*. Responses of these community members to the established treatments are explored in Rumschlag et al., 2020 and are not a focus of the current study.

We randomly assigned 14 treatments (12 pesticides, two controls) with four replicate mesocosms of each treatment, which resulted in 56 total mesocosms (Figure 1a). The 12 pesticide treatments were nested; treatments included two pesticide types (insecticide and herbicide), two classes within each pesticide type (organophosphate insecticide, carbamate insecticide, chloroacetanilide herbicide, and triazine herbicide), and three different pesticides in each of four classes (Figure 1a). Samples from two mesocosms were not processed for phytoplankton identification because of an error, so all analyses contain only 54 total mesocosms with three replicates for acetochlor and simazine treatments. At the start of the experiment, we applied a single dose of technical grade pesticides at environmentally relevant concentrations to mimic runoff of pesticides into freshwater systems following rainfall. To calculate environmentally relevant concentrations, we used U.S. Environmental Protection Agency's GENEEC v2 software to generate estimated environmental concentrations of pesticides, which has been used in US federal synthetic chemical registration and ecological risk assessment (US EPA Office, 2001). We acquired pesticides from ChemService. Nominal concentrations of pesticides (μg/L) were: 64 chlorpyrifos, 101 malathion, 171 terbufos, 91 aldicarb, 219 carbaryl, 209 carbofuran, 123 acetochlor, 127 alachlor, 105 metolachlor, 102 atrazine, 202 simazine, and 106 propazine. One hour after pesticides were applied, composite water samples were collected from mesocosms with the same pesticide treatment. These samples were shipped on ice to Mississippi State Chemical Laboratory to verify nominal concentrations. Measured concentrations of pesticides (μg/L) were: 60 chlorpyrifos, 105 malathion, 174 terbufos, 84 aldicarb, 203 carbaryl, 227 carbofuran, 139 acetochlor, 113 alachlor, 114 metolachlor, 117 atrazine, 180 simazine, and 129 propazine. The experimental design also included water and solvent (0.0001% acetone) controls (Figure 1a). The experiment ran for 4 weeks, from June to July.

2.2 | Measurement of experimental responses

To characterize the algal community, we collected phytoplankton from the entire water column by inserting an upright PVC pipe, measuring 10 cm in diameter and 60 cm in height in the center of each mesocosm. A 1-l subsample of the volume of water collected with the pipe was preserved with in a 1% solution of Lugol's. Phytoplankton samples were collected in week 4 of the experiment. All samples were analyzed using the Utermöhl technique at 200–400 magnification (Lund et al., 1958). At least 400 natural units (colonies, filaments, and unicells) were enumerated

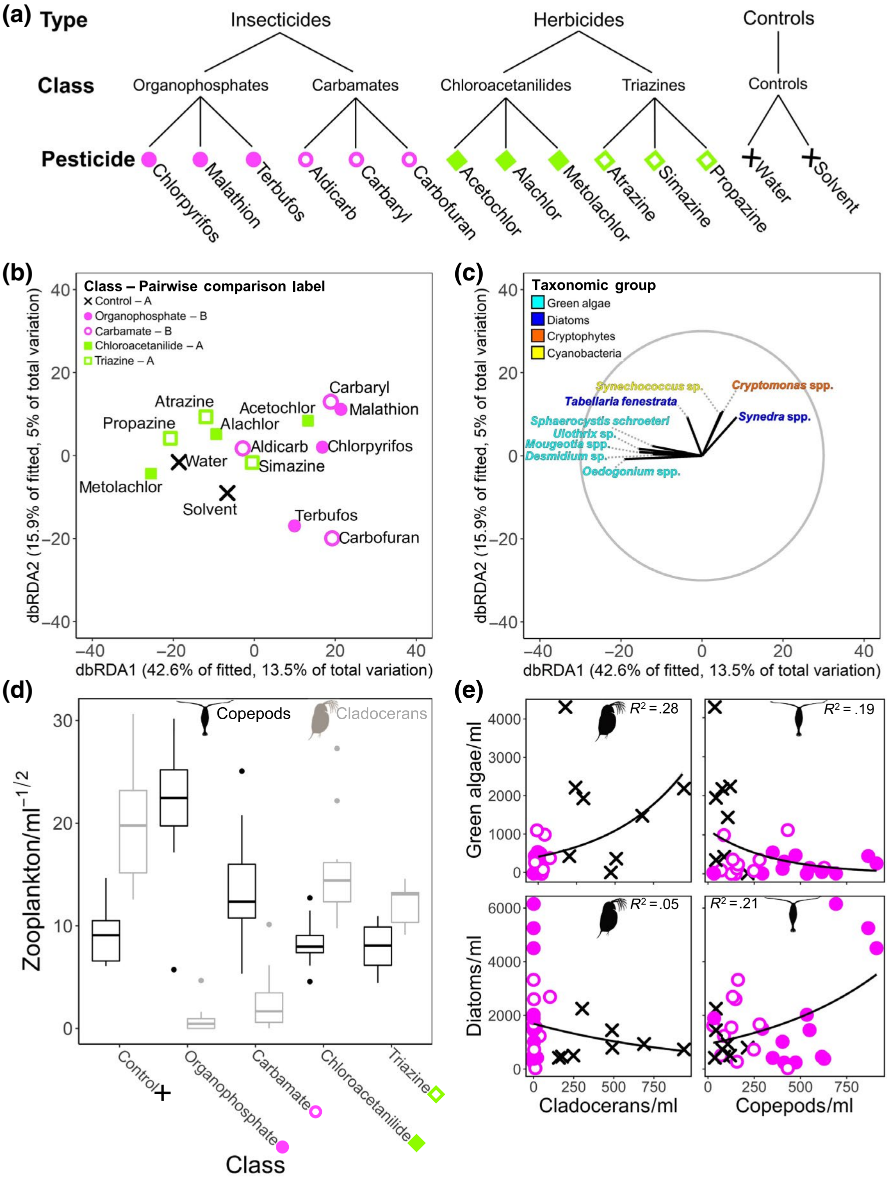

FIGURE 1 (a) Experimental design highlighting the nested structure of pesticide treatments. Each treatment was replicated four times with mesocosm as the replicate. (b) Distance-based redundancy analysis (dbRDA) plot of the phytoplankton community, showing differences among treatments by pesticide type. Points are the centroids of the 12 treatments. Treatments sharing the same letter are not different from each other in pairwise comparisons. (c) Vector overlay of phytoplankton species or genera colored by broad taxonomic group, showing that insecticides were associated with a decrease in green algae and modest increase in diatoms. The gray circle corresponds to vector lengths that would have a correlation coefficient of one with a given axis. (d) Cladoceran and copepod zooplankton densities in response to pesticide classes and the controls. Exposure to insecticides led to copepods becoming more abundant compared to cladocerans. In contrast, herbicides reduced total zooplankton, but the relative amounts of cladocerans and copepods remained similar to the controls. (e) Associations between densities of cladocerans or copepods and green algae or diatoms in insecticide and control treatments. Density of green algae was positively associated cladocerans and negatively associated with copepods, while diatoms were negatively associated with cladocerans and positively associated with copepods. In the plot of green algae and cladocerans, points have been jittered by 30 units in width and 50 units in height for ease of visualization. R^2 values are McFadden pseudo-R^2 values [Colour figure can be viewed at wileyonlinelibrary.com]

using standard keys with taxonomy updated as necessary (Guiry & Guiry, 2021; Prescott, 1962; Wehr & Kociolek, 2015). Densities per milliliter were calculated for a total of 74 genera and species of phytoplankton.

To characterize the zooplankton community, we collected zooplankton from the entire water column with a PVC pipe in the same manner as the phytoplankton samples; then, we capped the bottom and poured the water through 20 µm Nitex mesh. Two zooplankton samples were collected from each mesocosm, and these samples were combined and preserved in 70% ethanol. We counted and identified zooplankton to genera in 5 ml subsamples for each mesocosm with a zooplankton counting wheel (Wildlife Supply Company) and a dissecting microscope. Zooplankton sampling occurred in week 2 of the experiment.

To measure total abundance of phytoplankton, we took 10 ml water samples, filtered phytoplankton onto glass fiber filters (under low vacuum pressure, <10 psi; Whatman EPM 2000, 0.3 μm, 47 mm), and measured chlorophyll-*a* concentrations of each sample. We used an organic extraction procedure with a 50:50 mixture of 90% acetone to DMSO and measured chlorophyll-*a* concentrations using a standard fluorometric technique (Carrick et al., 1993). Chlorophyll-*a* was measured from water samples taken in week 2 of the experiment.

To measure ecosystem respiration, we measured dissolved oxygen (DO) at dusk and dawn on subsequent days using hand-held meters (YSI) during weeks 2 and 4 of the experiment. Respiration was calculated as DO at dusk minus DO at dawn from the following day. Three previous manuscripts, which use the same design as the current manuscript, also describe this experimental design and methods in detail (Rohr et al., 2008; Rumschlag et al., 2019, 2020). Timing of data collection for all endpoints was influenced by logistic constraints.

2.3 | Statistical analyses

To evaluate the consistency of the effects of pesticides within type, class, and individual pesticide on the algal community, we conducted a permutational multivariate analysis of variance (PERMANOVA; Table 1). This statistical model allowed us to attribute the variation explained in each pesticide level of organization (type, class, and individual pesticide), while accounting for the nested structure of our experimental design (Figure 1a). The predictors were the following random categorical terms: type (insecticide, herbicide), class (carbamate, organophosphate, chloroacetanilide, triazine) nested with type, and pesticide (12 total) nested within class within type. No controls were included because they were not hierarchically nested (Figure 1a). The multivariate response was a Bray–Curtis similarity matrix based on a community matrix of square root transformed densities of phytoplankton (abundance per ml) identified to genus or species at the end of the experiment. Across all treatments, 74 genera or species of phytoplankton were identified. The nested PERMANOVA used 9999 permutations and residuals under a reduced model. In addition, we evaluated pairwise differences between controls, carbamates, organophosphates, chloroacetanilides, and triazines with a multivariate comparison test using PERMANOVA (Figure 1b). In this statistical model, 9999 unrestricted permutations of raw data were used. In both the nested and pairwise PERMANOVAs, Type III partial sums of squares were evaluated.

To gauge how consistent the responses of phytoplankton community members are within broad taxonomic groups, we completed a second nested PERMANOVA on the algal community simplified to broad taxonomic groups (Table 1). This second nested PERMANOVA followed the same methods as the first, except that the multivariate response was based on densities of green algae, diatoms, cryptophytes, cyanobacteria, and euglenoids. This community matrix was generated by summing abundances of phytoplankton species and genera within their respective taxonomic groups. To determine how consistent the responses of phytoplankton were to pesticide exposures, we compared the relative amount of variation explained by the predictors and the residual variation in the two PERMANOVA models: the genera/species-level model and the model including the five taxonomic groups (Table 1).

To visualize (1) the consistency of the pesticide effects within type, class, and individual pesticide; (2) the consistency of responses of phytoplankton within broad taxonomic groups; and (3) how pesticides altered community composition of phytoplankton, we used a distance-based redundancy analysis (dbRDA, Figure 1b,c), which is an ordination technique that constrains the community response matrix by environmental variables, in this case the pesticide treatments. The dbRDA was based on Bray–Curtis similarities of the square root transformed densities of the more than 70 genera or species of phytoplankton. The underlying categorical predictors were organophosphate, carbamate, chloroacetanilide, triazine, and control. In the dbRDA plot, we show the centroid values for the 14 experimental treatments. Both PERMANOVA models and the dbRDA were first executed using PERMANOVA+for PRIMER version 7 (PRIMER-E Ltd, Plymouth, UK). Then, for ease of visualization

TABLE 1 Results of PERMANOVA models evaluating the effects of pesticides on the densities of algal communities. The multivariate response in the first model includes 74 species and genera. The multivariate response in the second model includes five broad taxonomic groups, including diatoms, green algae, cryptophytes, cyanobacteria, and euglenoids. *p* values were generated by Monte Carlo sampling. Variation explained is the proportion of the estimated component of variation for a given predictor relative to the model's total variation

Endpoints and source of variation	df	Pseudo *F*	*p*	Variation explained
Algal community by species and genera				
Type	1	5.08	.001	0.238
Class(Type)	2	0.68	.845	0.000
Pesticide(Class(Type))	8	1.17	.175	0.134
Residual	34			0.628
Algal community by taxonomic group				
Type	1	18.20	.009	0.218
Class(Type)	2	0.12	.971	0.000
Pesticide(Class(Type))	8	1.77	.052	0.242
Residual	34			0.539

of the dbRDA point and vector plots, data from PERMANOVA+for PRIMER were exported, and plots were made using "*ggplot2*" package in R.

Changes in zooplankton communities across controls and pesticide classes were evaluated using box-and-whisker plots (Figure 1d). Namely, we examined the changes in the relative abundance of two major taxonomic groups of zooplankton, cladocerans, and copepods, in response to classes of pesticides and the controls. To evaluate the associations between the densities of zooplankton and phytoplankton groups, we conducted four simple generalized linear regressions with Poisson distributions using the variation produced in the controls and the insecticide treatments (Figure 1e). In these models, the response was either density of green algae or diatoms and the predictors were either cladocerans or copepods. For each model, we evaluated Type II sums of squares, a Wald χ^2 test statistic, and a McFadden's pseudo-R^2.

To explore how exposure to pesticides altered abundance of broad taxonomic groups of phytoplankton, we examined box-and-whisker plots of densities of broad taxonomic groups of phytoplankton in response to classes of pesticides and the controls (Figure 2a). In addition, we examined box-and-whisker plots of chlorophyll-*a*, a metric of total phytoplankton abundance (Figure 2b). Since chlorophyll-*a* was measured soon after pesticide applications, we argue that chlorophyll-*a* measurements better reflect the immediate impacts of treatments on total abundance of phytoplankton compared to total phytoplankton density calculated from physical counts, which were measured at the end of the experiment.

To examine how respiration of aquatic systems is altered by pesticides over time, we compared the effects of pesticides by class to controls on respiration at 2 and 4 weeks using log response ratios (Figure 3a). For each time point, log response ratios of ecosystem respiration for individual mesocosms exposed to pesticides were calculated relative to the average of the control treatments (solvent and water controls) within a given spatial block. These calculations allow us to evaluate the magnitude of the difference in respiration of pesticide-treated mesocosms relative to the controls. We dropped observations in which DO at dawn was equal to DO at dusk, which generated an undefined value for the log response ratio ($n = 2$).

Next, to test how the community composition of phytoplankton was related to respiration measured at 2 weeks, we used non-metric multidimensional scaling (NMDS) ordination of broad taxonomic groups of phytoplankton densities based on Bray–Curtis similarities ("metaMDS" function, vegan package; Figure 3b). Following NMDS ordination, we fit respiration from week 2, using generalized additive models with the "ordisurf" function (vegan package), which is a test of how well the ordination of the phytoplankton community predicts respiration (Figure 3b). Finally, we assessed how the abundance of phytoplankton, as measured by chlorophyll-*a*, was related to respiration at 2 and 4 weeks using simple linear regressions (Figure 3c). For the two models, the response was respiration measured at either 2 or 4 weeks, and the predictor was log-transformed chlorophyll-*a*. We evaluated Type II sums of squares. All analyses and plots, with the exception of the PERMANOVA models, were completed in R version 3.6.1. Preliminary analyses revealed no significant influence of the spatial block, so the spatial block was not included in the analyses.

3 | RESULTS

3.1 | Pesticide types have consistent effects on broad taxonomic groups of phytoplankton

To evaluate the consistency of the effects of pesticides, we conducted a PERMANOVA that allowed us to attribute the variation explained in the phytoplankton community by pesticide type, class, and individual pesticide. For phytoplankton communities composed of more than 70 species and genera, pesticide type explained 24% of the variation in these communities (Table 1), which shows that the effects of pesticides on phytoplankton communities are generalizable to pesticide type.

To gauge the consistency in the responses of phytoplankton community members within broad taxonomic groups, we compared the genera/species-level PERMANOVA model to a model with the phytoplankton community simplified to broad taxonomic groups. Similar to the genera/species-level model, pesticide type explained 22% of the variation in the taxonomic groups (Table 1). Furthermore, the residual variation in the model including broad taxonomic groups of phytoplankton was less than the genera/species-level model (54% vs. 63% of residual variation, respectively, Table 1). Together, these results suggest that pesticides generally have similar effects on community members from the same broad taxonomic groups.

3.2 | Insecticides generate top-down effects on algal community composition and abundance which is associated with persistent increases in ecosystem respiration

We visualized the consistency in the effects of pesticides by type and the consistency in the responses of phytoplankton by broad taxonomic groups using dbRDA (Figure 1b,c). The dbRDA showed that insecticide exposures significantly decreased green algae and modestly increased diatoms (Figure 1b,c). These changes in community composition were likely the result of top-down effects of the zooplankton community. Insecticides had direct toxic effects on cladocerans, which led to a competitive release of copepods (Figure 1d). Across insecticides and controls, density of green algae was positively associated with cladocerans ($p < .001$, Wald $\chi^2 = 8495$, pseudo-$R^2 = .28$; Figure 1e top-left) and negatively associated with copepods ($p < .001$, Wald $\chi^2 = 4378$, pseudo-$R^2 = .19$; Figure 1e, top-right). At the same time, diatoms were negatively associated with cladocerans ($p < .001$, Wald $\chi^2 = 1455$, pseudo-$R^2 = .05$; Figure 1e, bottom-left) and positively associated with copepods ($p < .001$, Wald $\chi^2 = 8329$, pseudo-$R^2 = .21$; Figure 1e, bottom-right). In addition to changes in community composition, total

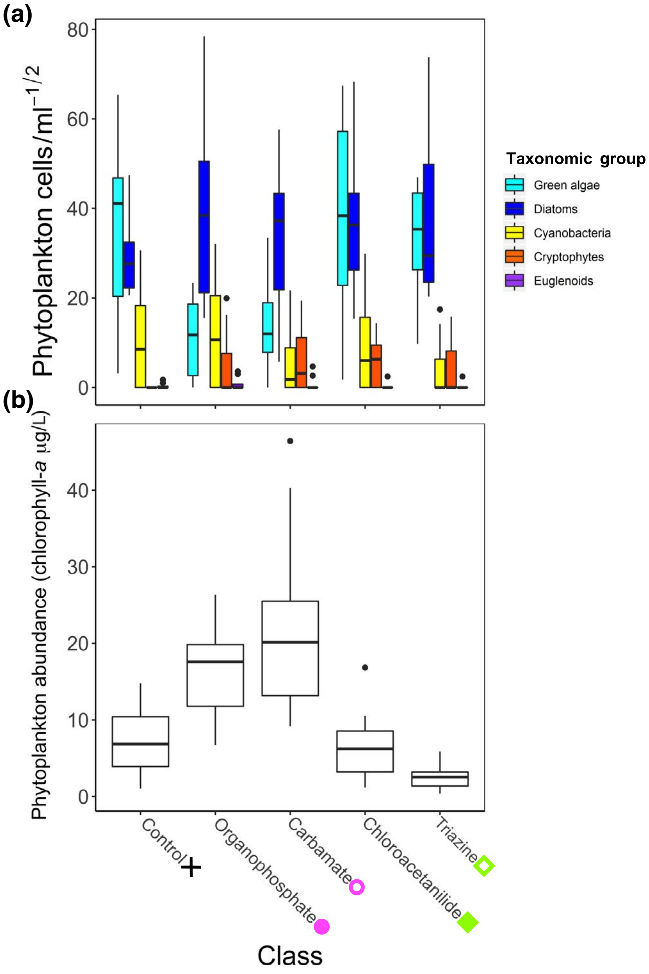

FIGURE 2 (a) Densities of broad taxonomic groups of phytoplankton in response to pesticide classes and controls at the end of the experiment. Exposure to insecticides resulted in a decrease in green algae and an increase in the mean abundance of diatoms relative to controls. (b) Total abundance of phytoplankton, as reflected by chlorophyll-*a* measurements, in response to pesticide classes and controls midway through the experiment. Insecticides were associated with increases in phytoplankton, while triazine herbicides were associated with decreases in phytoplankton relative to the control [Colour figure can be viewed at wileyonlinelibrary.com]

abundance of phytoplankton, as indicated by chlorophyll-*a*, increased with insecticide exposure (Figure 2b).

Comparisons of the effects of pesticides by class to controls using log response ratios demonstrated that respiration of the entire aquatic community increased significantly in response to insecticide exposure, and this effect persisted throughout the experiment (Figure 3a). The increase in ecosystem respiration was associated with both changes in community composition and total abundance of phytoplankton. For instance, the NMDS ordination of phytoplankton community composition and the vector overlay of ecosystem respiration at week 2 showed a significant association ($p < .001$, $F_{9,44} = 1.93$, $R^2 = .25$; Figure 3b). The increase in ecosystem respiration in insecticide treatments was associated with more diatoms and euglenoids and less green algae relative to all other treatments (Figure 3b). In addition, the greater total abundance of phytoplankton, represented by chlorophyll-*a*, in insecticide treatments was positively associated with ecosystem respiration in weeks 2 and 4 (week 2: $p < .001$, $F_{1,52} = 26.88$, $R^2 = .34$; week 4: $p < .001$, $F_{9,44} = 48.34$, $R^2 = .48$; Figure 3c).

3.3 | Herbicides decrease phytoplankton abundance which is associated with short-term decreases in ecosystem respiration

In contrast to the effect of insecticides, the dbRDA and pairwise comparisons demonstrated no difference in community composition of phytoplankton between the herbicides and the controls (Figures 1b and 2a). Instead, relative to controls, herbicide exposure resulted in a significant decrease in total abundance of phytoplankton, as indicated by chlorophyll-*a* (Figure 2b). Log response ratios showed that herbicides reduced ecosystem respiration, but these changes did not persist for the length of the experiment (Figure 3a). In addition, the effects of herbicides on ecosystem respiration varied by class, with triazines having a greater magnitude of effect compared to chloroacetanilides (Figure 3a). The NMDS ordination of the phytoplankton community composition and the vector overlay of ecosystem respiration at week 2 showed herbicides grouping with controls, suggesting no herbicide-induced changes in phytoplankton community composition could be linked to ecosystem respiration (Figure 3b). Instead, the lower total abundance of phytoplankton (represented by chlorophyll-*a*) in herbicide treatments was associated with lower ecosystem respiration in weeks 2 and 4 (Figure 3c).

4 | DISCUSSION

Synthetic chemicals represent a globally widespread disturbance that threatens freshwater ecosystems. Yet, what remains largely unknown is how consistent the responses of a diversity of community members are to an array of contaminants and how these changes in communities correspond to alterations in the retention and release of carbon in ponds. We evaluated the effects of 12 pesticides, nested in four pesticide classes and two pesticide types on pond ecosystems, which included diverse phytoplankton and zooplankton communities. Our results demonstrate that: (1) the effects of pesticides were consistent within herbicide and insecticide types and within broad taxonomic groups of phytoplankton, (2) herbicides decreased total phytoplankton abundance but had no effect on phytoplankton composition, (3) by shifting dominant zooplankton from cladocerans to copepods, insecticides indirectly increased phytoplankton abundance and altered phytoplankton community composition via a top-down effect, and (4) herbicides led to short-term decreases in ecosystem respiration that varied by herbicide classes, whereas insecticides led to persistent increases in ecosystem respiration.

First, our results suggest that prediction of the staggering number of possible direct and indirect effects associated with freshwater

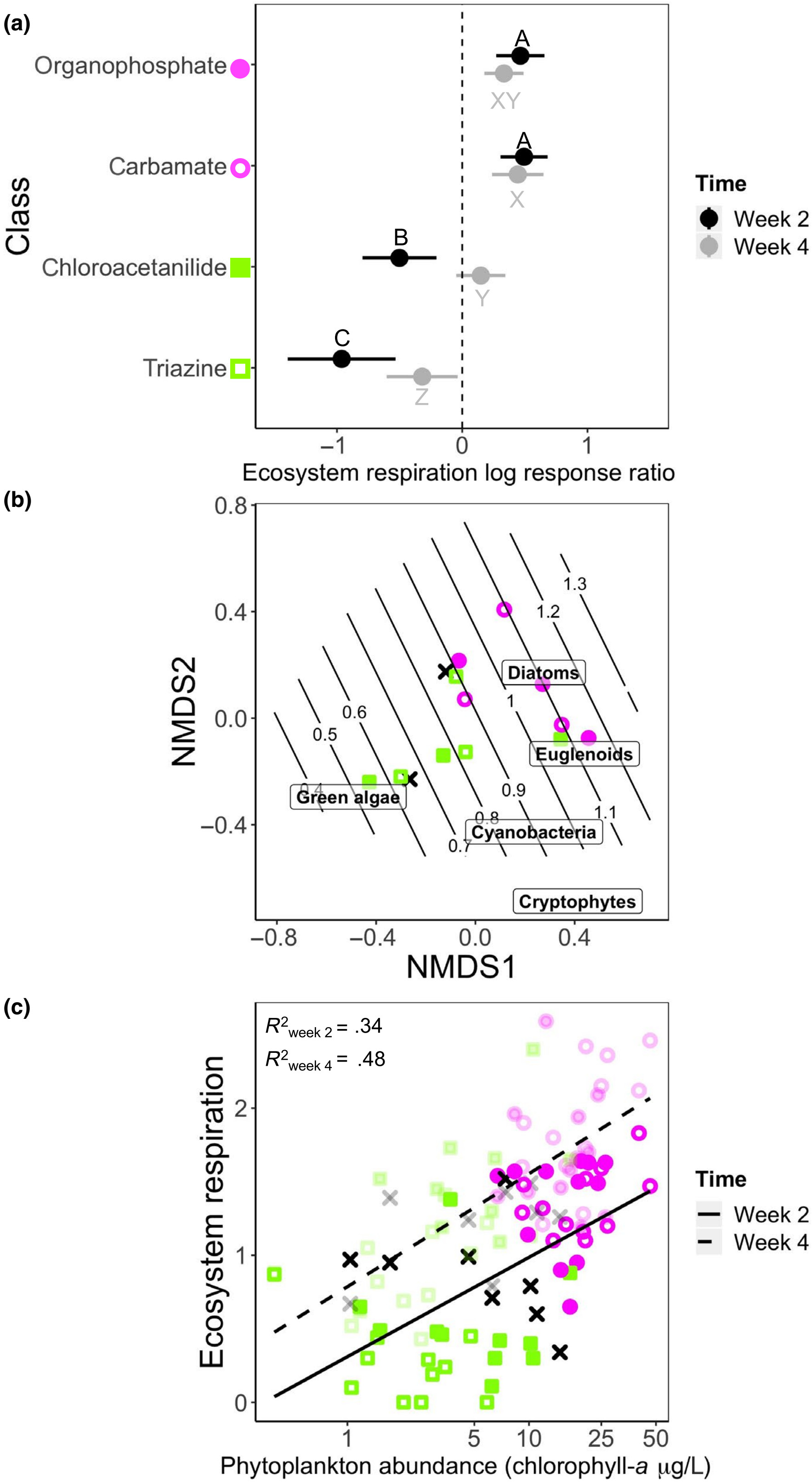

FIGURE 3 (a) Log response ratios and 95% confidence intervals showing the effect of pesticides by class on ecosystem respiration at week 2 and 4 relative to the controls. Pairwise comparisons were conducted within time points. Classes sharing the same letter are not different from each other. Insecticides resulted in an increase in ecosystem respiration that persisted through week 4. In contrast, herbicides were initially associated with a decrease in ecosystem respiration that did not persist to week 4. (b) NMDS of the phytoplankton community by broad taxonomic group ($k = 3$, stress = 0.10) and contour overlap of ecosystem respiration from week 2. This plot shows that the increase in ecosystem respiration in insecticide treatments was associated with more diatoms and euglenoids and less green algae, relative to all other treatments. Herbicide treatments grouped with controls, suggesting herbicide-induced changes in algal community composition are not linked to ecosystem respiration. (c) Regression plot showing a positive association between the abundance of phytoplankton, as measured by chlorophyll-*a*, and ecosystem respiration at weeks 2 and 4 suggesting that changes in the abundance of phytoplankton, as the result of herbicide and insecticide exposures, influenced ecosystem respiration. Associations at week 2 are shown with the solid line and opaque points. Associations at week 4 are shown with the dotted line and translucent points [Colour figure can be viewed at wileyonlinelibrary.com]

communities being exposed to thousands of synthetic chemicals could be simplified to groups of chemicals with similar environmental targets and to broad groups of taxonomically related organisms. Given that only 0.36% of the more than 100 million unique chemicals currently in existence have gone through regulation by a federal agency (Gessner & Tlili, 2016) and that freshwater systems are home to more than 125,000 described species (Balian et al., 2008; Dudgeon et al., 2006), society requires a risk assessment approach that can efficiently screen a vast number of chemicals against whole communities and ecosystems to accurately predict environmental safety; risk assessment using traditional approaches of testing a single compound against a single model organism could be extended to include tests of whole communities and ecosystems (Clements & Rohr, 2009; Rohr et al., 2006, 2016). Simplifying prediction to groups of chemicals with similar environmental targets and responses of related organisms would improve efficiency for federal regulating bodies around the world and allow for more resources to be devoted to looking for exceptions to general patterns (Clements & Rohr, 2009; Rohr et al., 2006, 2016).

The generalizable effects of insecticides and herbicides on algal and zooplankton communities that we found were consistent with previous studies. In the literature, insecticide exposure regularly reduce cladoceran zooplankton, which leads to a competitive release of copepods (Boone et al., 2005; Bridges & Boone, 2003; Brock, van Wijngaarden, et al., 2000; Hua & Relyea, 2014; Relyea, 2009; Relyea & Diecks, 2008). Additionally, these changes in the zooplankton community regularly have top-down effects on phytoplankton, consistent with the observed increase in total abundance of phytoplankton and a shift in the phytoplankton community observed in our study (Boone et al., 2005; Bridges & Boone, 2003; Brock, van Wijngaarden, et al., 2000; Hua & Relyea, 2014; Relyea, 2009; Relyea & Diecks, 2008). More specifically, we found that increased density of copepods was negatively associated with the density of green algae and positively associated with diatom density. Total phytoplankton abundance might have increased because, compared to cladocerans, copepods are less efficient phytoplankton feeders and have broader diets encompassing non-algal food sources (Sommer & Sommer, 2006). Changes in the phytoplankton community could have been the results of differences in feeding preferences of copepods versus cladocerans. Alternatively, insecticides may have indirectly altered the availability of nutrients for phytoplankton. Insecticides could have been directly toxic to insect predators, which in turn could have altered herbivory of snail and tadpoles and therefore, nutrient availability (Peacor & Werner, 2000). Herbicides did not change phytoplankton composition but decreased total phytoplankton abundance; herbicide-induced reductions in phytoplankton have been shown in other studies (Brock, Lahr, et al., 2000; Halstead et al., 2014, 2018; Rohr & Crumrine, 2005). Similar to other studies, the total abundance of zooplankton in herbicide-treated ponds likely indirectly decreased because of the decrease in phytoplankton, a common food source for all zooplankton (Noack et al., 2003; Relyea, 2009).

Few studies have attempted to evaluate how synthetic chemical disturbances in communities are associated with changes in ecosystem functions (Bernhardt et al., 2010; Rosi-Marshall & Royer, 2012; but see Halstead et al., 2014; McMahon et al., 2012; Rosi-Marshall et al., 2013; Rumschlag et al., 2020). Our study found that insecticides led to persistent, and non-trivial, increases in ecosystem respiration, while herbicides lead to short-term decreases in ecosystem respiration that varied with pesticide classes. Alterations in the abundance and composition of phytoplankton caused by exposure to insecticides and herbicides could have been linked to changes in ecosystem in three ways, either individually or in combination. First, phytoplankton may have contributed directly to ecosystem respiration. So, decreased phytoplankton abundance in herbicide treatments might have resulted in less total respiration, while increased phytoplankton abundance and altered composition in insecticide treatments might have resulted in more total respiration. Second, the abundance of phytoplankton may have affected the abundance of microbial decomposers, which in turn could have contributed to ecosystem respiration. Finally, the abundance of phytoplankton may have led to more and/or larger secondary and/or tertiary consumers, which contributed to ecosystem respiration.

The variation in the duration and magnitude of changes in ecosystem respiration by pesticide type and class could be explained by the environmental persistence of the pesticides and the generation time of the organism to which the pesticide is directly toxic. For instance, ecosystem respiration in ponds exposed to herbicides might have recovered more quickly because herbicides were directly toxic to phytoplankton communities, whose generation times are upwards of a day (Laws, 2013), and likely were able to rebound from the direct toxicity of herbicides. In contrast, ecosystem respiration in ponds exposed to insecticides might have been slower because direct toxicity affected zooplankton that have generation times of several weeks (Kalff, 2002). In addition to ecosystem respiration varying by pesticide type, ecosystem respiration also varied by herbicide class. Triazine herbicides had a greater and more persistent negative effect on ecosystem respiration than chloroacetanilide herbicides, likely because triazines persist in the environment longer than chloroacetanilides (soil half-lives of 110–146 days vs. 14–26 days, respectively [Pesticide Acton Network Pesticide Database]).

Pesticide-induced alterations to respiration found in our study suggest that chemical contamination has the potential to alter the ecosystem metabolism in freshwater systems. While the influence of nutrient subsidies (nitrogen, phosphorus, and carbon) on metabolism in lakes and streams is well studied (Stanley et al., 2016; Williamson et al., 2020; Woodward et al., 2012), few studies have examined the influence of chemical contaminants, such as pesticides, pharmaceuticals (but see Robson et al., 2020; Rosi-Marshall et al., 2013), or heavy metals (but see Carlisle & Clements, 2005), on components of the carbon cycle. In our study, while insecticides led to persistent increases in ecosystem respiration and a bloom in phytoplankton, insecticides could have led to carbon storage as biomass, release of atmospheric carbon, or no change in net primary productivity compared to controls. The outcome of the effect of insecticides on these patterns would depend on the size of the contribution of phytoplankton blooms to gross primary productivity. For instance, if gross primary productivity was greater or less than respiration, then carbon would have been stored as biomass or released, respectively. In contrast, we posit that herbicides could have led to a short-term release of atmospheric carbon because herbicides were associated with short-term decreases in respiration and phytoplankton abundance, which likely translated to reduced gross primary productivity. Future studies should investigate the effects of pesticides on the carbon cycle more holistically by directly evaluating changes to gross and net primary productivity and rates of carbon storage. These additional pieces of the carbon cycle would allow for the evaluation of how synthetic chemicals alter net fluxes and pools of carbon in aquatic systems. Runoff from spring application of pre-emergent herbicides on agricultural fields could possibly lead to short-term releases of atmospheric carbon in adjacent water bodies. Given the enormous amount of herbicides released in the environment annually (1.3 billion kg worldwide in 2012; Atwood & Paisley-Jones, 2017) and that inland freshwater systems account for 0.6 billion tons of carbon storage (more than all carbon buried in oceanic sediments, Aufdenkampe et al., 2011; Battin et al., 2009), we postulate that herbicide use could have profound impacts on the global carbon cycle.

Given that the production and application of synthetic chemicals has been increasing exponentially for decades (Gessner & Tlili, 2016), understanding the generalizable mechanisms by which synthetic chemicals can alter aquatic ecosystems is critical if our goal is efficiency in environmental risk assessment (Rohr & Crumrine, 2005; Rohr et al., 2006, 2016). Our results support the hypothesis that predicting the effects of synthetic chemicals on complex, diverse freshwater systems can be simplified by generalizing patterns to groups of chemicals that share similar targets and to groups of organisms that are taxonomically related. To better understand, regulate, and protect human and ecological health, policy makers should look to generalizable patterns so that efficiency in risk assessment to global change drivers can become a priority, which will free resources to look for exceptions to general patterns.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Jason T. Hoverman, Thomas R. Raffel, and Jason R. Rohr designed the experiment, Jason T. Hoverman, Thomas R. Raffel, and Hunter J. Carrick, conducted the experiment, Dale A. Casamatta conducted the identification and enumeration of phytoplankton, Samantha L. Rumschlag and Michael B. Mahon conducted the analyses, Samantha L. Rumschlag wrote the manuscript, and all authors contributed to editing the manuscript.

DATA AVAILABILITY STATEMENT

Data are available via figshare (https://doi.org/10.6084/m9.figshare.16840126.v1).

ORCID

Samantha L. Rumschlag https://orcid.org/0000-0003-3125-8402

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