1    **Consistent Effects of Pesticides on Community Structure and Ecosystem Function in**

2    **Freshwater Systems**

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19    Data Availability: Data will be made available via figshare upon acceptance of the publication.

ABSTRACT

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21    Predicting ecological effects of contaminants remains challenging because of the sheer number

22    of chemicals and their ambiguous role in biodiversity-ecosystem function relationships. We

23    evaluated responses of pond ecosystems to standardized concentrations of 12 pesticides, nested

24    in four pesticide classes and two pesticide types. We show consistent effects of herbicides and

25    insecticides on ecosystem function, but slightly less consistent effects on community

26    composition. Effects of pesticides on ecosystem functions were often mediated by changes to

27    biodiversity, and our analyses show that consistency in effects of pesticide types on functions

28    was driven by functional redundancy among species. These results suggest that risk assessment

29    of the thousands of registered chemicals on ecosystem responses could be simplified to a smaller

30    number of chemical groups and to groups of functionally redundant taxa.

31      Freshwater systems are the most biodiverse in the world and provide important

32  ecosystem services (*1*), yet they are imperiled by pesticide contamination (*2*). Two major

33  challenges, among many, impede prediction of responses of freshwater ecosystems to pesticides.

34  First, the extent to which individual pesticides have consistent effects on ecosystem functions

35  and biodiversity is unknown. In the U.S. and Europe, tens of thousands of synthetic chemicals

36  are registered, and in the U.S. >350 pesticides are applied annually (*3*). If the effects of pesticides

37  are consistent within 'pesticide classes' (those with similar chemical structures) or 'pesticide

38  types' (those targeting similar pests), then the complexity in predicting impacts of pesticides

39  could be markedly reduced (*4*). Such consistency would improve efficiency of risk assessment

40  and allow a greater focus on exceptions to general patterns. Second, the role of pesticides in

41  biodiversity-ecosystem function relationships has not been elucidated (*5*). Historically, random

42  and direct manipulations of single-trophic level communities and measurement of associated

43  ecosystem processes (*6*) have established causality between biodiversity and ecosystem function

44  (*7*). However, this approach overlooks the importance of anthropogenic factors, whose

45  influences on communities are far from random (*8*), alter multiple trophic levels (*9*), and occur

46  via direct and indirect pathways (*5*).

47      In an effort to suggest improvements to risk assessment, the objectives of the current

48  study were to: 1) evaluate the consistency of effects across pesticide types, classes, and

49  individual pesticides on ecosystem processes and communities, 2) assess whether the effects of

50  pesticides on ecosystem processes and communities were the result of sublethal, non-target

51  effects or changes in abundance of 'targeted taxa', and 3) determine if disruptions in ecosystem

52  processes from pesticides were mediated by changes in biodiversity. We propose three

53  hypotheses. First, ecosystem processes respond consistently to different pesticides within

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54     pesticide types because taxonomically related community members often have similar functional

55     roles within the ecosystem. So, reductions in the abundance of taxa of a single group (e.g., green

56     algae) might be specific to an individual pesticide or class, but these reductions would result in

57     similar effects on ecosystem function overall (e.g., photosynthesis) (*10*). Second, communities

58     respond consistently to pesticides within classes because of taxa-specific sensitivities to

59     pesticides (*11*). Third, disruptions in ecosystem processes are mediated by changes to

60     biodiversity.

61         We used 72 outdoor mesocosms to evaluate the effects of two control treatments (water

62     and solvent), four simulated-pesticide treatments, and 12 pesticides on tri-trophic temperate pond

63     communities. The pesticide treatments were nested in four classes (organophosphates,

64     carbamates, chloroacetanilides, triazines) and two types (insecticides and herbicides) (Fig. 1a,b).

65     To represent pesticide runoff following rainfall, pesticides were applied singly at the beginning

66     of the experiment at standardized environmentally relevant concentrations calculated using U.S.

67     EPA software. Simulated-pesticide treatments were top-down or bottom-up food web

68     manipulations that attempted to mimic direct (i.e. lethal) effects of actual herbicides and

69     insecticides on algae and zooplankton abundances, respectively. Mesocosm studies are an

70     efficient approach to toxicity testing as they provide toxicity data on multiple species

71     simultaneously under environmentally realistic conditions (Supplemental Text).

72         Pesticide type explained 46% of the variation in ecosystem function associated with the

73     pesticide treatments (Table S1, Fig. 1c). Herbicides were associated with a decrease in suspended

74     phytoplankton that led to increased abundance of attached periphyton, likely through an increase

75     in light availability (Fig. 1d,e). With decreased phytoplankton, pH at dusk and dissolved oxygen

76   at dawn and dusk decreased (Fig. 1d,e), reflecting reduced primary productivity relative to whole

77   system respiration, which might have been driven by the decomposition of dead phytoplankton.

78        In contrast to herbicide-exposed systems, insecticide-exposed systems had an increase in

79   phytoplankton, which shaded and thus decreased periphyton (Fig. 1d,e), an effect shown in other

80   studies (*11*). Increases in phytoplankton were likely driven by shifts in zooplankton communities

81   away from cladocerans and towards copepods (described below). The corresponding increase in

82   dissolved oxygen at dawn and dusk and in acidity at dawn reflects increased respiration and

83   primary production (Fig. 1d,e). While some variation in ecosystem responses was also explained

84   by individual pesticides, it was small relative to variation explained by pesticide type and was

85   driven by two pesticides (e.g., alidcarb within carbamates, chlorpyrifos within organophosphates;

86   Fig. 1c).

87        We tested for the effects of individual pesticides, classes, and types separately on the

88   single-trophic-level zooplankton community (six zooplankton genera) and on the tri-trophic

89   community (insect and salamander predators, snail and anuran herbivores, and periphyton and

90   phytoplankton primary producers). Similar to ecosystem function, pesticide type explained the

91   majority of the variance (44.2%) in the zooplankton community, followed by pesticide class

92   (18.8%) (Table S1). Distance-based redundancy analysis (dbRDA) showed that: 1) herbicide-

93   and insecticide-treated mesocosms had distinct zooplankton communities, 2) within their

94   respective pesticide types, organophosphate insecticides, chloroacetanilide herbicides, and

95   triazine herbicides caused further distinction in zooplankton communities, and 3) there was

96   relatively high multivariate dispersion within the carbamate class (Fig. 2a). In response to

97   insecticides, cladoceran zooplankton were virtually eliminated, likely leading to competitive

98   release of copepods (Fig. 2b,c). Reduced cladocerans, which are more efficient phytoplankton

99   grazers than copepods (*12*), likely drove the increased relative abundance of phytoplankton in the

100  algal community (Fig. 1c). In contrast to the changes in community composition associated with

101  insecticides, herbicides decreased zooplankton abundance with no apparent change in

102  composition (Fig. S3), likely by reducing phytoplankton (i.e., bottom-up effect). The stronger

103  bottom-up effect of triazines compared to chloroacetanilide herbicides on zooplankton was likely

104  because of longer environmental persistence (soil half-lives 110-146 d vs. 14-26 d, respectively

105  [Pesticide Action Network Pesticide Database]). Thus, consistent with the ecosystem function

106  results, these findings on the zooplankton community suggest that ecological risk assessment can

107  be largely simplified to generalized effects of pesticide type or class.

108         In the tri-trophic community, variation explained by pesticides was about equally

109  distributed among type, class, and individual pesticide (Table S1). The dbRDA showed that: 1)

110  herbicide- and insecticide-treated mesocosms had distinct communities, 2) within herbicides,

111  triazines and chloroacetanilides classes caused further distinction in communities, and 3) there

112  was relatively high multivariate dispersion in communities exposed to carbamate and

113  organophosphate insecticides (Fig. 3a). Overall, survival of predators was low with insecticides,

114  except for aldicarb (Fig. 3b,c). Amphibian and snail prey generally had greater positive

115  responses to insecticides compared to controls or herbicides (Fig. 3b,c), suggesting benefits from

116  a release from predators, a trend found in other studies (*13*). When we grouped responses of taxa

117  by functional role in the community (algae, herbivores, and predators), the amount of variance

118  accounted for by pesticide type (29%) was almost double the variance accounted for by either

119  pesticide class (17.6%) or individual pesticide (17.3%) (Table S1). From these results we

120  conclude that complexity in predicting the effects of pesticides on communities could be reduced

121  by evaluating responses of functional groups instead of individual taxonomic responses.

122     Next, we evaluated if the effects of pesticides occurred via changes in abundance of

123     'targeted taxa' by examining simulated pesticide treatments. This evaluation was motivated by a

124     call to uncover non-target effects and mechanisms by which anthropogenic drivers alter

125     biodiversity and ecosystem function via direct manipulations of taxa (*6*). With the exception of

126     the bottom-up simulated herbicide, the effects of simulated pesticide treatments on 'targeted

127     taxa' did not match the effects of pesticide classes (pair-wise comparisons Fig. 1-3), likely

128     because of difficulties in sustaining manipulations that matched the magnitude and specificity of

129     actual pesticides (see Supplemental Text and Fig. S3 for details) on species with short generation

130     times. While manipulating species composition has been critical historically in the study of

131     biodiversity-ecosystem function, the same approaches are likely not well-suited for 1) species

132     that can exhibit population dynamics in the timescale of an experiment because they should be

133     able to quickly recover from a manipulation and 2) studies of disturbance because matching the

134     complexity of the effects of actual disturbance is challenging.

135     Only the bottom-up simulated herbicide (i.e., triple shade cloth) was effective at reducing

136     the targeted taxa, as it significantly reduced algae (t=2.009, *p*=0.015, Fig. 1). When we compared

137     this treatment to triazine herbicides, there were minor differences in effects on ecosystem

138     function and community composition, but only in magnitude, not direction, and neither triazines

139     nor bottom-up simulated herbicides led to detectable lethal effects on non-target taxa. Bottom-up

140     simulated herbicides and triazines grouped together in the tri-trophic community cluster diagram

141     (Fig. 3c), and triazines were not different from bottom-up simulated herbicide treatments in the

142     zooplankton community analysis (Fig. 2c). These results suggest that the observed effects of

143     triazines on freshwater ecosystems were driven predominantly by direct toxicity to targeted taxa

144     and their associated indirect effects, rather than non-target effects.

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145    Path models revealed that effects of herbicides on ecosystem functions, such as primary

146    productivity and respiration, were mediated by their effects on biodiversity (Fig. 4a; via changes

147    in evenness of algae and not richness, Fig. S5). In contrast, although insecticides influenced

148    biodiversity (Fig. 4c), insecticides primarily altered ecosystem function via the direct path, which

149    captures both direct effects of pesticides (e.g., biogeochemical alterations to carbon cycle) and

150    indirect effects mediated by unmeasured aspects of biodiversity (e.g., microbiota). The absence

151    of a significant biodiversity-mediated effect of insecticides is likely because insecticides directly

152    reduced species (e.g., predators, zooplankton) that contributed little to the ecosystem functions

153    we measured (photosynthesis, respiration, decomposition). Given that species responses showed

154    greater variability to pesticides compared to ecosystem functions and pesticide-induced changes

155    to ecosystem functions were at times mediated by changes to biodiversity, the observed

156    consistency in the responses of ecosystem functions to pesticides within pesticide types is likely

157    driven by functional redundancies of species. Indeed, when we simplified our community by

158    functional roles, pesticide type explained almost twice the variance as pesticide class or

159    individual pesticide (Table S1).

160        Ecological risk assessment, made complex by tens of thousands of synthetic chemicals

161    and diverse species assemblages, could be simplified by assessing groups of chemicals that share

162    similar chemical structures or environmental targets and examining responses of functional

163    groups of taxa (*14*). A similar approach is taken by Quantitative Structure-Activity Relationships

164    (QSAR) (*15*), which attempts to assess toxicity of synthetic chemicals using information on

165    compound structure. The clustering of pesticides in this study was not consistent with predictions

166    from QSAR models, likely because predictions are based on few model organisms and do not

167    include indirect effects (Supplemental Text). Nevertheless, this study suggests that simplifying

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168   groups of commercial chemicals that share similar chemical structures or environmental targets

169   can be extended from individuals to ecosystems, which would improve predictions and provide

170   more time and resources to identify potential exceptions to general patterns. Before these

171   changes are adopted by risk managers, additional testing would be warranted to evaluate if the

172   patterns observed here are consistent across additional contaminants, biomes, and community

173   compositions and structures.

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232 **Figure 1. Experimental design and similarity of ecosystem responses by pesticide type.**

233 **A)** Food web diagram of experimental communities. **B)** Experimental design showing

234 hierarchical structure of treatments. Each treatment was replicated four times with mesocosm as

235 the replicate. **C)** Distance-based redundancy analysis (dbRDA) plot of multivariate ecosystem

236 responses showing differences among treatments grouped by pesticide type. Individual points are

237 the centroids of 18 treatments in the experiment. For pair-wise comparisons, treatments sharing

238 letters are not different from each other. **D)** Vector overlay of ecosystem responses for the

239 corresponding dbRDA plot. The gray circle corresponds to vector lengths that would have a

240 correlation coefficient of one with each axis. **E)** Cluster diagram of experimental treatments and

241 ecosystem-level responses showing grouping of experimental treatments according to pesticide

242 type.

243

244 **Figure 2. Zooplankton communities respond consistently to pesticides within type, class,**

245 **and individual pesticide. A)** dbRDA plot, **B)** vector overlay, and **C)** cluster diagram of

246 multivariate zooplankton densities showing differences among type, class, and individual

247 pesticide.

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249 **Figure 3. Insecticides generally reduce insect predators in tri-trophic communities,**

250 **resulting in increases in the survival and growth of their prey. A)** dbRDA plot, **B)** vector

251 overlay, and **C)** cluster diagram of multivariate community responses showing differences

252 among type, class, and individual pesticide. When responses within tri-trophic communities are

253 grouped by functional role (algae, herbivores, predators), type explains twice as much variation

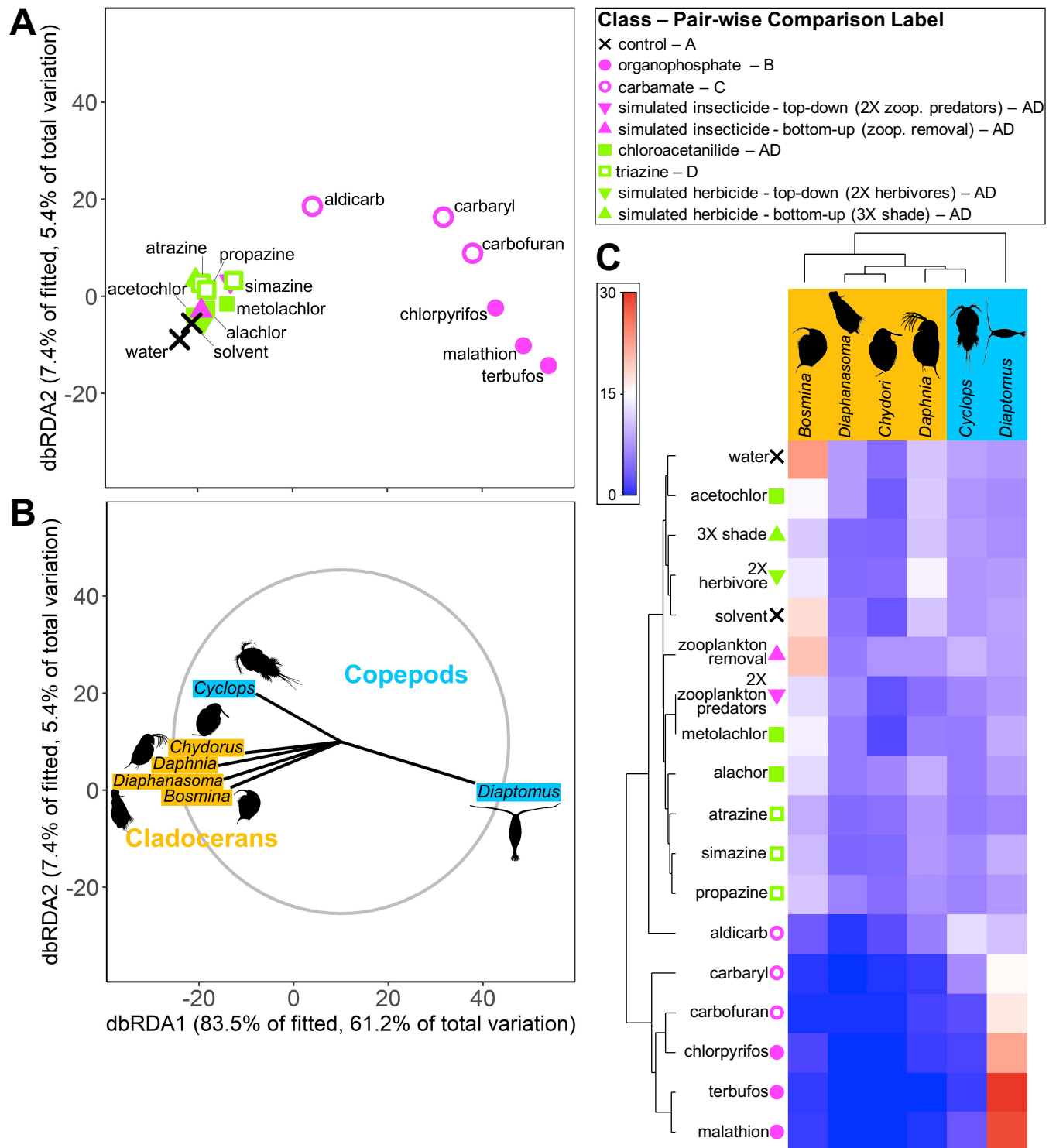254 as class and individual pesticide (Table S1).

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**Figure 4. Path models among pesticides, biodiversity, and ecosystem function.** Relationships

among Shannon diversity, ecosystem functions, and **A)** herbicides or **C)** insecticides, and

associated scatterplots of Shannon diversity and ecosystem functions for the **B)** herbicides and

controls and **D)** insecticides and controls. Herbicides affected ecosystem function by increasing

evenness and reducing primary productivity, whereas there was no detectable relationship for

insecticides. Solid arrows are significant paths, and dotted arrows are non-significant paths. P-

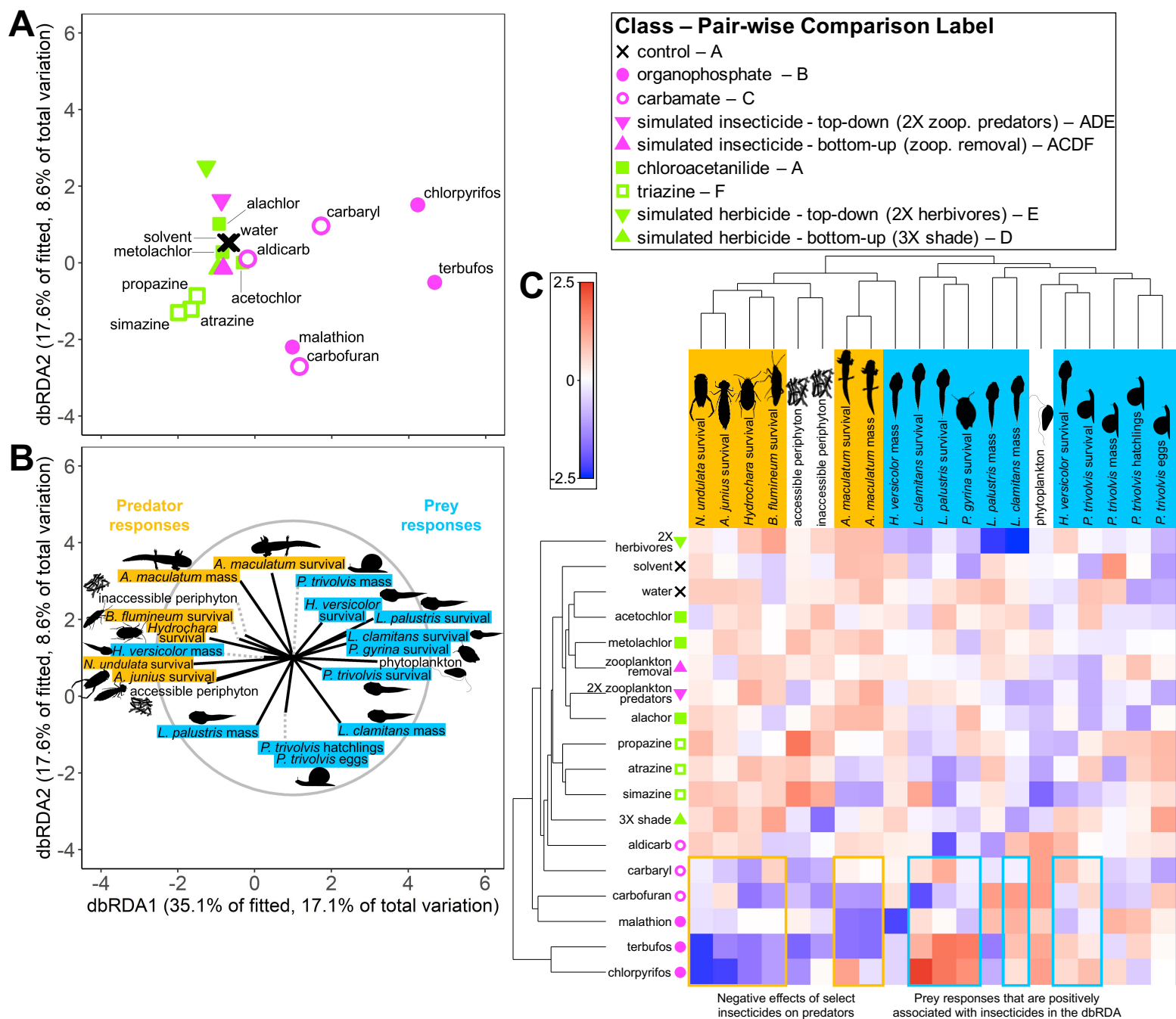values, standardized coefficients, and conditional $R^2$ values are provided.

263     Figure 1.
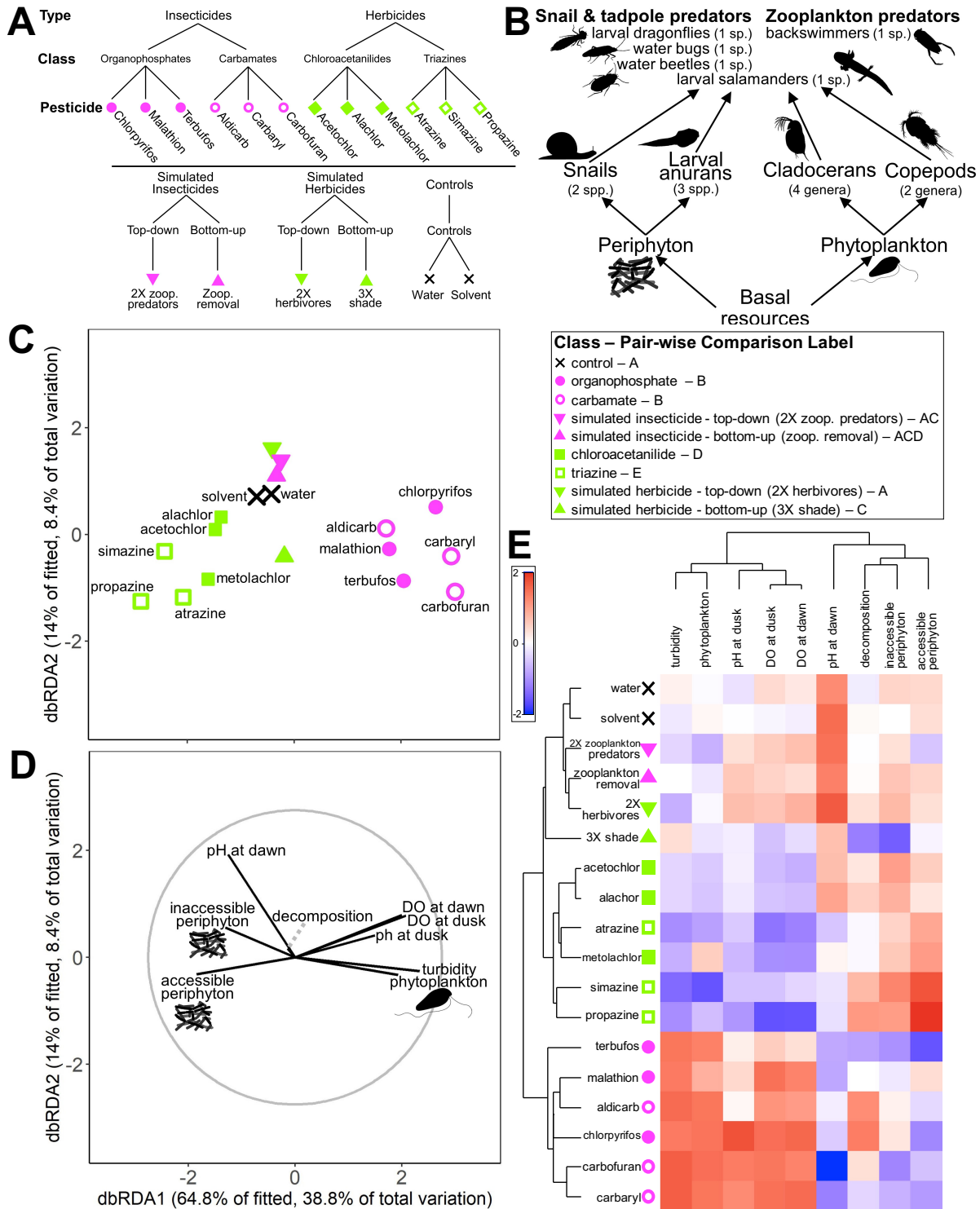
264     Figure 2.

265    Figure 3.

266    Figure 4.