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Supplementary Materials for

Consistent Effects of Pesticides on Community Structure and Ecosystem Function in Freshwater Systems

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- Materials and Methods
- Supplemental Text
- Figs. S1 to S5
- Tables S1 and S2
- References (16-18)

25 **Materials and Methods**

26 Experimental Design and Community Composition

27 We conducted a randomized-block experiment at the Russell E. Larsen Agricultural
28 Research Center (Pennsylvania Furnace, PA, USA) with replicated mesocosm ponds.
29 Mesocosms were 1,100-L cattle tanks covered with 60% shade cloth. The spatial block was
30 distance from a tree line in our mesocosm field. Three weeks before pesticide application, these
31 mesocosms were filled with 800 L water, 300 g mixed hardwood leaves, and inoculations of
32 zooplankton, periphyton, and phytoplankton homogenized from four local ponds. Just before
33 pesticide application on the same day, each tank received two snail, three larval anuran, one
34 larval dragonfly, one water bug, one water beetle, one larval salamander, and one backswimmer
35 species (11 *Helisoma (Planorbella) trivolvis*, 10 *Physa gyrina*; 20 *Hyla versicolor*, 20 *Lithobates*
36 *palustris*, 20 *Lithobates clamitans*; 2 *Anax junius*; 2 *Belostoma flumineum*; 5 *Hydrochara* sp.; 3
37 *Ambystoma maculatum*; 6 *Nototoca undulata*) (Fig. 1a). These community members naturally
38 coexist and were applied at naturally occurring densities (16). Initial conditions of some
39 mesocosms varied in simulated pesticide treatments (see below).

40 We randomly assigned 18 treatments (12 pesticides, 4 simulated pesticides, 2 controls)
41 with four replicate mesocosms of each treatment, which resulted in 72 total mesocosms (Fig.
42 S1b). The 12 pesticide treatments were nested; we included two pesticide types (insecticide,
43 herbicide), two classes within each pesticide type (organophosphate insecticide, carbamate
44 insecticide, chloroacetanilide herbicide, triazine herbicide), and three different pesticides in each
45 of four classes (Fig. S1b). To represent runoff of pesticides into freshwater systems following a
46 rainfall event, we applied single doses of technical grade pesticides at environmentally relevant
47 concentrations at the beginning of the experiment. To ensure our exposures represented
48 environmental relevance, we used estimated environmental concentrations of pesticides,
49 calculated by U.S. Environmental Protection Agency's GENEEC v2 software, Table S2). Our
50 design also included water and solvent (0.0001% acetone) controls (Fig. 1b). Pesticides were
51 obtained from ChemService (West Chester, PA, USA). Nominal concentrations of pesticides
52 ($\mu\text{g/L}$) were: 64 chlorpyrifos, 101 malathion, 171 terbufos, 91 aldicarb, 219 carbaryl, 209
53 carbofuran, 123 acetochlor, 127 alachlor, 105 metolachlor, 102 atrazine, 202 simazine, and 106
54 propazine. We collected composite water samples one hour after application to mesocosms and
55 shipped samples on ice to Mississippi State Chemical Laboratory to verify these nominal
56 concentrations. Measured concentrations of pesticides ($\mu\text{g/L}$) were: 60 chlorpyrifos, 105
57 malathion, 174 terbufos, 84 aldicarb, 203 carbaryl, 227 carbofuran, 139 acetochlor, 113 alachlor,
58 114 metolachlor, 117 atrazine, 180 simazine, and 129 propazine.

59 The four simulated pesticide treatments were top-down or bottom-up food web
60 manipulations intended to mimic effects of actual herbicides and insecticides on community
61 members. These manipulations occurred once and were concurrent with the timing of pesticide
62 applications. Top-down and bottom-up simulated insecticide treatments were designed to reduce
63 densities of zooplankton, simulating effects of insecticides on zooplankton survival. For top-
64 down simulated insecticides, we doubled the densities of zooplankton predators by including six
65 total *A. maculatum* larval salamanders and 12 *N. undulata* backswimmers per mesocosm. For
66 bottom-up simulated insecticides (i.e., direct manipulation of a lower arthropod trophic level),
67 we removed zooplankton with a vertical tow-net. Top-down and bottom-up simulated herbicides
68 were designed to reduce algae, simulating effects of herbicides on survival and growth of algae.
69 For top-down simulated herbicides, we doubled the densities of large herbivores to increase
70 grazing pressure by including 22 *H. trivolvis* snails, 20 *P. gyrina* snails, 40 *H. versicolor* larval

71 anurans, 40 *L. palustris* larval anurans, and 40 *L. clamitans* larval anurans per mesocosm. For
72 bottom-up simulated herbicides, we covered mesocosms in three sheets of 60% shade cloth in an
73 attempt to block light and reduce photosynthesis. The experiment ran for four weeks, from June
74 to July.

75

76 Measurements of Experimental Responses

77 During the experiment, we sampled periphyton using clay tiles (100 cm²) oriented
78 perpendicularly along the bottom of the mesocosm. Each mesocosm had two periphyton
79 measurements: ‘inaccessible periphyton’ taken from caged clay tiles that excluded herbivores
80 and ‘accessible periphyton’ taken from clay tiles that were uncaged allowing herbivore access.
81 We sampled phytoplankton from water samples taken 10 cm below the water surface. Periphyton
82 was scrubbed from tiles and phytoplankton from water samples (10 mL) were filtered onto glass
83 fiber filters (under low vacuum pressure, <10 psi; Whatman EPM 2000, 0.3 μm, 47 mm) to
84 estimate associated chlorophyll concentrations. The chlorophyll concentration of each filter was
85 determined using an organic extraction procedure with a 50:50 mixture of 90% acetone to
86 DMSO. We measured chlorophyll-*a* concentrations using a standard fluorometric technique. We
87 scored water clarity, a metric of light availability, on a scale from one (clear) to five (opaque)
88 blind to treatment. We measured pH and dissolved oxygen (DO) at dusk and dawn on subsequent
89 days using hand-held meters (YSI, Yellow Springs, OH, USA). We measured decomposition by
90 taking the dry mass of hardwood leaf packets in each mesocosm at the beginning and the end of
91 experiment. In addition, we sampled snail egg masses and hatchlings using two rectangular
92 pieces of Plexiglass (465 cm²) in each mesocosm, one hung on the side and one on the bottom of
93 the mesocosm. Zooplankton were collected from the entire water column by placing a PVC pipe
94 (10 cm diameter, 60 cm height) upright in the center of each tank, capping the bottom, and
95 pouring the water through a 20 μm Nitex mesh. We collected two samples of zooplankton from
96 each mesocosm, and we combined and preserved the samples in 70% ethanol. Zooplankton were
97 counted and identified in 5 mL subsamples for each mesocosm using a zooplankton counting
98 wheel (Wildlife Supply Company, Yulee, FL, USA) and a dissecting microscope. At the end of
99 the experiment, mesocosms were drained, and the remaining animals were counted, euthanized,
100 and preserved. Two previous manuscripts, which use the same design as the current manuscript,
101 also describe this experimental design and methods (4, 17).

102

103 Statistical Analyses

104 To test for the consistency of effects of type, class, and individual pesticide on aquatic
105 ecosystem processes and communities and to attribute the variation explained to each pesticide
106 level of organization while accounting for the nested structure of our experimental design
107 (Figure 1b), we completed permutational analyses of variance (PERMANOVA). For nested
108 PERMANOVA models, the predictors were the following random categorical terms: type
109 (insecticide, herbicide), class (carbamate, organophosphate, chloroacetanilide, triazine) nested
110 within type, and pesticide (12 in total) nested within class within type. These models did not
111 include controls or simulated pesticides because these treatments were not hierarchically nested
112 (Fig. S1b). We evaluated 9999 permutations using residuals under a reduced model. Following
113 nested PERMANOVAs, we used pair-wise multiple comparisons tests using PERMANOVAs to
114 evaluate differences among controls, organophosphates, carbamates, top-down simulated
115 insecticides, bottom-up simulated insecticides, chloroacetanilides, triazines, top-down simulated
116 herbicides, and bottom-up simulated herbicides. In these pair-wise comparisons, we evaluated

117 9999 unrestricted permutations of raw data. All PERMANOVAs also included spatial block as a
118 random predictor to account for variation in sunlight associated with distance from a tree line.
119 Preliminary analyses showed that exclusion of the block did not change the results. In all
120 PERMANOVAs, test statistics associated with Type III partial sums of squares were evaluated.

121 We conducted four nested PERMANOVAs. Our first nested PERMANOVA focused on
122 ecosystem processes and included the following responses: pH and dissolved oxygen taken both
123 at dawn and dusk, decomposition (percent mass loss of hardwood leaf packets), turbidity (water
124 clarity scores from 1 to 5), and densities of phytoplankton, accessible periphyton, and
125 inaccessible periphyton (measured via chlorophyll-*a*). The resemblance matrix for these
126 responses was constructed using a Euclidean distance matrix of log-transformed and normalized
127 values.

128 Our second and third nested PERMANOVAs focused on community structure. We
129 separated community members into two statistical models based on the forms of response
130 variables; those whose response variables were densities based on counts (zooplankton) and
131 those community members whose response variables were survival, mass, reproductive rates, or
132 density abstracted from chlorophyll measurements (insect predators, snail and tadpole
133 herbivores, and algae; termed the tri-trophic community). The multivariate response for the
134 zooplankton community included densities of *Daphnia*, *Diaphanasoma*, *Chydorus*, *Bosmina*,
135 *Diaptomus*, and *Cyclops*. Zooplankton community analyses were based on square-root
136 transformed densities using Bray-Curtis similarities. The multivariate response for the tri-trophic
137 community model included: survival (0 to 1) of all amphibian, snail, and insect community
138 members; average mass of surviving individuals for each amphibian species and *H. trivolvis*
139 snails; average number of hatchlings and eggs per surviving *H. trivolvis* snail; and densities of
140 phytoplankton and periphyton. Mass and reproductive rates were standardized to the number of
141 surviving individuals to account for the different densities added to each tank at the beginning of
142 the study (i.e. extra herbivores in top-down simulated herbicide treatment and extra predators in
143 bottom-up simulated insecticide treatment). Mass and reproductive rates of *P. gyrina* were not
144 included because of low survival across treatments. Survival rates were arc-sine square-root
145 transformed and normalized, and all other variables were log-transformed and normalized. Tri-
146 trophic community analyses were based on Euclidean distances.

147 Finally, our fourth nested PERMANOVA evaluated a simplified tri-trophic community.
148 We simplified the tri-trophic community responses into three functional roles within the
149 community: algae, herbivores, and predators. Tri-trophic community responses of individual taxa
150 were transformed and normalized as described previously, and then they were averaged
151 according to functional group. We averaged densities of periphyton and phytoplankton into a
152 single “algae” response, all amphibian and snail responses into a single “herbivore” response,
153 and all insect and salamander responses into a single “predator” response. The simplified tri-
154 trophic community model was based on Euclidean distances.

155 To visualize consistency of effects within type, class, and individual pesticides on
156 multivariate ecosystem and community responses and to compare pesticide effects to simulated
157 pesticides and controls, we used distance-based redundancy analyses (dbRDA) and two-way
158 cluster diagrams. The dbRDAs were based on appropriate resemblance matrices for ecosystem
159 and community responses as described above. The underlying categorical predictors in the
160 models for ecosystem processes and tri-trophic communities included: the spatial block,
161 organophosphate, carbamate, chloroacetanilide, triazine, top-down simulated insecticide, bottom-
162 up simulated insecticide, top-down simulated herbicide, bottom-up simulated herbicide, and

163 control. In the zooplankton analyses, all previous predictors were included except for spatial
164 block because it was not significant in the PERMANOVA test. In the dbRDA plots, when spatial
165 block was included in the ecosystem and tri-trophic community plots, we show the centroid
166 values for the 18 experimental replicates.

167 As an alternative to the dbRDAs presented in the main text, we also visualized the
168 consistency of effects within type, class, and individual pesticide on ecosystem, tri-trophic
169 community, and zooplankton responses and compared pesticide effects to simulated pesticides
170 and controls, using principal coordinates analyses (PCoA) (Figs. S2, S3, S5). PCoAs were based
171 on appropriate resemblance matrices as described previously. PCoAs were conducted in
172 PERMANOVA+ for PRIMER and resulting data were exported. Point and vector plots were
173 made using exported data and the ‘*ggplot2*’ package in R. Ellipses on point plots represent 95%
174 confidence intervals of groups based on standard errors and were made using the *ordiellipse*
175 function in the ‘*vegan*’ package.

176 For the two-way cluster diagrams, clusters of pesticide treatments were based on centroid
177 distances of the appropriate resemblance matrices. Clusters of multivariate responses were based
178 on Euclidean distance resemblance matrices of averaged treatment responses. Before averaging,
179 ecosystem and community responses were transformed and normalized as described previously.
180 In clustering of treatments and responses, the cluster mode was the group average. In the
181 PERMANOVAs for ecosystem processes and tri-trophic community responses, the effect of
182 block was significant (Table S1). Thus, we accounted for the effect of block by taking the
183 residuals of simple linear regressions with individual ecosystem or tri-trophic community
184 responses as the independent variable and block as the predictor in the generation of the shaded
185 values of the two-way cluster diagrams. Then, we averaged these block-adjusted treatment
186 responses with the ‘*shade plot*’ function in PRIMER. For the zooplankton community, the effect
187 of block was not significant in the PERMANOVA model (Table S1), so shaded values of the
188 two-way cluster diagrams were simply the averaged treatment responses. All PERMANOVA
189 models, pair-wise comparisons, dbRDAs, and two-way cluster diagrams were executed using
190 PERMANOVA+ for PRIMER version 7 (PRIMER-E Ltd, Plymouth, UK). For ease of
191 visualization of dbRDA and PCoA plots, data from PERMANOVA+ for PRIMER were
192 exported, and plots were made using ‘*ggplot2*’ package in R.

193 To compare the level of support for direct versus indirect biodiversity-mediated effects
194 by which pesticides might influence ecosystem processes, we performed path analyses using the
195 ‘*piecewiseSEM*’ package. We chose to use path analyses because they allowed us to test multiple
196 linked hypotheses via the consideration of multiple variance-covariance matrices in which
197 variables serve as both dependent and independent variables. In evaluating the effects of
198 herbicides and insecticides on ecosystem processes, we chose to compare two mechanistic paths:
199 the direct effects of herbicides or insecticides on ecosystem processes and the indirect effects in
200 which the effects of herbicides or insecticides are mediated by the impact of biodiversity on
201 ecosystem processes. The unit of replication was the mesocosm, and each path model contained
202 32 independent replicates. Within the path models, we accounted for the effect of spatial block
203 by using linear mixed effect models in which block was the random intercept term. For our
204 biodiversity metrics, we calculated Hill numbers of species richness ($q = 0$), Shannon diversity (q
205 $= 1$, exponent of Shannon index), and Simpson diversity ($q = 2$, inverse of Simpson index) (18).
206 Hill numbers are preferred over other diversity metrics because units of Hill numbers are
207 effective number of species as opposed to unitless metrics that are challenging to interpret (18).
208 Biodiversity metrics were calculated in PRIMER. We present the results of path analyses using

209 Shannon diversity in the main text, while results using species richness and Simpson diversity
210 are included in the Supplemental Information (Fig. S6). For all path models, ecosystem function
211 is the first axis from a principal coordinates analysis of the Euclidean resemblance matrix of log-
212 transformed and normalized ecosystem responses including: pH and dissolved oxygen taken both
213 at dawn and dusk, decomposition, and turbidity. Fit statistics indicate that all path models fit well
214 (Fisher's $C = 0$, p -value = 1).

215

216 **Supplemental Text**

217 Costs and Benefits of Mesocosm Studies

218 Using mesocosms studies in toxicity testing has previously been criticized because of
219 high costs compared to traditional single species toxicity tests like the LC50. While a mesocosm
220 study takes more time and money to conduct compared to a single LC50 study, it also provides
221 information on the toxicity to multiple organisms under more environmentally realistic
222 conditions. To properly consider the costs and benefits of a mesocosm experiment, an estimate
223 would need to consider both the abundance of toxicity data and the ecological realism of the
224 data. For instance, assume that the average mesocosm study provides toxicity information for 10
225 species, then this study should be compared to the costs of conducting 10 LC50 studies.
226 Additionally, the toxicity data from the mesocosm experiment should be of higher value because
227 it includes realistic ecological complexities (e.g. direct and indirect effects, recovery dynamics of
228 the populations and communities). In comparison, the LC50 study measures the toxicity of a
229 single organism under contrived lab conditions. When these two approaches are more
230 appropriately compared, the benefits of mesocosm experiments could outweigh the costs in
231 comparison to traditional toxicology studies.

232

233 Performance of Simulated Pesticide Treatments

234 With the exception of bottom-up simulated herbicide, the effects of simulated pesticides
235 did not match the effects of pesticide classes (pair-wise comparisons Fig. 1-3). These treatments
236 performed poorly likely because manipulating taxa did not match the magnitude or the
237 specificity of the long-term effect of pesticides. Top-down simulated herbicides (i.e., doubled
238 herbivores) were designed to reduce algae, but the added tadpoles and snails mostly feed on
239 periphyton, while the actual herbicides had a greater long-term net negative effect on
240 phytoplankton (Fig. 1). Top-down (i.e., doubled zooplankton predators) and bottom-up (i.e.,
241 zooplankton removal) simulated insecticides both failed to replicate the differential toxicity that
242 insecticides had on cladoceran versus copepod zooplankton (Fig. S3).

243

244 Evaluation of Acute Aquatic Toxicity Using QSAR Approaches

245 We completed analyses to evaluate if the consistency of pesticides on aquatic systems
246 observed in the current study could be predicted by QSAR methods based on the structure of the
247 pesticides alone. We used the QSAR Toolbox (<https://qsartoolbox.org/>) developed in partnership
248 with The Organisation for Economic Co-operation and Development (OECD). QSAR Toolbox is
249 a centralized, open-source software system for predicting toxicity of chemicals by applying a
250 category approach. One functionality of the software is the clustering of chemicals into similar
251 groups based on the predicted acute aquatic toxicity. The predicted acute aquatic toxicity is
252 based solely on the pesticide's chemical structure. We conducted three different analyses that
253 examined the clustering of our 12 pesticides. Below, we describe the three different analyses and
254 the results.

255
256 1) Acute Aquatic Toxicity Classification by Verhaar
257 Description: The Acute aquatic toxicity classification by Verhaar consists of parametric and
258 structural rules to mimic the Verhaar rules developed by Toxtree software. This system is
259 introduced for chemical categorization purposes or can be used for the prioritization of chemicals
260 for subsequent testing.

261
262 Results:

263 Group 1: All carbamates and organophosphates

264 Group 2: All triazines

265 Group 3: All chloroacetanilides

266

267 2) Acute Aquatic Toxicity by Mode of Action by OASIS

268 Description: This profile divides chemicals in different categories according to their acute toxic
269 mode of action (MOA). 2D structural information is used only to identify the MOA of
270 chemicals. Based on theoretical and empiric knowledge the following seven hierarchically
271 ordered MOA are distinguished: Aldehydes; alpha, beta-Unsaturated alcohols; Phenols and
272 Anilines; Esters; Narcotic Amines; Basesurface narcotics.

273

274 Results:

275 Group 1: All carbamates, organophosphates, and chloroacetanilides

276 Group 2: All triazines

277

278 3) Aquatic Toxicity Classification by ECOSAR

279 Description: The Aquatic Toxicity Classification by ECOSAR profiler consists of molecular
280 definitions to mimic the structural definitions of chemical classes within the U.S. Environmental
281 Protection Agency's Ecological Structure-Activity Program (ECOSAR™). ECOSAR™ contains
282 a library of class-based structural activity relationships for predicting aquatic toxicity, overlaid
283 with an expert decision tree based on expert rules for selecting the appropriate chemical class for
284 evaluation of the compound.

285

286 Results:

287 Group 1: All triazines

288 Group 2: All chloroacetanilides

289 Group 3: Carbofuran and carbaryl (both carbamates)

290 Group 4: Aldicarb (carbamate)

291 Group 5: Terbufos (organophosphate)

292 Group 6: Malathion (organophosphate)

293 Group 7: Chlorpyrifos (organophosphate)

294

295 So, the result is that the pesticide groups vary based on the underlying assumptions of the
296 model even though all three models are generally trying to predict pesticides that have similarity
297 in the aquatic toxicities. These groupings are not very consistent with the observed toxicities to
298 taxa in our study (tri-trophic and zooplankton communities). For instance, in the tri-trophic
299 community analyses, the pairwise comparisons would suggest that all four pesticide classes
300 behave differently. In the analyses of the zooplankton, the pairwise comparisons suggest that

301 chloroacetanilides and triazines should group together while organophosphates and carbamates
302 should form two additional separate groups.

303 The main issues with these groups of pesticides based on predicted toxicity is that they do
304 not consider the variation across responses of groups of taxa and they do not consider indirect
305 effects of pesticides. For instance, a QSAR model might predict direct effects of herbicides on
306 algae, but that model will not include the indirect effect of herbicides on total zooplankton
307 abundance.

308

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322 Anuran tadpole: created by Michael Mahon (vectorization), J.J. Harrison (photography), CC BY-
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333 *Diaptomus*: created by Michael Mahon (vectorization), NOAA Great Lakes Environmental
334 Research Lab (photography), CC BY-SA 2.0

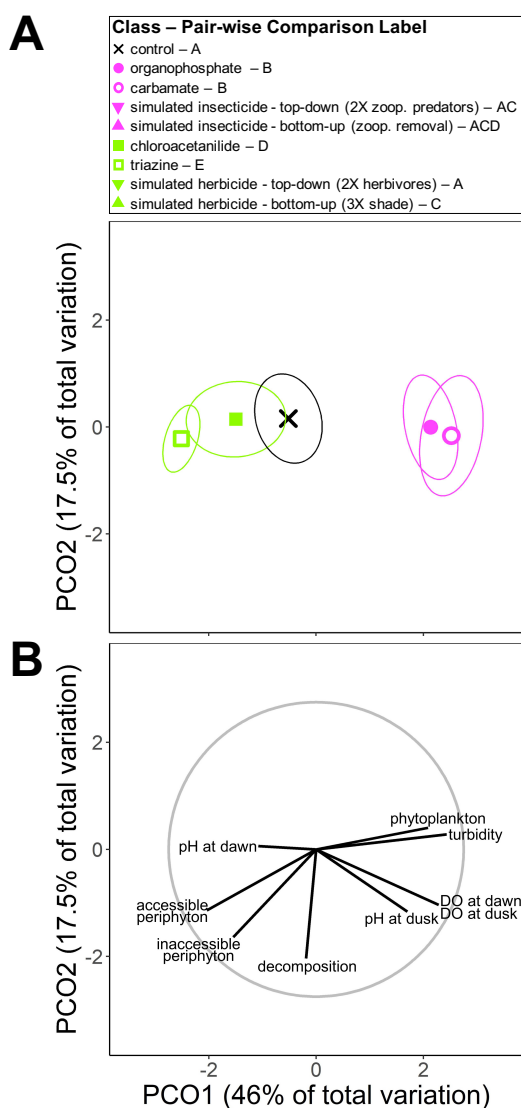
335 *Anax junius*: created by Michael Mahon (vectorization), J.J. Harrison (photography), CC BY 3.0

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339 *Ambystoma maculatum*: created by Jake Warner, CC0 1.0

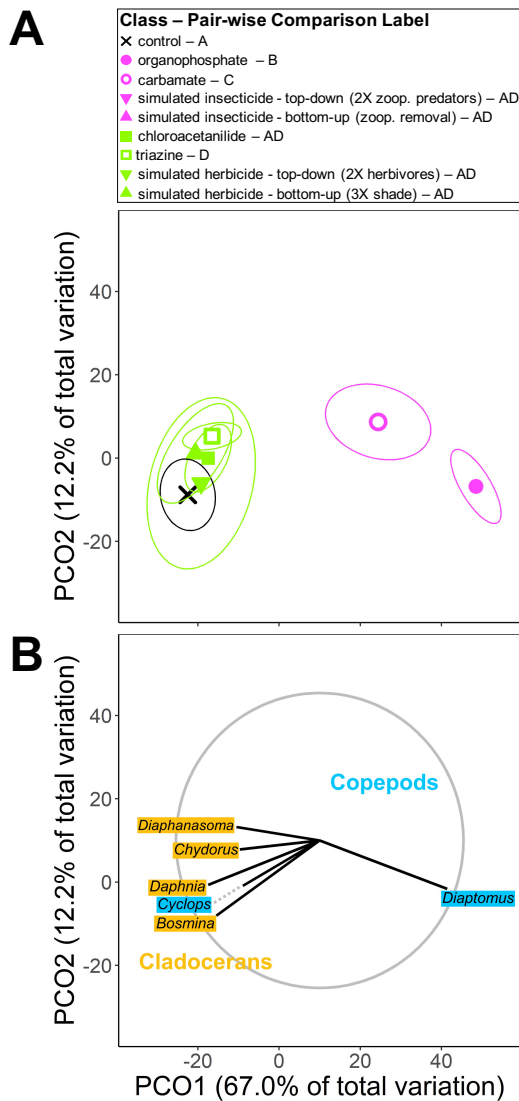
340 *Nototoca undulata*: created by Michael Mahon (vectorization); Christopher Johnson
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342

343 **Fig. S1 Principal coordinates analysis for multivariate ecosystem responses.**

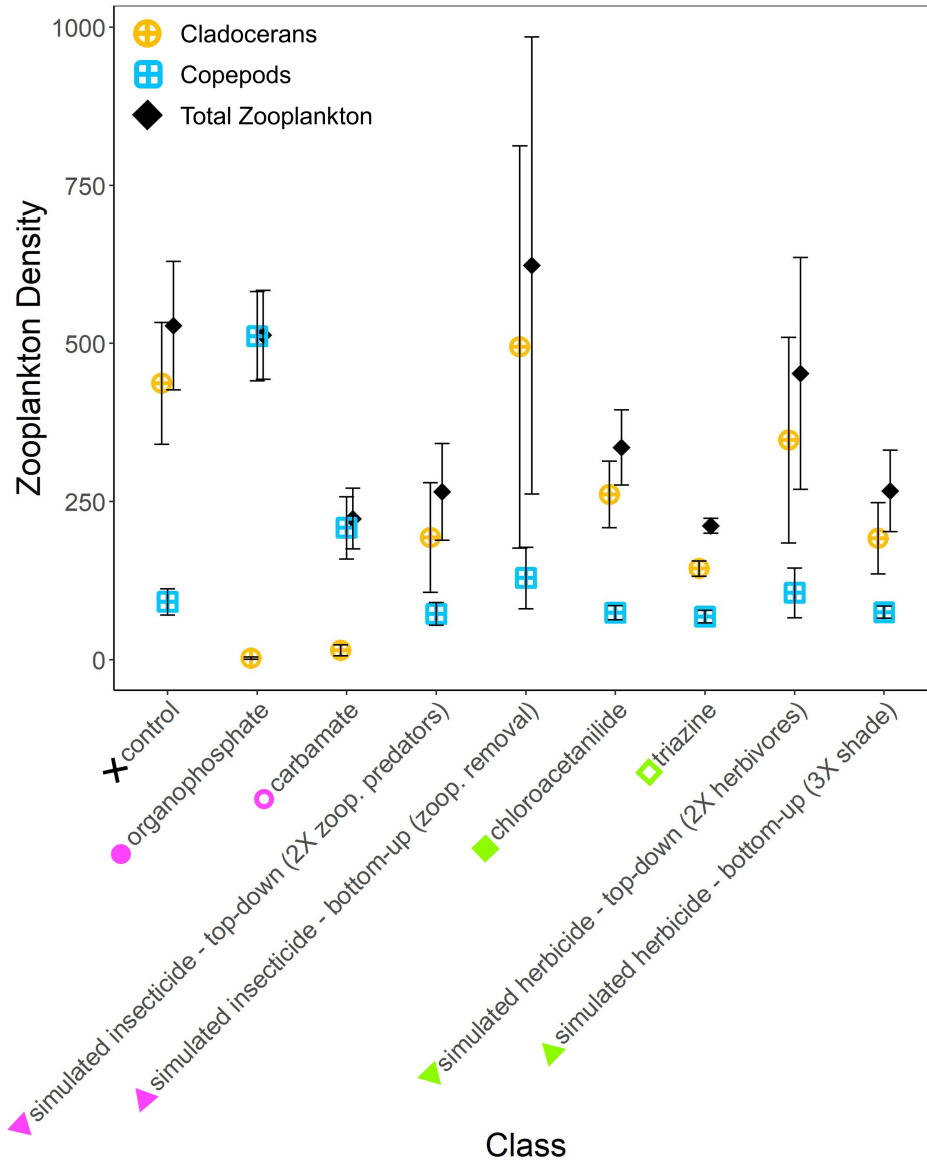
344 **A)** Principal coordinates analysis plot of multivariate ecosystem-level responses showing
 345 differences in pesticide treatments by type. Individual points are centroids for the representative
 346 treatment with ellipses based on 95% confidence intervals calculated using a standard error. All
 347 simulated herbicides and insecticides were different from corresponding pesticide treatments, so
 348 they were not included in the plot for ease of viewing. Pair-wise comparison labels are given in
 349 the figure legend. Treatments sharing letters are not different from each other. **B)** Vector overlay
 350 of log-transformed and normalized ecosystem-level responses for corresponding principal
 351 coordinates analysis plot. Gray circle shows relative vector distance lengths; the gray circle
 352 corresponds to vector lengths that would have a correlation coefficient of one.



353

354 **Fig. S2 Principal coordinates analysis for multivariate zooplankton responses.**

355 **A)** Principal coordinates analysis plot of multivariate zooplankton densities by genera showing
 356 differences in pesticide treatments by type, class, and individual pesticide. Individual points are
 357 centroids for the representative treatment with ellipses based on 95% confidence intervals
 358 calculated using a standard error. Simulated pesticides that were different from corresponding
 359 pesticide treatments, including bottom-up and top-down simulated insecticides, were not
 360 included in the plot for ease of viewing. Pair-wise comparison labels are given in the figure
 361 legend. Treatments sharing letters are not different from each other. **B)** Vector overlay of square-
 362 root transformed zooplankton densities by genera for corresponding principal coordinates
 363 analysis plot. Gray circle shows relative vector distance lengths; the gray circle corresponds to
 364 vector lengths that would have a correlation coefficient of one.

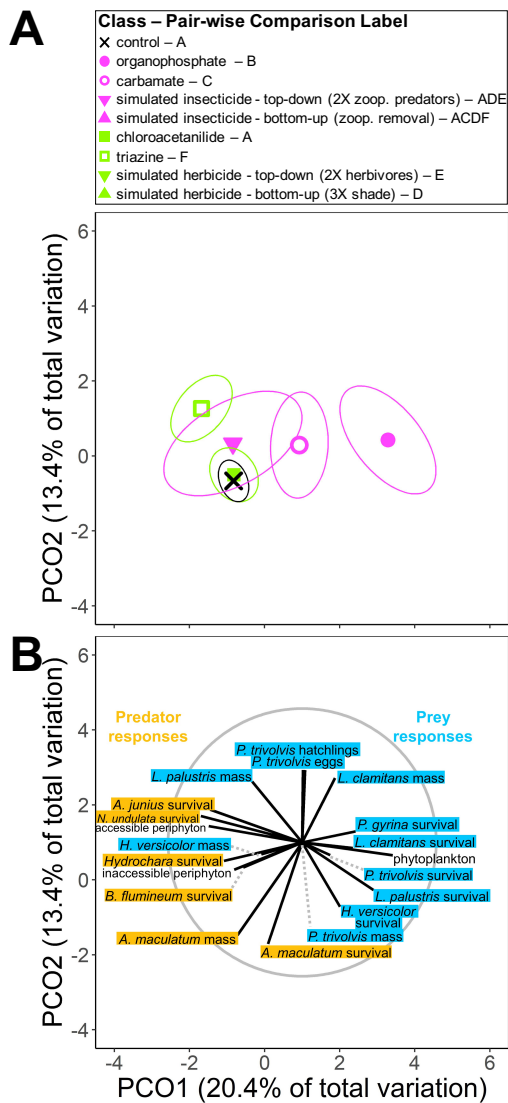


365

366 **Fig. S3 Zooplankton densities in response to experimental treatments.**

367 Densities of cladocerans, copepods, and total zooplankton in response to pesticide classes,
 368 simulated insecticides and herbicides, and the controls. The main impact of insecticides on
 369 zooplankton communities was a change in community composition with copepods becoming
 370 more abundant compared to cladocerans. In contrast, the main effect of herbicides on
 371 zooplankton was a decline in total abundance with no change in community composition; the
 372 relative amounts of cladocerans to copepods were comparable to the controls.

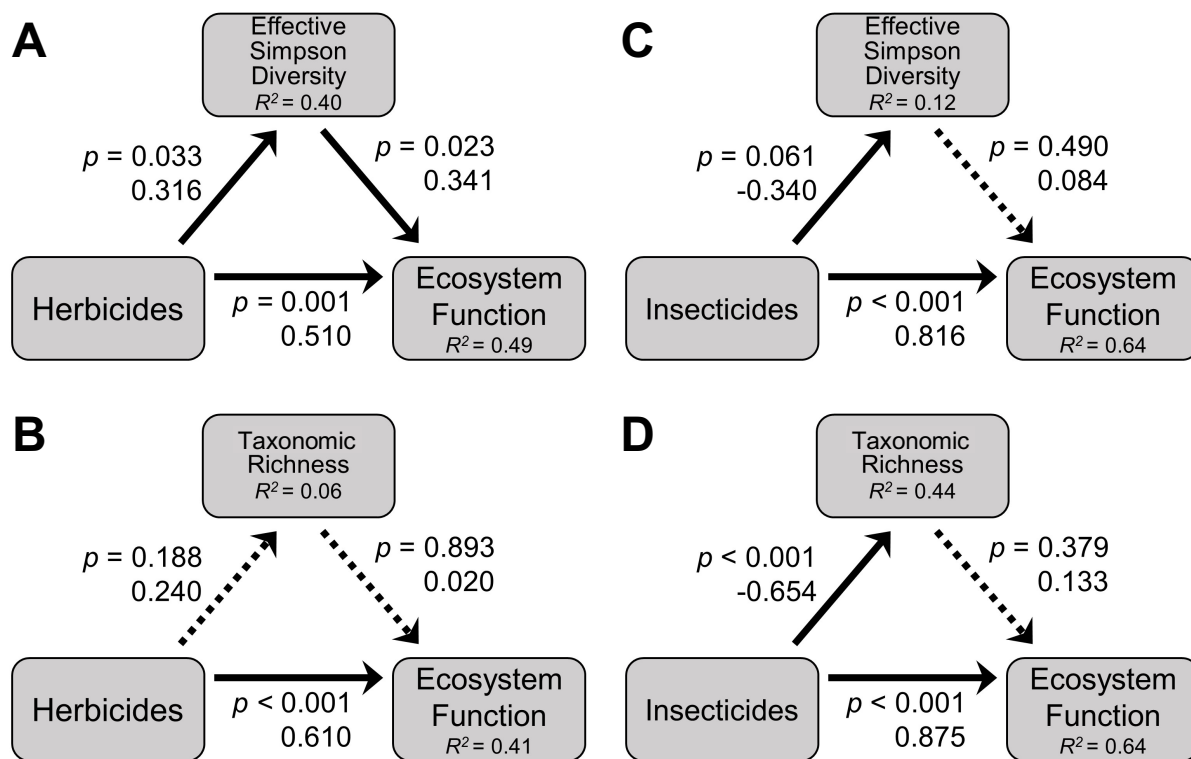
373



374

375 **Fig. S4 Principal coordinates analysis for multivariate community responses.**

376 **A)** Principal coordinates analysis plot of multivariate community-level responses showing
 377 differences in pesticide treatments by type, class, and individual pesticide. Individual points are
 378 centroids for the representative treatment with ellipses based on 95% confidence intervals
 379 calculated using a standard error. Simulated herbicides and insecticides that were different from
 380 corresponding pesticide treatments, including bottom-up simulated insecticide and top-down and
 381 bottom-up simulated herbicides, were not included in the plot for ease of viewing. Pair-wise
 382 comparison labels are given in the figure legend. Treatments sharing letters are not different
 383 from each other. **B)** Vector overlay of log-transformed and normalized community responses for
 384 corresponding principal coordinates analysis plot. Gray circle shows relative vector distance
 385 lengths; the gray circle corresponds to vector lengths that would have a correlation coefficient of
 386 one.



387

388 **Fig. S5 Relationships among pesticide types, diversity metrics, and ecosystem function.**
 389 Path analyses showing relationships among **A)** herbicides, Simpson diversity, and ecosystem
 390 function; **B)** herbicides, taxonomic richness, and ecosystem function; **C)** insecticides, Simpson
 391 diversity, and ecosystem function; and **D)** insecticides, taxonomic richness, and ecosystem
 392 function. For all path models, ecosystem function is the first axis from a principal coordinates
 393 analysis of the Euclidean resemblance matrix of log-transformed and normalized ecosystem
 394 responses including: pH and dissolved oxygen taken both at dawn and dusk, decomposition, and
 395 turbidity. Solid arrows are paths with $p < 0.07$, and dotted arrows are paths $p > 0.07$. Next to
 396 each path is the p-value and standardized coefficient. Next to each response is the conditional R^2 .

397 **Table S1.**

398 PERMANOVA models evaluating the effects of pesticides on multivariate responses including
 399 ecosystem responses (pH and DO at dawn and dusk; decomposition; turbidity; periphyton; and
 400 phytoplankton), zooplankton densities (densities of six genera), tri-trophic community responses
 401 (survival of all non-zooplankton species; average mass of surviving amphibians and *H. trivolvis*
 402 snails; average eggs and hatchlings of surviving *P. trivolvis* snails; periphyton; and
 403 phytoplankton), and simplified tri-trophic community (combined responses of algae, herbivores,
 404 and predators). All models account for the influence of a spatial block. *P* values were generated
 405 by Monte Carlo sampling and those less than 0.05 are bolded. Variation explained, represented
 406 as a proportion, is the estimated component of variation for a given predictor relative to the
 407 model's total variation excluding block. So, variation explained accounts for the influence of the
 408 spatial block.
 409

Endpoints and Source of Variation	<i>df</i>	Pseudo <i>F</i>	<i>p</i>	Variation Explained
Ecosystem				
Block	3	2.142	0.013	
Type	1	21.247	0.0004	0.461
Class(Type)	2	1.346	0.224	0.073
Pesticide(Class(Type))	8	1.838	0.004	0.146
Residual	33			0.319
Zooplankton community				
Block	3	1.5395	0.124	
Type	1	9.6265	0.020	0.442
Class(Type)	2	4.551	0.004	0.188
Pesticide(Class(Type))	8	1.8831	0.010	0.118
Residual	33			0.252
Tri-trophic community				
Block	3	1.915	0.005	
Type	1	2.849	0.038	0.222
Class(Type)	2	1.806	0.034	0.154
Pesticide(Class(Type))	8	2.111	0.0001	0.215
Residual	33			0.409
Simplified tri-trophic community				
Block	3	2.697	0.012	
Type	1	4.271	0.087	0.291
Class(Type)	2	2.484	0.071	0.176
Pesticide(Class(Type))	8	1.924	0.025	0.173
Residual	33			0.360

410

411 **Table S2.**

412 Model parameters of GENEEC version 2 used to generate environmentally relevant pesticide concentrations (Peak EEC [estimated
 413 environmental concentration]) used in the experiment.

	Triazine herbicides			Chloroacetanilide herbicides			Carbamate insecticides			Organophosphate insecticides		
	Atrazine	Propazine	Simazine	Acetochlor	Alachor	Metolachlor	Aldicarb	Carbaryl	Carbofuran	Chlorpyrifos	Malathion	Terbufos
Model Parameter Inputs												
Trade name	Aatrex	Milocep	Princel 4L	Harness	Bullet	Dual II Magnum	Temik	Sevin 80S	Furadan	Dursban 50W	Fyfanon ULV	Counter 15G
Crop	Corn	Sorghum	Corn	Corn	Corn/ sorghum	Corn	Potatoes	Corn/ sorghum	Tobacco/ Barley	Turfgrass	Mosquito control	Corn
Application Rate (lbs a.i./acre)	2	2	4.4	3	2.8125	2.3875	3	2	1.624	8	6	7.395
Number of applications	1	1	1	1	1	1	1	4	1	1	1	1
Days between applications	-	-	-	-	-	-	-	7	-	-	-	-
K _d	-	-	1.96 ^a	3.03 ^a	-	-	0.053 ^a	-	1.23 ^a	-	-	-
K _{oc}	100 ^a	65 ^d	-	-	170 ^a	200 ^a	30 ^a	300 ^a	-	6070 ^a	1248 ^b	500 ^a
Soil half-life (d)	300 ^b	231 ^c	100 ^e	84 ^a	49 ^c	56 ^c	72 ^a	21 ^a	120 ^a	30.5 ^b	6 ^c	5 ^b
Wetted application?	No	No	No	No	No	No	No	No	No	No	No	No
Application method	Ground spray	Ground spray	Ground spray	Ground spray	Ground spray	Ground spray	Granular (2 inches)	Ground spray	Aerial	Ground spray	Ground spray	Granular (surface)
No spray zone (ft)	0	0	0	0	0	0	0	0	0	0	0	0
Solubility (mg/L)	33	8.5 ^b	5 ^a	223	242	530	6000 ^a	40 ^a	320 ^a	2 ^a	130 ^a	5
Aquatic half-life (d)	742 ^c	462 ^f	700 ^e	12 ^g	98	-	10 ^a	10 ^a	57 ^a	-	-	3.5 ^c
Hydrolysis half-life (d)	-	-	-	-	-	210	-	-	-	78 ^c	147 ^c	-
Photolysis half-life (d)	335 ^d	-	-	-	-	71	12 ^b	45	5 ^b	28 ^c	-	-
Peak EEC (ppb)	102	106	202	123	127	105	91	219	209	64	101	171

414 a Exotoxnet

415 b USDA

416 c Spectrum Laboratories

417 d USEPA fact sheet

418 e Pesticide Action Network

419 f Two times the soil half-life

420 g <http://pmep.cce.cornell.edu/profiles/herb-growthreg/24-d-butylate/acetochlor/new-ai-acetochlor.html>