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Effect of interspecific competition on species sensitivity distribution models: analysis of plant responses to chemical 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 (HC₅). 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 HC₅ 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_5). 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 HC₅ 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 μ mol photons/m²/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 μ M, corresponding to, respectively, 51.5, 103, 206, 309, and 360 μ 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^{th} isoproturon concentration and j refers to the j^{th} repli-
253 cate, x_i is the isoproturon concentration, y_{ij} is the response level of the endpoint,
254 ϕ 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 `fitdistscens` 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			

Total selected	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 $[\text{maximum tested dose}; +\infty[$. 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.

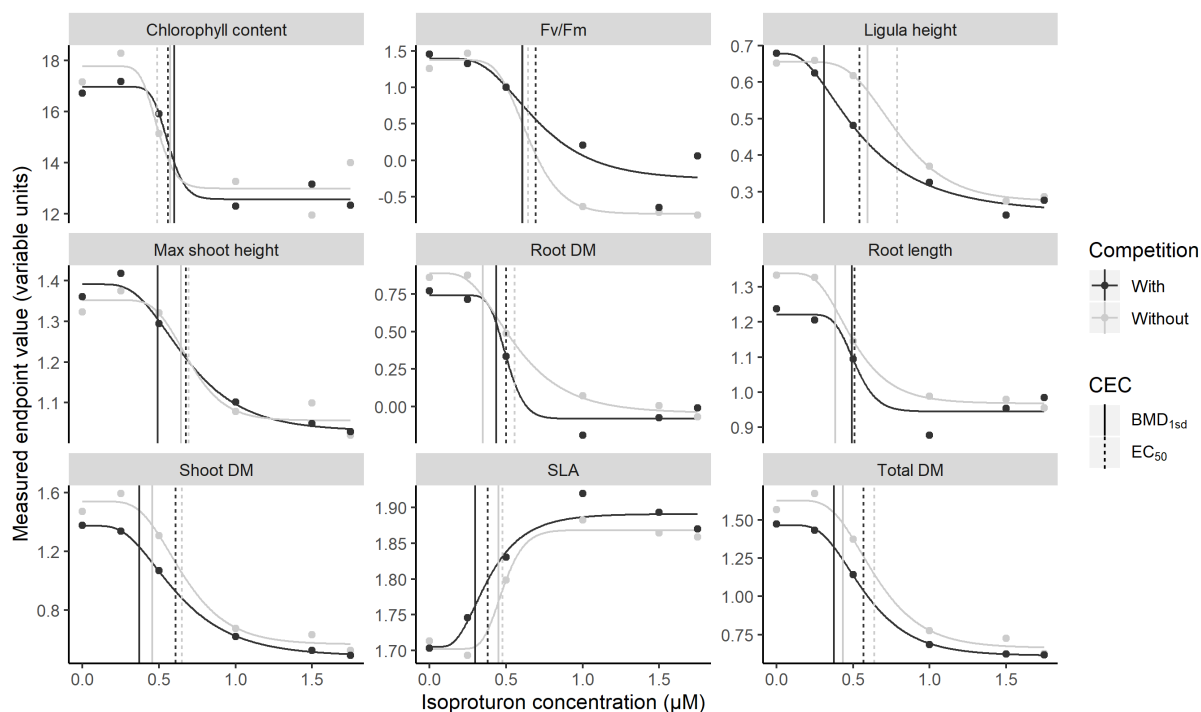
357 We calculated for each BMD_{Zsd} value the corresponding percentage of re-
358 sponse (i.e. the x in EC_x corresponding to each BMD_{Zsd} value). BMD_{1sd}
359 corresponded in average to $EC_{29.7}$ values (interquartile range of x equivalent
360 for BMD_{1sd} : [16.5, 38.3]) whereas BMD_{2sd} corresponded 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 EC₅₀ levels and the plain vertical lines the BMD_{1sd} levels. EC₁₀ and BMD_{2sd} 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 HC₅ 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.

	EC₁₀	EC₅₀	BMD_{1sd}	BMD_{2sd}
Min value	0.112/0.011 (11.13)	0.285/0.231 (1.23)	0.212/0.101 (2.11)	0.261/0.218 (1.19)
Mean value	0.197/0.090 (2.19)	0.435/0.388 (1.12)	0.291/0.196 (1.48)	0.494/0.371 (1.33)
Dry mass	0.125/0.077 (1.63)	0.180/0.130 (1.38)	0.219/0.113 (1.94)	0.215/0.187 (1.15)
ESD 5th percentile	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 HC₅ values (in μM) for the different CEC and multiple endpoints handling methods. The value before the slash is the HC₅ without competition, the value after the slash the HC₅ with competition and the value between brackets the ratio between the two HC₅.

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.

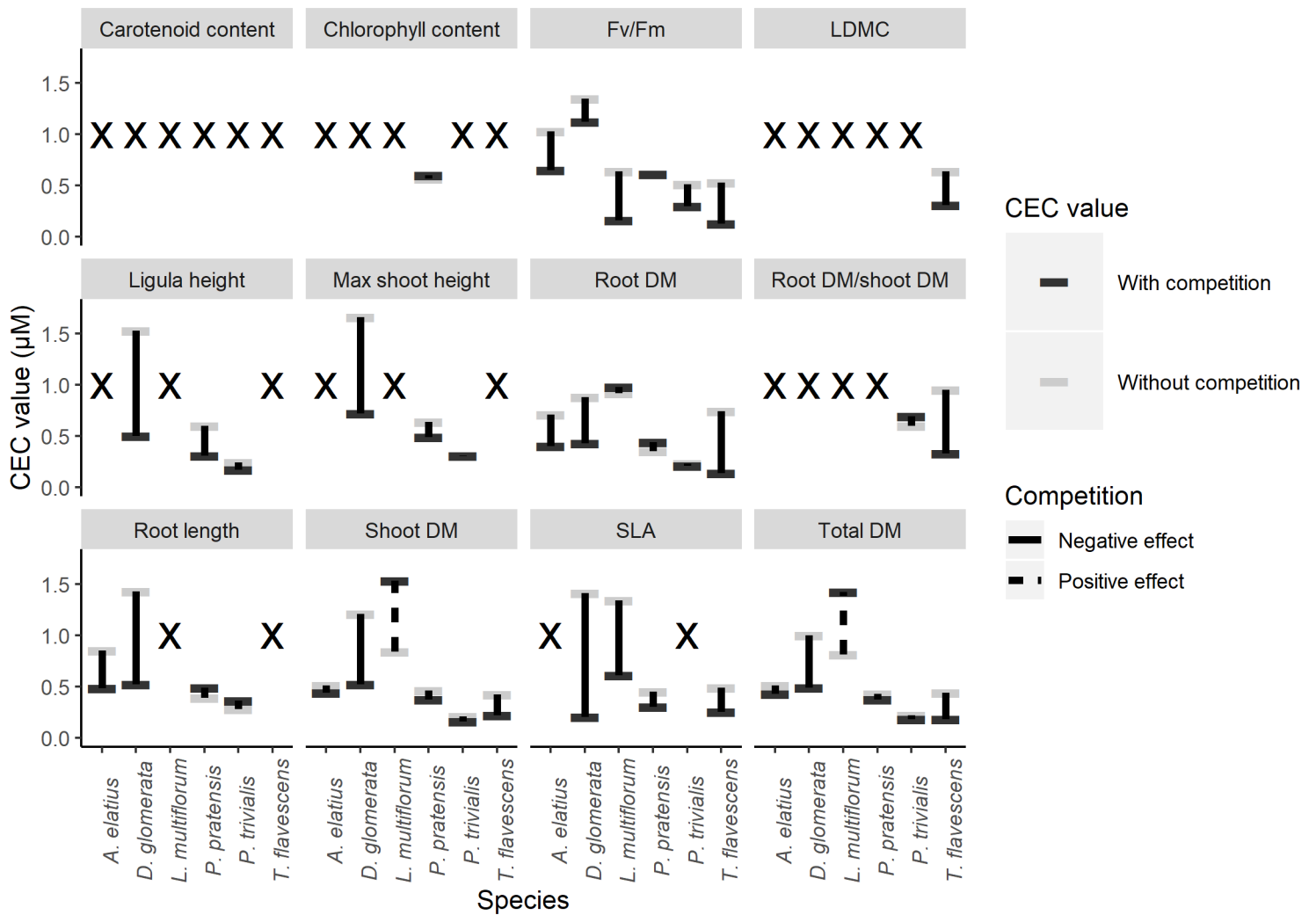


Figure 3: Comparison of calculated CEC values in presence of a competitor (y-axis) against the CEC values in absence of a competitor (x-axis) for the four types of calculated CECs. The different species and the different endpoints are respectively described by different point shapes and by their shade 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.

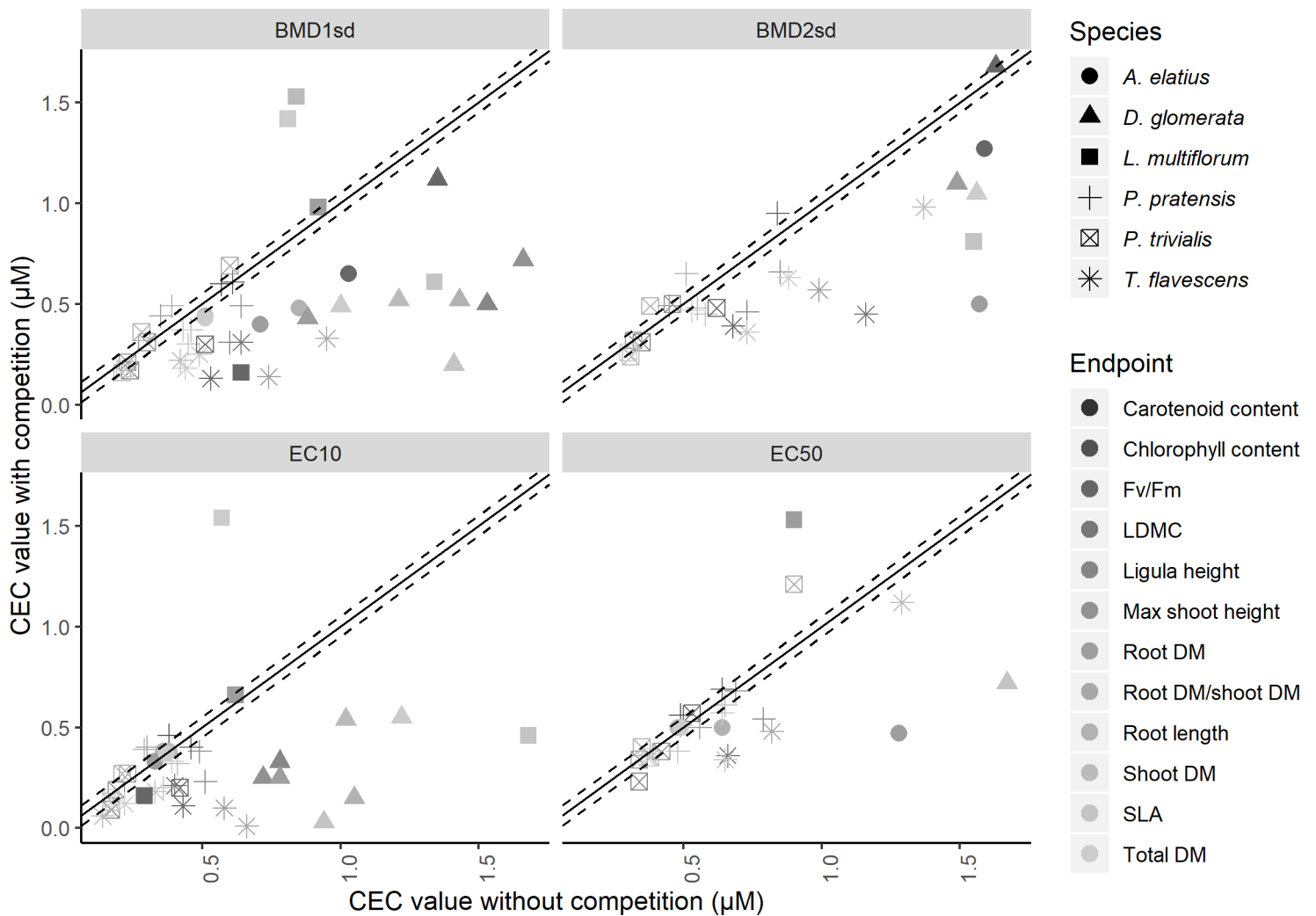


Figure 4: Example of Endpoint Sensitivity Distribution fits for BMD_{1sd} and the six tested species. The stairs represents the empirical cumulative distribution function of the data used to model the ESDs and the curves gives the ESDs themselves. In grey are the data without competition and in black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

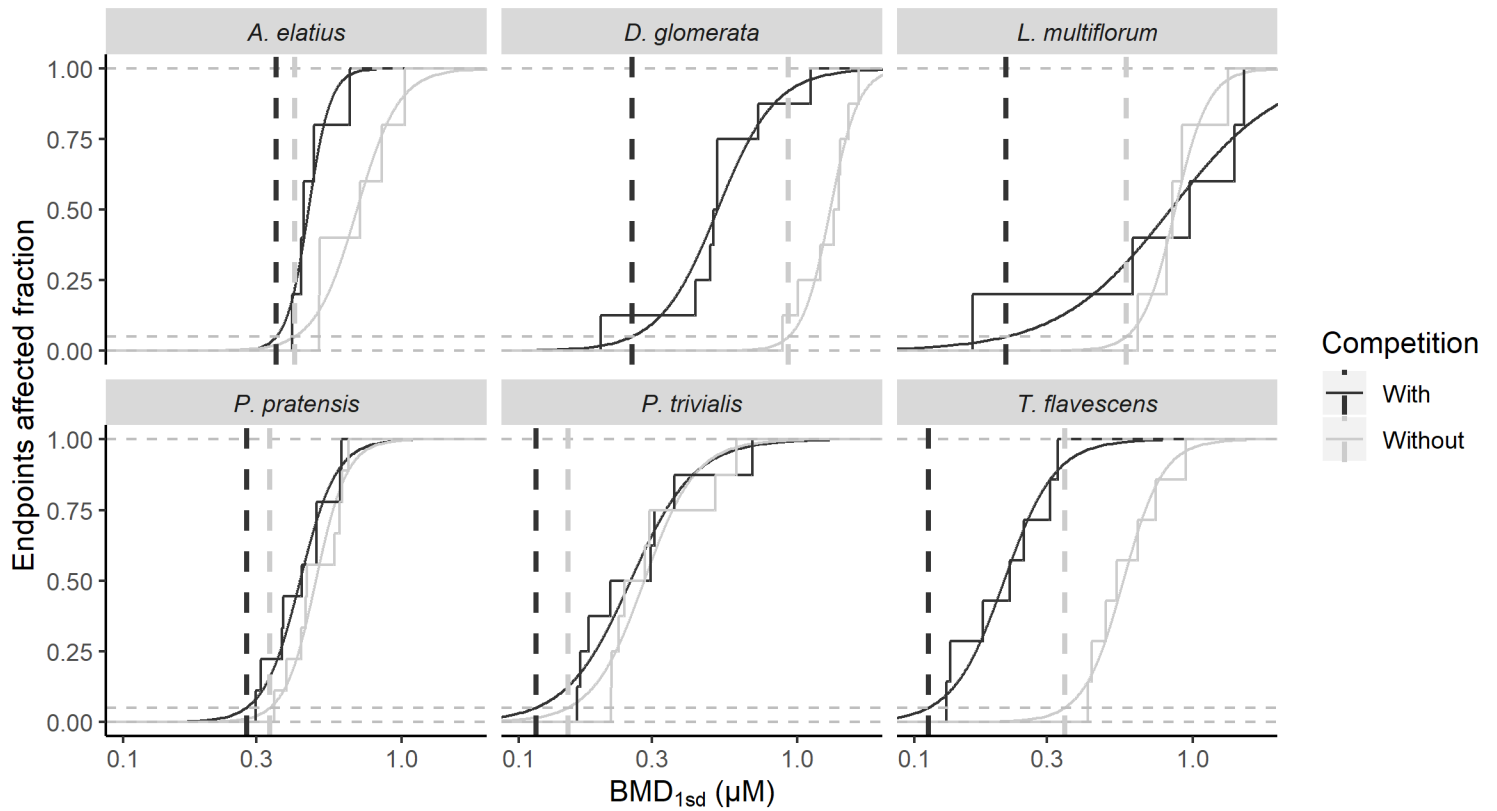
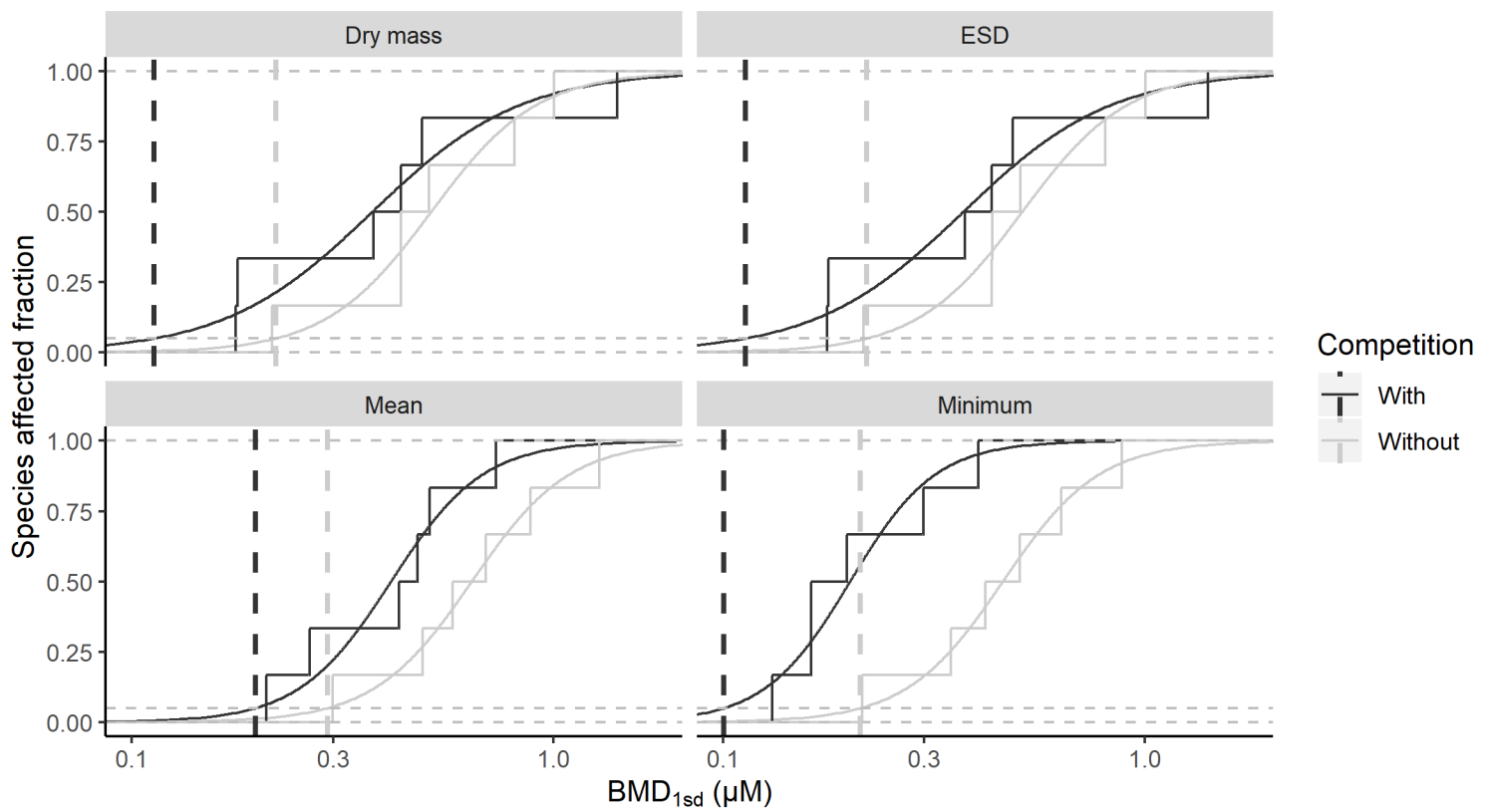


Figure 5: Example of Species Sensitivity Distribution fits produced with BMD_{1sd} . The stairs represents the empirical cumulative distribution function of the data used to model the SSDs and the curves give the SSDs themselves. In grey are the data without competition and in black the data with competition. 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 5th 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 5th 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 5th 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 (≤ 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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