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1 **Monoculture and co-culture tests of the toxicity of four typical**  
2 **herbicides on growth, photosynthesis**  
3 **and oxidative stress responses of the marine diatoms**  
4 ***Pseudo-nitzschia mannii* and *Chaetoceros decipiens***

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41 Sahraoui).

## 30 Abstract

1  
2  
3 31 The toxicity of four herbicides in mixture (alachlor, diuron, des-isopropyl-atrazine and  
4  
5 32 simazine) on the growth and the photosynthesis parameters of two marine diatoms *Pseudo-*  
6  
7 33 *nitzschia mannii* and *Chaetoceros decipiens* have been investigated for 9 days in monoculture  
8  
9  
10 34 and co-culture tests. The catalase (CAT) and guaiacol peroxidase (GPX) were also monitored  
11  
12 35 to assess the oxidative stress response. In single-species assays, while both species displayed  
13  
14  
15 36 no affected instantaneous growth rate by herbicides, their physiological responses were  
16  
17 37 different. Chl *a* content of *P. mannii* significantly decreased upon herbicide exposure, due  
18  
19  
20 38 probably to pigment destruction or inhibition of their synthesis. This decrease was associated  
21  
22 39 with a reduction in the chlorophyll fluorescence parameters ( $ABS_0/RC$ ,  $TR_0/RC$ ,  $ET_0/RC$  and  
23  
24 40  $DI_0/RC$ ). In contrast, *C. decipiens* maintained an effective photosynthetic performance under  
25  
26  
27 41 herbicide exposure, as Chl *a* per cell content and the specific energy fluxes per reaction center  
28  
29 42 remained unchanged relative to control values. GPX activity was significantly higher in  
30  
31  
32 43 contaminated *P. mannii* and *C. decipiens* monocultures than in controls at early herbicide  
33  
34 44 exposure (1 day), whereas a significant induction of CAT activity occurred later (from day 3  
35  
36 45 for *C. decipiens* and at day 9 for *P. mannii*) in response to herbicides. In control co-culture, *P.*  
37  
38  
39 46 *mannii* was eliminated by *C. decipiens*. As observed in the monoculture, the herbicides did not  
40  
41 47 affect the photosynthetic performance of *C. decipiens* in co-culture, but significantly reduced  
42  
43  
44 48 its instantaneous growth rate. The oxidative stress response in co-culture has similar trends to  
45  
46 49 that of *C. decipiens* in monoculture, but the interspecies competition likely resulted in higher  
47  
48  
49 50 CAT activity under herbicide exposure. Results of this study suggest that herbicide toxicity for  
50  
51 51 marine diatoms might be amplified by interspecies interactions in natural communities, which  
52  
53  
54 52 might lead to different physiological and growth responses.  
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55 *Key words:* diatom, herbicide, growth, photosynthesis, antioxidant enzyme activity.

## 56 **Introduction**

57       The expansion and intensification of agricultural activities over the last few decades has  
58 led to a substantial increase in herbicide use (Mhadhbi et al. 2019; de Suza et al. 2020). These  
59 toxicants are not readily adsorbed by soil (Moncada, 2005) and therefore they can reach rivers,  
60 lakes and then marine waters, through spraying, leaching and runoff (Carter, 2000; Ramezani,  
61 2008). Some herbicides, belonging to triazines and phenylureas are forbidden or restricted in  
62 several Mediterranean countries (e.g. atrazine, 2004/141/CE ; simazine 2004/247/CE and  
63 diuron, 2007/417/CE) but they are still found in aquatic ecosystems (Nödler et al., 2014;  
64 Bancon-Montigny et al. 2019). Herbicides are persistent pollutants in seawater, since they are  
65 weakly adsorbed by marine sediment (Thomas et al. 2002; Tissier et al. 2005). Moreover, their  
66 degradation is limited and can potentially produce more toxic and/or persistent secondary  
67 metabolites (Ortiz-Hernández et al. 2013). Therefore, herbicides constitute a potential threat for  
68 marine ecosystems and their phytotoxicity has already been demonstrated on several  
69 phototrophic organisms, including phytoplankton (e.g. glyphosate, Smedbol et al. 2017; Lam  
70 et al. 2020; triazine, Yang et al. 2019), microphytobenthic biofilms (e.g. diuron and atrazine,  
71 Legrand et al. 2006; glyphosate, Lima et al. 2014) and macrophytes (e.g. norflurazon and  
72 fluridone, Chalifour, 2014; paraquat, Moustakas et al. 2016; glyphosate, Mudge and Houlahan,  
73 2019). Most herbicides, including triazines and phenylureas, are photosynthetic inhibitors.  
74 Their active molecules bind to the D1 protein that blocks electron transport from photosystem  
75 II (Schuler and Rand, 2008), thus inhibiting Hill's reaction (Leboulanger *et al.* 2001; Dorigo *et*  
76 *al.* 2004; Macedo et al. 2008). This further provokes oxidative stress with reactive oxidative  
77 species (ROS) formation and accumulation leading to the damage of pigments, proteins, and  
78 lipids of the photosynthetic apparatus (Gomes and Juneau, 2017). Other herbicides belonging

79 to chloroacetamide family, such as alachlor and metalachlor, inhibit the biosynthesis of amino-  
80 acids and long chain fatty acids (Boger et al. 2003; Yang et al. 2010; Busi, 2014).

81 Several studies have assessed the impact of herbicides on benthic and planktonic  
82 microalgae, particularly on diatoms. These silicified microalgae are ubiquitous photosynthetic  
83 organisms in marine and fresh waters that account for 20% of global primary production and  
84 they are the major contributors to sedimentary organic matter (Nelson et al. 1995; Thornton,  
85 2014). Diatoms are at the base of marine food webs, particularly the herbivorous pathway  
86 (Sakka Halili et al. 2014; Meddeb et al. 2019) and thus the effects of toxicants on these primary  
87 producers can be cascaded to higher trophic levels consequently impacting the function of the  
88 whole ecosystem (Relyea, 2009; Pesce et al. 2011; Hashimoto et al. 2019). Diatoms quickly  
89 respond to environmental pollutants and hence they are often used as efficient bioindicators to  
90 assess the ecological status of aquatic ecosystem (Stevenson and Pan, 2010; Berthon et al. 2011;  
91 Pandey, 2017; Pandey et al. 2020). Exposure to herbicides at concentrations ranging from  $\mu\text{g}$   
92  $\text{L}^{-1}$  to  $\text{mg L}^{-1}$ , was often followed by either significant decrease in Chl *a*, growth rate,  
93 photosynthetic efficiency or lipid content for several marine diatoms (Schmitt-Jansen and  
94 Altenburger, 2005; Gatidou and Thomaidis, 2007; Bao et al. 2011; Ebenezer and Ki, 2013;  
95 Coquillé et al. 2018). Deformation of diatom frustules and DNA alteration can be also often  
96 observed (Schmitt-Jansen and Altenburger, 2005; Debenest et al. 2008; Roubex et al. 2011).  
97 However, it is possible to observe no adverse effects on diatoms by herbicides. For example,  
98 Debenest et al. (2010) have not detected any impact of atrazine ( $25 \mu\text{g L}^{-1}$ ) on a freshwater  
99 diatom community biomass and Hourmant et al. (2009) even found increase in pigment content,  
100 photosynthetic rate, and ATP synthesis for *Chaetoceros gracilis* exposed to bentazon with  
101 concentration of 0 to  $100 \mu\text{g L}^{-1}$ . In fact, there is a considerable variability in diatom species  
102 sensitivity to herbicides (Larras et al. 2014), even towards the same herbicide (Larras et al.  
103 2012).

104 Most of the data on the sensitivity of diatoms to herbicides are provided from single-  
1 species bioassays. These allow a wide assessment of herbicide impacts at the molecular, cellular  
2  
3  
4  
5 106 and population levels (Wood et al. 2016; 2019; Chamsi et al. 2019; Demailly et al. 2019).  
6

7 107 However, there are insufficient conclusive data in view of the effects of herbicides at the  
8  
9  
10 108 community level. In natural communities, interactions between coexisting species may alter  
11  
12 109 their ability to respond to perturbations. Indeed, it has been shown that diatoms respond  
13  
14 110 differently to environmental stress depending on whether they are tested in laboratory  
15  
16 111 monocultures, mixtures or in natural communities (Bérard et al. 1999; Schabhüttl et al. 2012;  
17  
18  
19 112 Koedooder et al. 2019).

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21  
22 113 The present study aimed to assess the toxicity of four herbicides (diuron, des-isopropyl-  
23  
24 114 atrazine (DIA), alachlor and simazine) in mixture on two ecologically important marine  
25  
26 115 diatoms: the pennate species *Pseudo-nitzschia manni* and the centric species *Chaetoceros*  
27  
28 116 *decipiens*. The first species, known as a neurotoxic producer, was recently reported in  
29  
30 117 Mediterranean waters (Sakka Hlaili et al. 2016) and the second is a potentially noxious diatom  
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32  
33 118 that can form intense blooms (Smayda, 2006; López-Cortés et al. 2015). These diatoms were  
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36 119 isolated from the Bizerte Lagoon (SW Mediterranean) in which the four herbicides are  
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38  
39 120 predominant ( $\sum_{\text{diuron+DIA+alachlor+simazine}}: 0.035 \mu\text{g L}^{-1}$ , Bancon-Montigny et al. 2019).  
40  
41  
42 121 Effects were evaluated with different physiological parameters Chl *a*, growth rate,  
43  
44 122 photosynthetic efficiency, and antioxidant enzyme activities, to better assess the herbicide  
45  
46 123 toxicity. Experiments were carried out either as single-species (monoculture) or two-species  
47  
48  
49 124 (co-culture) bioassays.

## 50 51 52 125 **Material and Methods**

### 53 54 126 **Microalgal isolation and culture conditions**

55  
56  
57 127 Cells of *Chaetoceros* (diameter: 24-27  $\mu\text{m}$ , length: 33-36  $\mu\text{m}$  ) and *Pseudo-nitzschia*  
58  
59 128 (width: 1.80-1.90  $\mu\text{m}$ , length: 32-37  $\mu\text{m}$ ) were collected in spring 2015 with a Hydrobios 23  
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65

129  $\mu\text{m}$  mesh plankton net by vertical hauls from 3 to 4 m depth to establish laboratory cultures of  
130 diatom species. Monoclonal cultures were obtained by single-cell micropipette isolation.  
131 Nonaxenic cultures were grown in f/2 medium (Guillard and Ryther, 1962) in 125 mL flasks  
132 incubated at 20°C and with a photon flux density of 100  $\mu\text{E m}^{-2} \text{ s}^{-1}$ , provided by cool-white  
133 fluorescent tubes, with a 12:12 h light:dark cycle in temperature-controlled chamber  
134 (Aqualytic, Dortmund, Germany). Diatom cultures were acclimated to these experimental  
135 conditions for a minimum of 4-5 generations before dilution with fresh media and the start of  
136 the experiments. For this, early exponentially growing cells were transferred on new f/2 media,  
137 every 8 days of incubation. Inoculations were carried out in a laminar flow hood and using  
138 sterile laboratory material to avoid contamination. All Flasks were swirled by hand at least  
139 twice daily, and their positions on the incubator shelves were changed arbitrarily at least once  
140 each day to moderate any discontinuities in irradiance or temperature within the incubator.

### 141 **Species identification**

142 Identification of the two diatom isolates up to the genus level was made by light  
143 microscopy under a Zeiss AxioCam HRc light microscope (Zeiss, Oberkochen, Germany).  
144 Then, in a second step, transmission electron microscopy was used to identify the two diatoms  
145 at the species level. For this, 20 mL subsamples of cultures were filtered then washed with  
146 250 mL of distilled water through a 3  $\mu\text{m}$  pore size polytetrafluoroethylene (PTFE) membrane  
147 using a Millipore vacuum filtration apparatus. The diatom frustules were then re-suspended in  
148 5 mL distilled water and cleaned by adding 10 mL each of concentrated sulfuric and nitric acid  
149 in a boiling water bath for 60 min. Frustules were then washed once more with 250 mL of  
150 distilled water in the filtration apparatus and re-suspended in a final volume of 5 mL. For  
151 electron microscopy examination, one drop of cleaned sample was diluted 20:1 (for cultures)  
152 and single drops were dried onto 400-mesh copper grids bearing pure carbon support films, and  
153 examined using a Tecnai G2 TEM operating at 60 kV, at the Electron Microscopy Unit (Faculty



154 of Sciences of Bizerte, Tunisia). The species *Pseudo-nitzschia mannii* and *Chaetoceros*  
155 *decipiens* Cleve were identified following identification keys of Amato and Montresor (2008)  
156 and of Lee et al. (2014), respectively.

### 157 **Chemicals preparation**

158 The herbicides used in this study are representative of two different modes of action and  
159 have different solubility  $K_{ow}$  values (Table 1). Stock solutions of herbicides (all dissolved in  
160 sterilized Milli-Q water) were used to prepare an herbicide mixture (diuron, DIA, alachlor and  
161 simazine) containing  $\sim 1 \text{ mg l}^{-1}$  of each herbicide.

### 162 **Experimental design**

163 Herbicide contamination experiments were carried out on single-species bioassays  
164 (monoculture of *Pseudo-nitzschia mannii* or *Chaetoceros decipiens*), as well as on a co-culture  
165 of both species. Bioassays were conducted in triplicate in 2 L glass Erlenmeyers flasks,  
166 containing 1000 mL of F/2 medium and inoculated with diatom strain cultures in the  
167 exponential growth phase. Cultures were set at the initial cell densities of  $5 \times 10^3 \text{ mL}^{-1}$  for *P.*  
168 *mannii* and  $5 \times 10^2 \text{ cells mL}^{-1}$  for *C. decipiens*, respecting the *in situ* concentrations during  
169 bloom periods (Sahraoui et al., 2009; 2012). For each species in monoculture or co-culture, two  
170 treatments were carried out: control without any herbicide (treatment C) and treatment  
171 contaminated with the herbicide blend solution to have the final concentration of  $\sim 1 \mu\text{g L}^{-1}$  of  
172 each herbicide (treatment Herb). The concentration of total herbicides in contaminated flasks  
173 was  $4.64 \mu\text{g L}^{-1}$ . The chosen herbicide concentration represents  $\sim 5$  times the maximum level  
174 observed in Bizerte Lagoon watershed environment ( $1.246 \mu\text{g L}^{-1}$ , Mhadhbi et al. 2019).

175 All treatments (in mono- and co-cultures) were incubated for 9 days in the temperature-  
176 controlled chamber under the same light and temperature conditions described above.  
177 Subsamples were taken from all treatments at six time points (t= 0, 1, 2, 3, 6 and 9 days) for  
178 growth, physiological, photochemical and biochemical analyses.

179 **Laboratory analyses**

180 **Chlorophyll *a***

181 For chlorophyll *a* (Chl *a*), 10 ml samples were filtered through Whatman GF/F filters.  
182 Pigment concentrations were determined using the standard spectrophotometric method  
183 (Parsons et al. 1984), following extraction with 10 ml 90% acetone overnight at 4°C in the dark.

184 **Cell density and growth rate calculation**

185 Samples (1.5 ml) used to determine cell density were immediately fixed with 3% acid  
186 Lugol's solution. Counts were done in triplicate on 30 µl aliquots under an inverted microscope  
187 at magnification of x 40. The instantaneous specific growth rate ( $\mu_{\text{inst}}$ , d<sup>-1</sup>) was determined by  
188 linear regression of the natural logarithm (ln) of cell count vs time over the sampling days.

189 **Measurements of photosynthetic parameters**

190 Samples (10 ml) were dark-adapted for 15 min prior to fluorescence measurements. The  
191 AquaPen AP-P100 (Photon Systems Instruments, Czech Republic) was used to measure the  
192 rapid polyphasic Chl *a* fluorescence rise during 3 sec by using a LED blue emitter light intensity  
193 of 3000 µE m<sup>-2</sup>s<sup>-1</sup>.

194 Fluorescence yield (F) at investigated fluorescence transients was determined according  
195 to Strasser and Strasser (1995). The fluorescence yield at 50 µs was considered as O transient  
196 (F<sub>50µs</sub>) when all reaction centers of PSII are open. The maximal fluorescence yield (F<sub>M</sub>) was  
197 determined at P transient and the variable fluorescence yields related to J and I transients were  
198 determined at 2 ms and 30 ms, respectively. Photosynthetic parameters indicating PSII activity  
199 (ABS<sub>0</sub>/RC: absorption of photons per active reaction center (RC); TR<sub>0</sub>/RC: trapped energy flux  
200 per RC; ET<sub>0</sub>/RC: electron transport flux per RC and DI<sub>0</sub>/RC: non-photochemical energy  
201 dissipation per active reaction centre) were evaluated following to Strasser et al. (1999).

202 **Detection of antioxidant enzyme activities**

203 Samples (100 ml) were collected by centrifugation at 6000 g for 30 min at 4°C and then  
204 broken down in the phosphate buffer (pH = 7.0) by sonication. Samples were observed under a  
205 microscope to make sure the breaking ratio was more than 95%. Finally, the sonicated solution  
206 was centrifuged again at 6000 g for 30 min at 4°C and the supernatants were collected to  
207 measure the antioxidant enzyme activities.

208 Catalase activity was determined according to the method of Beers and Sizer (1952) by  
209 following the H<sub>2</sub>O<sub>2</sub> disappearance kinetic for 5 minutes at 240 nm. The reaction medium  
210 contained 50 mM phosphate buffer at pH 7.5 and 10 mM H<sub>2</sub>O<sub>2</sub>. The extinction coefficient was  
211 0.036 mM<sup>-1</sup> cm<sup>-1</sup> and the reaction was initiated by the addition of the protein extract.

212 The guaiacol peroxidase (GPX) activity was determined according to the method of  
213 Jiang and Penner (2015) based on the increase in the absorbance at 470 nm due to the  
214 polymerization of guaiacol to tetraguaiacol in the presence of hydrogen peroxide for 5 minutes.  
215 The reaction medium contained 0.1 M sodium phosphate at pH 6.5, 0.135 mM H<sub>2</sub>O<sub>2</sub> and 3.6  
216 mM guaiacol. The molar extinction coefficient of tetraguaiacol was 26.6 mM<sup>-1</sup> cm<sup>-1</sup>. The  
217 reaction was initiated by the addition of the protein extract.

### 218 **Statistical analyses**

219 Mean and standard deviation values were calculated for each treatment from three  
220 independent replicate cultures and graphed. A two-way ANOVA was used to test the effect of  
221 herbicide mixture and culture mode on the instantaneous growth rate of *P. mannii* and *C.*  
222 *decipiens* maintained in monoculture and co-culture. The normality of data distribution  
223 (Kolmogorov–Smirnov test) and homogeneity of variance (Bartlett–Box test) were respected  
224 and differences were accepted as significant when  $p < 0.05$ .

225 For each experiment (*P. mannii* monoculture, *C. decipiens* monoculture and *P. mannii* +  
226 *C. decipiens* co-culture), Student's *t*-tests were performed to detect significant differences in  
227 Chl *a* cell content, photosynthetic parameters and antioxidant enzyme activities between control

228 and herbicide treatments and for comparisons between the different cultures (with a  $p$  value  
1  
2 229  $<0.05$  considered as statistically significant) after having checked the homoscedasticity with  
3  
4  
5 230 Fisher's F-test).

6  
7 231 The statistical analyses were performed with the software XLSTAT 18.

## 9 232 RESULTS

### 11 233 Response of diatom growth

#### 13 234 Instantaneous growth rate

14  
15  
16 235 At day 1, the instantaneous growth rate of *P. mannii* monoculture was about  $\sim 1.10 \text{ d}^{-1}$  for  
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18  
19 236 the control and the herbicide treatments (Fig. 1a). Values then increased to about  $1.25 \text{ d}^{-1}$  at day  
20  
21  
22 237 2 and then decreased to  $\sim 0.39 \text{ d}^{-1}$  at day 9 for both treatments. The herbicide mixture had no  
23  
24 238 significant effect on the growth of *P. mannii* maintained in monoculture (two-way ANOVA,  $P$   
25  
26 239  $> 0.05$ , Fig. 1a). However, the co-culture had a negative effect on the growth of *P. mannii*,  
27  
28  
29 240 which displayed constantly negative values over the experiment for the control treatment ( $-0.35$   
30  
31 241 to  $-0.07 \text{ d}^{-1}$ ) (two-way ANOVA,  $P < 0.05$ , Fig. 1a). Negative growth rates were also registered  
32  
33  
34 242 for *P. mannii* contaminated co-culture, with similar values to those of the control co-culture.  
35  
36 243 The herbicide mixture did not affect the growth of *C. decipiens* in monoculture (two-way  
37  
38  
39 244 ANOVA,  $P > 0.05$ , Fig. 1b) since comparable instantaneous growth rate values were registered  
40  
41 245 between the control and the herbicide treatment (from  $\sim 0.5$  at day 1 to  $\sim 0.2$  at day 9). In control  
42  
43  
44 246 co-culture, *C. decipiens* maintained similar growth rates to those of the control monoculture  
45  
46 247 (two-way ANOVA,  $P > 0.05$ , Fig. 1b). Conversely, the herbicide mixture has significantly  
47  
48  
49 248 impacted *C. decipiens* when the species was growing in co-culture (two-way ANOVA,  $P <$   
50  
51 249  $0.05$ , Fig. 1b) and its instantaneous growth rate values were reduced by 35.41 %, 41.39 % and  
52  
53 250 61.11 % compared to the control co-cultures, at days 3, 6 and 9, respectively.

#### 54 55 251 Chlorophyll $a$ content

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1  
2 252 Under monoculture conditions, the Chl *a* per cell content of *P. mannii* (initially of  
3 253  $9.34 \pm 0.4$   $\mu\text{g cell}^{-1}$ ) increased significantly in the control to reach a maximum on day 3  
4  
5 254 ( $18.77 \pm 5.75$   $\mu\text{g cell}^{-1}$ ) and then decreased sharply to attain  $2.09 \pm 0.01$   $\mu\text{g cell}^{-1}$  at the end of  
6  
7 255 the experiment (Fig. 2a). The herbicide mixture induced a significant reduction in Chl *a* cell  
8  
9 256 content for *P. mannii* monoculture (Student's *t*-test,  $P < 0.05$ ) and values decreased by 55-89%  
10  
11 257 compared to the control after 2-3 days. Conversely, the addition of herbicides did not provoke  
12  
13 258 any significant effect on the specific biomass of *C. decipiens* in monoculture (Student's *t*-test,  
14  
15 259  $P > 0.05$ , Fig. 2b) whose mean values varied in a range from  $31.76 \pm 7.77$  to  $53.61 \pm 7.90$   $\mu\text{g}$   
16  
17 260  $\text{cell}^{-1}$ .

18  
19 261 For the diatom co-culture under control conditions, the initial Chl *a* per cell content ( $9.86$   
20  
21 262  $\pm 0.8$   $\mu\text{g cell}^{-1}$ ) slightly diminished at the beginning (until day 3) to increase thereafter  
22  
23 263 significantly by 2 to 3-fold compared to the initial level (Fig. 2c). The herbicide mixture induced  
24  
25 264 a significant increase in Chl *a* cell content compared to the control (Student's *t*-test,  $P < 0.05$ )  
26  
27 265 with the most important rise at day 3 (increase by 8 folds compared to the control) (Fig. 2c).

### 28 266 **Physiological response of diatoms**

#### 29 267 **Photosynthetic parameters**

30  
31 268 Whether for the diatom monocultures or co-culture, the maximum quantum yield of PSII  
32  
33 269 ( $F_v/F_m$ ) did not show any significant difference between the control and herbicide treatment  
34  
35 270 (Student's *t*-test,  $P > 0.05$ , Fig. 3a). This parameter declined from the beginning ( $\sim 0.7 - 0.8$   
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37 271 a.u.) to the end of the experiment, reaching values of -20 to -30 % relative to the initial level  
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39 272 (Fig. 3a).

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41 273 For *P. mannii* in monoculture, the herbicide mixture induced a significant sharp decrease  
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43 274 of  $ABS_0/RC$  (Student's *t*-test,  $P < 0.05$ , Fig. 3b). This reduction was noticeable from day 1,  
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45 275 such that values of this parameter (in the range of 1.38-1.98 a.u. for control) approached the  
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47 276 zero at day 2 (-98.01 % relative to control) Values recovered to control levels at day 3 to  
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278 decrease again to approximately -50 % relative to the control at day 9. This ratio was unchanged  
279 by the herbicide mixture in the monoculture of *C. decipiens* (Student's *t*-test,  $P > 0.05$ , Fig. 3b)  
280 with values ranging in general between 2 and 3 a.u. For the diatom co-culture, there was also  
281 no significant effect of the herbicide mixture on  $ABS_0/RC$  (Student's *t*-test,  $P > 0.05$ , Fig 3b)  
282 and values were often in the range of 2 to 3 a.u.

283 Likewise, the ratios of trapping of excitation energy per reaction centre ( $TR_0/RC$ ) and  
284 electron transport per active reaction centre ( $ET_0/RC$ ) were significantly reduced by herbicide  
285 for *P. mannii* monoculture (Student's *t*-test,  $P < 0.05$ , Fig. 3c, d) as both parameters decreased  
286 at day 2 by 80% and 89 % relative to control, respectively. Conversely,  $TR_0/RC$  and  $ET_0/RC$   
287 were unchanged by the herbicide mixture in *C. decipiens* monoculture (Student's *t*-test,  $P >$   
288 0.05) with values mostly of about 1.5-1.8 a.u. and 1.12-2.1 a.u., respectively (Fig. 3c, 3d). For  
289 the diatom co-culture, there was no significant effect of the herbicide mixture on both ratios  
290 (Student's *t*-test,  $p > 0.05$ , Fig. 3c, d).

291 Herbicide treatment significantly impacted the dissipated energy flux per active reaction  
292 center ( $DI_0/RC$ ) for *P. mannii* monoculture (Student's *t*-test,  $P < 0.05$ , Fig. 3e), causing 33-  
293 98% reduction relative to control. Conversely, no significant effect was observed for this  
294 physiological parameter for the *C. decipiens* monoculture (range of values: 0.78-1.31 a.u.) nor  
295 for the diatom co-culture (range of values: 0.74-1.18 a.u.) (Student's *t*-test,  $P > 0.05$ , Fig. 3e).

### 296 **Antioxidant enzyme activity**

297 For the *P. mannii* monoculture, the catalase activity was relatively low in the control  
298 (range of values: 0.42 – 1.05 U  $mg^{-1}$  Protein, Fig 4a). The herbicide mixture did not affect the  
299 enzyme activity (Student's *t*-test,  $P > 0.05$ ) except at the end of the experiment where a great  
300 increase was observed (roughly 20 times the control value, Fig. 4a). In the *C decipiens* control  
301 monoculture the catalase activity was significantly greater than that of *P. mannii* control  
302 monoculture (range of values: 7.14-10.52 U  $mg^{-1}$  Protein) (Student's *t*-test,  $P < 0.05$ ). Exposure

303 to herbicide resulted in a significant increase in catalase activity (Student's *t*-test,  $P < 0.05$ ,  
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2 304 Fig.4b) and values increased by 3-5 folds relative to control after 3-days treatment. The same  
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5 305 response to herbicides was observed for the diatom co-culture (Student's *t*-test,  $P < 0.05$ ,  
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7 306 Fig. 4c) as catalase activity increased by 4-7 folds in comparison to control after 3-days  
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9 307 treatment.

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12 308 Overall, the GPX activity, measured in monocultures and co-culture, was not affected by  
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14 309 the herbicide mixture addition (Student's *t*-test,  $P > 0.05$ , Fig. 4d, e, f) except on day 1, when  
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16 310 the enzyme showed 2-3 folds higher activity in herbicide treatment. The GPX activity values  
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18 311 generally varied within the range of 1.83-22.41 U mg<sup>-1</sup> Protein, for all type of cultures (Fig. 4d,  
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20 312 e, f).

## 24 313 DISCUSSION

### 26 314 Herbicide Toxicity towards diatoms in monoculture

#### 29 315 Growth and Chl *a* cell content

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33 316 Our findings showed that the herbicide mixture did not significantly affect the  
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35 317 instantaneous growth rate of *P. mannii* and *C. decipiens* grown as monocultures ( $P > 0.05$ , Fig.  
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37 318 1a, b). Literature data on the effect of herbicides on diatom growth are highly contrasted.  
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39 319 Indeed, it seems difficult to distinguish a general response of diatoms to herbicide exposure.  
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41 320 Previously, authors have reported a decrease in growth for freshwater diatoms in response to  
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43 321 herbicides, such as atrazine (1-1000 µg L<sup>-1</sup>: Larras et al. 2013; 500 µg L<sup>-1</sup>: Wood et al. 2014),  
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45 322 metolachlor (5-30 µg L<sup>-1</sup>: Roubex et al. 2011), isoproturon (5-20 µg L<sup>-1</sup>: Pérès et al. 1996) and  
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47 323 diuron (10 µg L<sup>-1</sup>: Demailly et al. 2019). All these authors have used herbicides at higher  
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49 324 concentrations than used in this study ( $\Sigma$  herbicides ~ 5 µg L<sup>-1</sup>), which could explain the lack  
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51 325 of herbicide impact on the growth of *P. mannii* and *C. decipiens* monocultures. However, other  
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53 326 studies have reported positive impact of herbicides on diatom growth (50 µg L<sup>-1</sup> atrazine,  
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55 327 Kalopesa et al., 2008; 30 µg L<sup>-1</sup> isoproturon, Debenest et al. 2009; and 7 µg L<sup>-1</sup> diuron, Ricart  
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328 et al. 2009). In our experiment, *P. mannii* and *C. decipiens*, growing as monocultures, seemed  
329 to tolerate the herbicide mixture at the tested concentration. Both species originated from  
330 Bizerte Lagoon, where noticeable contamination by herbicides has been reported for waters and  
331 sediment (Pringault et al. 2016; Bancon-Montigny et al. 2019; Mhadhbi et al. 2019). The  
332 ecosystem has long been under the influence of water runoff from adjacent land areas used for  
333 agricultural crops cultivation with intensive use of herbicides (Ben Salem et al. 2017).  
334 Consequently, it is likely that *P. mannii* and *C. decipiens* isolated from this contaminated  
335 ecosystem might have developed tolerance to herbicides through adaptation mechanisms that  
336 make them physiologically able to resist to these pollutants.

337 In monoculture, the Chl *a* per cell content of *P. mannii* was significantly affected by Herb  
338 treatment ( $P < 0.05$ , Fig. 2a), although growth was not affected (Fig. 1a). This suggests that the  
339 per-cell Chl *a* content would decrease under herbicide contamination probably due to inhibition  
340 of pigment synthesis or pigment destruction. In fact, herbicides, particularly the PSII inhibitors  
341 (e.g. DIA, diuron and simazine), are known to act by binding to the exchangeable quinone site  
342 in the PSII reaction center, thus blocking electron transfer (Gomes and Juneau, 2017; Thomas  
343 et al. 2020). The excitation of the PSII complex is therefore directly transferred to O<sub>2</sub>, resulting  
344 in the formation of singlet oxygen causing uncontrolled oxidation of membranes components  
345 in particular chlorophyll pigments, including Chl *a* (Thomas et al. 2020). Furthermore, PSII  
346 inhibiting herbicides would be responsible for the accumulation of compounds such as  
347 polyenes, phytols and phytophloenes that inhibit chlorophyll synthesis in microalgae (Thomas  
348 et al. 2020). Conversely to *P. mannii*, herbicides did not cause any effect on Chl *a* cell content  
349 of *C. decipiens* monoculture ( $P > 0.05$ , Fig. 2b), suggesting that the two species have different  
350 levels of tolerance to herbicides. In fact, studies carried out on *Chaetoceros* species reported  
351 different results. An increase in Chl *a* content was observed for *C. gracilis* exposed to 50 to  
352 60 µg l<sup>-1</sup> of Bentazon, while a drastic decrease was found for *Chaetoceros* sp. as a short-term



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2 354 response to 2,4-D dimethylamine (50 to 300 mg l<sup>-1</sup>) and endosulfan (20 to 100 mg l<sup>-1</sup>) exposure  
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### 5 355 **Photosynthetic response and antioxidant enzymes activity**

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8 356 Whether for *P. mannii* or for *C. decipiens* grown in monoculture our results showed that  
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10 357 the Fv/Fm ratio has been unaltered by the tested herbicide mixture ( $P < 0.05$ , Fig 3.a),  
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12 358 suggesting a relative integrity of photosystem II under the experimental conditions. However,  
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14 359 herbicide-induced changes were clearly visible in all the other OJIP parameters (ABS<sub>0</sub>/RC,  
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16 360 TR<sub>0</sub>/RC, ET<sub>0</sub>/RC and DI<sub>0</sub>/RC) for *P. mannii* monoculture (Fig. 3). Several authors have already  
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18 361 pointed out that Fv/Fm is probably not the best parameter to evaluate toxic effects of herbicides  
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20 362 due to its relatively low sensitivity (e.g. Juneau et al. 2007; Kargar et al. 2019). Fv/Fm measures  
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22 363 the optimal photosynthetic efficiency and does not reflect the true nature of PSII-activity under  
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24 364 normal light conditions (Juneau et al. 2007). The decrease in the photosynthetic parameters in  
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26 365 *P. mannii* monoculture may indicate an increase in activated fraction of RCs or in antenna size  
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28 366 by *P. mannii* to counterbalance the chlorophyll pigment deterioration by herbicides (cf. Fig. 2a)  
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30 367 to maintain an efficient light energy transfer. In fact, these ratios are influenced by the ratio of  
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32 368 active/inactive RCs of light harvesting complex (LHC) and as the number of active centers  
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34 369 increased, they decreased (Mathur et al. 2011; Kumar et al. 2019). None of the specific energy  
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36 370 fluxes per RC were affected by herbicides for *C. decipiens* monoculture ( $P > 0.05$ , Fig 3),  
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38 371 suggesting that the species could maintain an effective photosynthetic performance under  
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40 372 herbicide exposure, which could explain the lack of effect on its Chl *a* content. Therefore, *C.*  
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42 373 *decipiens* seemed to be physiologically more tolerant to herbicides than *P. mannii*. This could  
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44 374 be due to the antenna organization, which differs significantly between pennate and centric  
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46 375 diatoms (Gundermann et al. 2019).  
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58 376 The enzymes involved in oxidative damage repair have significantly increased under  
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60 377 herbicide contamination. However, this stimulation was different between the diatom species.  
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378 Indeed, the CAT activity has increased for *P. mannii* only by the end of the experience, whereas  
379 *C. decipiens* showed a considerably higher CAT response to the herbicide mixture ( $P < 0.05$ ,  
380 Fig. 4). CAT enzyme has been possibly activated to regulate toxic levels of reactive oxygen  
381 species (ROS) which production was probably induced by herbicides (Bai et al. 2015; Thomas  
382 et al. 2020). For *C. decipiens*, the eventual oxidative damage could be likely induced by the  
383 Very-Long-Chain Fatty Acid (VLCFA) inhibitor (alachlor) as the PSII inhibiting herbicides has  
384 not impacted its photosynthetic performance ( $P > 0.05$ , Fig 3). It is widely known that the  
385 toxicity of chloroacetamides like alachlor is linked to the oxidation of cell components with the  
386 increase of ROS and lipid peroxidation (Maronić et al. 2018). At short term (day 1), the  
387 herbicide mixture promptly stimulated the GPX activity for *P. mannii* and *C. decipiens* (Fig.  
388 4), probably indicating an oxidative short-term stress in both species under herbicide exposure,  
389 as it has been observed with other chemical toxic compounds (Manimaran et al. 2012, Nguyen  
390 Deroche et al. 2012). In summary, it seems that the antioxidant enzymatic response to  
391 herbicides could provide an efficient protection to *C. decipiens*, probably resulting in the  
392 maintenance of its photosynthetic apparatus integrity and Chl *a* per-cell content. On the other  
393 hand, the antioxidant defense system of *P. mannii* was only triggered at the short time scale  
394 which might result in significant effects on the photosynthetic apparatus integrity.

### Herbicide toxicity towards diatoms in co-culture

#### Growth and Chl *a* cell content

Under control conditions, diatom species in co-culture have evolved in a different way  
than when maintained in monoculture, with a slower growth for *C. decipiens* and a decline for  
*P. mannii*. (two-way ANOVA,  $P < 0.05$ , Fig. 1). This suggests that *C. decipiens* achieved  
growth advantage by dominating *P. mannii* due to either a better competition for resources (e.g.,  
nutrients, light) and/or a chemically mediated interference (i.e. allelopathy). In our study, the  
mono- and co-cultures bioassays were performed under similar physico-chemical conditions,

403 using the same non-limiting nutrient concentrations, that the exclusion of *P. mannii* in co-  
404 culture might probably be explained by allelopathy. Allelopathy was found to play a significant  
405 role in dominance, succession, and formation of natural diatom communities (Leflaive and Ten  
406 Hage, 2009; Zhang et al. 2014). In addition, the small size of *P. mannii* may increase its  
407 sensitivity to allelopathic interactions due to a high surface/volume ratio (Lyzkowski and  
408 Karp-Boss, 2014).

409 The single-species exposure to the herbicide mixture, at the tested concentration, showed  
410 that *P. mannii* and *C. decipiens* were tolerant, with no significant impact on their instantaneous  
411 growth rates (two-way ANOVA,  $P > 0.05$ , Table 2). However, growth of *C. decipiens* in co-  
412 culture was significantly impacted by herbicides (two-way ANOVA,  $P < 0.05$ , Table 2),  
413 suggesting that its resistance to herbicide exposure could be modulated when the species  
414 competes in co-culture. This trend may be explained by the classical trade-off theory between  
415 tolerance ability and competitive-response ability in phytoplankton (Mohr, 2019; Lüring,  
416 2021).

417 The differences in sensitivity to herbicides among *P. mannii* and *C. decipiens* under co-  
418 culture conditions might be also explained by differences at the genetic level or in the trophic  
419 mode. In fact, some authors have observed that herbicides may cause a decrease in growth of  
420 some diatoms, specifically from the centric genera, whereas it has the opposite effect on pennate  
421 diatoms (Tang et al., 1997; Bérard et al., 2004). In this sense, Larras et al. (2014) have found a  
422 strong phylogenetic signal for diatom sensitivity to herbicides with a major separation within  
423 the phylogenetic tree. These authors observed that the most sensitive species were mainly  
424 centrics and araphid diatoms whereas the most resistant species were mainly pennates. Other  
425 authors asserted that sensitivity differences among diatom species to herbicides is more likely  
426 related to their trophic mode (Larras et al. 2012). Indeed it was argued that heterotrophic  
427 diatoms were more tolerant than autotrophic diatoms species to PSII inhibitors (Pérès et al.

1996; Bérard et al. 1998; Debenest et al. 2009). For this assumption, authors explained that conversely to autotrophic species, facultative or obligatory heterotrophic species (eventually the case of *P. mannii* in this study) could metabolize other substrata to decrease their dependence on photosynthesis (Larras et al. 2012) and thus to prevent effects of PSII inhibitor herbicides. Heterotrophy has been clearly evidenced for several species of the genus *Pseudo-nitzschia* (Mengelt and Prezelin, 2005, Loureiro et al. 2009, Melliti Ben Garali et al. 2016).

In our experiment, the growth of *P. mannii* was inhibited by *C. decipiens* which dominated the co-culture, substantially on days 6 and 9. The Herbicide induced increase of Chl *a* cell content seemed then to be likely related to *C. decipiens* (Fig. 2). This suggest that the competitive advantage of *C. decipiens* might not affect its Chl *a* response to herbicides. Increased Chl *a* content in response to herbicides has been noted for several microalgae, including *C. gracilis*, contaminated by PSII inhibitors such as diuron (Magnusson et al. 2008; Stachowski-Haberkorn et al. 2013), atrazine (Adler et al. 2007), bentazon (Mine and Matsunaka, 1975), isoproturon and terbutryn (Rioboo et al. 2002). This reaction, also known as “greening”, is widely described as an increase in pigment in antennae in order to maximize light harvesting as a strategy to counterbalance the herbicide action (Magnusson et al. 2008; Ricart et al. 2009; Mansano et al. 2017) and hence maintain an efficient conversion of light energy into chemical energy (Coquillé et al. 2014). This would allow an adaptation of photosynthetic metabolism to herbicide effects.

### **Photosynthetic response and antioxidant enzymes activity**

Maximum quantum yield of PSII ( $F_v/F_m$ ) as well as all the energy fluxes per RC ( $ABS_0/RC$ ,  $TR_0/RC$ ,  $ET_0/RC$  and  $DI_0/RC$ ) were unchanged by the herbicide mixture for the diatom co-culture ( $P > 0.05$ , Fig. 3), closely retracing evolution of these parameters for *C. decipiens* (Fig. 3), which has by far dominated the diatom co-culture. So, as for Chl *a*, the competitive advantage of *C. decipiens* on co-culture did not also influence its photosynthesis

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453 response to the herbicide mixture. As observed for *P. mannii* or *C. decipiens* monocultures, the  
454 GPX activity was stimulated only after one day of contamination in co-culture (Fig. 4),  
455 suggesting that this enzyme could be considered as the antioxidant defense at early exposure to  
456 herbicides. There was a significant increase of CAT activity by herbicides in co-culture ( $P <$   
457  $0.05$ , Fig. 4), especially at late herbicide exposure, similarly to that observed for *C. decipiens*  
458 monoculture, providing additional support that the physiological response of the co-culture to  
459 herbicides was likely attributed to *C. decipiens*. However, CAT activity levels were  
460 significantly higher than those measured for *C. decipiens* monoculture (Student's  $t$ -test,  $P <$   
461  $0.05$ , Fig. 4) and values at days 3 and 6 were 1.2 to 2 folds higher than in *C. decipiens*  
462 monoculture, probably suggesting a higher CAT response due to the competitive stress. These  
463 results provided further evidence that GPX and CAT enzymes were triggered by the herbicide-  
464 induced oxidative stress, at early and tardy exposure, in order to repair damage and keep the  
465 diatom in good physiological state during most period of herbicide contamination.

## 466 CONCLUSION

467 The study found that response to herbicides differ between the two marine diatoms and  
468 between the culture conditions. The diatom *C. decipiens* was more sensitive to herbicides in co-  
469 culture than in the single species-pollutant experiment, which suggest that herbicide pollution  
470 in natural environments might considerably alter the diatom populations and phytoplankton  
471 communities. We link altered growth of *C. decipiens* to a modified physiological profile under  
472 herbicide conditions, thereby suggesting that chemical pollutants effects are not only reflected  
473 by changes in cell densities but also by changes in the physiology of the cells. In order to attain  
474 higher comparability with natural conditions, further studies should focus on the effects of  
475 herbicide pollution and other abiotic fluctuations and their interactions with competition  
476 between diatoms. More complex diatom combinations could be also tested in order to identify

477 composite ecosystem responses and acquire approaches to apprehend changes that might take  
478 place under future environmental scenarios.

## 479 **DECLARATIONS**

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## 483 **COMPLIANCE WITH ETHICAL STANDARDS**

484 Conflict of interest: the authors have no competing interests to declare that are relevant to the  
485 content of this article.

## 486 **AVAILABILITY OF DATA AND MATERIAL**

487 All data and materials as well as software application or custom code comply with field  
488 standards.

## 489 **ETHICS APPROVAL**

490 This article does not contain any studies with animals performed by any of the authors

## 491 **AUTHOR CONTRIBUTIONS**

492 IS, SMB, ZC and ASH contributed to the study conception and design. Laboratory analyses  
493 were performed by IS and ZC and CG. The first draft of the paper was written by IS. ASH  
494 and OP commented on previous versions of the paper. All authors read and approved the final  
495 paper.

## 497 **REFERENCES**

- 498 Adler NE, Schmitt-Jansen M, Altenburger R (2007) Flow cytometry as a tool to study phytotoxic  
499 modes of action. *Environ Toxicol Chem* 26:297-306
- 500 Amato A, Montresor M (2008) Morphology phylogeny and sexual cycle of *Pseudo-nitzschia*  
501 *mannii* sp nov (Bacillariophyceae): a pseudo-cryptic species within the *P*  
502 *pseudodelicatissima* complex. *Phycologia* 47(5):487-497
- 503 Bai X, Sun C, Xie J, Song H, Zhu Q, Su Y, Qian H, Fu Z (2015) Effects of atrazine on photosynthesis  
504 and defense response and the underlying mechanisms in *Phaeodactylum tricorutum*. *Environ Sci*  
505 *Pollut R* 22 (22):17499-17507. <http://doi.org/10.1007/s11356-015-4923-7>
- 506 Bancon-Montigny M, Gonzalez C, Delpoux S, Avenzac M, Spinelli S, Mhadhbi T, Mejri K, Sakka  
507 Hlaili A, Pringault O (2019) Seasonal changes of chemical contamination in coastal waters during  
508 sediment resuspension. *Chemosphere* 235:651-661. [http://doi.org/10.1016/j.chemosphere.2019.06](http://doi.org/10.1016/j.chemosphere.2019.06.213)  
509 213

- 510 Bao VWW, Leung KMY, Qiu J-W, Lam MHW (2011) Acute toxicities of five commonly used  
1 511 antifouling booster biocides to selected subtropical and cosmopolitan marine species. *Mar Pollut*  
2 512 *Bull* 62:1147-1151
- 3  
4 513 Beers RFJr, Sizer IW (1952) A spectrophotometric method for measuring the breakdown of hydrogen  
5 514 peroxide by catalase. *J Biol Chem* 195:133-140
- 6  
7 515 Ben Salem F, Said O, Duran R, Grunberger O (2017) First Survey of Agricultural Pesticides Used for  
8 516 Crops in Ichkeul Lake-Bizerte Lagoon Watershed (Tunisia). *Environ Sci Ind J* 13(6)156
- 9  
10 517 Bérard A, Pelte T, Menthon E, Druart JC, Bourrain X (1998) Characterisation of phytoplankton from  
11 518 two limnic systems contaminated by a herbicidal photosynthetic inhibitor The PICT method  
12 519 (pollution-induced community tolerance): application and significance. *Ann Limnol Int J Lim* 34  
13 520 (3):269-282
- 14 521 Bérard A, Leboulanger C, Pelte T (1999) Tolerance of *Oscillatoria limnetica* Lemmermann to atrazine  
15 522 in natural phytoplankton populations and in pure culture: influence of season and temperature.  
16 523 *Arch Environ Contam Toxicol* 37:472-479
- 17  
18 524 Bérard A, Dorigo U, Mercier I, Becker-van Slooten K, Grandjean D, Leboulanger C (2004)  
19 525 Comparison of the ecotoxicological impact of the triazines Irgarol 1051 and atrazine on  
20 526 microalgal cultures and natural microalgal communities in Lake Geneva. *Chemosphere* 53:935-  
21 527 44. [http://doi.org/10.1016/S0045-6535\(03\)00674-X](http://doi.org/10.1016/S0045-6535(03)00674-X)
- 22  
23 528 Berthon V, Bouchez A, Rimet F (2011) Using diatom life-forms and ecological guilds to assess  
24 529 organic pollution and trophic level in rivers: a case study of rivers in south-eastern France.  
25 530 *Hydrobiologia* 673:259-271. <http://doi.org/10.1007/s10750-011-0786-1>
- 26  
27 531 Böger P (2003) Mode of Action for Chloroacetamides and Functionally Related Compounds. *J Pest*  
28 532 *Sci* 28:324-329 <http://doi.org/10.1584/jpestics.28.324>
- 29  
30 533 Busi R (2014) Resistance to herbicides inhibiting the biosynthesis of Very Long Chain Fatty Acids.  
31 534 *Pest Manag Sci* 70 (9):1378-1384 <http://doi.org/10.1002/ps.3746>
- 32  
33 535 Carter A (2000) How pesticides get into water - And proposed reduction measures. *Pestic Outlook*  
34 536 11:149-156 <http://doi.org/10.1039/b006243j>
- 35  
36 537 Chalifour A, Arts MT, Kainz MJ, Juneau P (2014) Combined effect of temperature and bleaching  
37 538 herbicides on photosynthesis pigment and fatty acid composition of *Chlamydomonas reinhardtii*.  
38 539 *Eur J Phycol* 49(4):508-515
- 39 540 Chamsi O, Pinelli E, Faucon B, Perrault A, Lacroix L, Sánchez-Pérez JM, Charcosset J-Y (2019)  
40 541 Effects of herbicide mixtures on freshwater microalgae with the potential effect of a safener. *Ann*  
41 542 *Limnol Int J Lim* 55 (3). <http://doi.org/10.1051/limn/2019002>
- 42  
43 543 Coquillé N, Jan G, Moreira A, Morin S (2014) Use of diatom motility features as endpoints of  
44 544 metolachlor toxicity. *Aquat Toxicol* 158:202-210. <http://doi.org/10.1016/j.aquatox.2014.11.021>
- 45  
46 545 Coquillé N, Ménard D, Rouxel J, Dupraz V, Melissa E, Pardon P, Budzinski H, Morin S, Parlanti E,  
47 546 Stachowski-Haberkorn S (2018) The influence of natural dissolved organic matter on herbicide  
48 547 toxicity to marine microalgae is species dependent. *Aquat Toxicol* 198:103-117. <http://doi.org/10.1016/j.aquatox.2018.02.019>
- 49 548  
50  
51 549 De Souza R, Seibert D, Quesada H, Bassetti FJ, Fagundes-Klen M, Bergamasco R (2020) Occurrence  
52 550 impacts and general aspects of pesticides in surface water: A review *Process. Saf Environ Prot*  
53 551 135:22-37. <http://doi.org/10.1016/j.psep.2019.12.035>
- 54  
55 552 Debenest T, Silvestre J, Coste M, Delmas F, Pinelli E (2008) Herbicide effects on freshwater benthic  
56 553 diatoms: Induction of nucleus alterations and silica cell wall abnormalities. *Aquat Toxicol* 88 (1):  
57 554 84-94
- 58  
59 555 Debenest T, Pinelli E, Coste M, Silvestre J, Mazzella N, Madigou C, Delmas F (2009) Sensitivity of  
60 556 freshwater periphytic diatoms to agricultural herbicides. *Aquat Toxicol* 93:11-17
- 61  
62  
63  
64  
65

- 557 Debenest T, Silvestre J, Coste M, Pinelli E (2010) Effects of pesticides on freshwater diatoms. Rev  
1 558 Environ Contam Toxicol 203:87-103
- 2 559 Demailly F, Elfeky I, Malbezin L, Le Guédard M, Eon M, Bessoule JJ, Feurtet-Mazel A, Delmas F,  
3 560 Mazzella N, Gonzalez P, Morin S (2019) Impact of diuron and S-metolachlor on the freshwater  
4 561 diatom *Gomphonema gracile*: Complementarity between fatty acid profiles and different kinds of  
5 562 ecotoxicological impact-endpoints. Sci Total 20 (688):960-969. <http://doi.org/10.1016/j.scitotenv>  
6 563 2019 06 347
- 7 564 Dorigo U, Bourrain X, Bérard A, Leboulanger C (2004) Seasonal changes in the sensitivity of river  
8 565 microalgae to atrazine and isoproturon along a contamination gradient. Sci Total Environ 318:  
9 566 101-114 [http://doi.org/10.1016/S0048-9697\(03\)00398-X](http://doi.org/10.1016/S0048-9697(03)00398-X)
- 10 567 Ebenezer V, Ki J-S (2013) Physiological and biochemical responses of the marine dinoflagellate  
11 568 *Prorocentrum minimum* exposed to the oxidizing biocide chlorine. Ecotox Environ Safe 92:  
12 569 129-134. 10. <http://doi.org/10.1016/j.ecoenv> 2013 03 014i
- 13 570 Finizio A, Vighi M, Sandroni D (1997). Determination of N-octanol/water partition coefficient (Kow)  
14 571 of pesticide critical review and comparison of methods. Chemosphere 34: 131-161.  
15 572 [http://doi.org/10.1016/S0045-6535\(96\)00355-4](http://doi.org/10.1016/S0045-6535(96)00355-4).
- 16 573 Gatidou G, Thomaidis NS, 2007. Evaluation of single and joint toxic effects of two antifouling  
17 574 biocides their main metabolites and copper using phytoplankton bioassays. Aquat Toxicol 15 85  
18 575 (3):184-191 <http://doi.org/10.1016/j.aquatox> 2007 09 002 Epub 2007 Sep 6 PMID: 17942164
- 19 576 Gomes M, Juneau P (2017) Temperature and Light Modulation of Herbicide Toxicity on Algal and  
20 577 Cyanobacterial Physiology. Front Environ Sci 5 50. <http://doi.org/10.3389/fenvs> 2017 00050
- 21 578 Guillard RRL, Ryther JH (1962) Studies on Marine Planktonic Diatoms I *Cyclotella nana* Hustedt and  
22 579 *Detonula confervacea* (Cleve) Gran. Can J Microbiol 8:229-239. [http://doi.org/10.1139/m62-](http://doi.org/10.1139/m62-029)  
23 580 [029](http://doi.org/10.1139/m62-029)
- 24 581 Gundermann K, Wagner V, Mittag M, Büchel C (2019) Fucoxanthin-chlorophyll protein complexes of  
25 582 the centric diatom *Cyclotella Meneghiniana* differ in Lhcx1 and Lhcx6\_1 content. Plant Physiol  
26 583 179 (4):1779-1795. <http://doi.org/10.1104/pp.18.01363> Epub 2019 Feb 7
- 27 584 Hashimoto K, Eguchi Y, Oishi H, Tazunoki Y, Tokuda M, Sánchez-Bayo F, Goka K, Hayasaka D  
28 585 (2019) Effects of a herbicide on paddy predatory insects depend on their microhabitat use and an  
29 586 insecticide application. Ecol Appl 29 (6) 01945. <http://doi.org/10.1002/eap.1945>
- 30 587 Hii Y, Shia K, Chuah T-S, Hing LS (2009) Physiological responses of *Chaetoceros sp.* and  
31 588 *Nannochloropsis sp.* to short-term 2,4-D dimethylamine and endosulfan exposure. Aquat Ecosyst  
32 589 Health 12:375-389. <http://doi.org/10.1080/14634980903347662>
- 33 590 Hourmant A, Amara A, Pouline P, Durand G, Arzul G, Quiniou F (2009) Effect of Bentazon on  
34 591 Growth and Physiological Responses of Marine Diatom: *Chaetoceros gracilis*. Toxicol Mech  
35 592 Method 19:109-115. <http://doi.org/10.1080/15376510802290892>
- 36 593 Jiang S, Penner MH (2015) Selective oxidation of enzyme extracts for improved quantification of  
37 594 peroxidase activity. Anal Biochem 1(476): 20-25. <http://doi.org/10.1016/j.ab> 2015 01 017 Epub  
38 595 2015 Jan 29 PMID: 25640588
- 39 596 Juneau P, Qiu B, Deblois C (2007) Use of chlorophyll fluorescence as a tool for determination of  
40 597 herbicide toxic effect. Review Toxicol Environ Chem 89:609-625. [http://doi.org/10](http://doi.org/10.1080/02772240701561569)  
41 598 [1080/02772240701561569](http://doi.org/10.1080/02772240701561569)
- 42 599 Kalopesa E, Nikolaidis G, Menkissoglu-Spiroudi, U (2008). Atrazine effects on growth of the diatom  
43 600 *Rhizosolenia setigera* (Ehrenberg) brightwell. Fresenius Environ Bull 17 :1932-1937.
- 44 601 Kargar M, Ghorbani R, Rashed M, Mohammad H, Rastgoo M (2019) Chlorophyll Fluorescence - a  
45 602 Tool for Quick Identification of Accase and ALS Inhibitor Herbicides Performance Planta  
46 603 Daninha 37. <http://doi.org/10.1590/s0100-83582019370100132>
- 47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65



- 604 Koedooder C, Stock W, Willems A, Mangelinckx S, De Troch M, Vyverman W, Sabbe K (2019)  
1 605 Diatom-Bacteria Interactions Modulate the Composition and Productivity of Benthic Diatom  
2 606 Biofilms *Front Microbiol.* <http://doi.org/10.1038/fmicb.2019.01255>
- 3  
4 607 Kumar K, Young-Seok H, Kyung-Sil C, Jeong-Ae K, Taejun H (2009) Chlorophyll fluorescence  
5 608 based copper toxicity assessment of two algal species. *Toxicol Environ Health Sci* 1(1).  
6 609 <http://doi.org/10.1007/BF03216459>
- 7  
8 610 Lam C, Kurobe T, Lehman P, Berg M, Hammock B, Stillway M, Pandey P, Teh S (2020) Toxicity of  
9 611 herbicides to cyanobacteria and phytoplankton species of the San Francisco Estuary and  
10 612 Sacramento-San Joaquin River Delta California USA. *J Environ Sci Health Part A* 55:1-12  
11 613 <http://doi.org/10.1080/10934529.2019.1672458>
- 12  
13 614 Larras F, Bouchez A, Rimet F, Montuelle B (2012) Using bioassays and species sensitivity  
14 615 distributions to assess herbicide toxicity towards benthic diatoms. *Plos One* 8 :1-9
- 15  
16 616 Larras F, Lambert A-S, Pesce S, Rimet F, Bouchez A, Montuelle B (2013) The effect of temperature  
17 617 and a herbicide mixture on freshwater periphytic algae. *Ecotoxicol Environ Safe* 98 :162-170.  
18 618 <http://doi.org/10.1016/j.ecoenv.2013.09.007>
- 19  
20 619 Larras F, Keck F, Montuelle B, Rimet F, Bouchez A (2014) Linking Diatom Sensitivity to Herbicides  
21 620 to Phylogeny: A Step Forward for Biomonitoring? *Environ Sci Technol* 48(3):1921-1930.  
22 621 <http://doi.org/10.1021/es4045105>
- 23  
24 622 Leboulanger C, Rimet F, Lacotte M, Bérard A (2001) Effect of atrazine and nicosulfuron on  
25 623 freshwater microalgae. *Environ Int* 26 (3):131-135. [http://doi.org/10.1016/S0160-4120\(00\)00100-](http://doi.org/10.1016/S0160-4120(00)00100-8)  
26 624 8
- 27  
28 625 Lee SD, Joo H, Lee J (2014) Critical criteria for identification of the genus *Chaetoceros*  
29 626 (Bacillariophyta) based on setae ultrastructure II Subgenus *Hyalochaete*. *Phycologia* 53:614-638.  
30 627 <http://doi.org/10.2216/14-51R21>
- 31  
32 628 Leflaive J, Ten-Hage L (2009) Chemical interactions in diatoms: Role of polyunsaturated aldehydes  
33 629 and precursors. *New Phytol* 184:794-805. <http://doi.org/10.1111/j.1469-8137.2009.03033.x>
- 34  
35 630 Legrand H, Herlory O, Jean-marc G, Gerard B, Richard P (2006) Inhibition of microphytobenthic  
36 631 photosynthesis by the herbicides atrazine and diuron. *Cah Biol Mar* 47 (1):39-45
- 37  
38 632 Lima IS, Baumeier NC, Rosa RT, Campelo PM, Rosa EA (2014) Influence of glyphosate in  
39 633 planktonic and biofilm growth of *Pseudomonas aeruginosa*. *Braz J Microbiol* 45(3):971-975  
40 634 <http://doi.org/10.1590/s1517-83822014000300029>
- 41  
42 635 López-Cortés D, Nuñez-Vazquez E, Band-Schmidt C, Gárate-Lizárraga I, Hernandez F, Bustillos J  
43 636 (2015) Mass fish die-off during a diatom bloom in the Bahía de La Paz Gulf of California.  
44 637 *Hidrobiologica* 25:39-48
- 45  
46 638 Loureiro S, Jauzein C, Garcés E, Collos Y, Camp J, Vaqué D (2009) The significance of organic  
47 639 nutrients to *Pseudo-nitzschia delicatissima* (Bacillariophyceae) nutrition. *J Plankton Res* 31(4):  
48 640 399-410
- 49  
50 641 Lürling M (2021) Grazing resistance in phytoplankton. *Hydrobiologia* 848:237-249. <http://doi.org/10.1007/s10750-020-04370-3>
- 51  
52 643 Lyczkowski ER, Karp-Boss L (2014) Allelopathic effects of *Alexandrium fundyense* (Dinophyceae)  
53 644 on *Thalassiosira cf. gravida* (Bacillariophyceae): a matter of size. *J Phycol* 50(2):376-387.  
54 645 <http://doi.org/10.1111/jpy.12172> Epub 2014 Mar 15 PMID: 26988194
- 55  
56 646 Macedo RS, Lombardi AT, Omachi CY, Rörig LR (2008) Effects of the herbicide bentazon on growth  
57 647 and photosystem II maximum quantum yield of the marine diatom *Skeletonema costatum*.  
58 648 *Toxicol In Vitro* 22 (3):716-22. <http://doi.org/10.1016/j.tiv.2007.11.012> Epub 2007 Nov 23  
59 649 PMID: 18180139
- 60  
61  
62  
63  
64  
65

- 650 Magnusson M, Heimann K, Negri A (2008) Comparative effects of herbicides on photosynthesis and  
1 651 growth of tropical estuarine microalgae. *Mar Pollut Bull* 56:1545-1552. <http://doi.org/10.1016/j.marpolbul.2008.05.023>  
2 652
- 3  
4 653 Manimaran K, Panneerselvam K, Ashokkumar S, Prabu VA, Sampathkumar P (2012) Effect of copper  
5 654 on growth and enzyme activities of marine diatom *Odontella mobiliensis*. *Bull Environ Contamin Toxicol* 88:30-37. <http://doi.org/10.1007/s00128-011-0427-4>  
6 655
- 7 656 Mansano A, Moreira R, Dornfeld H, Freitas E, Vieira E, Sarmento H, Rocha O, Seleglim M (2017)  
8 657 Effects of diuron and carbofuran and their mixtures on the microalgae *Raphidocelis subcapitata*.  
9 658 *Ecotox Environ Safe* 142:312-321. <http://doi.org/10.1016/j.ecoenv.2017.04.024>
- 10  
11 659 Maronić SD, Ivna Š, Horvatić J, Pfeiffer T, Stević F, Waeg G, Jaganjac M (2018) S-metolachlor  
12 660 promotes oxidative stress in green microalga *Parachlorella kessleri* - A potential environmental  
13 661 and health risk for higher organisms. *Sci Total Environ* 637-638: 41-49. <http://doi.org/10.1016/j.scitotenv.2018.04.433>  
14 662
- 15  
16 663 Mathur S, Allakhverdiev S, Jajoo A (2011) Analysis of high temperature stress on the dynamics of  
17 664 antenna size and reducing side heterogeneity of Photosystem II in wheat leaves (*Triticum*  
18 665 *aestivum*). *Biochim Biophys Acta* 1807:22-29. <http://doi.org/10.1016/j.bbabi.2010.09.001>
- 19  
20 666 Meddeb M, Niquil N, Grami B, Mejri K, Haraldsson M, Chaalali A, Pringault O, Sakka Hlaili A  
21 667 (2019) A new type of plankton food web functioning in coastal waters revealed by coupling  
22 668 Monte Carlo Markov Chain Linear Inverse method and Ecological Network Analysis. *Ecol Indic*  
23 669 104:67-85. <http://doi.org/10.1016/j.ecolind.2019.04.077>
- 24  
25 670 Melliti Ben Garali S, Sahraoui I, de la Iglesia P, Chalghaf M, Diogène J, Ksouri J, Sakka Hlaili A  
26 671 (2016) Effects of nitrogen supply on *Pseudo-nitzschia calliantha* and *Pseudo-nitzschia cf seriata*:  
27 672 field and laboratory experiments. *Ecotoxicol* 25(6):1211-1225. <http://doi.org/10.1007/s10646-016-1675-1> Epub 2016 May 25 PMID: 27225994  
28 673
- 29  
30 674 Melliti Ben Garali S, Sahraoui I, de la Iglesia P, Chalghaf M, Diogène J, Ksouri J, Sakka Hlaili A  
31 675 (2020) Factors driving the seasonal dynamics of *Pseudo-nitzschia* species and domoic acid at  
32 676 mussel farming in the SW Mediterranean Sea. *Chem Ecol* 36(1):66-82. <http://doi.org/10.1080/02757540.2019.1676417>  
33 677
- 34  
35 678 Mengelt C, Prézelin B (2005) A Potential Novel Link Between Organic Nitrogen Loading and  
36 679 *Pseudo-nitzschia spp* Blooms California and the World Ocean - Proceedings of the Conference  
37 680 [http://doi.org/10.1061/40761\(175\)78](http://doi.org/10.1061/40761(175)78)
- 38  
39 681 Mhadhbi T, Pringault O, Nouri H, Spinelli S, Hamouda B, Gonzalez C (2019) Evaluating polar  
40 682 pesticide pollution with a combined approach: a survey of agricultural practices and POCIS  
41 683 passive samplers in a Tunisian lagoon watershed. *Environ Sci Pollut Res* 26(1):342-361.  
42 684 <http://doi.org/10.1007/s11356-018-3552-3>
- 43  
44 685 Mine A, Shooichi M (1975) Mode of action of bentazon: Effect on photosynthesis. *Pest Biochem*  
45 686 *Physiol* 5(5):444-450. [http://doi.org/10.1016/0048-3575\(75\)90017-6](http://doi.org/10.1016/0048-3575(75)90017-6)
- 46  
47 687 Mohr MP (2019) Defense mechanisms in phytoplankton: traits and trade-offs Technical University of  
48 688 Denmark 99 p
- 49  
50 689 Moncada A (2005) Environmental fate of diuron Environmental Monitoring Branch Department of  
51 690 Pesticide Regulation (<http://www.cdpr.ca.gov/docs/empm/pubs/fatememo/diuron.pdf>)
- 52  
53 691 Moustakas M, Malea P, Zafeirakoglou A, Sperdoulis I (2016) Photochemical changes and oxidative  
54 692 damage in the aquatic macrophyte *Cymodocea nodosa* exposed to paraquat-induced oxidative  
55 693 stress. *Pestic Biochem Physiol* 126:28-34. <http://doi.org/10.1016/j.pestbp.2015.07.003> Epub 2015  
56 694 Jul 20 PMID: 26778431
- 57  
58 695 Mudge J, Houlahan J (2019) Wetland macrophyte community response to and recovery from direct  
59 696 application of glyphosate-based herbicides. *Ecotoxicol Environ Safe* 183:109475 <http://doi.org/10.1016/j.ecoenv.2019.109475>  
60 697
- 61  
62  
63  
64  
65

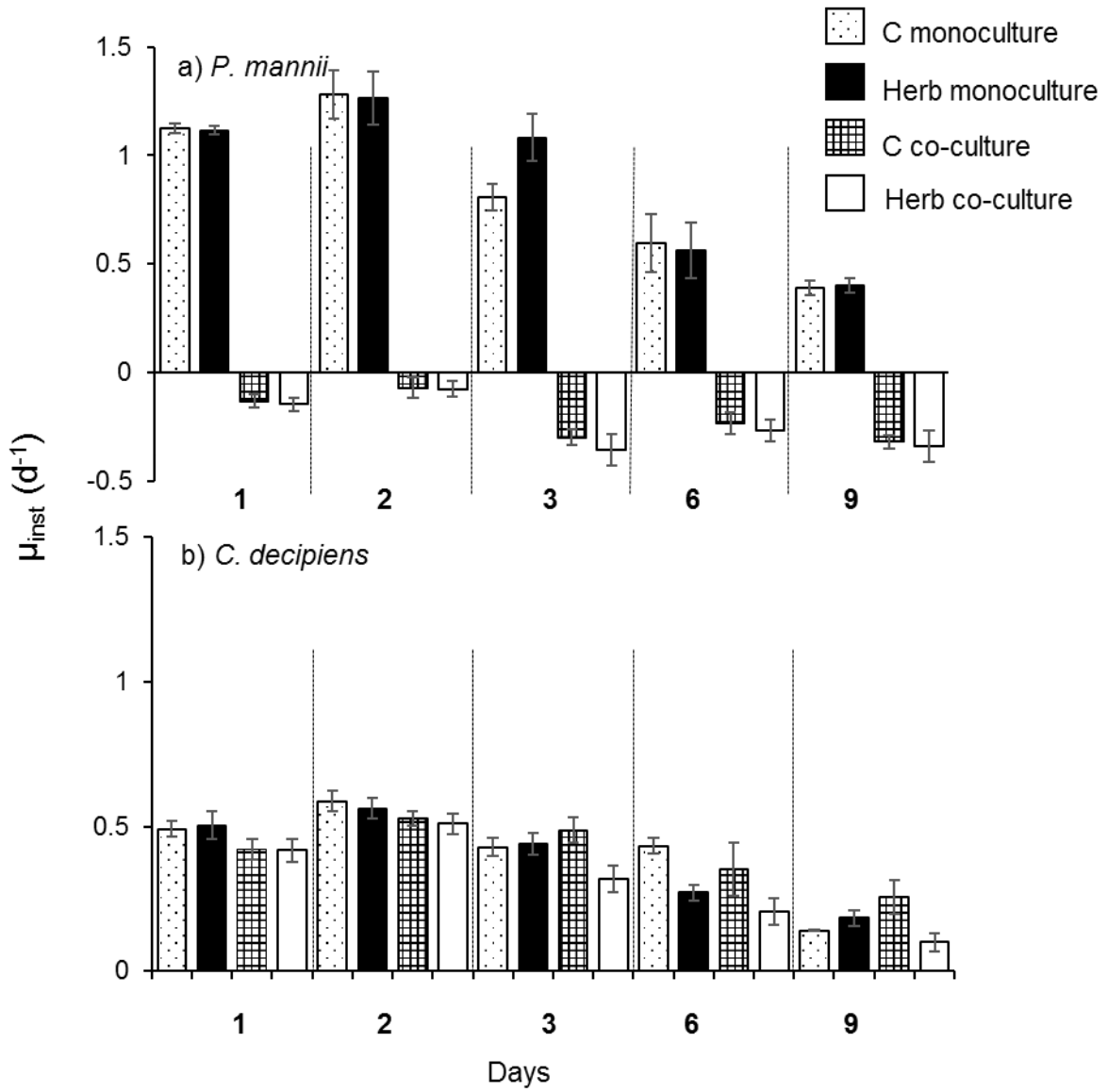
- 698 Nelson DM, Treguer P, Brzezinski MA, Leynaert A, Queguiner B (1995) Production and dissolution  
1 699 of biogenic silica in the ocean: revised global estimates comparison with regional data and  
2 700 relationship to biogenic sedimentation. *Global Biogeochem Cycles* 9:359-72
- 3  
4 701 Nguyen DN, Caruso A, Thi TL, Bui T, Schoefs B, Gérard T, Manceau A (2012) Zinc affects  
5 702 differently growth photosynthesis antioxidant enzyme activities and phytochelatin synthase  
6 703 expression of four marine diatoms. *Sci World J.* 2012:982957. <http://doi.org/10>  
7 704 [1100/2012/982957](http://doi.org/10.1100/2012/982957)
- 8  
9 705 Nödler K, Voutsas D, Licha T (2014). Polar organic micropollutants in the coastal environment of  
10 706 different marine systems. *Mar. Pollut. Bull* 85:50-59. [http://dx.doi.org/10.](http://dx.doi.org/10.1016/j.marpolbul.2014.06.024)  
11 707 [1016/j.marpolbul.2014.06.024](http://dx.doi.org/10.1016/j.marpolbul.2014.06.024).
- 12 708 Ortiz-Hernandez L, Enrique S-S, Castrejón-Godínez M, Dantan E, Popoca E (2013) Mechanisms and  
13 709 strategies for pesticide biodegradation: Opportunity for waste soils and water cleaning. *Revista*  
14 710 *Internacional de Contaminacion Ambiental* 29:85-104
- 15  
16 711 Pandey L (2017) The use of diatoms in ecotoxicology and bioassessment: Insights advances and  
17 712 challenges. *Water Res* 115:1-20
- 18  
19 713 Pandey L, Sharma YC, Park J, Choi S, Lee H, Lyu J, Han T (2020) Evaluating features of periphytic  
20 714 diatom communities as biomonitoring tools in fresh brackish and marine waters 194:67-77  
21 715 [http://doi.org/10 1016/j.aquatox 2017 11 003](http://doi.org/10.1016/j.aquatox.2017.11.003)
- 22  
23 716 Parsons TR, Maita Y, Lalli CM (1984) *A Manual of Chemical and Biological Methods for Seawater*  
24 717 *Analysis* Pergamon Press Oxford 173 pp
- 25  
26 718 Pérès F, Florin D, Grollier T, Feurtet-Mazel A, Coste M, Ribeyre F, Michalet R, Boudou A (1996)  
27 719 Effects of the phenylurea herbicide isoproturon on periphytic diatom communities in freshwater  
28 720 indoor microcosm. *Environ Pollut* 94:141-52. [http://doi.org/10 1016/S0269-7491\(96\)00080-2](http://doi.org/10.1016/S0269-7491(96)00080-2)
- 29  
30 721 Pesce S, Bouchez A, Montuelle B (2011) Effects of organic herbicides on phototrophic microbial  
31 722 communities in freshwater ecosystems. *Rev Environ Contamin Toxicol*  
32 723 214:87-124. [http://doi.org/10 1007/978-1-4614-0668-6\\_5](http://doi.org/10.1007/978-1-4614-0668-6_5)
- 33  
34 724 Pringault O, Lafabrie C, Avezac M, Bancon-Montigny C, Carre C, Chalghaf M, Delpoux S, Duvivier  
35 725 A, Elbaz-Poulichet F, Gonzalez C, Got P, Leboulanger C, Spinelli S, Sakka Hlaili A, Bouvy M  
36 726 (2016) Consequences of contaminant mixture on the dynamics and functional diversity of  
37 727 bacterioplankton in a southwestern Mediterranean coastal ecosystem. *Chemosphere* 144:1060-  
38 728 1073. [http://doi.org/10 1016/j.chemosphere 2015 09 093](http://doi.org/10.1016/j.chemosphere.2015.09.093)
- 39  
40  
41 729 Ramezani S, Saharkhiz M, Ramezani F, Mohammad Hossein F (2008) Use of essential oils as  
42 730 bioherbicides. *J Essent Oil-Bear Plants* 11:319-327. [http://doi.org/10 1080/0972060X 2008](http://doi.org/10.1080/0972060X.2008.10643636)  
43 731 [10643636](http://doi.org/10.1080/0972060X.2008.10643636)
- 44  
45 732 Ricart M, Barceló D, Geiszinger A, Guasch H, Alda MLD, Romaní AM, Vidal G, Villagrasa M,  
46 733 Sabater S (2009) Effects of low concentrations of the phenylurea herbicide diuron on biofilm  
47 734 algae and bacteria. *Chemosphere* 76:1392-1401
- 48  
49 735 Roubex V, Mazzella N, Méchin B, Coste M, Delmas F (2011) Impact of the herbicide metolachlor on  
50 736 river periphytic diatoms: Experimental comparison of descriptors at different biological  
51 737 organization levels. *Intern J Limnol* 47 :239-249. [http://doi.org/10 1051/limn/2011009](http://doi.org/10.1051/limn/2011009)
- 52  
53 738 Sahraoui I, Sakka Hlaili A, Hadj Mabrouk H, Léger C, Bates SS (2009) Blooms of the diatom  
54 739 genus *Pseudo-nitzschia* H Peragallo in Bizerte lagoon (Tunisia SW Mediterranean). *Diatom Res*  
55 740 24(1):175-190. [http://doi.org/10 1080/0269249X 2009 9705789](http://doi.org/10.1080/0269249X.2009.9705789)
- 56  
57 741 Sahraoui I, Grami B, Bates SS, Bouchouicha D, Chikhaoui MA, Mabrouk HH, Hlaili AS (2012)  
58 742 Response of potentially toxic *Pseudo-nitzschia* (Bacillariophyceae) populations and domoic acid

- 743 to environmental conditions in a eutrophied SW Mediterranean coastal lagoon (Tunisia). *Estuar*  
1 744 *Coast* 102:95-104. <https://doi.org/10.1016/j.ecss.2012.03.018>
- 2  
3 745 Sakka Hlaili A, Niquil N, Legendre L (2014) Planktonic food webs revisited: Reanalysis of results  
4 746 from the linear inverse approach *Prog Oceanogr.* 120:216-229. [https://doi.org/10.1016/j.pocean](https://doi.org/10.1016/j.pocean.2013.09.003)  
5 747 2013 09 003
- 6  
7 748 Sakka Hlaili A, Sahraoui I, Bouchouicha-Smida D, Melliti Garali S, Ksouri J, Chalghaf M, Bates SS,  
8 749 Lundholm N, Kooistra W, de La Iglesia P, Diogène J (2016) Toxic and potentially toxic diatom  
9 750 blooms in Tunisian (SW Mediterranean) waters: Review of ten years of investigations. *Adv*  
10 751 *Environ Res* 48:51-69 Nova Science Publishers 572 Inc (Daniels J A editor)
- 11  
12 752 Schabhüttl S, Peter H, Weigelhofer G, Hein T, Weigert A, Striebel M (2012) Temperature and species  
13 753 richness effects in phytoplankton communities. *Oecologia* 171 (2):527-536. [http://doi.org/10](http://doi.org/10.1007/s00442-012-2419-4)  
14 754 1007/s00442-012-2419-4
- 15  
16 755 Schmitt M, Altenburger R (2005) Predicting and observing responses of algal communities to  
17 756 photosystem II-herbicide exposure using pollution-induced community tolerance and species-  
18 757 sensitivity distributions. *Environ Toxicol Chem* 24:304-312. <http://doi.org/10.1897/03-647.1>
- 19  
20 758 Schuler LJ, Rand GM (2008) Aquatic risk assessment of herbicides in freshwater ecosystems of South  
21 759 Florida. *Arch Environ Contam Toxicol* 54(4):571-83. <http://doi.org/10.1007/s00244-007-9085-2>  
22 760 Epub 2007 Dec 19 PMID: 18094912
- 23  
24 761 **Sherwani S I, Arif I A, Khan H A (2015). Modes of Action of Different Classes of Herbicides.**  
25 762 **<http://doi.org/10.5772/61779>.**
- 26  
27 763 Smayda TJ (2006) Autecology of bloom forming microalgae: Extrapolation of laboratory results to  
28 764 field populations and the Redfield - Braarud debate revisited p 215-270 In D V Subba Rao [ed ]  
29 765 *Algal Cultures Analogues of Blooms and Application Science Publishers Enfield NH*
- 30  
31 766 Smedbol É, Lucotte M, Labrecque M, Lepage L, Juneau P (2017) Phytoplankton growth and PSII  
32 767 efficiency sensitivity to a glyphosate-based herbicide (Factor 540®). *Aquat Toxicol* 192:265-273  
33 768 <http://doi.org/10.1016/j.aquatox.2017.09.021> Epub 2017 Sep 28 PMID: 28992597
- 34  
35 769 Stachowski-Haberkorn S, Jérôme M, Rouxel J, Khelifi C, Rincé M, Burgeot T (2013)  
36 770 Multigenerational exposure of the microalga *Tetraselmis suecica* to diuron leads to spontaneous  
37 771 long-term strain adaptation *Aquat Toxicol* 140 380-388
- 38  
39 772 Stevenson R, Pan Y (2010) Assessing environmental conditions in rivers and streams with diatoms  
40 773 *The Diatoms: Applications for the Environmental and Earth Sciences Second Edition*  
41 774 <http://doi.org/10.1017/CBO9780511763175.005>
- 42  
43 775 Strasser BJ, Strasser RJ (1995) Measuring fast fluorescence transients to address environmental  
44 776 questions: The JIP-Test In: Mathis P Ed *photosynthesis: from light to biosphere* KAP Press  
45 777 Dordrecht. 977-980. [http://doi.org/10.1007/978-94-009-0173-5\\_1142](http://doi.org/10.1007/978-94-009-0173-5_1142)
- 46  
47 778 Strasser RJ, Srivastava A, Tsimilli-Michael M (1999) Screening the vitality and photosynthetic  
48 779 activity of plants by fluorescent transient In Behl RK Punia MS and Lather BPS (eds) *Crop*  
49 780 *Improvement for Food Security: 72-115* SSARM Hisar India
- 50  
51 781 Tang J, Hoagland KD, Siegfried BD (1997) Differential toxicity of atrazine to selected freshwater  
52 782 algae. *Bull Environ Contam Toxicol* 59:631-637
- 53  
54 783 Thomas KV, McHugh M, Waldock M (2002) Antifouling paint booster biocides in UK coastal waters:  
55 784 Inputs occurrence and environmental fate. *Sci Tot Environ* 293:117-127
- 56  
57 785 Thomas M, Flores F, Kaserzon S, Reeks T, Negri A (2020) Toxicity of the herbicides diuron  
58 786 propazine tebuthiuron and haloxyfop to the diatom *Chaetoceros muelleri*. *Sci Reports* 10 (1)  
59 787 19592. <http://doi.org/10.1038/s41598-020-76363-0>
- 60  
61  
62  
63  
64  
65

- 788 Thornton D (2014) Dissolved organic matter (DOM) release by phytoplankton in the contemporary  
1 789 and future ocean. *Eur J Phycol* 49:20-46. <http://doi.org/10.1080/09670262.2013.875596>
- 2 790 **Thurman E, Meyer, M (1996). Herbicide Metabolites in Surface Water and Groundwater: Introduction**  
3 **and Overview. ACS Symposium Series. 630. x1-15. 10.1021/bk-1996-0630.ch001.**  
4
- 5 792 Tissier C, Morvan C, Bocquené G, Grossel H, James A, Marchand M (2005) Les substances  
6 793 prioritaires de la Directive cadre sur l'eau (DCE) Fiches de synthèse Rapport IFREMER  
7 794 ([http://www.ifremer.fr/delpc/pdf/RAPPORT\\_FICHES33\\_SUBSTANCES.pdf](http://www.ifremer.fr/delpc/pdf/RAPPORT_FICHES33_SUBSTANCES.pdf))  
8
- 9 795 Wood R, Mitrovic S, Kefford B (2014) Determining the relative sensitivity of benthic diatoms to  
10 796 atrazine using rapid toxicity testing: A novel method. *Sci Tot Environ* 485-486:421-427.  
11 797 <http://doi.org/10.1016/j.scitotenv.2014.03.115>
- 12 798 Wood R, Mitrovic S, Lim R, Kefford B (2016) How benthic diatoms within natural communities  
13 799 respond to eight common herbicides with different modes of action. *Sci Tot Environ* 557-558:  
14 800 636-643. <http://doi.org/10.1016/j.scitotenv.2016.03.142>
- 15 801 Wood R, Mitrovic S, Lim R, Warne M, Dunlop J, Kefford B (2019) Benthic diatoms as indicators of  
16 802 herbicide toxicity in rivers - A new species at Risk (SPEAR herbicides) index. *Ecol Indic* 99:  
17 803 203-213. <http://doi.org/10.1016/j.ecolind.2018.12.035>
- 18 804 Yang X, Guschina I, Hurst S, Wood S, Langford M, Hawkes T, Harwood J (2010) The action of  
19 805 herbicides on fatty acid biosynthesis and elongation in barley and cucumber. *Pest Manage Sci* 66:  
20 806 794-800. <http://doi.org/10.1002/ps.1944>
- 21 807 Yang L, Li H, Zhang Y, Jiao N (2019) Environmental risk assessment of triazine herbicides in the  
22 808 Bohai Sea and the Yellow Sea and their toxicity to phytoplankton at environmental  
23 809 concentrations. *Environ Int* 133(Pt A):105175. <http://doi.org/10.1016/j.envint.2019.105175>
- 24 810 Zhang Y, Wang J, Tan L (2014) The study on the influencing factors of *Chaetoceros curvisetus*  
25 811 allelopathic effect on *Skeletonema costatum* and the primary research on the properties of  
26 812 *Chaetoceros curvisetus* allelochemical. *Acta Oceanol Sinica* 36:123-129
- 27 813 Zhang Y, Wang J, Tan L (2019) Characterization of allelochemicals of the diatom *Chaetoceros*  
28 814 *curvisetus* and the effects on the growth of *Skeletonema costatum*. *Sci Tot Environ* 660:269-276.  
29 815 <http://doi.org/10.1016/j.scitotenv.2019.01.056>
- 30  
31  
32  
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1 **Figure 1**

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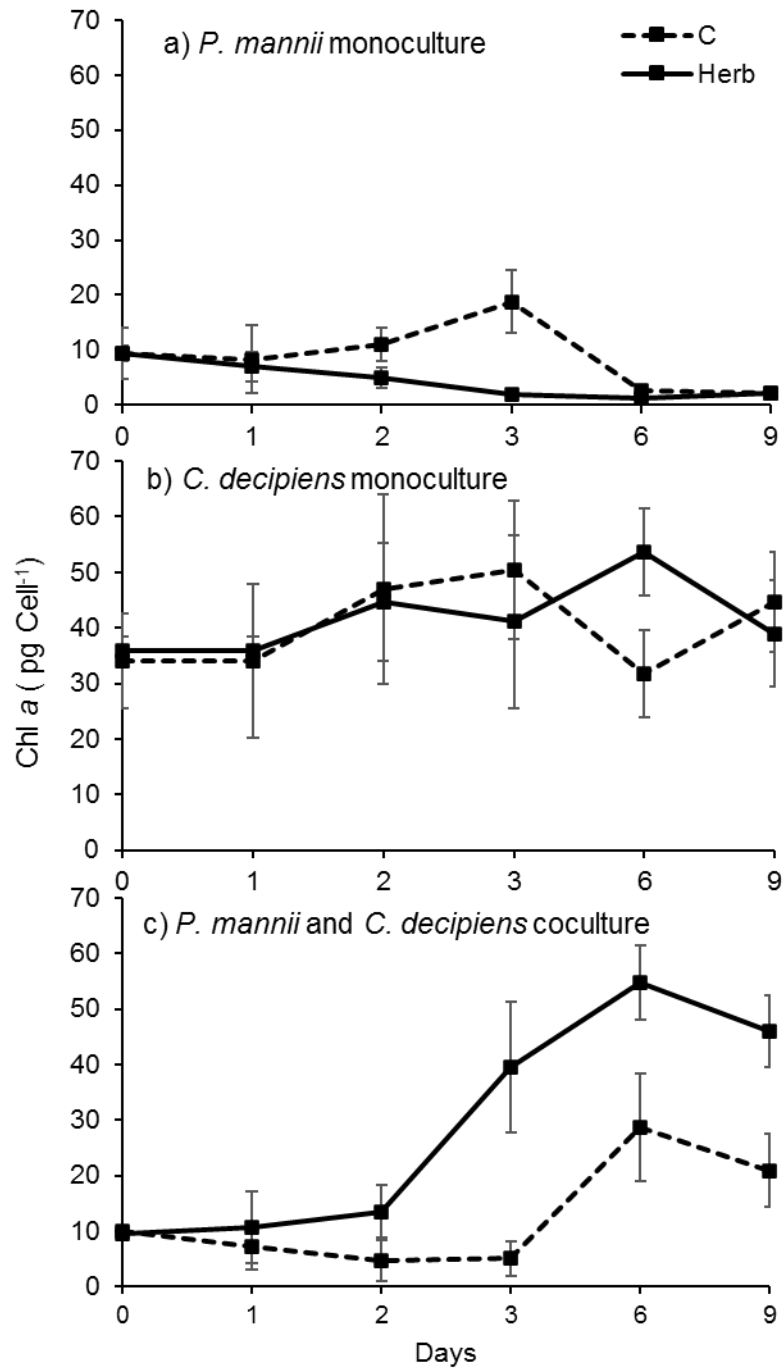
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21 **Figure 2**



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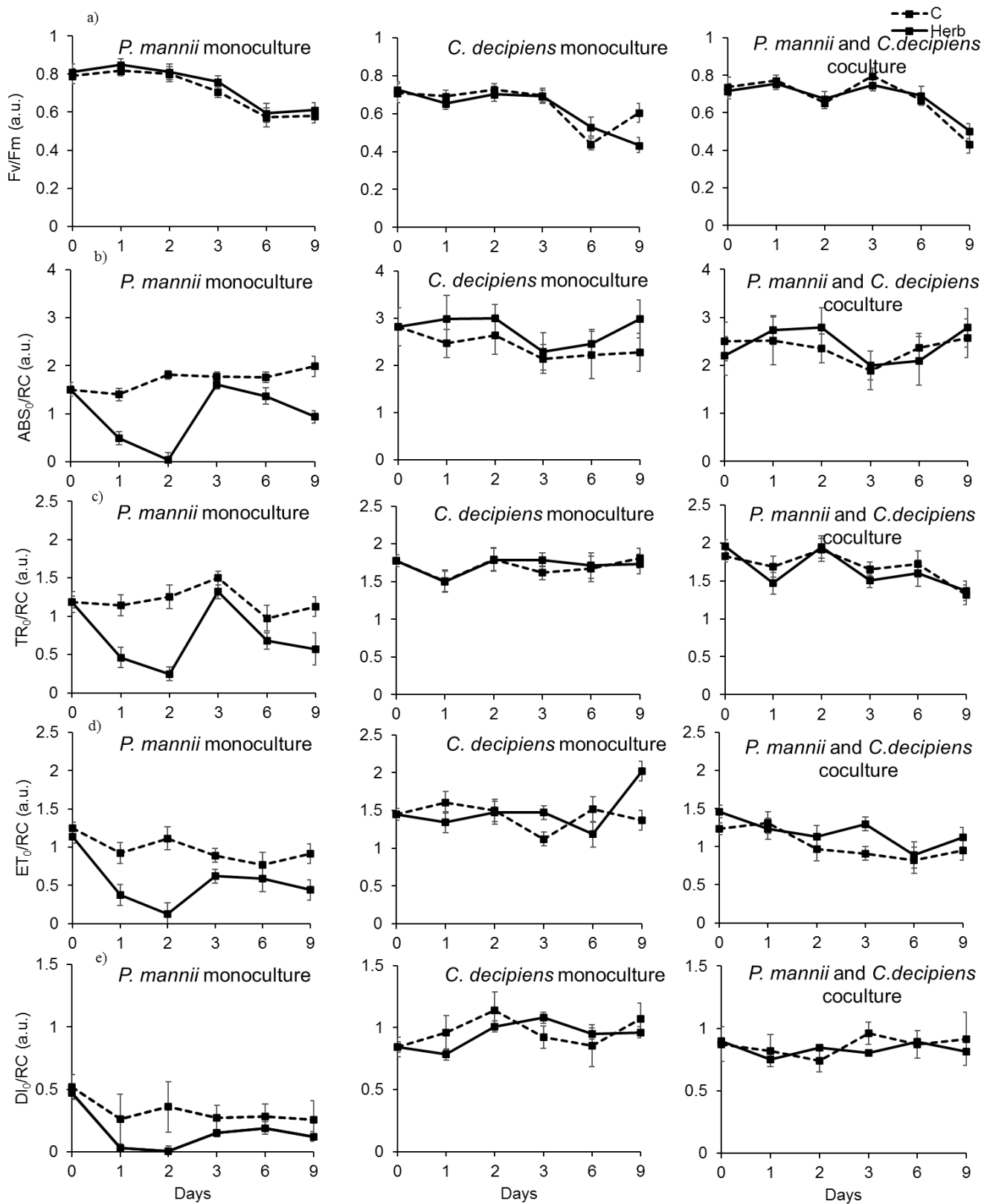
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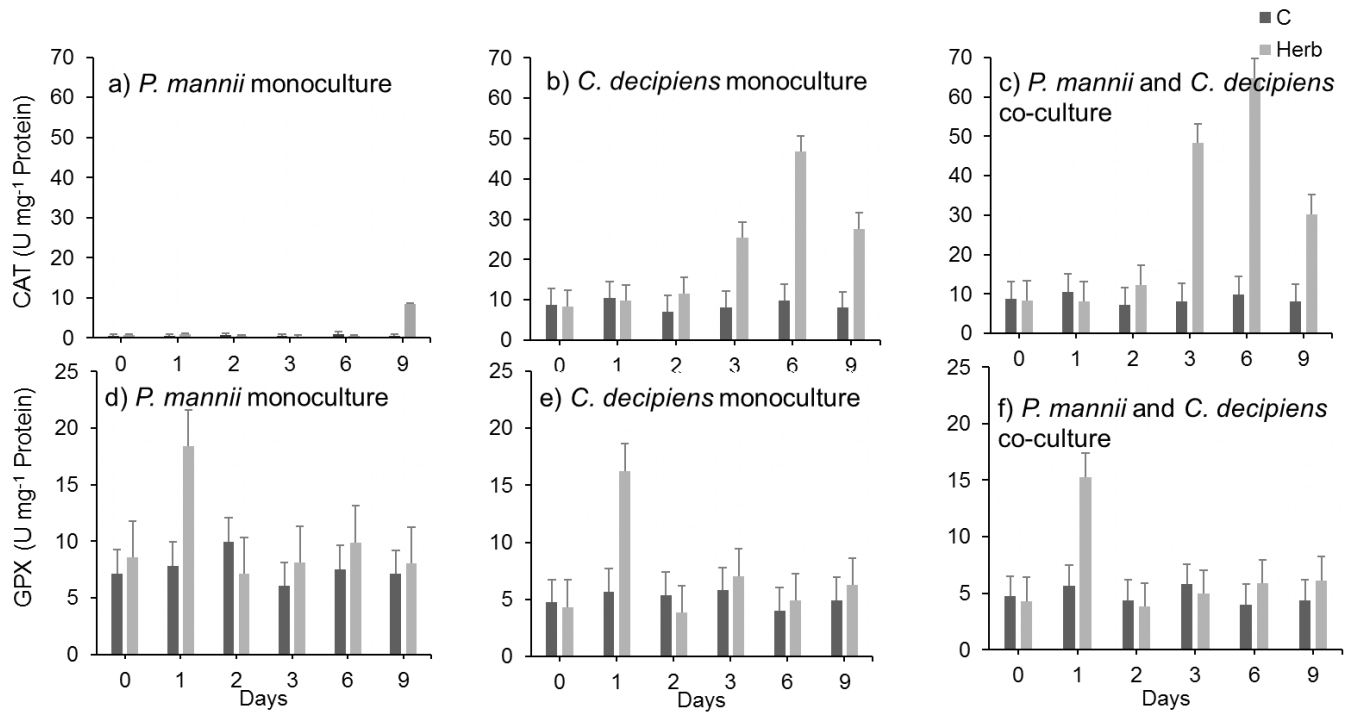
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Figure 3





37 **Figure 4**



**Table 1** Chemical family, physical proprieties and action modes of the different tested herbicides.

Herbicides	Chemical family <sup>a</sup>	Log K <sub>ow</sub> <sup>b</sup>	Solubility (mg L <sup>-1</sup> ) <sup>b, c</sup>	Mode of action/Site of action <sup>a</sup>
Alachlor	Chloroacetamide	3.52	210 ± 38.62	Growth inhibitor/ Very-Long-Chain Fatty Acid (VLCFA) Inhibitors
DIA	Triazine	1.15	3200	Photosynthesis inhibitor/ PSII inhibitor
Diuron	Phenylurea	2.68	47.30 ± 30	Photosynthesis inhibitor/ PSII inhibitor
Simazine	Triazine	2.18	6.43 ± 4.29	Photosynthesis inhibitor/ PSII inhibitor

<sup>a</sup> Sherwani *et al.*, 2015, <sup>b</sup> Finizio *et al.*, 1997, <sup>c</sup> Thurman and Meyer, 1996

**Table 2** F ratios of two-way ANOVA analysis testing for effects of treatment (control/herbicide) and culture mode (monoculture/co-culture) on instantaneous growth rates (IGR) of *P. mannii* and *C. decipiens*

	Treatment		Culture mode		Treatment x Culture mode	
	F	<i>Prob</i>	F	<i>Prob</i>	F	<i>Prob</i>
IGR <i>P. mannii</i>	1.943	0.170	<b>152.611</b>	<b>0.000</b>	1.769	0.152
IGR <i>C. decipiens</i>	1.196	0.281	<b>11.687</b>	<b>0.001</b>	<b>19.078</b>	<b>0.000</b>



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**Suppl Mtrls (not cover letter - place cover letter in  
"comments")**

Figure captions (Sahraoui et al.).docx