

## RESEARCH OUTPUTS / RÉSULTATS DE RECHERCHE

### Community composition modifies direct and indirect effects of pesticides in freshwater food webs

Zhao, Qinghua ; De Laender, Frederik; Van den Brink, Paul J.

*Published in:*  
Science of the Total Environment

*DOI:*  
[10.1016/j.scitotenv.2020.139531](https://doi.org/10.1016/j.scitotenv.2020.139531)

*Publication date:*  
2020

*Document Version*  
Peer reviewed version

[Link to publication](#)

*Citation for pulished version (HARVARD):*  
Zhao, Q, De Laender, F & Van den Brink, PJ 2020, 'Community composition modifies direct and indirect effects of pesticides in freshwater food webs', *Science of the Total Environment*, vol. 739, 139531.  
<https://doi.org/10.1016/j.scitotenv.2020.139531>

#### General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

#### Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

1 **Community composition modifies direct and indirect effects of pesticides in freshwater**  
2 **food webs**

3 Quinghua Zhao<sup>a\*</sup>, Frederik De Laender<sup>b</sup> and Paul J. Van den Brink<sup>a,c</sup>

4 <sup>a</sup> Aquatic Ecology and Water Quality Management group, Wageningen University and  
5 Research, P.O. Box 47, 6700 AA Wageningen, The Netherlands

6 <sup>b</sup> Research Unit of Environmental and Evolutionary Biology, Namur Institute of Complex  
7 Systems, and Institute of Life, Earth, and the Environment, University of Namur, Rue de  
8 Bruxelles 61, 5000, Namur, Belgium

9 <sup>c</sup> Wageningen Environmental Research, Wageningen University and Research, P.O. Box 47,  
10 6700 AA Wageningen, The Netherlands

11 \*Corresponding author, Email: qinghua.zhao@wur.nl

12

13 **Abstract**

14 For environmental risk assessment, the effects of pesticides on aquatic ecosystems are often  
15 assessed based on single species tests, disregarding the potential influence of community  
16 composition. We, therefore, studied the influence of changing the horizontal (the number of  
17 species within trophic levels) and vertical composition (number of trophic levels) on the  
18 ecological effects of the herbicide linuron and the insecticide chlorpyrifos, targeting producers  
19 and herbivores, respectively. We tested how adding, to a single primary producer, 4 selected  
20 competing producer species, 0-1-4 selected herbivore species, and one selected predator  
21 species resulting in 1, 2 and 3 trophic levels, changes the effects of the two pesticides.

22 Linuron decreased producer biovolume less (17%) when the 4 producers were added,  
23 because insensitive producers compensated for the loss of sensitive producers. However,  
24 linuron decreased producer biovolume 42% and 32% more as we increased the number of

25 herbivore species from 0 to 4 and as we increased trophic levels from 1 to 3, respectively. The  
26 indirect negative effect of linuron on herbivore biovolume was 11% and 15% lower when more  
27 producer and herbivores were added, respectively. Adding a predator increased this indirect  
28 negative effect by 22%.

29 Chlorpyrifos decreased herbivore biovolume about 10% less when adding multiple herbivore  
30 or producer species. However, adding a predator magnified the direct negative impact on  
31 herbivores (13%). Increasing the number of producer, herbivore species and adding trophic  
32 levels increased the indirect positive impact on producer biovolume (between 10% and 35%).

33 Our study shows that changing horizontal composition can both increase and decrease the  
34 effects of the selected pesticides, while changing vertical composition by adding number of  
35 trophic levels always increased these effects. Therefore, single species sensitivity will not  
36 always represent a worst case estimate of ecological effects. Protecting the most sensitive  
37 species may not ensure protection of ecosystems.

38 **Keywords:**

39 Producers, Herbivores, Vertical composition, Linuron, Chlorpyrifos

40

41 **1. Introduction**

42 Ecological risk assessment of chemicals is mainly based on the results of single-species  
43 laboratory tests performed with algae, daphnia and fish, representing a limited set of standard  
44 test species (Artigas et al., 2012; Brock et al., 2006; Rohr et al., 2016). However, community  
45 composition in natural ecosystems often is more complex and how to address this difference

46 in community composition is considered one of the most difficult challenges in ecotoxicology  
47 (De Laender and Janssen, 2013; Rohr et al., 2016; Van den Brink et al., 2018). Community  
48 composition in natural systems can be characterised in two dimensions: the number of species  
49 within trophic levels (horizontal composition) and number of trophic levels (vertical  
50 composition). Both dimensions of composition could influence the effects of chemicals on  
51 aquatic communities (Baert et al., 2016; De Laender et al., 2015; Zhao et al., 2019). Recent  
52 work showed that the two dimensions had contrasting effects on the short-term stability of  
53 whole food webs (using total biomass as a proxy) after pesticide exposure (Zhao et al., 2019).  
54 However, how the two dimensions influence direct and indirect effects of chemicals after  
55 prolonged exposure is at present unknown.

56 The direct negative effects of herbicides on population size of primary producers (hereafter  
57 named 'producers') can be smaller when more producer species are added (Baert et al., 2016).  
58 A more diverse producers' community can include both sensitive and tolerant producers  
59 (Baert et al., 2016). When environmental stressors reduce the population of sensitive  
60 producers, negative interactions among producers result in competitive release, so that  
61 reductions in populations of sensitive species can be compensated by an increase of tolerant  
62 species (Baert et al., 2016; De Laender et al., 2016; Gonzalez and Loreau, 2009). In contrast,  
63 the direct negative effects of herbicides on producer populations can be larger as more  
64 herbivore species are added, because herbicides and herbivore grazing could interact to  
65 aggravate the herbicide effects (Halstead et al., 2014; Rohr et al., 2006; Rohr and Crumrine,  
66 2005). Conversely, the presence of a predator could suppress the herbivore population  
67 (Anderson et al., 1996; Pace et al., 1999), and the resulting decrease in grazing pressure could  
68 alleviate the direct negative effects of herbicides on producers.

69 The direct negative effects of insecticides on herbivores (population size) can be smaller as  
70 more herbivore species are added, again due to compensation. A more diverse herbivores'  
71 community can include both sensitive and tolerant herbivores, while more intolerant  
72 herbivores have larger probability to be included (Becker and Liess, 2017). Insecticides  
73 decrease the populations of sensitive herbivores, resulting in its resource (producers) being  
74 released from grazing, which in turn can result in an increase of tolerant herbivores via an  
75 increase of food resources (Rohr and Crumrine, 2005). The indirect benefit of insecticides on  
76 tolerant herbivores can thus compensate the decline of sensitive herbivores . The insecticide  
77 can also be hypothesized to affect herbivores less when more producer species are added,  
78 because of an increased probability that an edible producer would occur that promotes  
79 herbivore growth (Haddad et al., 2011). However, the insecticide could affect herbivore  
80 population size more severely when a predator is present, because of synergistic interactions  
81 between the insecticide and predation (Beketov and Liess, 2006; Relyea and Mills, 2001;  
82 Trekels et al., 2013). For example, Relyea and Mills (2001) reported that the pesticide carbaryl  
83 was 4 times more toxic to the prey (tadpoles) when a predator (*Ambystoma maculatum*) was  
84 present. Some studies, however, showed that interactions between insecticides and presence  
85 of a predator on herbivores can be additive or antagonistic (Campero et al., 2007; Janssens  
86 and Stoks, 2017, 2013; Trekels et al., 2011).

87 The indirect effects of pesticides are also expected to depend on horizontal and vertical  
88 composition. Herbicides could indirectly decrease herbivore population size, due to a  
89 decrease in edible producer biomass (Bracewell et al., 2019; Fleege et al., 2003; Preston,  
90 2002). We expect that the herbicides could decrease herbivores even more when a predator  
91 is present, due to an increase of both bottom-up and top-down control (Clements and Rohr,  
92 2009; Rohr et al., 2006; Rohr and Crumrine, 2005). In addition, insecticides could, indirectly,

93 induce an increase of producer population size, because of the top-down induced release of  
94 producers (Clements and Rohr, 2009; Halstead et al., 2014; Rohr et al., 2006; Rohr and  
95 Crumrine, 2005). It is thus expected that the release of producers could be stronger when a  
96 predator is present as this will serve as an extra top-down effect.

97 To test these hypotheses, we conducted microcosm experiments mimicking planktonic food  
98 webs in which we added 4 selected competing producer species to a single producer, 0, 1 or  
99 4 selected herbivore species, and one selected predator species. By doing so we also changed  
100 vertical composition (1, 2 and 3 trophic levels). We then tested whether horizontal and vertical  
101 composition influences either the effects of the herbicide linuron or the effect of insecticide  
102 chlorpyrifos

103

## 104 **2. Materials and Methods**

### 105 *2.1. Experimental conditions*

106 We experimentally tested the effect of horizontal and vertical composition on simple food  
107 webs exposed to pesticides. The experiments, which lasted for 21 days, were performed in  
108 900 mL glass jars, filled with 500 ml WC medium and contained in a water bath at constant  
109 temperature ( $19.9\text{ }^{\circ}\text{C} \pm 0.8\text{ }^{\circ}\text{C}$ ) and a light regime of 12h: 12h (light: dark). The light intensity  
110 at the surface (measured with a LI-COR LI-250A, LI-COR Biosciences, Lincoln, USA) was  $120$   
111  $\mu\text{mol m}^{-2}\text{ s}^{-1}$ , and was created using Ceramalux® Phillips 430 Watt High Pressure Sodium Non-  
112 Cycling Lamps.

### 113 *2.2. Organisms*

114 We obtained all producers and herbivores from cultures present at the Aquatic Ecology and  
115 Water Quality Management group of Wageningen University. Five green algae (*Scenedesmus*  
116 *acutus*, *Chlorella vulgaris*, *Desmodesmus pannonicus*, *Raphidocelis subcapitata* and  
117 *Scenedesmus obliquus*) were randomly selected as producers while four cladoceran (*Daphnia*  
118 *magna*, *Daphnia pulex*, *Daphnia lumholtzi* and *Moina macrocopa*) species were randomly  
119 selected as herbivores. All these organisms were collected from Dutch lakes or ditches and  
120 then cultivated in the lab. The algae were cultured in WC medium under continuous light. The  
121 herbivores were cultured in RT medium using a natural day/night light rhythm (Tollrian, 1993)  
122 and fed with algae *C. vulgaris* at  $10^{-5}$  cell ml<sup>-1</sup> day<sup>-1</sup>. One individual of *Chaoborus obscuripes*  
123 was selected as a predator. *C. obscuripes* was collected from Sinderhoeve Experimental  
124 Station ([www.sinderhoeve.org](http://www.sinderhoeve.org); Renkum, The Netherlands). Before addition, *C. obscuripes* was  
125 kept in a 5 L plastic bucket with 1.5 L pond water and 1.5 L WC medium, stored in a fridge (4-  
126 7 °C) to slower the moulting and fed with cladocerans every three days. Before the  
127 experiments started, herbivores and predators were separately moved into WC medium to  
128 starve for 24 h, so that their guts were cleared of pre-fed food.

### 129 2.3. Experimental setup

130 To a single randomly selected primary producer (*R. subcapitata*), we added 4 producers, 0, 1,  
131 or 4 selected herbivores, and one selected predator resulting in 1, 2 and 3 trophic levels  
132 (vertical composition). The other four producers were *S. acutus*, *C. vulgaris*, *D. pannonicus* and  
133 *S. obliquus*. The single herbivore was *M. macrocopa* (randomly assigned). The other three  
134 herbivores were *D. magna*, *D. pulex*, and *D. lumholtzi*. The predator was *C. obscuripes*.

135 We adopted a design where we manipulated horizontal and vertical composition, as well as  
136 the exposure to contaminants. To manipulate composition, we crossed horizontal

137 composition of the producers (two levels; 1 or 5 species) and horizontal composition of the  
138 herbivores (three levels; 0, 1 or 4 species), resulting in 6 food-web structures. When  
139 consumers were present, we also manipulated the presence of a predator (absent or present),  
140 resulting in 4 more food-web structures. We therefore also manipulated vertical composition  
141 (1, 2 and 3 trophic levels). This gives a total of 10 different food-web structures (Table S1). To  
142 manipulate exposure to contaminants, we either exposed these compositions to the  
143 insecticide chlorpyrifos (0 and 1  $\mu\text{g l}^{-1}$ ), or the herbicide linuron (0 and 100  $\mu\text{g l}^{-1}$ ). The 0  $\mu\text{g l}^{-1}$   
144 linuron and chlorpyrifos treatments served as controls. The nominal concentration of 100  $\mu\text{g l}^{-1}$   
145 linuron was chosen because it is higher than the 72d  $\text{EC}_{50}$  for relative growth inhibition of 6  
146  $\mu\text{g l}^{-1}$  for *Scenedesmus acutus* (Snel et al., 1998) and lower than the 21 days NOEC value (180  
147  $\mu\text{g l}^{-1}$ ) for reproduction of *D. magna* (Crane et al., 2007). It was expected that the  
148 concentration had no direct toxic effect on herbivores but only on producers (Cuppen et al.,  
149 1997; Slijkerman et al., 2005). The nominal chlorpyrifos concentration of 1  $\mu\text{g l}^{-1}$  is the 48h  $\text{LC}_{50}$   
150 value for *D. magna* (Kersting and van Wijngaarden, 1992), so that treatment effects were  
151 supposed to not completely eliminate the herbivores and allow recovery (Daam et al., 2008;  
152 Van den Brink et al., 1996).

153 We replicated each treatment four times, leading to 10 food web structures x 2 contaminants  
154 x 2 treatments (control and contaminant treatment) x 4 replicates = 160 vessels in total. The  
155 initial total biovolume of producer and herbivores was always 25  $\text{mm}^3$  and 0.2  $\text{mm}^3$ ,  
156 respectively, regardless of producer and herbivores richness. For the systems with all three  
157 trophic levels, we added one individual of the predator *C. obscuripes* to each system. We made  
158 sure the predators used in the experiments had a mean ( $\pm\text{SD}$ ) individual body length of 10.46  
159  $\pm 0.11$  mm to avoid a bias introduced by body size-dependent feeding rates.

160 *2.4. Chemical application and analysis*

161 All stock solutions for linuron and chlorpyrifos were created in a same way that 5 mL of stock  
162 solution was diluted with WC medium to reach the desired concentration. For the stock  
163 solution of linuron, we diluted the commercial product Afalon® Flow with a linuron  
164 concentration of 450 mg ml<sup>-1</sup> to 10 µg ml<sup>-1</sup>. The stock solution of chlorpyrifos was achieved by  
165 diluting a commercial formulation Dursban® 4E, with a chlorpyrifos concentration of 480 mg  
166 ml<sup>-1</sup> to 0.1 µg ml<sup>-1</sup>. Then 5 mL of stock solution was added into the system. Each system was  
167 filled WC medium up to 500 ml and stirred 15 seconds immediately before the start of the  
168 experiment.

169 To monitor the chemical degradation during the experiment samples were taken after 1h, 2,  
170 4, 6, 14 and 21 days of exposure. In order to analytically verify the linuron concentration of  
171 each experimental jar, 2 mL of water sample was added to 0.5 ml methanol. The chemical  
172 concentration was analysed according to Van den Brink et al. (1997), through Agilent  
173 Technologies LC-QQQ Mass spectrometer with a binary pump, Bin Pump, model G1312A, with  
174 MilliQ + 0.1% fatty acid as solvent A and methanol + 0.1 % fatty acid as solvent B with a ratio  
175 of 20:80. For the chlorpyrifos analysis, 8 mL samples were taken from each system and then 2  
176 ml *n*-hexane was added, followed by vortex for 1 minute under 1000 revolution per second. A  
177 1mL subsample was transferred to a GC vial, then followed by GC and electron capture  
178 detection to determine the exact concentration of chlorpyrifos (Rubach et al., 2011).

179 *2.5. Ecological endpoints*

180 We estimated the biovolume and composition of producers and herbivores, and biovolume of  
181 predators in each replicate on day 2, 4, 6, 14 and 21 day after the beginning of the experiment.  
182 At each sampling day, we first sampled the controls followed by the exposure jars to prevent

183 cross contamination. Producer biovolume ( $\text{mm}^3 \text{ l}^{-1}$ ) was measured with a CASY® Cell Counter  
184 model TT (innovates AG CASY®- Technology). In order to estimate algae composition, 900  $\mu\text{l}$   
185 algae samples were stained with 100  $\mu\text{l}$  lugol preservative for microscopic enumeration of  
186 algal cells using an inverted light-microscope (Nikon Eclipse E100 microscope with a DS-2Mv-  
187 L2 camera; Nikon Corp., Tokyo, Japan) at 200 magnification. Herbivore biovolume ( $\text{mm}^3 \text{ l}^{-1}$ ) in  
188 each replicate was calculated as abundance (individual  $\text{l}^{-1}$ ) times individual biovolume ( $\text{mm}^3$   
189 individual $^{-1}$ ). The individual biovolume ( $\text{mm}^3$  individual $^{-1}$ ) of herbivores and predators were  
190 measured by a formula  $0.074 * L^{2.92}$  (L is length in mm) (Horn, 1991), where the body length  
191 was estimated using light microscopy Olympus szx10 (Olympus Corp, Tokyo, Japan) at 10  
192 magnification. Abundance and composition was recorded after sucking all individuals into an  
193 inverted 10 mL serological pipette to put into 50 ml culture dish that filled with 20 ml WC  
194 medium. Afterwards herbivores and predators were put back in their beakers for next  
195 sampling.

## 196 2.6. Data analyses

197 Biovolume or abundance were used to calculate the effect sizes for the producers, herbivores  
198 and the predator, while chlorophyll  $a$  was used to compute effect size for photosynthetic  
199 capacity. Effects sizes were calculated by dividing the value for the treatment by the mean of  
200 control so that an effect size smaller than 1 indicates a negative impact of the chemical on the  
201 producers, herbivores or the predator, a 1 no effect, while effect sizes larger than 1 indicates  
202 a positive impact. To each of chemicals, three-way ANOVA's were used to estimate the effects  
203 of the horizontal composition of producers and herbivores, the vertical composition and with  
204 all combination of interactions on the effect sizes of producers (abundance, biovolume,  
205 chlorophyll  $a$ ) and herbivores (abundance or biovolume) on sampling days 2, 4, 6, 14 and 21,

206 respectively, yielding 50 three-way ANOVA's (5 response variables × 5 sample days × 2  
207 pesticides). We adopted the same approach for the effect sizes of predator biovolume.  
208 However, note that by definition, in the case of the presence of a predator, the vertical  
209 composition was always three, so we could only analyse the effects of horizontal composition,  
210 yielding 10 two-way ANOVA's (1 response variables × 5 sample days × 2 pesticides). Normality  
211 of model residuals was verified by the QQ-plot (Fig. S1–S2).

212 The effects of herbicide (insecticide) on producers (herbivores) were the largest on day 6 (see  
213 results section). We used raw data (biovolume, density or chlorophyll *a*) on this day to  
214 understand the interactions between treatments. The raw data were natural log-transformed  
215 prior to analysis. For each pesticide data set, we applied four-way ANOVA's to estimate the  
216 effect of horizontal composition of producers and herbivores, vertical composition, pesticide  
217 and their pairwise interactions, on (1) producers (abundance, biovolume and chlorophyll *a*)  
218 and (2) herbivores (abundance and biovolume), respectively, yielding 10 four-way ANOVA's (5  
219 response variables × 2 pesticides). Normality of model residuals was verified by the QQ-plot  
220 (Fig. S3–S4). We adopted the same statistical approach for the effect on predator biovolume.  
221 However, note that by definition, vertical composition is always three when a predator is  
222 present, so we could only analyse the effects of horizontal composition, yielding 2 three-way  
223 ANOVA's (1 response variable × 2 pesticides). Normality of model residuals was again verified  
224 by the quantile–quantile (QQ) plot (Fig. S3–S4). Finally, to evaluate the effects on community  
225 composition on day 6, the day with the maximum effects, we again used four-way ANOVA's  
226 to test the effect of horizontal composition of producers, herbivores and vertical composition,  
227 pesticide and their pairwise interactions, on ln(biovolume) of (1) the producer species (*R.*  
228 *subcapitata*, i.e. the single producer treatment) and (2) the herbivore species (*M. macrocopa*,  
229 i.e. the single herbivore treatment). For the other four producer species (*S. acutus*, *C. vulgaris*,

230 *D. pannonicus* and *S. obliquus*), a three-way ANOVA's was used to test the effect of horizontal  
231 composition of herbivores, vertical composition, pesticides and their pairwise interactions on  
232  $\ln(\text{biovolume})$ , because the horizontal composition of producers was always five. Similarly, we  
233 used three-way ANOVA's to test the effect of horizontal composition of producers, vertical  
234 composition, pesticide and their pairwise interactions on  $\ln(\text{biovolume})$  of each of the rest  
235 three herbivore species (*D. magna*, *D. pulex*, and *D. lumholtzi*), because the horizontal  
236 composition of herbivores was always four. The analysis of community composition yielded  
237 18 ANOVA's (9 response variables  $\times$  2 pesticides). Normality of model residuals was again  
238 verified by the quantile–quantile (QQ) plot (Fig. S5–S6).

239

### 240 **3. Results and discussion**

#### 241 *3.1. Pesticide concentration*

242 The mean start concentrations for linuron and chlorpyrifos in the experimental systems were  
243 94.2 ( $\pm$  8.4)% and 87.8 ( $\pm$  9.4)% of the nominal concentrations, respectively (Fig 1). The  
244 dissipation half-life (DT50) for linuron could not be calculated ( $>$  21 d; Fig 1), while the DT50  
245 of chlorpyrifos was between 5-8 days. The observed persistence of linuron and chlorpyrifos  
246 were in line with those observed in other planktonic systems by Daam et al. (2008, 2009) and  
247 Daam and Van den Brink (2007) who reported DT50 values of  $>$  21 days for linuron and 6-10 d  
248 for chlorpyrifos.

#### 249 *3.2. Effect of linuron*

##### 250 *3.2.1 Influence of community composition on direct effects*

251 Throughout experiments, the direct negative effect of the herbicide linuron on producer  
252 biovolume was, on average, 17% smaller when the 4 producer species were added (Fig. 2a).  
253 However, this direct negative effect was 42% larger when the number of herbivore species  
254 was increased from 0 to 4 and 32% larger when vertical composition was changed from 1 to 3  
255 (Fig. 2b-c). On day 6, linuron had its maximum effect on producer biovolume (Fig. 2a-c). The  
256 negative effect of linuron on producer biovolume was larger when adding more herbivore  
257 species and when vertical composition was higher, regardless of the composition of the  
258 producer community (Fig. 3a-b). The negative effects were strongest when the number of  
259 producer species was lowest, the number of herbivore species highest, and vertical  
260 composition equal to 2 (Fig. 3a). These trends were similar when using chlorophyll *a*  
261 (photosynthetic capacity) as an endpoint (Fig S7. a-c; Fig S8. a-b).

262 Adding producers decreased the direct negative effect on producer biovolume (Fig. 2a) and  
263 chlorophyll *a* (Fig S7. a-c), due to the decrease of sensitive chlorophytes biovolume (e.g. *R.*  
264 *subcapitata* and *C. vulgaris*) compensated by other tolerant chlorophytes (e.g. *S. obliquus*) (Fig.  
265 4a). *R. subcapitata*, previously known as *Selenastrum capricornutum* and *Pseudokirchneriella*  
266 *subcapitata*, has a 5d EC<sub>50</sub> of 67 µg l<sup>-1</sup> based on abundance (USEPA, 2020), explaining its  
267 decrease in biovolume (Fig. 4a). *C. vulgaris* and to a lesser extent *S. acutus* also show a  
268 decrease in biovolume, which can be explained by their 7d EC<sub>50</sub> of 50 µg l<sup>-1</sup> also based on  
269 abundance and 3d EC<sub>50</sub> of 8.9 µg l<sup>-1</sup> based on population growth rate, respectively (Stephenson  
270 and Kane, 1984; USEPA, 2020). The other two species, *D. pannonicus* and *S. obliquus*, showed  
271 no response or an increase in biovolume (Fig. 4a) and, unfortunately, no sensitivity data is  
272 available, but *S. obliquus* became relatively abundant in small plankton dominated  
273 microcosms stressed by 150 µg l<sup>-1</sup> linuron (Daam and Van den Brink, 2007). Some semi-field  
274 experiments also showed that linuron had both positive and negative effects on Chlorophytes

275 (Daam et al., 2009; Slijkerman et al., 2005; Van den Brink et al., 1997). For example, Daam et  
276 al. (2009) reported that some of chlorophytes (e.g., *Coelastrum cambricum* and *Pediastrum*  
277 *duplex*) decreased in population size, which was compensated by increases of other  
278 chlorophytes (e.g., *Ankistrodesmus falcatus*, *Oocystis pusilla* and *Oocystis lacustris*).

279 In contrast, the negative impact of linuron on producers was larger when more herbivores  
280 were added (Fig. 2b), due to a larger suppression of producer biovolume (e.g. *C. vulgaris*) (Fig.  
281 4a) when multiple herbivores were present. Multiple herbivore species more effectively  
282 reduce producer population sizes than a single herbivore species because of larger  
283 consumption rates (Duffy et al., 2003; Naeem and Li, 1998).

284 The presence of a predator (*C. obscuripes*) decreased the biovolume of the herbivores (Fig. 3c-  
285 d; Fig. 4b), as has also been reported by Black and Dodson (1990) and Hebert and Grewe  
286 (1985), due to predation. The presence of the predator hence alleviated the grazing pressure  
287 on producers, which made the direct negative effect of linuron on producers smaller than the  
288 treatments with producers and herbivores only, i.e. vertical composition=2, (Fig. 2c). However,  
289 the presence of the predator did not eliminate all herbivores. Thus, the herbivores still  
290 consumed producers (e.g., especially small sized *C. vulgaris*) (Fig. 4a). Hence, the presence of  
291 a predator and herbivores still made the negative effect of linuron on producers larger than  
292 the negative effect of linuron on producers in treatments where only producers were present,  
293 i.e. when vertical composition was equal to 1, (Fig. 2c).

### 294 3.2.2 Influence of community composition on indirect effects

295 The herbicide-induced decrease of producers led to indirect negative effects on herbivore  
296 biovolume (Fig. 2d-f). Some semi-field experiments found both negative and positive impacts  
297 of linuron on herbivores (Cuppen et al., 1997; Daam et al., 2009). For example, Cuppen et al.

298 (1997) reported negative effects of linuron on Rotatoria but positive effects on Copepoda.  
299 They attributed these negative and positive linuron effects to the preferred resources of these  
300 herbivores, i.e. diatoms for Rotatoria and *Chlamydomonas* for Copepoda, respectively, the  
301 latter showing a large increase in the linuron stressed systems (Van den Brink et al., 1997).  
302 Here, we only found negative effects of linuron on herbivores, which can be attributed to  
303 herbivores consuming all producer species and the overall decrease in biovolume of the algae  
304 species (Fig. 4a) as no adaptation was found like as by Van den Brink et al. (1997).

305 In addition, the indirect negative effect of linuron on herbivore biovolume was 11% smaller  
306 when 4 producers were added and 15% smaller when the number of herbivore species was  
307 increased from 1 to 4 (Fig. 2d-e). This was because adding more producers and herbivores  
308 caused an increase of the absolute biovolume of herbivores on day 6 (Fig. 4b). However, the  
309 indirect negative effect of linuron on herbivores was 22% larger when vertical composition  
310 was changed from 2 to 3 (Fig. 2f), because predation decreased the absolute biovolume of  
311 each herbivore species (Fig. 4b). On day 6, linuron also had its maximum effect on herbivore  
312 biovolume (Fig. 2d-f). The negative effect of linuron on herbivore biovolume was smaller when  
313 more producers and herbivores were present, independent of vertical composition (Fig 3c-d).  
314 The negative effects were smallest when the number of producer species was highest, the  
315 number of herbivore species highest and the vertical composition equal to 2 (Fig 3c). We  
316 detected qualitatively identical results (single and interactive effects) using abundance as a  
317 proxy (Fig S7d–7i; Fig. S8), even though the magnitude of decreases and increases was smaller.  
318 We did not detect significant effect of composition on the predator biovolume (Table S2).

### 319 3.3. Effect of chlorpyrifos

#### 320 3.3.1 Influence of community composition on direct effects

321 As found for linuron, the direct effect of chlorpyrifos also depended on horizontal and vertical  
322 composition. The direct negative effect on herbivore biovolume was, on average, 7% smaller  
323 when the number of herbivore species was increased from 1 to 4 and 12% smaller when 4  
324 producers were added, while the negative direct effect was 13% larger when vertical  
325 composition was changed by adding a predator (Fig. 5a-c). On day 6, chlorpyrifos also had its  
326 maximum effect on herbivore biovolume (Fig. 5a-c). The negative effect of chlorpyrifos on  
327 herbivore biovolume was smaller with adding more producers and more herbivores across  
328 any level of vertical composition (Fig 6a-b). The negative effects were smallest when the  
329 number of producer species was highest, the number of herbivore species highest, and the  
330 vertical composition equal to 2 (Fig 6a).

331 The negative direct effect of chlorpyrifos on herbivores was smaller when adding more  
332 herbivores (Fig. 5b), which was associated with the loss of sensitive herbivores (e.g. *M.*  
333 *macrocopa*, *D. magna* and *D. pulex*) being compensated by other more tolerant herbivores  
334 (e.g. *D. lumholtzi*) (Fig, 7a). Only for *D. magna*, *M. macrocopa* and *D. pulex*, single species  
335 toxicity values could be found with 2-6d EC<sub>50</sub> values of 0.20, 0.27 and 0.21 µg l<sup>-1</sup>, respectively  
336 (Na et al., 2012; USEPA, 2020), explaining their decrease. The compensation of sensitive  
337 species by more tolerant ones has been shown previously (Daam et al., 2008; Van Wijngaarden  
338 et al., 2005). For example, Daam et al. (2008) showed that the decrease of Cladocera (e.g.  
339 *Streblocerus pygmaeus*) by chlorpyrifos was compensated by other tolerant Cladocera (e.g.  
340 *Dunhevedia crassa*).

341 In addition, we found that the direct negative effect of chlorpyrifos on the herbivore  
342 population was smaller when adding more of its resource (i.e. producer) (Fig. 5a), due to  
343 higher producers increasing the biovolumes of herbivores (e.g. *D. lumholtzi*) (Fig. 7a). However,

344 the presence of a predator *C. obscuripes* made the negative effect of chlorpyrifos on  
345 herbivores larger (Fig. 5c; Fig. 6a-b), as expected (Relyea and Mills, 2001; Van den Brink et al.,  
346 2017). For example, Relyea and Mills (2001) showed that, if a predator (*Ambystoma*  
347 *maculatum*) was present, the pesticide carbaryl was 4 times more toxic to the prey (tadpoles).  
348 Predation and chlorpyrifos has similar effects, and can produce synergistic effects when  
349 combined (Relyea and Mills, 2001).

### 350 3.3.2 Influence of community composition on indirect effects

351 The chlorpyrifos-induced decrease of herbivores resulted in indirect positive effects on  
352 producer biovolume (Fig. 5d-f), as found by Daam and Van den Brink (2007). This indirect  
353 positive effect was 10% stronger when 4 producers were added (10% stronger), the number  
354 of herbivore species was increased from 1 to 4 (35% stronger) and vertical composition was  
355 changed from 1 to 3 (33% stronger) (Fig. 5d-f). On day 6, chlorpyrifos also had a maximum  
356 effect on producer biovolume (Fig. 5d-f). The positive effect of chlorpyrifos on producer  
357 biovolume was highest when the number of producer species was highest, more trophic levels  
358 were present and the number of herbivore species equal to 1 (Fig 6c-d). The indirect positive  
359 effect on chlorophyll *a* was qualitatively similar (Fig. S9a-9c; Fig. S10a-b), but no significant  
360 effect of composition on predator biovolume was detected (Table S3). Again, we found  
361 qualitatively identical results (single and interactive effects) using abundance as proxy (Fig.  
362 S9d–9j; Fig. S10c-f).

363 The positive effect on producer biovolume (and chlorophyll *a*) can be understood from the  
364 release of grazing. The decrease of herbivores especially promoted the growth of its producer  
365 food source (e.g. *D. pannonicus*) (Fig. 7b). The increase was reinforced by adding producers,  
366 herbivores and trophic levels (Fig. 7b), making the positive effect on producer biovolume (and

367 chlorophyll  $a$ ) larger (Fig. 5d-f; Fig. S9). Previous studies only reported chlorpyrifos-induced  
368 increase of producers (Daam et al., 2008; Daam and Van den Brink, 2007). For example, Daam  
369 and Van den Brink (2007) showed that chlorpyrifos application decreased herbivore  
370 abundances (Cladocera) and consequently caused an increase in chlorophyll  $a$  levels. We  
371 further showed that the increase of producers could be reinforced by both horizontal and  
372 vertical composition, as explained above.

### 373 3.4. Compositional and diversity effects

374 It should be noted that the compositional effects we report on here cannot be readily  
375 translated to diversity effects. In order to directly test for horizontal and vertical diversity  
376 effects, composition should be replicated within each diversity level, as done in biodiversity-  
377 ecosystem function research (De Laender et al., 2016). Instead, our design should be  
378 understood as a test of the effects of embedding a reference set of selected single producer  
379 and consumer species (*R. subcapitata* and *M. macrocopa* respectively) into a community of  
380 increasing complexity. While we selected this reference set randomly, future works could base  
381 this selection on occurrence frequency. *C. vulgaris* and *D. pulex*, for example, are dominant  
382 green alga and Cladocera in many natural lakes and ponds, and could therefore have been  
383 chosen as our reference set (Cohen and Post, 1993; Hassan and Alkam, 2008; Hebert and  
384 Finston, 2001; Steiner, 2002; Sze, 1980; Tiwari and Chauhan, 2006; Wen et al., 2005).  
385 Speculating how such an alternative selection of the reference set would have changed our  
386 results is not straightforward, as this will depend on both the sensitivity and the ecology of  
387 the species (Baert et al., 2017). When based solely on sensitivity arguments, we do not expect  
388 selecting this different reference set would have changed our results considerably. Indeed,  
389 toxicity data suggest that the consumers *D. pulex* and *M. macrocopa* have similar sensitivities

390 for the chlorpyrifos, as do the producers *R. subcapitata* and *C. vulgaris* herbicide linuron (Table  
391 S4). We further expect that, again based on sensitivity arguments only, our conclusions  
392 regarding composition effects will also be valid when we should have selected other species  
393 (i.e. the producer *S. acutus* and the herbivore *D. magna*), as their sensitivity is similar to that  
394 of our chosen reference set (Table S4).

395

## 396 **5. Conclusions**

397 Our experiment and analyses demonstrate that the direct and indirect effect of pesticides on  
398 aquatic ecosystems depends on horizontal and vertical composition. From these results, the  
399 following main conclusions can be drawn: (1) changing horizontal composition by adding  
400 species to our reference species increased or decreased the (in)direct effect of pesticides,  
401 depending on the type of pesticide used; (2) changing vertical composition by adding trophic  
402 levels always made (in)direct effects larger, regardless of the type of pesticide used. One  
403 important implication of our results is that the effects of pesticides on single species do not  
404 always correspond to worst-case scenarios and that protecting the most sensitive species does  
405 not protect the whole ecosystem. Given that community composition of natural systems  
406 widely varies between and within systems, we call for more research on how horizontal and  
407 vertical composition and diversity affect food-web resistance and resilience. Such studies will  
408 improve our understanding of the interaction between toxicological and ecological  
409 mechanisms, which is greatly needed to improve our understanding of the environmental  
410 impacts of chemicals and their risk assessment (Van den Brink et al., 2018).

411

412 **Declaration of Competing Interest**

413 The authors declare that we have no conflicts of interest to this work and there are no  
414 competing financial interests. We declare that all authors agreed to the contents in  
415 manuscript.

416 **Acknowledgement**

417 We thank Silke Vollbrecht, Frits Gillissen and Marlies Vollebregt for their assistance during the  
418 experiments. We thank Sanne van den Berg, Anna Huang, Lara Schuijt, Markus Hermann and  
419 Annika Mangold-Döring for valuable suggestions. We also thank the cooperation of all other  
420 participants in this study. QHZ is supported by the China Scholarship Council (No.  
421 201606190229).

422 **References**

- 423 Anderson, J.S., Teutsch, M., Dong, Z., Delgado-lo, F., Nu-, F., Vera, J.C., Hajra, A., Schro, E.,  
424 Ried, T., Liu, P.P., Collins, F.S., 1996. A meta-analysis of the freshwater trophic cascade.  
425 Proc. Natl. Acad. Sci. 93, 7723–7726. <https://doi.org/10.1073/pnas.93.15.7723>
- 426 Artigas, J., Arts, G., Babut, M., Caracciolo, A.B., Charles, S., Chaumot, A., Combourieu, B.,  
427 Dahllöf, I., Despréaux, D., Ferrari, B., Friberg, N., Garric, J., Geffard, O., Gourlay-Francé,  
428 C., Hein, M., Hjorth, M., Krauss, M., De Lange, H.J., Lahr, J., Lehtonen, K.K., Lettieri, T.,  
429 Liess, M., Lofts, S., Mayer, P., Morin, S., Paschke, A., Svendsen, C., Usseglio-Polatera, P.,  
430 Van den Brink, N., Vindimian, E., Williams, R., 2012. Towards a renewed research  
431 agenda in ecotoxicology. Environ. Pollut. 160, 201–206.  
432 <https://doi.org/10.1016/j.envpol.2011.08.011>
- 433 Baert, J.M., De Laender, F., Janssen, C.R., 2017. The Consequences of Nonrandomness in  
434 Species-Sensitivity in Relation to Functional Traits for Ecosystem-Level Effects of  
435 Chemicals. Environ. Sci. Technol. 51, 7228–7235.  
436 <https://doi.org/10.1021/acs.est.7b00527>
- 437 Baert, J.M., De Laender, F., Sabbe, K., Janssen, C.R., 2016. Biodiversity increases functional  
438 and compositional resistance, but decreases resilience in phytoplankton communities.  
439 Ecology 97, 3433–3440. <https://doi.org/10.1002/ecy.1601>
- 440 Becker, J.M., Liess, M., 2017. Species Diversity Hinders Adaptation to Toxicants. Environ. Sci.  
441 Technol. 51, 10195–10202. <https://doi.org/10.1021/acs.est.7b02440>
- 442 Beketov, M.A., Liess, M., 2006. The influence of predation on the chronic response of  
443 Artemia sp. populations to a toxicant. J. Appl. Ecol. 43, 1069–1074.  
444 <https://doi.org/10.1111/j.1365-2664.2006.01226.x>
- 445 Black, A.R., Dodson, S.I., 1990. Demographic costs of Chaoborus-induced phenotypic

446 plasticity in *Daphnia pulex*. *Oecologia* 83, 117–122.  
447 <https://doi.org/10.1007/BF00324642>

448 Bracewell, S., Verdonshot, R.C.M., Schäfer, R.B., Bush, A., Lapen, D.R., Van den Brink, P.J.,  
449 2019. Qualifying the effects of single and multiple stressors on the food web structure  
450 of Dutch drainage ditches using a literature review and conceptual models. *Sci. Total*  
451 *Environ.* 684, 727–740. <https://doi.org/10.1016/j.scitotenv.2019.03.497>

452 Brock, T.C., Arts, G.H., Maltby, L., Van den Brink, P.J., 2006. Aquatic risks of pesticides,  
453 ecological protection goals, and common aims in European Union Legislation. *Integr.*  
454 *Environ. Assess. Manag.* 2, e20–e46. <https://doi.org/10.1002/ieam.5630020402>

455 Campero, M., Slos, S., Ollevier, F., Stoks, R., 2007. Sublethal pesticide concentrations and  
456 predation jointly shape life history: Behavioral and physiological mechanisms. *Ecol.*  
457 *Appl.* 17, 2111–2122. <https://doi.org/10.1890/07-0442.1>

458 Clements, W.H., Rohr, J.R., 2009. Community responses to contaminants: using basic  
459 ecological principles to predict ecotoxicological effects. *Environ. Toxicol. Chem.* 28,  
460 1789–1800. <https://doi.org/10.1897/09-140.1>

461 Cohen, I., Post, A.F., 1993. The heterotrophic connection in a photoautotrophic *Chlorella*  
462 *vulgaris* dominant in wastewater oxidation ponds. *Water Sci. Technol.* 27, 151–155.  
463 <https://doi.org/10.2166/wst.1993.0546>

464 Crane, M., Maycock, D., Watts, C.D., Atkinson, C., Johnson, I., 2007. Proposed EQS for Water  
465 Framework Directive Annex VIII substances: 2,4- dichlorophenol.

466 Cuppen, J.G.M., Van den Brink, P.J., Hartgers, E.M., Fettweis, U., Crum, S.J.H., Van Donk, E.,  
467 Brock, T.C.M., 1997. Sensitivity of macrophyte-dominated freshwater microcosms to  
468 chronic levels of the herbicide linuron. *Ecotoxicol. Environ. Saf.* 38, 13–24.  
469 <https://doi.org/10.1006/eesa.1997.1555>

470 Daam, M.A., Rodrigues, A.M.F., Van den Brink, P.J., Nogueira, A.J.A., 2009. Ecological effects  
471 of the herbicide linuron in tropical freshwater microcosms. *Ecotoxicol. Environ. Saf.* 72,  
472 410–423. <https://doi.org/10.1016/j.ecoenv.2008.07.009>

473 Daam, M.A., Van den Brink, P.J., 2007. Effects of chlorpyrifos, carbendazim, and linuron on  
474 the ecology of a small indoor aquatic microcosm. *Arch. Environ. Contam. Toxicol.* 53,  
475 22–35. <https://doi.org/10.1007/s00244-006-0001-y>

476 Daam, M.A., Van den Brink, P.J., Nogueira, A.J.A., 2008. Impact of single and repeated  
477 applications of the insecticide chlorpyrifos on tropical freshwater plankton  
478 communities. *Ecotoxicology* 17, 756–771. <https://doi.org/10.1007/s10646-008-0227-8>

479 Darbro, J.M., Johnson, P.H., Thomas, M.B., Ritchie, S.A., Kay, B.H., Ryan, P.A., 2012. Effects of  
480 *Beauveria bassiana* on survival, blood-feeding success, and fecundity of *Aedes aegypti*  
481 in laboratory and semi-field conditions. *Am. J. Trop. Med. Hyg.* 86, 656–664.  
482 <https://doi.org/10.4269/ajtmh.2012.11-0455>

483 De Laender, F., Janssen, C.R., 2013. Brief communication: The ecosystem perspective in  
484 ecotoxicology as a way forward for the ecological risk assessment of chemicals. *Integr.*  
485 *Environ. Assess. Manag.* 9, e34–e38. <https://doi.org/10.1002/ieam.1428>

486 De Laender, F., Morselli, M., Baveco, H., Van den Brink, P.J., Di Guardo, A., 2015.  
487 Theoretically exploring direct and indirect chemical effects across ecological and  
488 exposure scenarios using mechanistic fate and effects modelling. *Environ. Int.* 74, 181–  
489 190. <https://doi.org/10.1016/j.envint.2014.10.012>

490 De Laender, F., Rohr, J.R., Ashauer, R., Baird, D.J., Berger, U., Eisenhauer, N., Grimm, V.,  
491 Hommen, U., Maltby, L., Melià, C.J., Pomati, F., Roessink, I., Radchuk, V., Van den  
492 Brink, P.J., 2016. Reintroducing Environmental Change Drivers in Biodiversity–

493 Ecosystem Functioning Research. *Trends Ecol. Evol.* 31, 905–915.  
494 <https://doi.org/10.1016/j.tree.2016.09.007>

495 Duffy, J.E., Cardinale, B.J., France, K.E., McIntyre, P.B., Thébault, E., Loreau, M., 2007. The  
496 functional role of biodiversity in ecosystems: Incorporating trophic complexity. *Ecol.*  
497 *Lett.* 10, 522–538. <https://doi.org/10.1111/j.1461-0248.2007.01037.x>

498 Duffy, J.E., Paul, J., Elizabeth, A., 2003. Grazer diversity effects on ecosystem functioning in  
499 seagrass beds. *Ecol. Lett.* 6, 637–645.

500 Fleeger, J.W., Carman, K.R., Nisbet, R.M., 2003. Indirect effects of contaminants in aquatic  
501 ecosystems. *Sci. Total Environ.* 317, 207–233. [https://doi.org/10.1016/S0048-](https://doi.org/10.1016/S0048-9697(03)00141-4)  
502 [9697\(03\)00141-4](https://doi.org/10.1016/S0048-9697(03)00141-4)

503 Gonzalez, A., Loreau, M., 2009. The Causes and Consequences of Compensatory Dynamics in  
504 Ecological Communities. *Annu. Rev. Ecol. Evol. Syst.* 40, 393–414.  
505 <https://doi.org/10.1146/annurev.ecolsys.39.110707.173349>

506 Haddad, N.M., Crutsinger, G.M., Gross, K., Haarstad, J., Tilman, D., 2011. Plant diversity and  
507 the stability of foodwebs. *Ecol. Lett.* 14, 42–46. [https://doi.org/10.1111/j.1461-](https://doi.org/10.1111/j.1461-0248.2010.01548.x)  
508 [0248.2010.01548.x](https://doi.org/10.1111/j.1461-0248.2010.01548.x)

509 Halstead, N.T., McMahan, T.A., Johnson, S.A., Raffel, T.R., Romansic, J.M., Crumrine, P.W.,  
510 Rohr, J.R., 2014. Community ecology theory predicts the effects of agrochemical  
511 mixtures on aquatic biodiversity and ecosystem properties. *Ecol. Lett.* 17, 932–941.  
512 <https://doi.org/10.1111/ele.12295>

513 Hassan, F.M., Alkam, F., 2008. Phytoplankton and related nutrients in sawa lake, iraq. *J.*  
514 *Dohuk Univ.* 11, 67–76. <https://doi.org/10.1016/B978-0-12-410416-7.00009-4>

515 Hebert, P.D.N., Finston, T.L., 2001. Macrogeographic patterns of breeding system diversity in  
516 the *Daphnia pulex* group from the United States and Mexico. *Heredity (Edinb.)* 87, 153–  
517 161. <https://doi.org/10.1046/j.1365-2540.2001.00885.x>

518 Hebert, P.D.N., Grewe, P.M., 1985. Chaoborus-induced shifts in the morphology of *Daphnia*  
519 *ambigua*. *Limnol. Oceanogr.* 30, 1291–1297. <https://doi.org/10.4319/lo.1985.30.6.1291>

520 Horn, W., 1991. The influence of biomass and structure of the crustacean plankton on the  
521 water transparency in the Saidenbach storage reservoir. *Hydrobiologia* 225, 115–120.  
522 <https://doi.org/10.1007/BF00028390>

523 Janssens, L., Stoks, R., 2017. Chlorpyrifos-induced oxidative damage is reduced under  
524 warming and predation risk: Explaining antagonistic interactions with a pesticide.  
525 *Environ. Pollut.* 226, 79–88. <https://doi.org/10.1016/j.envpol.2017.04.012>

526 Janssens, L., Stoks, R., 2013. Synergistic effects between pesticide stress and predator cues:  
527 Conflicting results from life history and physiology in the damselfly *Enallagma*  
528 *cyathigerum*. *Aquat. Toxicol.* 132–133, 92–99.  
529 <https://doi.org/10.1016/j.aquatox.2013.02.003>

530 Kersting, K., van Wijngaarden, R., 1992. Effects of chlorpyrifos on a microecosystem. *Environ.*  
531 *Toxicol. Chem.* 11, 365–372. <https://doi.org/10.1002/etc.5620110310>

532 Na, W., Lili, L., Kaifeng, S., Shunshan, D., 2012. Analysis of structure -activity relationship and  
533 toxicity of organophosphorus pesticide to plankton. *Ecol. Environ. Sci.* 21, 118–123.

534 Naeem, S., Li, S., 1998. Consumer species richness and autotrophic biomass. *Ecology* 79,  
535 2603–2615. [https://doi.org/10.1890/0012-9658\(1998\)079\[2603:CSRAAB\]2.0.CO;2](https://doi.org/10.1890/0012-9658(1998)079[2603:CSRAAB]2.0.CO;2)

536 Pace, M.L., Cole, J.J., Carpenter, S.R., Kitchell, J.F., 1999. Trophic cascades revealed in diverse  
537 ecosystems. *Trends Ecol. Evol.* 14, 483–488.

538 Preston, B.L., 2002. Indirect effects in aquatic ecotoxicology: Implications for ecological risk  
539 assessment. *Environ. Manage.* 29, 311–323. <https://doi.org/10.1007/s00267-001-0023->

540 1

541 Relyea, R.A., Mills, N., 2001. Predator-induced stress makes the pesticide carbaryl more  
542 deadly to gray treefrog tadpoles (*Hyla versicolor*). *Proc. Natl. Acad. Sci. U. S. A.* 98,  
543 2491–2496. <https://doi.org/10.1073/pnas.031076198>

544 Rohr, J.R., Crumrine, P.W., 2005. Effects of an herbicide and an insecticide on pond  
545 community structure and processes. *Ecol. Appl.* 15, 1135–1147.  
546 <https://doi.org/10.1890/03-5353>

547 Rohr, J.R., Kerby, J.L., Sih, A., 2006. Community ecology as a framework for predicting  
548 contaminant effects. *Trends Ecol. Evol.* 21, 606–613.  
549 <https://doi.org/10.1016/j.tree.2006.07.002>

550 Rohr, J.R., Salice, C.J., Nisbet, R.M., 2016. The pros and cons of ecological risk assessment  
551 based on data from different levels of biological organization. *Crit. Rev. Toxicol.* 46,  
552 756–784. <https://doi.org/10.1080/10408444.2016.1190685>

553 Rubach, M.N., Crum, S.J.H., Van den Brink, P.J., 2011. Variability in the dynamics of mortality  
554 and immobility responses of freshwater arthropods exposed to chlorpyrifos. *Arch.*  
555 *Environ. Contam. Toxicol.* 60, 708–721. <https://doi.org/10.1007/s00244-010-9582-6>

556 Slijkerman, D.M.E., Moreira-Santos, M., Jak, R.G., Ribeiro, R., Soares, A.M.V.M., Van Straalen,  
557 N.M., 2005. Functional and structural impact of linuron on a freshwater community of  
558 primary producers: The use of immobilized algae. *Environ. Toxicol. Chem.* 24, 2477–  
559 2485. <https://doi.org/10.1897/04-658R.1>

560 Snel, J.F.H., Vos, J.H., Gylstra, R., Brock, T.C.M., 1998. Inhibition of photosystem II (PSII)  
561 electron transport as a convenient endpoint to assess stress of the herbicide linuron on  
562 freshwater plants. *Aquat. Ecol.* 32, 113–123. <https://doi.org/10.1023/A:1009971930626>

563 Srivastava, D.S., Bell, T., 2009. Reducing horizontal and vertical diversity in a foodweb  
564 triggers extinctions and impacts functions. *Ecol. Lett.* 12, 1016–1028.  
565 <https://doi.org/10.1111/j.1461-0248.2009.01357.x>

566 Steiner, C.F., 2002. Context-dependent effects of *Daphnia pulex* on pond ecosystem  
567 function: Observational and experimental evidence. *Oecologia* 131, 549–558.  
568 <https://doi.org/10.1007/s00442-002-0934-4>

569 Stephenson, R.R., Kane, D.F., 1984. Persistence and Effects of Chemicals in Small Enclosures  
570 in Ponds R. *Arch. Environ. Contam. Toxicol.* 13, 313–326.

571 Sze, P., 1980. Seasonal succession of phytoplankton in Onondaga Lake, New York (USA).  
572 *Phycologia* 19, 54–59.

573 Tiwari, A., Chauhan, S.V.S., 2006. Seasonal phytoplanktonic diversity of Kitham lake, Agra. *J.*  
574 *Environ. Biol.* 27, 35–38.

575 Tollrian, R., 1993. Neckteeth formation in *Daphnia pulex* as an example of continuous  
576 phenotypic plasticity: morphological effects of *Chaoborus* kairomone concentration and  
577 their quantification. *J. Plankton Res.* 15, 1309–1318.  
578 <https://doi.org/10.1093/plankt/15.11.1309>

579 Trekels, H., Van de Meutter, F., Stoks, R., 2013. Predator cues magnify effects of the  
580 pesticide endosulfan in water bugs in a multi-species test in outdoor containers. *Aquat.*  
581 *Toxicol.* 138–139, 116–122. <https://doi.org/10.1016/j.aquatox.2013.04.008>

582 Trekels, H., Van de Meutter, F., Stoks, R., 2011. Effects of species-specific interactions with  
583 predation risk on the relative species sensitivities to a pesticide in water boatmen  
584 (*Corixidae*). *Oikos* 120, 897–905. <https://doi.org/10.1111/j.1600-0706.2010.18852.x>

585 USEPA, 2020. Sustainable Materials Management: The Road Ahead; U.S., Environmental  
586 Protection Agency: Washington, DC, June 2009. <https://doi.org/10.1021/es202079y>

587 Van den Brink, P.J., Boxall, A.B.A., Maltby, L., Brooks, B.W., Rudd, M.A., Backhaus, T.,  
588 Spurgeon, D., Verougstraete, V., Ajao, C., Ankley, G.T., Apitz, S.E., Arnold, K., Brodin, T.,  
589 Cañedo-Argüelles, M., Chapman, J., Corrales, J., Coutellec, M.A., Fernandes, T.F., Fick, J.,  
590 Ford, A.T., Giménez Papiol, G., Groh, K.J., Hutchinson, T.H., Kruger, H., Kukkonen, J.V.K.,  
591 Loutseti, S., Marshall, S., Muir, D., Ortiz-Santaliestra, M.E., Paul, K.B., Rico, A., Rodea-  
592 Palomares, I., Römbke, J., Rydberg, T., Segner, H., Smit, M., van Gestel, C.A.M., Vighi,  
593 M., Werner, I., Zimmer, E.I., van Wensem, J., 2018. Toward sustainable environmental  
594 quality: Priority research questions for Europe. *Environ. Toxicol. Chem.* 37, 2281–2295.  
595 <https://doi.org/10.1002/etc.4205>

596 Van den Brink, P.J., Hartgers, E.M., Fettweis, U., Crum, S.J.H., Van Donk, E., Brock, T.C.M.,  
597 1997. Sensitivity of macrophyte-dominated freshwater microcosms to chronic levels of  
598 the herbicide linuron. *Ecotoxicol. Environ. Saf.* 38, 13–24.  
599 <https://doi.org/10.1006/eesa.1997.1555>

600 Van den Brink, P.J., Klein, S.L., Rico, A., 2017. Interaction between stress induced by  
601 competition, predation, and an insecticide on the response of aquatic invertebrates.  
602 *Environ. Toxicol. Chem.* 36, 2485–2492. <https://doi.org/10.1002/etc.3788>

603 Van den Brink, P.J., Van Wijngaarden, R.P.A., Lucassen, W.G.H., Brock, T.C.M., Leeuwangh,  
604 P., 1996. Effects of the insecticide Dursban® 4E (active ingredient chlorpyrifos) in  
605 outdoor experimental ditches: II. Invertebrate community responses and recovery.  
606 *Environ. Toxicol. Chem.* 15, 1143–1153. <https://doi.org/10.1002/etc.5620150719>

607 Van Wijngaarden, R.P.A., Brock, T.C.M., Douglas, M.T., 2005. Effects of chlorpyrifos in  
608 freshwater model ecosystems: The influence of experimental conditions on  
609 ecotoxicological thresholds. *Pest Manag. Sci.* 61, 923–935.  
610 <https://doi.org/10.1002/ps.1084>

611 Wang, S., Brose, U., 2018. Biodiversity and ecosystem functioning in food webs: the vertical  
612 diversity hypothesis. *Ecol. Lett.* 21, 9–20. <https://doi.org/10.1111/ele.12865>

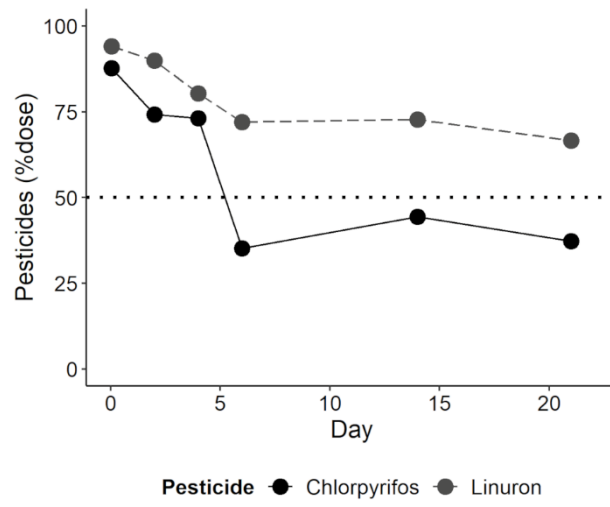
613 Wen, Z., Mian-Ping, Z., Xian-Zhong, X., Xi-Fang, L., Gan-Lin, G., Zhi-Hui, H., 2005. Biological  
614 and ecological features of saline lakes in northern Tibet, China. *Hydrobiologia* 541, 189–  
615 203. <https://doi.org/10.1007/s10750-004-5707-0>

616 Zhao, Q., Van, P.J. den B., Carpentier, C., Wang, Y.X.G., Rodriguez-Sanchez, P., Xu, C.,  
617 Vollbrecht, S., Gillissen, F., Vollebregt, M., Wang, S., Laender, F. De, 2019. Horizontal  
618 and vertical diversity jointly shape food web stability against small and large  
619 perturbations. *Ecol. Lett.* 22, 1152–1162. <https://doi.org/10.1111/ele.13282>

620  
621

622

623 **Figures**



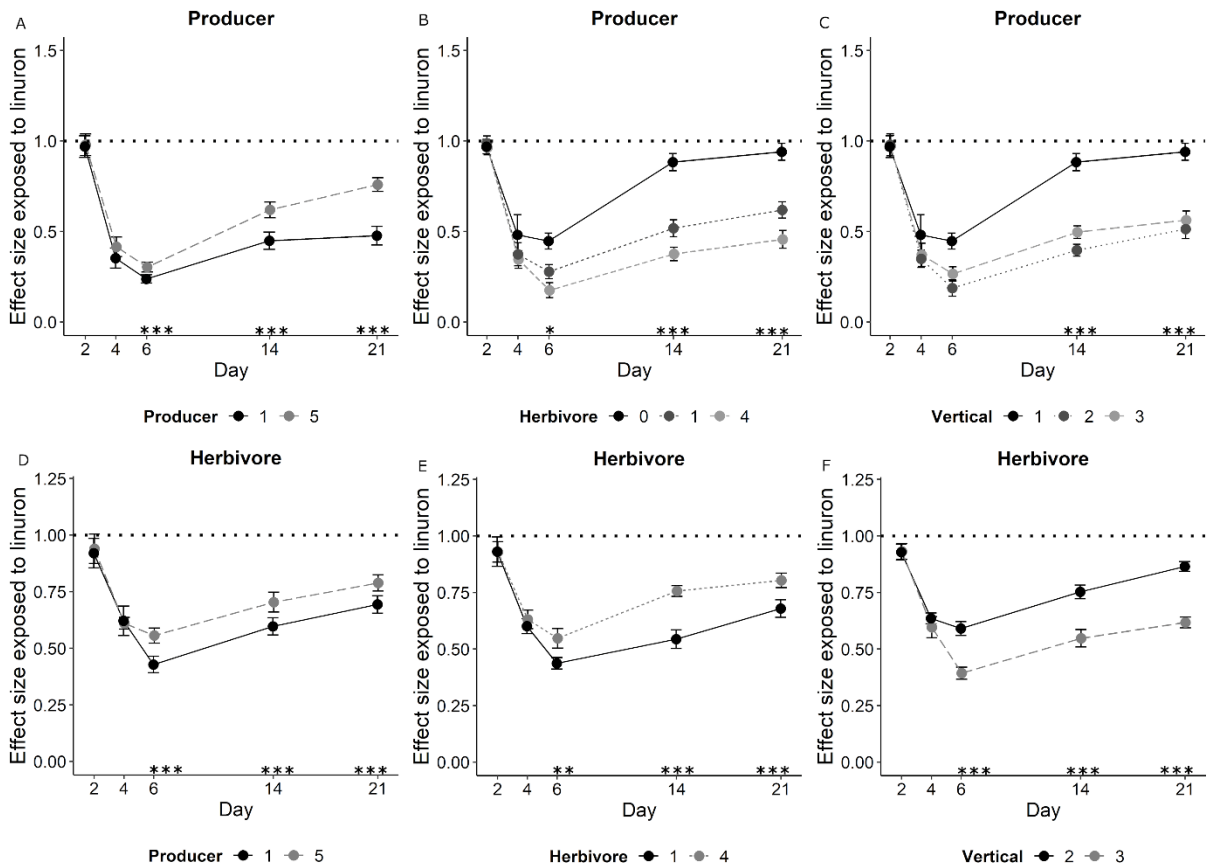
624

625 **Figure 1.** Concentration of linuron and chlorpyrifos in systems on sample 1h and day 2, 4, 6,

626 14 and 21.

627

628



630

631

**Figure 2.** The effects of horizontal composition of producer and herbivore, and vertical

632

composition on effect sizes (biovolume as proxy) of producers (A-C), herbivores (D-F) after

633

exposure to linuron. Plotted are sample mean  $\pm$  1 SE. An effect size is 1 (treatment = control)

634

indicating no effect of linuron, smaller than 1 (treatment < control) indicating a negative effect

635

of linuron, and bigger than 1 (treatment > control) indicating a positive impact. The bigger

636

deviation from effect size 1 (dash line) indicates larger effect of linuron. The effect size with 1

637

and 5 producers (A and D) was visualized by averaging effect sizes of all treatments with 1 and

638

5 producers, respectively, similar manipulation for the effect size under 0, 1 and 4 herbivores

639

species (B and E) and for the effect size under 1, 2 and 3 vertical composition (C and F).

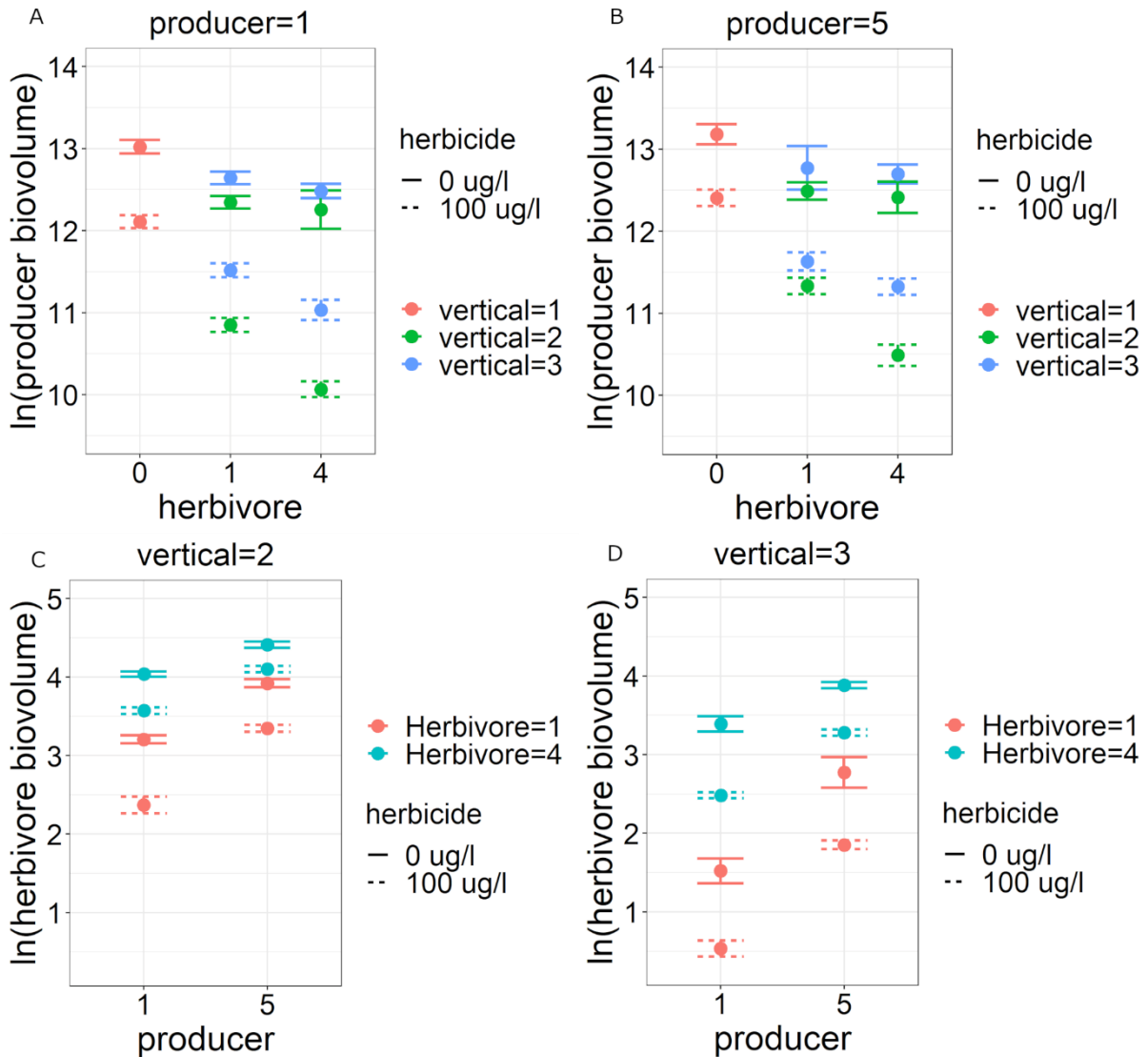
640

Detailed statistical results are listed in Table S5.1-S5.2. (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ).

641

642

643



644

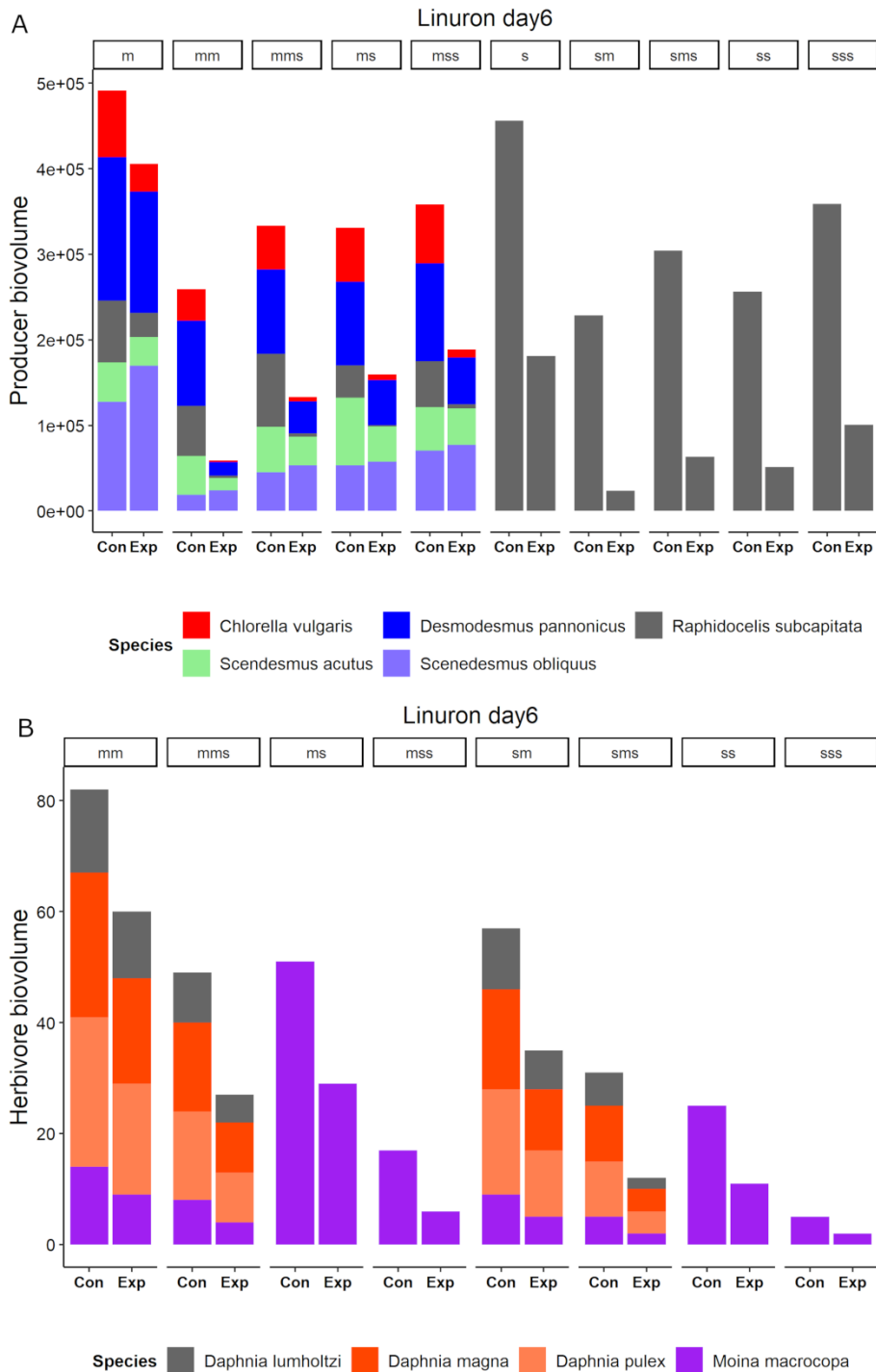
645 **Figure 3.** The interactive effects of horizontal (producers and herbivores) and vertical  
646 composition, herbicide linuron on  $\ln(\text{producer biovolume})$  (a, b) and on  $\ln(\text{herbivore}$   
647  $\text{biovolume})$  (c, d). Plotted are sample mean  $\pm$  1 SE. Solid error bars indicate linuron  
648 concentration of  $0 \mu\text{g l}^{-1}$ , while dashed ones stand for linuron concentration of  $100 \mu\text{g l}^{-1}$ .  
649 Detailed statistical results are listed in Table S6.

650

651

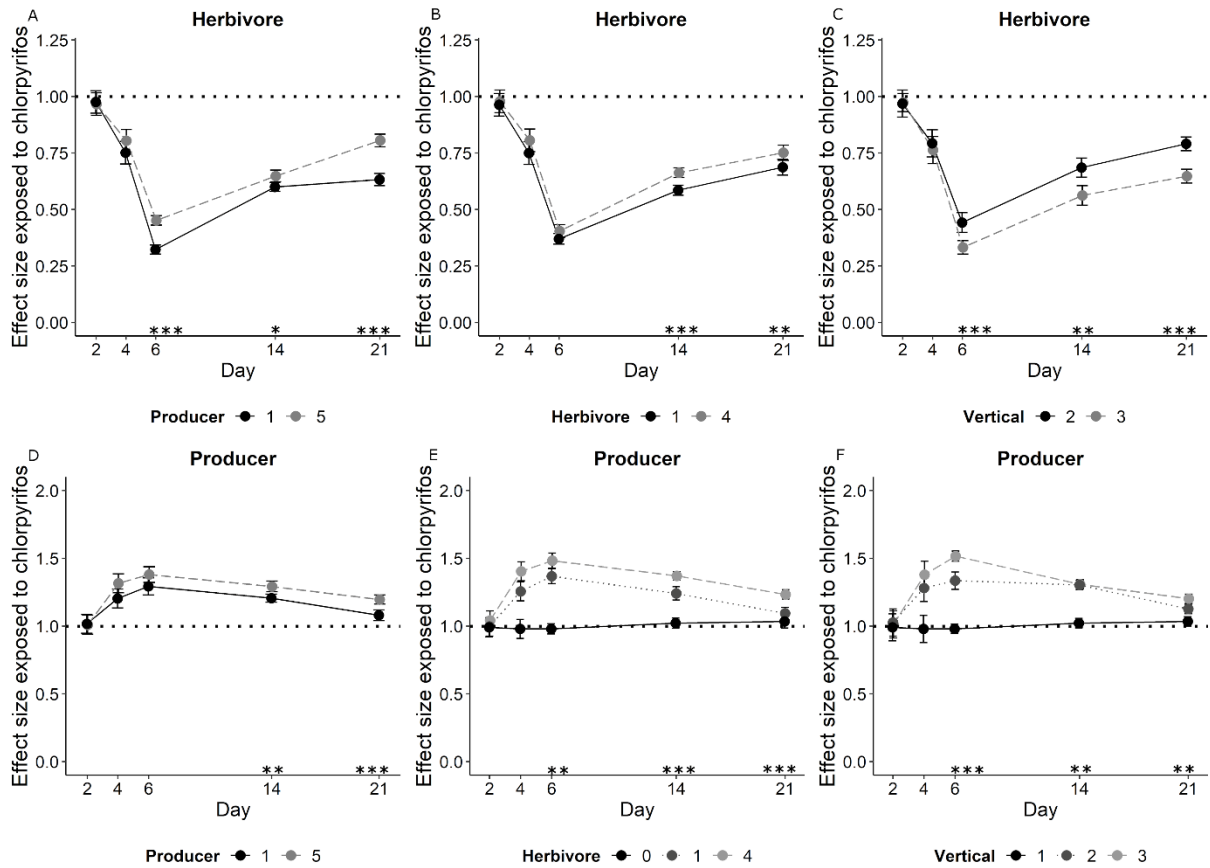
652

653



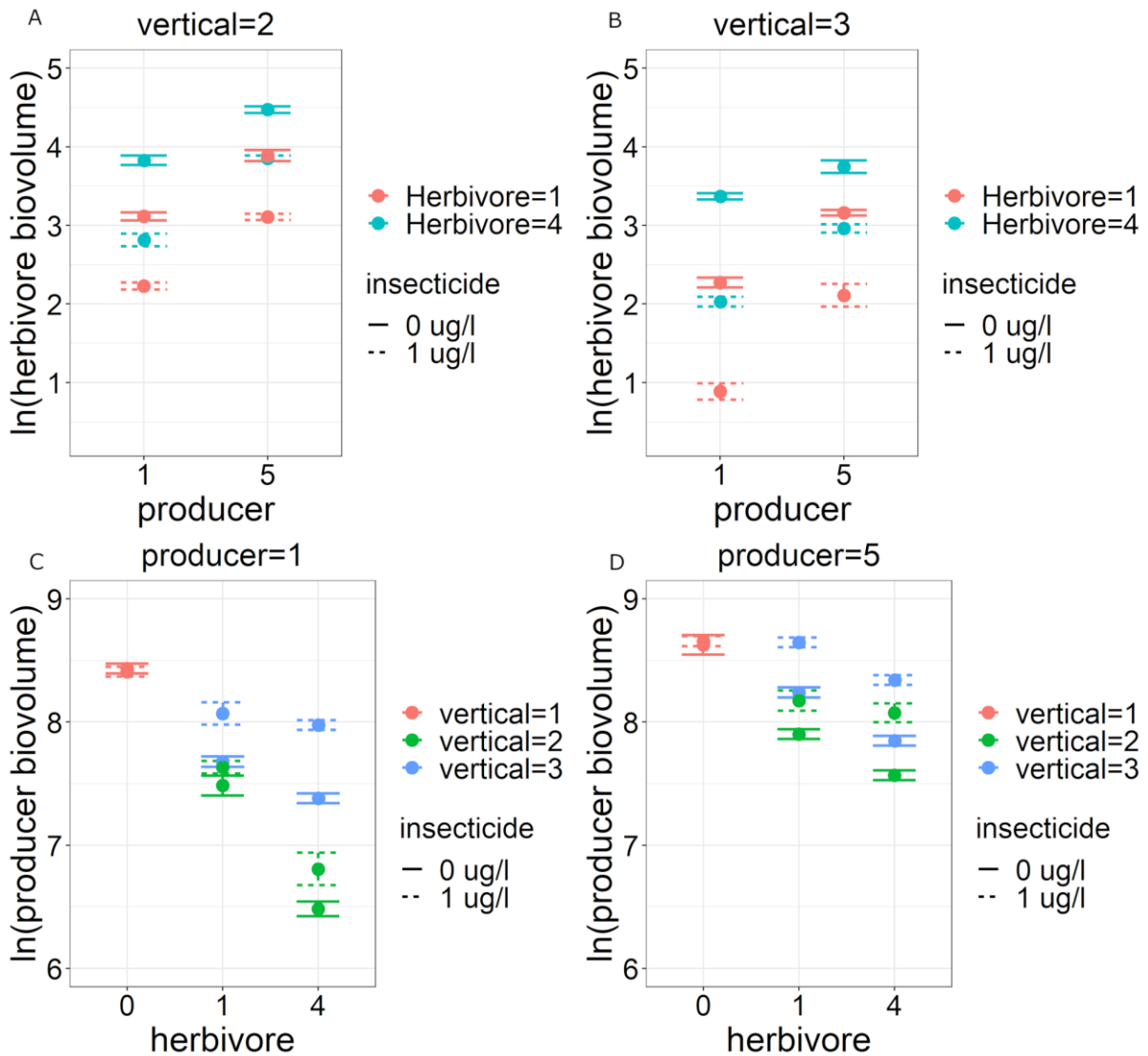
654

655 **Figure 4.** Species biovolume in the ten community types after linuron exposure on day 6. Con  
 656 represents control group, and Exp stands for exposure. Ten treatments include: s, single algae;  
 657 ss, single algae-single herbivore; sss, single algae-single herbivore-single predator; sm, single  
 658 algae-multiple herbivores; sms, single algae-multiple herbivores-single predator; m, multiple  
 659 algae; ms, multiple algae-single herbivore; mss, multiple algae-single herbivore-single  
 660 predator; mm, multiple algae-multiple herbivores; mms, multiple algae-multiple herbivores-  
 661 single predator). Detailed statistical results are listed in Table S7.1-7.2.



662  
 663 **Figure 5.** The effects of horizontal composition of producer and herbivore, and vertical  
 664 composition on effect sizes (biovolume as proxy) for herbivores (A-C), producers (D-F) after  
 665 exposure to chlorpyrifos. Plotted are sample mean  $\pm$  1 SE. An effect size is 1 (treatment =  
 666 control) indicating no effect of chlorpyrifos, smaller than 1 (treatment < control) indicating a  
 667 negative effect of chlorpyrifos, and bigger than 1 (treatment > control) indicating a positive  
 668 impact. The effect sizes with 1 and 5 produces (A and D) was visualized by averaging effect  
 669 size of all treatments with 1 and 5 producers, respectively, similar manipulation for the effect  
 670 size under 0, 1 and 4 herbivores (B and E) and for the effect size under 1, 2 and 3 vertical  
 671 composition (C and F). The bigger deviation from effect size 1 (dash line) indicates larger effect  
 672 of chlorpyrifos. Detailed statistical results are listed in Table S8.1-S8.2. (\* $P < 0.05$ , \*\*  $P < 0.01$ ,  
 673 \*\*\*  $P < 0.001$ ).

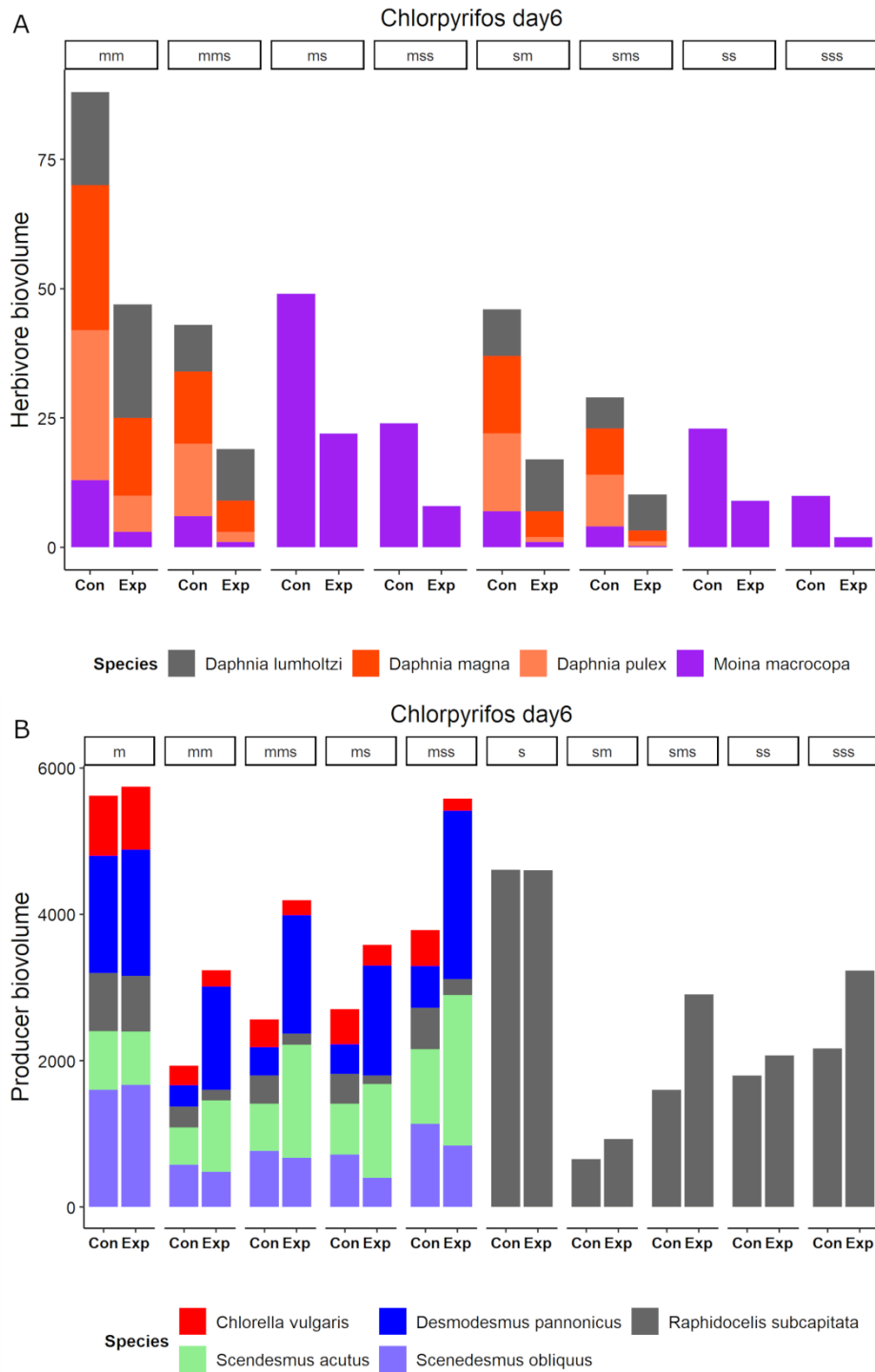
674



676

677 **Figure 6.** The interactive effects of horizontal (producer and herbivore) and vertical  
 678 composition, insecticide chlorpyrifos on  $\ln(\text{herbivore biovolume})$  (a, b) and on  $\ln(\text{producer}$   
 679  $\text{biovolume})$  (c, d). Plotted are sample mean  $\pm$  1 SE. Solid error bars indicate chlorpyrifos  
 680 concentration of  $0 \mu\text{g l}^{-1}$ , while dashed ones stand for chlorpyrifos concentration of  $1 \mu\text{g l}^{-1}$ .  
 681 Detailed statistical results are listed in Table S6.

682



683

684 **Figure 7.** Species absolute biovolume in the ten community types after chlorpyrifos exposure

685 on day 6. Con represents control group, and Exp stands for exposure. Ten treatments are same

686 as Figure 4. Detailed statistical results are listed in Table S9.1-9.2.

687