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# Effect of interspecific competition on species sensitivity distribution models: analysis of plant responses to chemical stress

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*Keywords:* Species Sensitivity Distribution, environmental risk assessment, isoproturon, herbicide, biotic interaction, multi-stress

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## 1        **ABSTRACT**

2        Species Sensitivity Distributions (SSD) are widely used in environmental risk  
3        assessment to predict the concentration of a contaminant that is hazardous for  
4        5% of species (HC<sub>5</sub>). They are based on monospecific bioassays conducted in  
5        the laboratory and thus do not directly take into account ecological interactions.  
6        This point, among others, is accounted for in environmental risk assessment  
7        through an assessment factor (AF) that is applied to compensate for the lack of  
8        environmental representativity. In this study, we aimed to assess the effects of  
9        interspecific competition on the responses towards isoproturon of plant species  
10       representative of a vegetated filter strip community, and to assess its impact on  
11       the derived SSD and HC<sub>5</sub> values. To do so, we realized bioassays confronting six  
12       herbaceous species to a gradient of isoproturon exposure in presence and absence  
13       of a competitor. Several modelling approaches were applied to see how they  
14       affected the results, using different critical effect concentrations and investigating  
15       different ways to handle multiple endpoints in SSD. At the species level, there  
16       was a strong trend toward organisms being more sensitive to isoproturon in

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17 presence of a competitor than in its absence. At the community level, this trend  
18 was also observed in the SSDs and  $HC_5$  values were always lower in presence of  
19 a competitor (1.12 to 11.13 times lower, depending on the modelling approach).  
20 Our discussion questions the relevance of SSD and AF as currently applied in  
21 environmental risk assessment.

## 22 1. Introduction

23 Long-term anthropogenic activities disrupt the environment. Due to the  
24 complexity of the interactions between living organisms, the accurate evaluation  
25 of the effects of these disturbances is delicate. Environmental Risk Assessment  
26 (ERA) is used to quantify the risk posed by contaminants and other environ-  
27 mental perturbations to living organisms. It mainly consists in the derivation  
28 of Predicted Environmental Concentration (PEC) and their comparison to Pre-  
29 dicted No Effect Concentration (PNEC) to assess the risk of chemicals on the  
30 environment (Amiard and Amiard-Triquet [2015]). Assessment factors (AF) are  
31 applied to secure the procedure. Such AF take into account the lack of real-  
32 ism of some conditions of classical ecotoxicological experiments and thus aim  
33 to extrapolate to realistic environmental conditions by including biotic interac-  
34 tions, intraspecific variability or intra and interlaboratory variability (Amiard  
35 and Amiard-Triquet [2015]). The different ERA methods are divided in 4 tiers  
36 (Aagaard et al. [2013]), the higher tiers using more ecologically relevant data.  
37 Results from methods of higher tiers (*i.e.* more environmentally relevant meth-  
38 ods) are divided by lower AF than those obtained by lower tier methods.

39 The Species Sensitivity Distribution (SSD; Kooijman [1987], Posthuma et al.  
40 [2001]) is a tier 2 ERA method made to produce PNEC at the community level  
41 for a given contaminant using data obtained from monospecific tier 1 bioassays.  
42 The SSD is a distribution of CECs (Critical Effect Concentrations such as Ef-  
43 fective Concentrations (EC) or Benchmark Doses (BMD)) obtained for different  
44 species for a given contaminant. The species under study have to be chosen to

45 be consistent with the mode of action of the studied contaminant and represen-  
46 tative of the considered environment (Van den Brink et al. [2006]). The SSD  
47 can lead to the derivation of the Hazardous Concentration for five percent of  
48 the species (HC<sub>5</sub>). This value, after application of an AF (ranging from 1 to 5  
49 for SSDs; Amiard and Amiard-Triquet [2015]), is commonly used in SSD-based  
50 ERA (for example in the United States of America: Stephan et al. [1985] and  
51 the European Union: European Chemical Bureau [2003]). The SSD method is  
52 however based on several assumptions listed in Forbes and Calow [2002] that  
53 still need to be tested.

54 In particular, very few attempts have been realized to test the effects of  
55 biotic interactions on organisms response against contaminants. In the field, or-  
56 ganisms are however affected by different types of biotic interactions that can be  
57 intra or interspecific. Those interactions can have positive (comensalism, sym-  
58 biosis or facilitation for example) or negative (competition for example) effects  
59 on the organism fitness. Biotic interactions can therefore lead to modifications  
60 of the effects of contaminants the organisms are exposed to. For example, Foit  
61 et al. [2012] used a dose-response design to test the effects of intra and inter-  
62 specific competition on *Daphnia magna* and *Culex pipiens molestus* response  
63 to fenvalerate. They found that competition tended to increase the toxic effect  
64 of fenvalerate on the two tested organisms and modified community dynam-  
65 ics, meaning that toxic exposure might disturb natural community for a longer  
66 period than predicted by monospecific bioassays alone. Gust et al. [2016] inves-  
67 tigated the effects of intraspecific competition on *Daphnia magna*'s tolerance to  
68 copper and lead using a dose-response design. They concluded that intraspe-  
69 cific competition tended to increase *Daphnia magna* mortality caused by Cu  
70 and Pb. Viaene et al. [2015] modeled the effects of intraspecific competition,  
71 interspecific competition by *B. calyciflorus* and predation by *Chaoborus* sp. (at  
72 low and high densities plus control for each type of biotic interaction) on the  
73 response of *Daphnia magna* against pyrene. They found that predation had the  
74 highest negative effect on daphnia density under chemical stress and that com-  
75 petition and predation tended to have antagonistic effects on daphnia tolerance

76 to pyrene. One of the hypothesis for this unexpected effect was that pyrene  
77 would have modified the community composition, leading to reduced negative  
78 biotic interactions. These studies all used macroinvertebrate species and mostly  
79 *Daphnia magna*, and their conclusions need to be extended to other taxonomic  
80 groups. For plants, which represent a key compartment of the ecosystems, very  
81 few studies have been conducted to test interaction effects between biotic inter-  
82 actions, such as competition, and chemical stress responses. Such a question is  
83 however essential for sessile organisms like plants, since they need to be toler-  
84 ant to all local negative environmental conditions to survive and maintain their  
85 population. Results mainly highlighted the existence of interactions between  
86 chemical stress responses and competitive interactions between plant species  
87 (Boutin et al. [2019], Damgaard et al. [2014]), reinforcing the need to take into  
88 account such biotic interactions in ERA procedures.

89 To our knowledge the effects of biotic interactions on SSD results were in-  
90 vestigated in only one study by De Laender et al. [2008]. In this study, the  
91 effects of ecological interactions on SSD results were assessed by a mechanistic  
92 dynamic ecosystem model involving two phytoplankton, three zooplankton and  
93 one fish compartments and interspecific interactions between them (predation,  
94 competition...). A toxic effect submodel, embedded in the ecosystem model, de-  
95 scribed the effects of the toxicants on the parameters of the ecosystem model  
96 (potentially affected parameters were mortality rate for zooplankton and fish  
97 and photosynthesis rate for phytoplankton). One thousand theoretical contami-  
98 nants were used with randomized 10% effect concentration ( $EC_{10}$ ) values drawn  
99 from a log-normal distribution (SSD without taking ecological interactions into  
100 account). Those  $EC_{10}$  values were used in the mechanistic dynamic ecosys-  
101 tem model, thus simulating the subsequent modifications of the ecosystem fate.  
102 New  $EC_{10}$  values derived from those simulations were calculated to obtain SSD  
103 taking ecological interactions into account. For about 25% of those 1000 con-  
104 taminants (254) taking into account biotic interactions led to a change in mean  
105 (190), standard deviation (94) or both (30) of the SSD compared to the one  
106 derived from  $EC_{10}$  produced without taking into account those interactions.

107 Moreover, results showed that this trend was higher for herbicides, implying,  
108 that the derived HC<sub>5</sub> would have great chances to be different. However, this  
109 work needs to be confirmed by data-based studies and to be extended to other  
110 types of communities.

111 Large amount of pesticides are used worldwide to treat a wide diversity of  
112 human-made systems. This is the case of croplands to ensure high agricultural  
113 productivity, but also of vegetated sport fields and green spaces, to control  
114 specific plant composition (Alavanja [2009]). Pesticides have effects on the tar-  
115 geted organisms within the treated areas but also on non-targeted organisms  
116 outside those areas (Crone et al. [2009]). Indeed, due to drift, leaching, and  
117 runoff, those contaminants are found in terrestrial (Geiger et al. [2010]) but  
118 also largely in aquatic ecosystems (Anderson et al. [2015], Annett et al. [2014],  
119 McMahon et al. [2012]). Isoproturon, for example, is a substituted urea photo-  
120 synthesis inhibitor, which is often used as a pre- and post-emergence herbicide in  
121 cereal crops. Before its banning in the EU in 2017 because of potential ground-  
122 water contamination and risks to aquatic life (European Commission [2016]),  
123 isoproturon showed a median concentration of 0.02 mg/kg of soil in investigated  
124 European agricultural soils (Silva et al. [2019]). The degradation and fate of  
125 isoproturon has also been studied. Johnson et al. [1994] showed that 7 days  
126 after isoproturon treatment, concentrations were about 9 mg/kg of soil, after 78  
127 days this concentration was about 1 mg/kg of soil and after 155 days this con-  
128 centration was above 0.5 mg/kg of soil. More generally, isoproturon was also  
129 found to contaminate rivers from one year to another (Dragon et al. [2019]),  
130 and to persist in the soil for at least 3 years after application allowing it to be  
131 mobilised by increasing rainwater (Johnson et al. [2001]). It is thus clear that  
132 a wide diversity of communities, within or outside pesticide-treated areas, are  
133 exposed to numerous pesticides, with concentrations ranging from residual to  
134 acute levels. But these communities, associating several species, are also the  
135 site of numerous biotic interactions, such as competition. This is particularly  
136 the case for plant communities, which, as sessile organisms, may thus be sub-  
137 mitted simultaneously to both pesticide exposure and potentially strong biotic

138 interactions. Plant communities are therefore a relevant community model to  
139 test the assumption: "Interactions between species do not influence the sen-  
140 sitivity distribution" highlighted by Forbes and Calow [2002] about the SSD  
141 method. Considering the studies listed above, we expected that 1) interspecific  
142 competition would have a negative effect on organism responses to toxicants in  
143 our study and that 2) the protective concentration for 95% of species would be  
144 lowered by interspecific competition. To test these assumptions, a dataset of  
145 monospecific bioassays results on 6 herbaceous species exposed to isoproturon  
146 in presence and absence of competition was produced. We then built SSDs  
147 with and without competition and compared them. For this purpose, we tried  
148 to characterize the effects of interspecific competition on SSD results using a  
149 variety of CECs as well as endpoints and ways to handle them.

## 150 2. Materials and methods

### 151 2.1. Dataset

#### 152 2.1.1. Tested species and competitor species

153 Six herbaceous grass species were chosen as representative of a model vege-  
154 tated filter strip community. They were selected to represent the natural plant  
155 diversity existing in terms of isoproturon tolerance and of competitive abil-  
156 ity: *Dactylis glomerata*, *Lolium multiflorum*, *Arrhenatherum elatius*, *Trisetum*  
157 *flavescens*, *Poa pratensis*, *Poa trivialis*. *Bromus erectus* was chosen as a com-  
158 petitor species to ensure a competitor pressure as constant as possible along  
159 the experiment duration. All the seeds were obtained from the Phytosem seed  
160 company (Gap, Hautes-Alpes, France).

#### 161 2.1.2. Experimental design

162 The pesticide used for exposure was isoproturon, a phenyl-urea photosystem  
163 II inhibitor. Despite its recent banning in EU, isoproturon environmental per-  
164 sistence, use in some no-EU countries, and common mode of action, similar to  
165 numerous worldwide used herbicides, still make this molecule a relevant pesti-  
166 cide model to study (Alberto et al. [2018], Eker [2019], Johnson et al. [2001]).

167 Ecotoxicological bioassays were realized for the six tested species and the two  
168 competition modalities (absence or presence of the competitor). Experiments  
169 were realized in microcosms under controlled conditions (20°C ; 16 hours day  
170 of light at 120 $\mu$ mol photons/m<sup>2</sup>/s). Three-liter-round microcosms (20 cm di-  
171 ameter, 12.9cm height) containing inert sterilized vermiculite as substrate were  
172 vegetalised by transferring seedlings at 2-leaves phenological stage previously  
173 grown from seeds in the absence of chemical stress. For each microcosm, one  
174 seedling of the tested species was placed at the center of the enclosure, and  
175 for the competition treatment, 37 seedlings of *Bromus erectus* were planted  
176 according to a standardized hexagonal pattern corresponding to 3 circles of,  
177 respectively, 6, 12, and 19 seedlings at equal distance (Birch et al. [2007]). Mi-  
178 crocosm contamination with isoproturon began after a 4-days acclimatization.  
179 Plant exposure to isoproturon was carried out by substrate watering in order to  
180 induce root chemical exposure. Continuous chemical stress was performed by  
181 watering the device with 150 mL of contaminated nutritive solution [Hoagland  
182 basal salt mix (No2, Caisson Laboratories, Smithfield, UT, USA) at 0.82 g/L,  
183 pH6] twice a week during the 25 days experiment. Five isoproturon (Cluzeau  
184 Indo Labo, Sainte Foy la Grande, France) concentrations (0.25, 0.5, 1, 1.5 and  
185 1.75  $\mu$ M, corresponding to, respectively, 51.5, 103, 206, 309, and 360  $\mu$ g/L) plus  
186 a control were used. For each concentration (6 concentrations including the  
187 control) and each competition modality (absence or presence of competitor),  
188 eight replicates were realized, leading to 96 microcosms per tested species.

### 189 2.1.3. Studied endpoints and metrics

190 At the end of the experiment, eleven endpoints were measured on the tested  
191 species, and an additional metric of dry mass (DM) ratio was calculated. These  
192 endpoints were chosen in order to detect the effects of the different treatments  
193 (herbicide exposure, competition) applied to the tested species in light of the  
194 literature. In addition to global traits (DM traits, shoot height, root length),  
195 some traits were preferentially chosen for their responses to herbicide (meximum  
196 efficiency of photosystem II determined from Fv/Fm, pigment contents), and to



197 competition (root/shoot DM ratio, Specific Leaf Area (SLA), Leaf Dry Mat-  
198 ter Content (LDMC), and ligula height corresponding to stem height). Fv/Fm  
199 chlorophyll fluorescence and pigment contents were measured as previously de-  
200 scribed in Serra et al. [2013]. SLA and LDMC were quantified as described  
201 in Cornelissen et al. [2003]. *Bromus* shoot dry biomass was also weighted, for  
202 each microcosm, at the end of the experiment. Analysis of these DM data  
203 showed that mean *Bromus* shoot DM was similar between species and isopro-  
204 turon treatments, thus allowing to compare all treatment modalities carried out  
205 in the experiment.

## 206 2.2. Data analysis

207 Critical Effect Concentration values are the elementary components neces-  
208 sary to build SSD. Those CECs were obtained following a stepwise procedure: 1)  
209 transformation of data for some endpoints, 2) selection of responsive endpoints  
210 for a given species, 3) fitting of concentration-response curves, 4) derivation of  
211 CEC values from the fits. The whole modelling process was implemented under  
212 the R environment (version 3.5.2; R Core Team [2019]).

### 213 2.2.1. Data transformations

214 Concentration-response curves fitting using non-linear regression assumes a  
215 Gaussian error model. Accordingly, the first step was to apply a transforma-  
216 tion on response for some endpoints as an attempt to improve homoscedastic-  
217 ity and residuals normality. For each endpoint, we built two ANOVA models  
218 where measured values were explained by species, competition modality and iso-  
219 proturon concentration combinations so that the remaining variability is only  
220 inter-replicate variability: one model was built with raw data and the other  
221 after transformation of the data.

222 We then visually inspected the residuals of the models to see if the trans-  
223 formation improved homoscedasticity and residuals normality and in that case,  
224 we kept it. Different transformations were tested, depending on the considered  
225 endpoint. For the Fv/Fm chlorophyll fluorescence endpoint, which is a propor-

226 tion, we applied a logit transformation ( $\log(p/(1-p))$ ) to change its scale, after  
227 having previously changed the 0 values (3 out of 471 Fv/Fm measurements) to  
228 0,01 (corresponding to about one fourth of the lowest non-zero value of 0,0395).  
229 For every other endpoint, a log transformation was tested.

### 230 *2.2.2. Selection of responsive endpoints*

231 Some of the measured endpoints did not present any variation against the  
232 isotroturon treatment. Concentration-response modelling was meaningless in  
233 such cases and led to numerical issues. A selection step was therefore applied  
234 to keep "responsive" endpoints for each species i.e. those that have exhibited  
235 variations according to isotroturon concentration. With six species, eleven end-  
236 points and two competition modalities, we had 132 subdatasets. It would have  
237 been complicated to inspect visually every subdataset to find the responsive  
238 ones. We thus decided to test for their responsivity numerically using the same  
239 procedure as in Larras et al. [2018]. We used a linear trend test to assess the  
240 significance of a regression line linking the endpoint values to the isotroturon  
241 concentrations. A Benjamini-Hochberg correction was applied on the p-values  
242 to reduce false positive selection, due to the high number of realized tests. A  
243 0.05 default threshold was used as false discovery rate. This procedure led to  
244 select different endpoints for each species. However, for a given species, an end-  
245 point was selected only if it was responsive with and without competition to  
246 enable a comparison of SSDs in both situations.

### 247 *2.2.3. Concentration-Response Curves modelling*

248 A concentration-response relationship was fitted on data for each selected  
249 endpoint, species and competition modality combination. Non-linear regressions  
250 were realized with the drc R package (version 3.0-1; Ritz et al. [2015]) using a  
251 log-probit model (see equation 1).

$$y_{ij} = c + (d - c) * \phi(b * (\log(x_i) - \log(e))) \quad (1)$$

252 where  $i$  refers to the  $i^{\text{th}}$  isoproturon concentration and  $j$  refers to the  $j^{\text{th}}$  repli-  
253 cate,  $x_i$  is the isoproturon concentration,  $y_{ij}$  is the response level of the endpoint,  
254  $\phi$  is the cumulative probability density of the normal law,  $e$  is the concentra-  
255 tion at which the maximum slope occurs (equal to the  $EC_{50}$ ;  $> 0$ )  $b$  is a shape  
256 parameter. if  $b < 0$ ,  $c$  is the response level at high concentrations and  $d$  is the  
257 response level at low concentrations. if  $b > 0$ ,  $c$  is the response level at low  
258 concentrations and  $d$  is the response level at high concentrations. If  $b = 0$ , the  
259 whole model becomes a constant model set at the arithmetical mean between  $c$   
260 and  $d$ .

#### 261 2.2.4. Critical Effect Concentration derivation

262 For each dose-response curve, two types of CEC were calculated. First, the  
263 Effective Concentration which leads to  $x\%$  of maximum effect (i.e. between the  
264 parameters  $c$  and  $d$ ) ( $EC_x$ ), was calculated. It is the most commonly used CEC  
265 in ecotoxicology as it begins to be widely accepted that NOEC (No Observed  
266 Effect Concentration) and LOEC (Low Observed Effect Concentration) suffer  
267 from some important weaknesses (Jager [2012]). Although any  $x$  value could  
268 be used, we have here studied  $EC_{50}$ , as it is the most widely used value in  
269 ecotoxicological studies and it is a direct parameter of the log-probit model we  
270 used, and  $EC_{10}$ , as this value is often used as a no-effect concentration proxy in  
271 risk assessment (Iwasaki et al. [2015]).

272 Secondly, we calculated the Benchmark Doses ( $BMD_{Zsd}$ ) as an alternative to  
273  $EC_x$ . This CEC, described in the EFSA guidance (EFSA Scientific Committee  
274 et al. [2017]), has the advantage to take into account data variability. Indeed,  
275 the  $BMD_{Zsd}$  is the concentration at which the Benchmark Response ( $BMR_{Zsd}$ ) is  
276 reached, the latter being equal to a change of  $z$  times the model residual standard  
277 deviation from the control mean. The  $z$  of  $BMD_{Zsd}$  is therefore theoretically  
278 speaking close to the  $x$  of  $EC_x$  as it defines the level considered to have a critical  
279 effect. The EFSA guidance proposes to use a  $z$  value of 1 (EFSA Scientific  
280 Committee et al. [2017]), but we also calculated  $BMD_{2sd}$ . This latter would  
281 correspond to a change up to one of the bounds of the 95% confidence interval

282 around the predicted value at the control (Larras et al. [2018]).

### 283 2.2.5. SSD modelling

284 SSDs were built *via* three different scenarios that are commonly used in  
285 scientific publications and regulatory texts. A fourth approach, adapted to our  
286 multiple endpoint dataset but not used in regulatory texts has also been tested.

287 1) In a first scenario, the geometric mean of the obtained CECs for the different  
288 endpoints has been used for each species. This approach has the advantage to  
289 use all the information available for the different endpoints. This is also the  
290 most commonly used approach in SSDs in the literature (Xu et al. [2015]). 2)  
291 In a second scenario, we used the lowest CEC value obtained for each species.  
292 This method is very protective, but it is only using a single value, thus making  
293 it sensitive to potential outliers. 3) In a third scenario, only the total dry mass  
294 endpoint was considered for each species. This was a responsive endpoint for all  
295 of the species under study and is usually measured in ecotoxicological studies  
296 on herbaceous grass species (Del Signore et al. [2016]). 4) The fourth approach  
297 did not directly use CEC values. We first built sensitivity distributions of the  
298 different endpoints for each species (Arts et al. [2008], Hanson and Solomon  
299 [2002]) and called them "Endpoint Sensitivity Distribution" (ESD) by analogy  
300 to SSDs. We then calculated the fifth percentile of these distributions for each  
301 species as a protective concentration for 95% of the endpoints and used these  
302 values to build the SSDs themselves. This ESD-using approach was considered  
303 to be a good compromise between the two first approaches (*i.e.* the geometric  
304 mean approach and the lowest value approach) as it takes into account all of  
305 the available datapoints (like the geometric mean approach) but gives a more  
306 protective result (like the lowest value approach) without being too sensitive to  
307 outliers.

308 SSDs have been modeled with log-logistic distributions. The fits were done  
309 using the `fitdistcens` function from the `fitdistrplus` R package (version 1.0-14;  
310 Delignette-Muller and Dutang [2015]) to integrate censored data and  $HC_5$  values  
311 were derived.

312 **3. Results**

313 *3.1. Data transformation*

314 After applying the procedure to chose which data to transform, the logit  
 315 transformation was applied to Fv/Fm chlorophyll fluorescence and the log trans-  
 316 formation to every other endpoints except chlorophyll and carotenoid contents.

317 *3.2. Selection of responsive endpoints*

318 Table 1 presents the results of the plant species screening and endpoint re-  
 319 sponsiveness. Five to nine endpoints were selected depending on the species.  
 320 Some endpoints were selected for all of the species. It is the case for Fv/Fm  
 321 chlorophyll fluorescence and the dry masses of root, shoot and total. In con-  
 322 trast, some endpoints were not responsive to isoproturon exposure, such as  
 323 LDMC and pigment contents (Table 1). We noticed that some endpoints for  
 324 some species were not selected because of the very high variability between  
 325 replicates (for chlorophyll and carotenoid contents for example). In total, 42  
 326 couples of (species, endpoint) were selected, leading to the construction of 84  
 327 concentration-response curves (with and without competitor).

Species	<i>P. trivialis</i>	<i>P. pratensis</i>	<i>T. flavescens</i>	<i>A. elatius</i>	<i>L. multiflorum</i>	<i>D. glomerata</i>
Ligula height	X	X				X
Max shoot height	X	X				X
Root length	X	X		X		X
LDMC			X			
SLA		X	X		X	X
Fv/Fm	X	X	X	X	X	X
Chlorophyll		X				
Carotenoid						
Root DM	X	X	X	X	X	X
Shoot DM	X	X	X	X	X	X
Total DM	X	X	X	X	X	X
Root DM/shoot DM	X		X			

<b>Total selected</b>	8	9	7	5	5	8
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Table 1: Responsive endpoints for the different tested species. An "X" indicates that the endpoint is considered responsive for the given species. DM stands for dry mass.

328 *3.3. Concentration-response curves modelling*

329 Figure 1 shows an example of curve fitting for *P. pratensis* for the differ-  
330 ent selected endpoints. An example of fit for another species can be found in  
331 SI (A). No numerical errors were encountered with this fitting procedure and  
332 the models have been fitted correctly in every case. The different fits have  
333 been visually checked and were consistent with the data. For every endpoint,  
334 the concentration-response curve is decreasing with increasing isoproturon con-  
335 centrations, the only exception being SLA whose value is increasing with the  
336 isoproturon concentration.

337 *3.4. Critical Effect Concentration derivation*

338 Figure 1 also shows an example of CEC calculation for *P. pratensis*. The  
339 values of the different CECs calculated for the selected endpoints for each species  
340 can be found in SI (B). For  $BMD_{2sd}$ , a value could not always be calculated  
341 as the  $BMR_{Zsd}$  was sometimes beyond the asymptote for high concentrations,  
342 meaning that the amplitude of the response was in that case lower than 2 (for  
343  $BMD_{2sd}$ ) times the model's residual standard deviation. This happened 12 times  
344 on 84  $BMD_{2sd}$  calculations and never happened for  $BMD_{1sd}$  calculations even if  
345 it was conceptually possible. As the  $EC_x$  effect level is a percentage of maximum  
346 effect (between the two asymptotes), it was successfully calculated in every case.  
347 There were however cases where the calculated  $EC_x$  or  $BMD_{Zsd}$  were above the  
348 maximum concentrations. We considered those values as censored values in the  
349 interval [maximum tested dose;  $+\infty$  [. This happened 3 times among the 84  
350 calculated  $EC_{10}$ , 20 times among the 84 calculated  $EC_{50}$  and 10 times among  
351 the 84 calculated  $BMD_{2sd}$ . This did not happen for  $BMD_{1sd}$ . Figure 2 shows the  
352 values of  $BMD_{1sd}$  calculated for the different species and endpoints (an example

353 for another CEC given in SI (C)). In most cases, the calculated CEC values were  
354 lower in presence of a competitor, thus showing that interspecific competition  
355 had a negative impact on organisms tolerance on most of the studied species  
356 and endpoints.

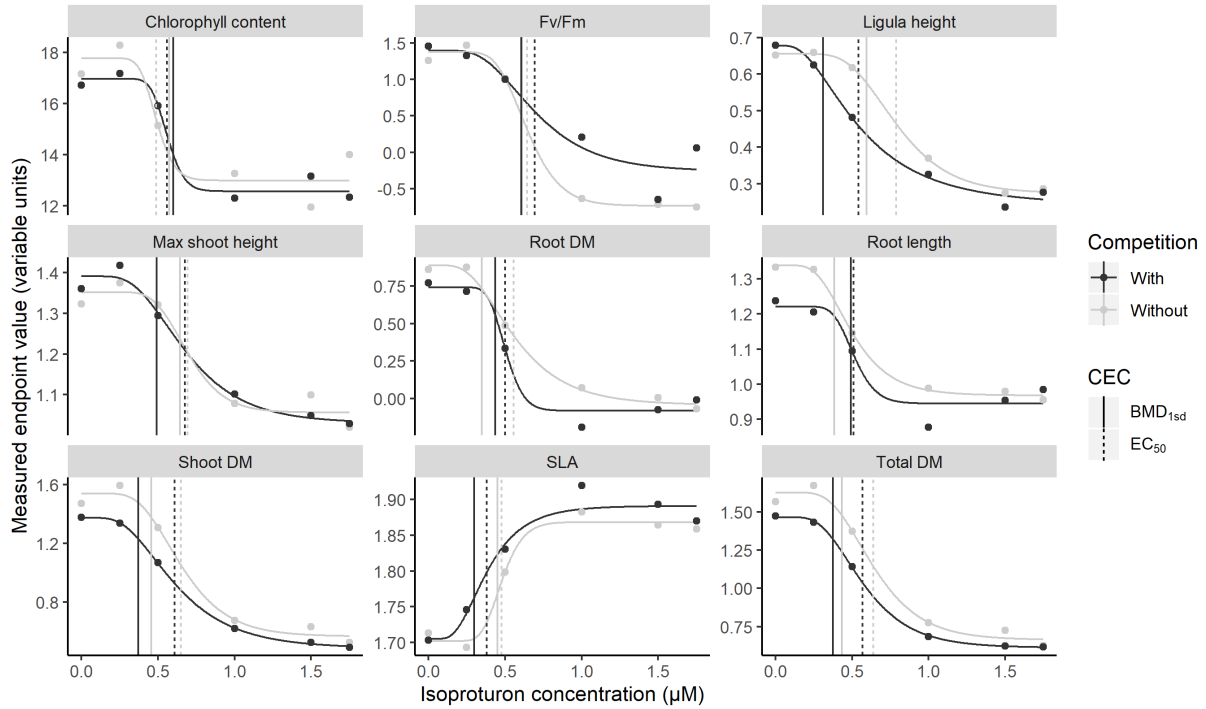
357 We calculated for each  $BMD_{Zsd}$  value the corresponding percentage of re-  
358 sponse (i.e. the  $x$  in  $EC_x$  corresponding to each  $BMD_{Zsd}$  value).  $BMD_{1sd}$   
359 corresponded in average to  $EC_{29.7}$  values (interquartile range of  $x$  equivalent  
360 for  $BMD_{1sd}$ : [16.5, 38.3]) whereas  $BMD_{2sd}$  corresponded in average to  $EC_{49.0}$   
361 values (interquartile range of  $x$  equivalent for  $BMD_{2sd}$ : [32.4, 64.3]).

362 Figure 3 summarizes the CEC values calculated for the different CEC types,  
363 species and endpoints and displays the value with competition against the value  
364 without competition. All CEC being pooled, about 10% of points are between  
365 the two dashed lines, thus showing that, in this case, competition had here mi-  
366 nor or no effect (less than a 5% variation in one way or another). Many more  
367 points are below the dashed lines (70%) than above (20%), thus showing that  
368 interspecific competition had mostly a negative effect on organism tolerance to  
369 chemical stress in our experiment. This is consistent with the hypothesis stated  
370 in introduction. This trend toward negative effect can be seen for every calcu-  
371 lated CECs and every species and endpoints tested even if it is not systematic.  
372 This trend toward negative effect however seems less visible for  $EC_{50}$  (44%)  
373 than for other CECs (69% for  $EC_{10}$ , 73% for  $BMD_{1sd}$  and 74% for  $BMD_{2sd}$ ).

### 374 3.5. ESD and SSD modelling

375 An example of the fitted ESD for the different species for  $BMD_{1sd}$  is shown  
376 in Figure 4. An exemple of figure for another CEC can be found in SI (D).  
377 Figure 5 shows examples of SSDs calculated from  $BMD_{1sd}$  and using the different  
378 methods for handling multiple endpoints (minimum, mean, total dry mass and  
379 "ESD"). An example of SSD obtained for another CEC is shown in SI (E). Table  
380 2 summarizes the shifts between  $HC_5$  values with and without competition  
381 through the ratios between  $HC_5$  values without competition and  $HC_5$  values  
382 with competition. We can see in this table that every shift ratio was above one,

Figure 1: Examples of concentration-response fits for *P. pratensis*. The points represent the mean of data for each concentration and the curves the fitted models. Data without competition and with competition are highlighted in grey and black respectively. The dashed vertical lines represent the EC<sub>50</sub> levels and the plain vertical lines the BMD<sub>1sd</sub> levels. EC<sub>10</sub> and BMD<sub>2sd</sub> are not displayed here for reasons of clarity. DM stands for dry mass.



383 thus showing that the interspecific competition tended to lower plant tolerance  
 384 at the community level. We can also see that the competition effect can lower  
 385 the HC<sub>5</sub> up to 11-fold, which strongly surpasses the Assessment Factor of 5  
 386 typically used in SSD-based ERA. This however happened only once and for  
 387 the least robust method.



	<b>EC<sub>10</sub></b>	<b>EC<sub>50</sub></b>	<b>BMD<sub>1sd</sub></b>	<b>BMD<sub>2sd</sub></b>
<b>Min value</b>	0.112/0.011 (11.13)	0.285/0.231 (1.23)	0.212/0.101 (2.11)	0.261/0.218 (1.19)
<b>Mean value</b>	0.197/0.090 (2.19)	0.435/0.388 (1.12)	0.291/0.196 (1.48)	0.494/0.371 (1.33)
<b>Dry mass</b>	0.125/0.077 (1.63)	0.180/0.130 (1.38)	0.219/0.113 (1.94)	0.215/0.187 (1.15)
<b>ESD 5<sup>th</sup> percentile</b>	0.084/0.022 (3.84)	0.156/0.121 (1.29)	0.159/0.096 (1.66)	0.167/0.126 (1.33)

Table 2: Summary of HC<sub>5</sub> values (in  $\mu\text{M}$ ) for the different CEC and multiple endpoints handling methods. The value before the slash is the HC<sub>5</sub> without competition, the value after the slash the HC<sub>5</sub> with competition and the value between brackets the ratio between the two HC<sub>5</sub>.

Figure 2:  $BMD_{1sd}$  values for the different species and endpoints. A black "X" indicates that the endpoint was not selected for the considered species and that no CEC values was calculated. The grey marks are the CEC values without competition and the black ones are the values with competition. The lines linking those two marks for a given species and endpoint combination are solid lines when competition had a negative effect on organisms tolerance (CEC with competition lower than CEC without competition) and are dashed lines when competition had a positive effect on organisms tolerance (CEC with competition higher than CEC without competition), thus leading to rather facilitation for the species. DM stands for dry mass.

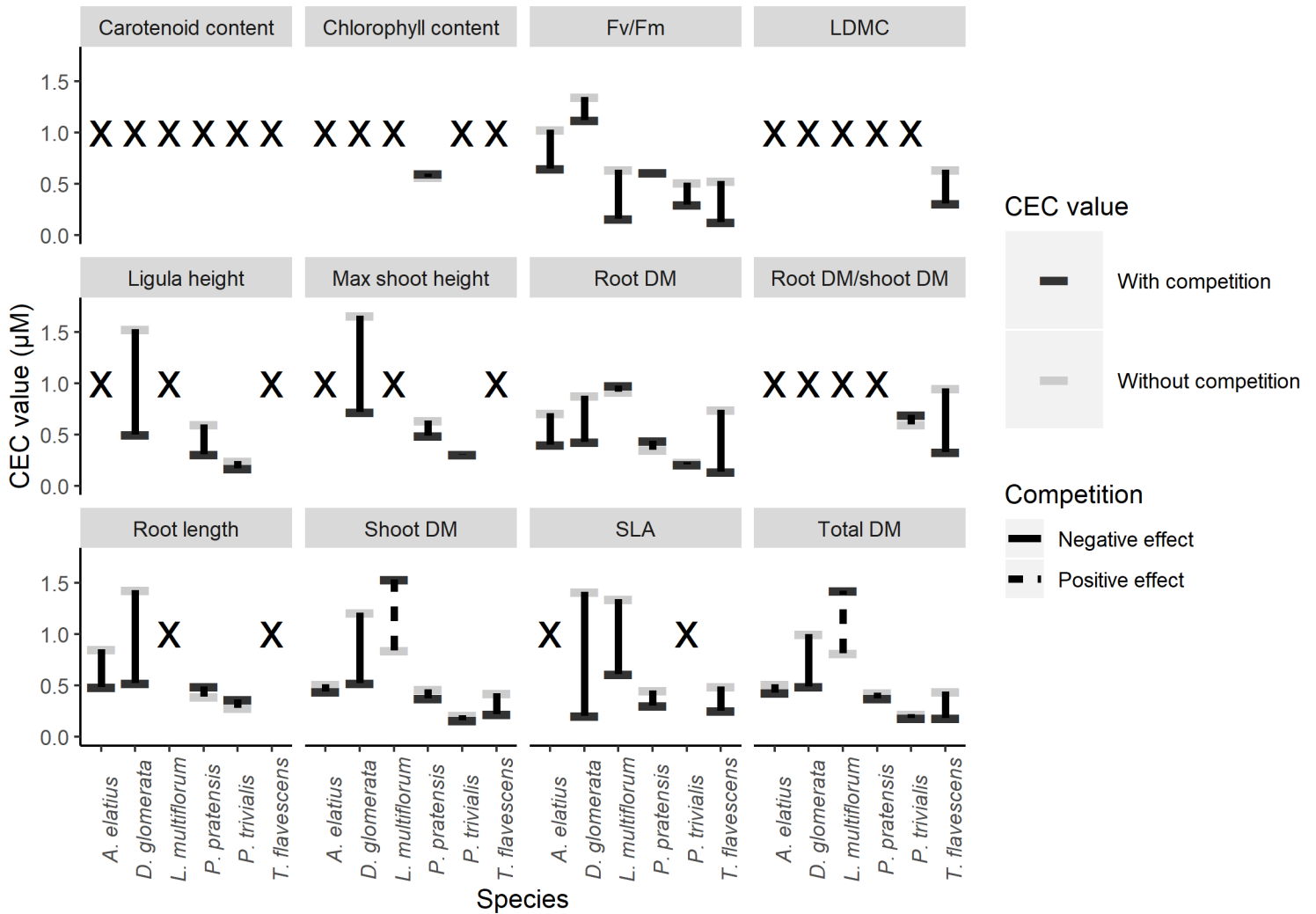


Figure 3: Comparison of calculated CEC values in presence of a competitor (y-axis) against the CEC values in absence of a competitor (x-axis) for the four types of calculated CECs. The different species and the different endpoints are respectively described by different point shapes and by their shade of grey. The black solid line represents the first bisector and the two black dashed lines give a 5 percent variation above and below the first bisector. We considered that the points situated between those two dashed lines showed no variation with regard to the competition modality. DM stands for dry mass.

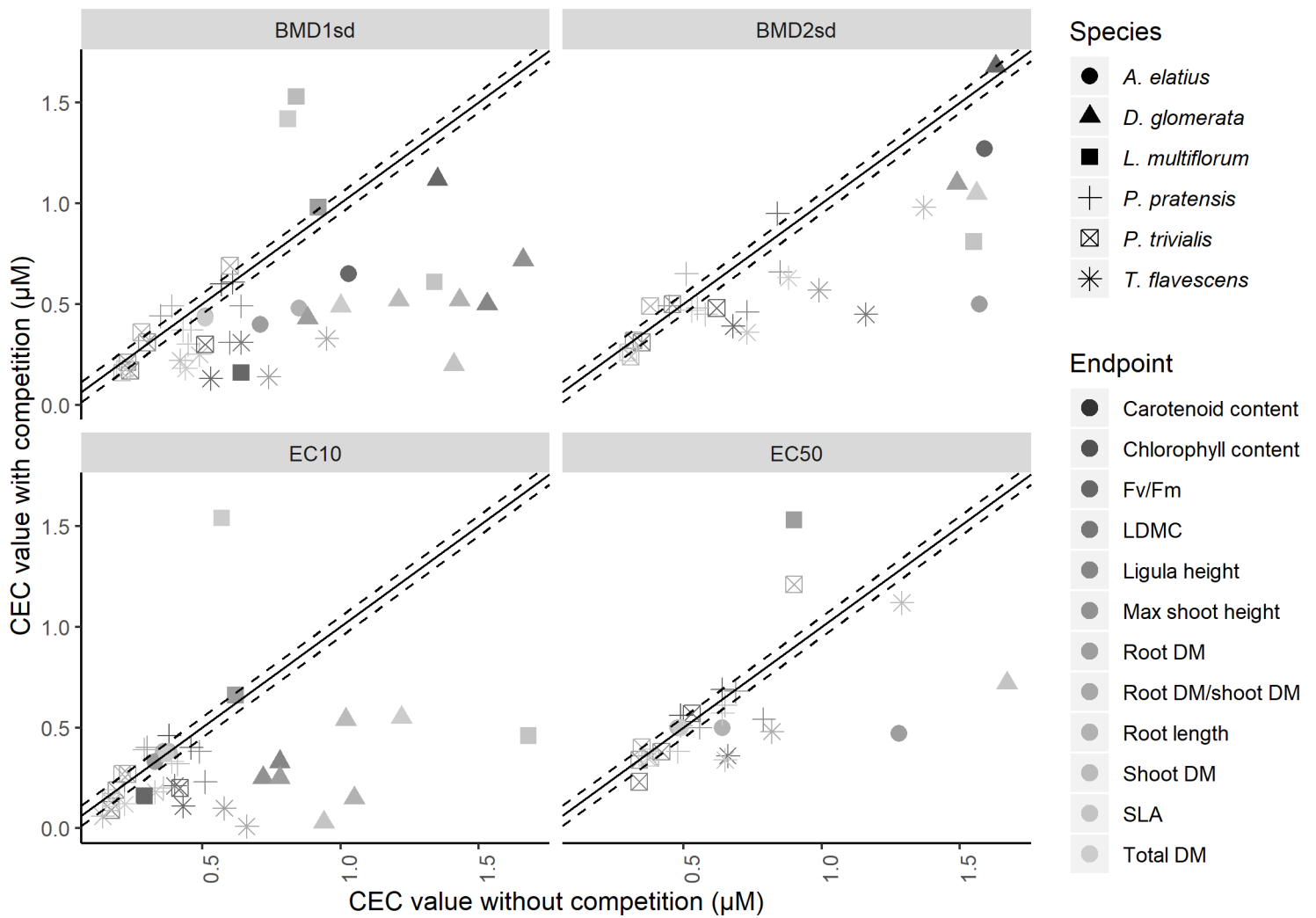


Figure 4: Example of Endpoint Sensitivity Distribution fits for  $BMD_{1sd}$  and the six tested species. The stairs represents the empirical cumulative distribution function of the data used to model the ESDs and the curves gives the ESDs themselves. In grey are the data without competition and in black the data with competition. The vertical dashed lines represent the fifth percentile.

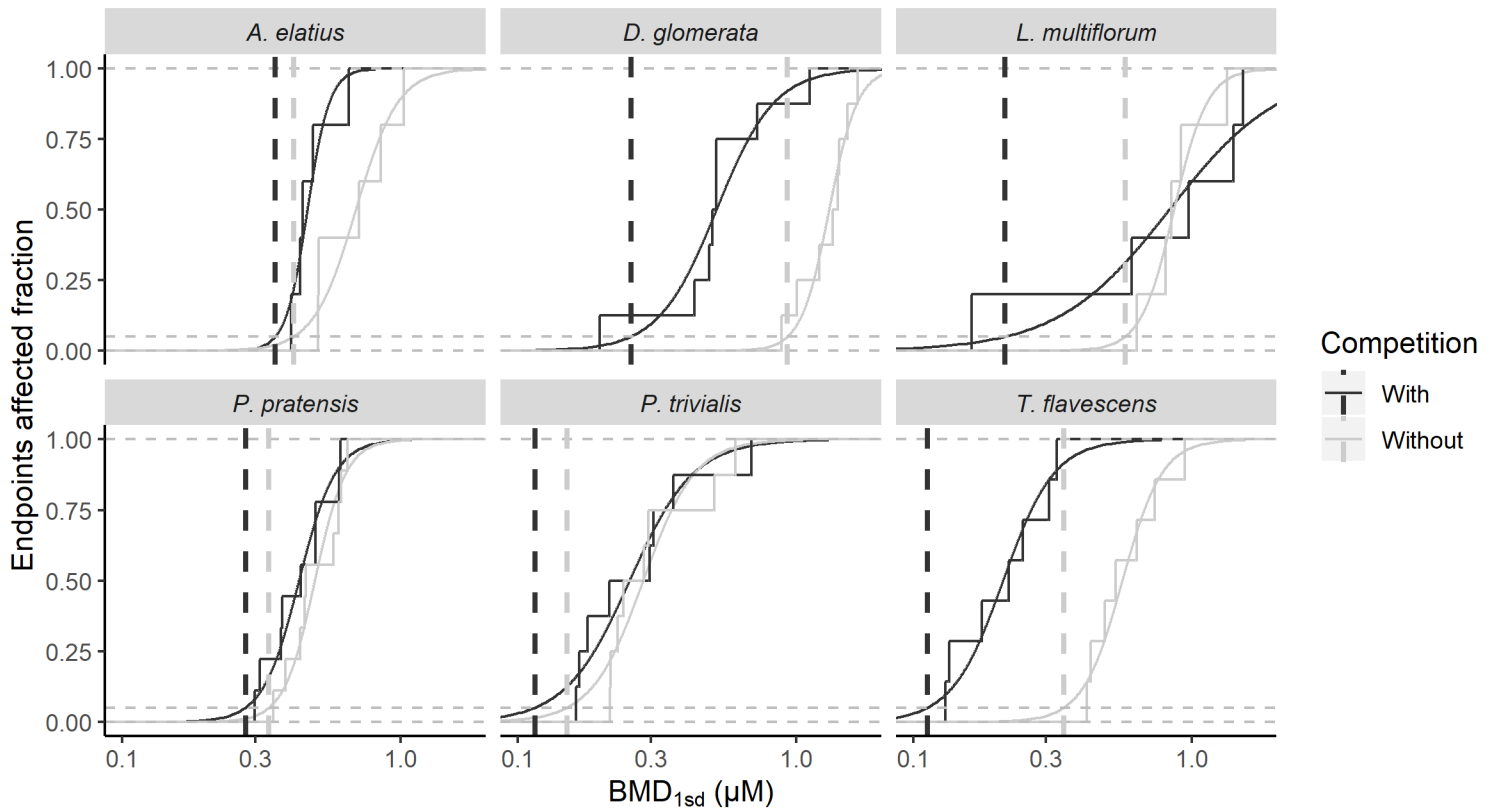
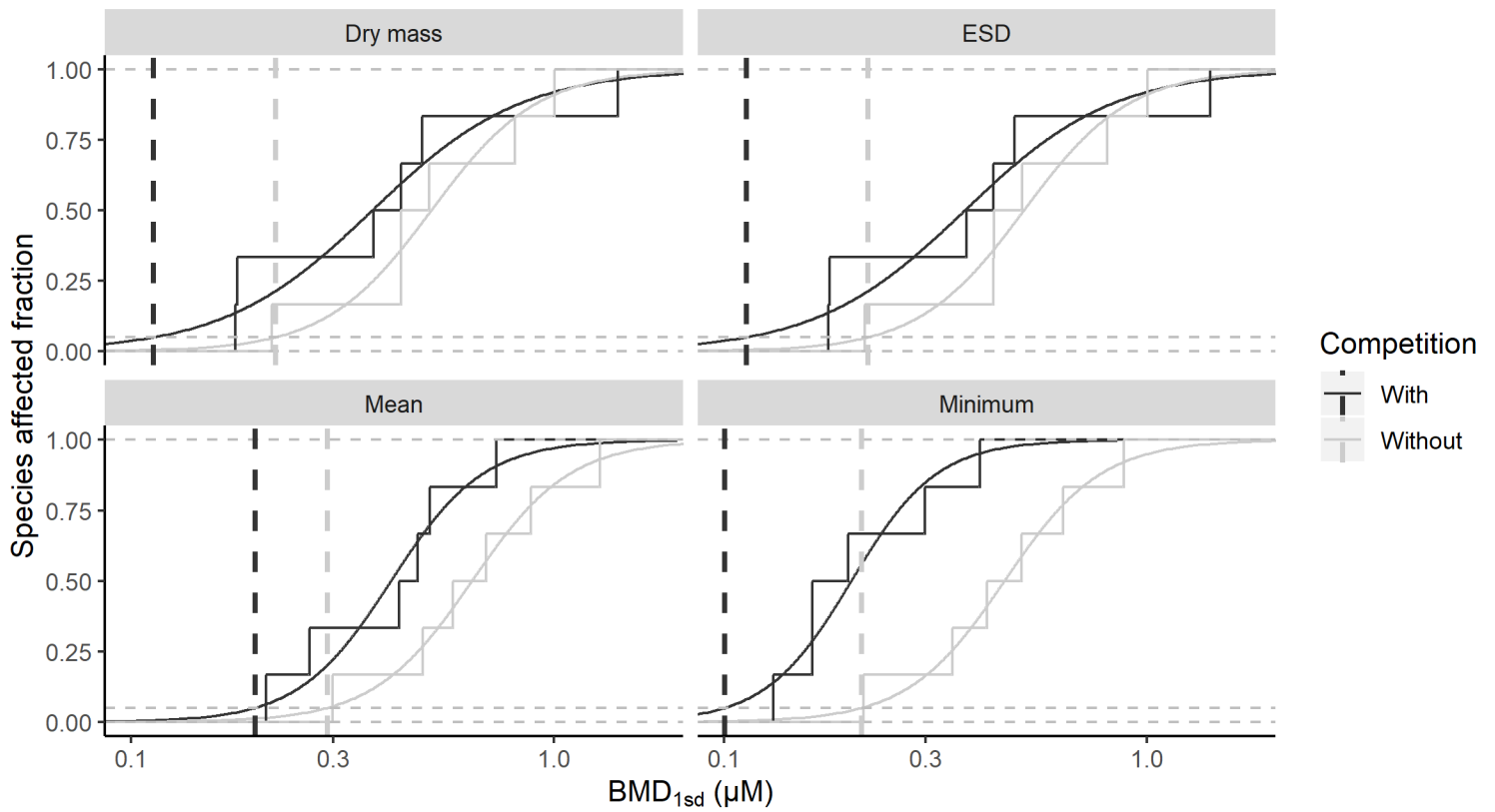


Figure 5: Example of Species Sensitivity Distribution fits produced with  $BMD_{1sd}$ . The stairs represents the empirical cumulative distribution function of the data used to model the SSDs and the curves give the SSDs themselves. In grey are the data without competition and in black the data with competition. The vertical dashed lines represent the  $HC_5$  levels. Each graphic displays a way to handle multiple endpoints: "Dry mass" is the SSD produced using the total dry mass for each species, "ESD" is the SSD produced using the 5<sup>th</sup> percentile from Endpoint Sensitivity Distribution of each species, "Mean" is the SSD produced using the mean value for each species and "Minimum" is the SSD produced using the minimum value for each species.



## 388 4. Discussion

### 389 4.1. Ecotoxicological relevance of bioassays

390 Usual ecotoxicological designs for plants only monitor very few endpoints  
391 as root length (ISO/TC 190/SC 4 Caractérisation biologique [2012]) or mass  
392 and seedling emergence (OCDE [2006]) and consider they are either represen-  
393 tative of the organism health (total dry mass for example) or they are primary  
394 maker in response to contamination compared to the other possible endpoints  
395 and are thus involved in the plant health (Krewski et al. [2011]). Among the  
396 eleven (or twelve if including the DM ratio, cf Table 1) endpoints measured, dry  
397 mass endpoints were found responsive to chemical stress for every species, thus  
398 reflecting plant health in accordance with the literature. In this study, CECs  
399 related to dry masses were often the lowest among the different endpoints for  
400 each species (Figure 2), advocating for their use in environmental risk assess-  
401 ment. The Fv/Fm photosystem II efficiency endpoint was also responsive to  
402 herbicide exposure. This endpoint, with regards to the mode of action of iso-  
403 proturon which specifically inhibits photosystem II reaction center (Grouselle  
404 et al. [1995]), is thus a relevant early endpoint to monitor such specific con-  
405 taminations in plants. In our study, pigment content endpoints exhibited low  
406 responsiveness compared to previous works using photosystem II inhibitor her-  
407 bicides (Ramel et al. [2009], Sulmon et al. [2004]). LDMC was also found weakly  
408 responsive to chemical exposure, confirming that this parameter was not rele-  
409 vant for plant ecotoxicity tests. Finally, the responsiveness of Root/Shoot DM  
410 ratio, SLA, and of length endpoints was found to be species specific underlying  
411 the involvement of species specific pattern of organ allocation in response to  
412 stress (Eziz et al. [2017], Xiong et al. [2018]).

413 A similar pattern of responses was observed for some of the endpoints. These  
414 different groups of endpoints could be discriminated based on their responsive-  
415 ness in the different species as well as the ranking of species in terms of tolerance.  
416 A first group contained the ligula height, the maximum shoot height and the  
417 root length. They were indeed selected for the same species: *D. glomerata*, *P.*

418 *pratensis*, *P. trivialis* (root length was also selected for *A. elatius*) (Table 1).  
419 These endpoints also shared the same tolerance ranking for concerned species:  
420 *D. glomerata* > *P. pratensis* > *P. trivialis* (Figure 2). A second group gath-  
421 ered the dry masses (root, shoot and total) and concerned all species (Table  
422 1). Again, a common tolerance ranking was observed: *L. multiflorum* > *D.*  
423 *glomerata* > *A. elatius* > *P. pratensis* > *P. trivialis*. The tolerance values of *T.*  
424 *flavescens* was however more variable between those different endpoints. Shoot  
425 and total dry masses were also the only endpoints where a clear positive effect  
426 of competition on tolerance was visible on Figure 2 for *L. multiflorum*.

427 Our experimental design allowed to test the effects of competition on the  
428 responses of tested species to chemical stress. As *Bromus erectus* was pre-  
429 identified as the most tolerant to isoproturon and the most competitive species,  
430 it was used as competitor to ensure a constant competition pressure. The com-  
431 petition tested was thus interspecific competition. One could possibly argue  
432 that the competition effects identified could not be differentiated from intraspe-  
433 cific competition. Indeed, an interesting experimental design would have been  
434 to also test intraspecific competition by exposing the tested species in presence  
435 of other individuals of the same species. However, aside from the fact that it  
436 would have considerably overburdened the experimental design, it would then  
437 have been impossible to interpret correctly the results as the different species  
438 display different competitive abilities and isoproturon tolerances. At high iso-  
439 proturon concentrations, species with low tolerance would have been highly  
440 affected, thus resulting in a reduced competition pressure.

#### 441 4.2. Relevance of SSD approaches in the context of ERA

442 The number of species used in this study is under the classical SSD standards.  
443 However, 6 species are enough regarding regulatory guidance of some countries  
444 such as Australia and New Zealand (Australian and New Zealand Environment  
445 and Conservation Council and Agriculture and Resource Management Council  
446 of Australia and New Zealand [2000]; minimum 5 species). Forbes and Calow  
447 [2002] have also proposed that the quality of data is at least as important as its

448 quantity and the fact that the six species studied here come from a consistent  
449 community and that several endpoints have been measured for each may be  
450 arguments in favor of the quality of our dataset. Finally, the built SSDs are not  
451 intended to have a direct regulatory use but to address the effect of interspecific  
452 competition on SSD. Even with only six species, our study was able to provide  
453 some insight about this question.

454 Our study used a great number of endpoints. Such approach provides new  
455 insights to assess the importance of measured endpoints in ERA through our  
456 innovative proposal for handling multiple endpoints. Indeed, by mixing several  
457 endpoints at once in our SSD building, we challenged their relevance and their  
458 relative individual sensitivity. It seems important to note that the effect of using  
459 different endpoints and ways to combine them into SSDs have not been studied  
460 yet (Del Signore et al. [2016]). Our results showed that  $HC_5$  calculated with  
461 the ESD 5<sup>th</sup> percentile was the lowest for 7 of the 8 CEC types and competition  
462 modalities (Table 2). Its constructions should also ensure that it is robust  
463 towards potential outliers. Regulatory guidances usually propose to use the  
464 minimum values or the geometric mean of the different values for each species  
465 to build SSDs, the latter being the most commonly used in practice. In light  
466 of our work, the 5<sup>th</sup> percentile from ESD method we investigated here could  
467 be proposed as a decent alternative. This method is however not applicable  
468 if the number of endpoints is low ( $\leq 5$ ). Such a method would also allow to  
469 construct more realistic SSD, adjustable to target communities, and suitable to  
470 the diversity of contaminants and related mixtures found in ecosystems. Indeed,  
471 using a larger set of endpoints enables to include general, species specific, and  
472 also contaminant specific endpoints, regarding both community diversity and  
473 contaminant mode of action.

474 Another issue is the question of the "non-responsive" endpoints. We made  
475 the choice to exclude them from the analysis, considering that they would not  
476 present a response to the isoproturon concentration, even above the maximum  
477 concentration of our experimental design. Excluding those data from our dataset  
478 could lead to possible biases. Indeed, this exclusion implies that they are not



479 relevant for our study whereas they may be only sparsely or not at all sensitive,  
480 thus meaning we may underestimate the total tolerance of tested species and  
481 related community.

482 An interesting continuation of this work would be to set up this experimen-  
483 tation again using other pesticides, especially not banned ones as isoproturon  
484 is. This would be of great help to assess if our conclusions on isoproturon could  
485 be extended to other compounds or if the responses we observed were specific,  
486 notably concerning the selection of "responsive" endpoints. It would also sup-  
487 port the potential need to measure more endpoints to assess the plant health  
488 and give new insights on the ESD method we propose.

489 Interspecific competition had a negative effect on organism responses to  
490 chemical stress, as shown by the different CEC values calculated. It can also  
491 be seen in Table 2 that this negative effect was propagated from single CEC  
492 values to  $HC_5$  produced from SSDs. The effect of interspecific competition we  
493 evidenced is robust, since  $HC_5$  values always exhibited the same trend, even  
494 if its intensity depended on the CEC that was used and on the multiple end-  
495 points handling method. Those results are consistent with the fact that, in  
496 SSDs, biotic interactions should have important effects on organism responses  
497 to herbicides and other pesticides targeting low trophic levels (De Laender et al.  
498 [2008]). Competition effect is not taken into account in the first two tiers of ERA  
499 as the first consists in using the lowest CEC of classical bioassays and the sec-  
500 ond in incorporating those bioassay results in predictive models. It is however  
501 included in the other tiers as those tiers are not based on monospecific bioas-  
502 says. The third ERA tier uses results from experiments in mesocosms where  
503 different species coexist. Those species are therefore interacting with each other  
504 and biotic interactions are occurring. The fourth ERA tier uses data from real  
505 ecosystems and is therefore considered as the most environmentally relevant by  
506 integrating real interactions and sources of variability. Those two tiers how-  
507 ever consider data from specific environments and conclusions can be difficult  
508 to expand to other situations.

## 509 **5. Conclusion**

510 This study showed that competitive interactions affected, both at the species  
511 level (bioassays results) and the community level (SSD results), the responses of  
512 plants to isoproturon. Such results thus highlight the relevance of accounting for  
513 biotic interactions to construct SSD models in a context of community dynamics  
514 prediction and of ERA. SSDs could thus be used more efficiently to design and  
515 predict the evolution of key plant communities, such as vegetated filter strips.  
516 They are indeed natural or sown plant communities whose function is to protect  
517 aquatic ecosystems by preventing pesticide leaching from crops to surrounding  
518 rivers. Installation of these grass strips between croplands and rivers is now  
519 regulatory (European Council [1991]). In this context, SSD models could be a  
520 relevant tool to predict plant community dynamics under conditions of recurrent  
521 pesticide exposures, in order to design and maintain functional buffer grass  
522 strips. More data are however necessary to assess these points and research  
523 must be carried on those topics.

## 524 **Associated content**

525 Supporting information. SI A: Example of concentration-response fits for  
526 *L. multiflorum*; SI B: Table of all the CEC values calculated; SI C: Graphical  
527 summary of  $EC_{50}$  values; SI D: ESD produced with  $EC_{50}$  values; SI E: SSD  
528 produced with  $EC_{50}$  values.

529 Figures equivalent to Fig 1 but for other species and figures equivalent to  
530 Fig 2, 4 and 5 but for other CEC are available upon request to the authors.

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