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

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Abstract

Species Sensitivity Distributions (SSD) are widely used in environmental risk assessment to predict the concentration of a contaminant that is hazardous for 5% of species (HC_5). They are based on monospecific bioassays conducted in the laboratory and thus do not directly take into account ecological interactions. This point, among others, is accounted for in environmental risk assessment through an assessment factor (AF) that is applied to compensate for the lack of environmental representativity. In this study, we aimed to assess the effects of interspecific competition on the responses towards isoproturon of plant species representative of a vegetated filter strip community, and to assess its impact on the derived SSD and HC_5 values. To do so, we realized bioassays confronting six herbaceous species to a gradient of isoproturon exposure in presence and absence of a competitor. Several modelling approaches were applied to see how they affected the results, using different critical effect concentrations and investigating different ways to handle multiple endpoints in SSD. At the species level, there was a strong trend toward organisms being more sensitive to isoproturon in

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

1. Introduction

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

The Species Sensitivity Distribution (SSD; Kooijman [1987], Posthuma et al. [2001]) is a tier 2 ERA method made to produce PNEC at the community level for a given contaminant using data obtained from monospecific tier 1 bioassays. The SSD is a distribution of CECs (Critical Effect Concentrations such as Effective Concentrations (EC) or Benchmark Doses (BMD)) obtained for different species for a given contaminant. The species under study have to be chosen to be consistent with the mode of action of the studied contaminant and representative of the considered environment (Van den Brink et al. [2006]). The SSD can lead to the derivation of the Hazardous Concentration for five percent of the species (HC_5). This value, after application of an AF (ranging from 1 to 5 for SSDs; Amiard and Amiard-Triquet [2015]), is commonly used in SSD-based ERA (for example in the United States of America: Stephan et al. [1985] and the European Union: European Chemical Bureau [2003]). The SSD method is however based on several assumptions listed in Forbes and Calow [2002] that still need to be tested.

In particular, very few attempts have been realized to test the effects of biotic interactions on organisms response against contaminants. In the field, organisms are however affected by different types of biotic interactions that can be intra or interspecific. Those interactions can have positive (comensalism, symbiosis or facilitation for example) or negative (competition for example) effects on the organism fitness. Biotic interactions can therefore lead to modifications of the effects of contaminants the organisms are exposed to. For example, Foit et al. [2012] used a dose-response design to test the effects of intra and interspecific competition on *Daphnia magna* and *Culex pipiens molestus* response to fenvalerate. They found that competition tended to increase the toxic effect of fenvalerate on the two tested organisms and modified community dynamics, meaning that toxic exposure might disturb natural community for a longer period than predicted by monospecific bioassays alone. Gust et al. [2016] investigated the effects of intraspecific competition on *Daphnia magna*'s tolerance to copper and lead using a dose-response design. They concluded that intraspecific competition tended to increase *Daphnia magna* mortality caused by Cu and Pb. Viaene et al. [2015] modeled the effects of intraspecific competition, interspecific competition by *B. calyciflorus* and predation by *Chaoborus* sp. (at low and high densities plus control for each type of biotic interaction) on the response of *Daphnia magna* against pyrene. They found that predation had the highest negative effect on daphnia density under chemical stress and that competition and predation tended to have antagonistic effects on daphnia tolerance to pyrene. One of the hypothesis for this unexpected effect was that pyrene would have modified the community composition, leading to reduced negative biotic interactions. These studies all used macroinvertebrate species and mostly *Daphnia magna*, and their conclusions need to be extended to other taxonomic groups. For plants, which represent a key compartment of the ecosystems, very few studies have been conducted to test interaction effects between biotic interactions, such as competition, and chemical stress responses. Such a question is however essential for sessile organisms like plants, since they need to be tolerant to all local negative environmental conditions to survive and maintain their population. Results mainly highlighted the existence of interactions between chemical stress responses and competitive interactions between plant species (Boutin et al. [2019], Damgaard et al. [2014]), reinforcing the need to take into account such biotic interactions in ERA procedures.

To our knowledge the effects of biotic interactions on SSD results were investigated in only one study by De Laender et al. [2008]. In this study, the effects of ecological interactions on SSD results were assessed by a mechanistic dynamic ecosystem model involving two phytoplancton, three zooplancton and one fish compartments and interspecific interactions between them (predation, competition...). A toxic effect submodel, embedded in the ecosytem model, described the effects of the toxicants on the parameters of the ecosystem model (potentially affected parameters were mortality rate for zooplancton and fish and photosynthesis rate for phytoplancton). One thousand theoretical contaminants were used with randomized 10% effect concentration (EC_{10}) values drawn from a log-normal distribution (SSD without taking ecological interactions into account). Those EC_{10} values were used in the mechanistic dynamic ecosystem model, thus simulating the subsequent modifications of the ecosystem fate. New EC_{10} values derived from those simulations were calculated to obtain SSD taking ecological interactions into account. For about 25% of those 1000 contaminants (254) taking into account biotic interactions led to a change in mean (190), standard deviation (94) or both (30) of the SSD compared to the one derived from EC_{10} produced without taking into account those interactions.

Moreover, results showed that this trend was higher for herbicides, implying, that the derived HC_5 would have great chances to be different. However, this work needs to be confirmed by data-based studies and to be extended to other types of communities.

Large amount of pesticides are used worldwide to treat a wide diversity of human-made systems. This is the case of croplands to ensure high agricultural productivity, but also of vegetated sport fields and green spaces, to control specific plant composition (Alavanja [2009]). Pesticides have effects on the targeted organisms within the treated areas but also on non-targeted organisms outside those areas (Crone et al. [2009]). Indeed, due to drift, leaching, and runoff, those contaminants are found in terrestrial (Geiger et al. [2010]) but also largely in aquatic ecosystems (Anderson et al. [2015], Annett et al. [2014], McMahon et al. [2012]). Isoproturon, for example, is a substituted urea photosynthesis inhibitor, which is often used as a pre- and post-emergence herbicide in cereal crops. Before its banning in the EU in 2017 because of potential groundwater contamination and risks to aquatic life (European Commission [2016]), isoproturon showed a median concentration of 0.02 mg/kg of soil in investigated European agricultural soils (Silva et al. [2019]). The degradation and fate of isoproturon has also been studied. Johnson et al. [1994] showed that 7 days after isoproturon treatment, concentrations were about 9 mg/kg of soil, after 78 days this concentration was about 1 mg/kg of soil and after 155 days this concentration was above 0.5 mg/kg of soil. More generally, isoproturon was also found to contaminate rivers from one year to another (Dragon et al. [2019]), and to persist in the soil for at least 3 years after application allowing it to be mobilised by increasing rainwater (Johnson et al. [2001]). It is thus clear that a wide diversity of communities, within or outside pesticide-treated areas, are exposed to numerous pesticides, with concentrations ranging from residual to acute levels. But these communities, associating several species, are also the site of numerous biotic interactions, such as competition. This is particularly the case for plant communities, which, as sessile organisms, may thus be submitted simultaneously to both pesticide exposure and potentially strong biotic interactions. Plant communities are therefore a relevant community model to test the assumption: "Interactions between species do not influence the sensitivity distribution" highlighted by Forbes and Calow [2002] about the SSD method. Considering the studies listed above, we expected that 1) interspecific competition would have a negative effect on organism responses to toxicants in our study and that 2) the protective concentration for 95% of species would be lowered by interspecific competition. To test these assumptions, a dataset of monospecific bioassays results on 6 herbaceous species exposed to isoproturon in presence and absence of competition was produced. We then built SSDs with and without competition and compared them. For this purpose, we tried to characterize the effects of interspecific competition on SSD results using a variety of CECs as well as endpoints and ways to handle them.

2. Materials and methods

2.1. Dataset

2.1.1. Tested species and competitor species

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

2.1.2. Experimental design

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

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

2.1.3. Studied endpoints and metrics

At the end of the experiment, eleven endpoints were measured on the tested species, and an additional metric of dry mass (DM) ratio was calculated. These endpoints were chosen in order to detect the effects of the different treatments (herbicide exposure, competition) applied to the tested species in light of the literature. In addition to global traits (DM traits, shoot height, root length), some traits were preferentially chosen for their responses to herbicide (meximum efficiency of photosystem II determined from Fv/Fm, pigment contents), and to competition (root/shoot DM ratio, Specific Leaf Area (SLA), Leaf Dry Matter Content (LDMC), and ligula height corresponding to stem height). Fv/Fm chlorophyll fluorescence and pigment contents were measured as previously described in Serra et al. [2013]. SLA and LDMC were quantified as described in Cornelissen et al. [2003]. Bromus shoot dry biomass was also weighted, for each microcosm, at the end of the experiment. Analysis of these DM data showed that mean *Bromus* shoot DM was similar between species and isoproturon treatments, thus allowing to compare all treatment modalities carried out in the experiment.

2.2. Data analysis

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

2.2.1. Data transformations

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

We then visually inspected the residuals of the models to see if the transformation improved homoscedasticity and residuals normality and in that case, we kept it. Different transformations were tested, depending on the considered endpoint. For the Fv/Fm chlorophyll fluorescence endpoint, which is a proportion, we applied a logit transformation ($log\left(p/\left(1-p\right)\right)$) to change its scale, after having previously changed the 0 values (3 out of 471 Fv/Fm measurements) to 0,01 (corresponding to about one fourth of the lowest non-zero value of 0,0395). For every other endpoint, a log transformation was tested.

2.2.2. Selection of responsive endpoints

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

2.2.3. Concentration-Response Curves modelling

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

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

where i refers to the i$^{\text{th}}$ isoproturon concentration and j refers to the j$^{\text{th}}$ replicate, x_i is the isoproturon concentration, y_{ij} is the response level of the endpoint, ϕ is the cumulative probability density of the normal law, e is the concentration at which the maximum slope occurs (equal to the EC_{50}; > 0) b is a shape parameter. if $b < 0$, c is the response level at high concentrations and d is the response level at low concentrations. if $b > 0$, c is the response level at low concentrations and d is the response level at high concentrations. If $b = 0$, the whole model becomes a constant model set at the arithmetical mean between c and d.

2.2.4. Critical Effect Concentration derivation

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

Secondly, we calculated the Benchmark Doses (BMD_Zsd) as an alternative to EC_x. This CEC, described in the EFSA guidance (EFSA Scientific Committee et al. [2017]), has the advantage to take into account data variability. Indeed, the BMD_Zsd is the concentration at which the Benchmark Response (BMR_Zsd) is reached, the latter being equal to a change of z times the model residual standard deviation from the control mean. The z of BMD_Zsd is therefore theoretically speaking close to the x of EC_x as it defines the level considered to have a critical effect. The EFSA guidance proposes to use a z value of 1 (EFSA Scientific Committee et al. [2017]), but we also calculated BMD_2sd. This latter would correspond to a change up to one of the bounds of the 95% confidence interval around the predicted value at the control (Larras et al. [2018]).

2.2.5. SSD modelling

SSDs were built *via* three different scenarios that are commonly used in scientific publications and regulatory texts. A fourth approach, adapted to our multiple endpoint dataset but not used in regulatory texts has also been tested. 1) In a first scenario, the geometric mean of the obtained CECs for the different endpoints has been used for each species. This approach has the advantage to use all the information available for the different endpoints. This is also the most commonly used approach in SSDs in the literature (Xu et al. [2015]). 2) In a second scenario, we used the lowest CEC value obtained for each species. This method is very protective, but it is only using a single value, thus making it sensitive to potential outliers. 3) In a third scenario, only the total dry mass endpoint was considered for each species. This was a responsive endpoint for all of the species under study and is usually measured in ecotoxicological studies on herbaceous grass species (Del Signore et al. [2016]). 4) The fourth approach did not directly use CEC values. We first built sensitivity distributions of the different endpoints for each species (Arts et al. [2008], Hanson and Solomon [2002]) and called them "Endpoint Sensitivity Distribution" (ESD) by analogy to SSDs. We then calculated the fifth percentile of these distributions for each species as a protective concentration for 95% of the endpoints and used these values to build the SSDs themselves. This ESD-using approach was considered to be a good compromise between the two first approaches (*i.e.* the geometric mean approach and the lowest value approach) as it takes into account all of the available datapoints (like the geometric mean approach) but gives a more protective result (like the lowest value approach) without being too sensitive to outliers.

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

3. Results

3.1. Data transformation

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

3.2. Selection of responsive endpoints

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

Species	***P. trivialis***	***P. pratensis***	***T. flavescens***	***A. elatius***	***L. multiflorum***	***D. glomerata***
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

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.

3.3. Concentration-response curves modelling

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

3.4. Critical Effect Concentration derivation

Figure 1 also shows an example of CEC calculation for *P. pratensis.* The values of the different CECs calculated for the selected endpoints for each species can be found in SI (B). For $\mathrm{BMD_{2sd}}$, a value could not always be calculated as the $\mathrm{BMR_{Zsd}}$ was sometimes beyond the asymptote for high concentrations, meaning that the amplitude of the response was in that case lower than 2 (for $\mathrm{BMD_{2sd}}$) times the model's residual standard deviation. This happened 12 times on 84 $\mathrm{BMD_{2sd}}$ calculations and never happened for $\mathrm{BMD_{1sd}}$ calculations even if it was conceptually possible. As the $\mathrm{EC_x}$ effect level is a percentage of maximum effect (between the two asymptotes), it was successfully calculated in every case. There were however cases where the calculated $\mathrm{EC_x}$ or $\mathrm{BMD_{Zsd}}$ were above the maximum concentrations. We considered those values as censored values in the interval [maximum tested dose; $+\infty$ [. This happened 3 times among the 84 calculated $\mathrm{EC_{10}}$, 20 times among the 84 calculated $\mathrm{EC_{50}}$ and 10 times among the 84 calculated $\mathrm{BMD_{2sd}}$. This did not happen for $\mathrm{BMD_{1sd}}$. Figure 2 shows the values of $\mathrm{BMD_{1sd}}$ calculated for the different species and endpoints (an example for another CEC given in SI (C)). In most cases, the calculated CEC values were lower in presence of a competitor, thus showing that interspecific competition had a negative impact on organisms tolerance on most of the studied species and endpoints.

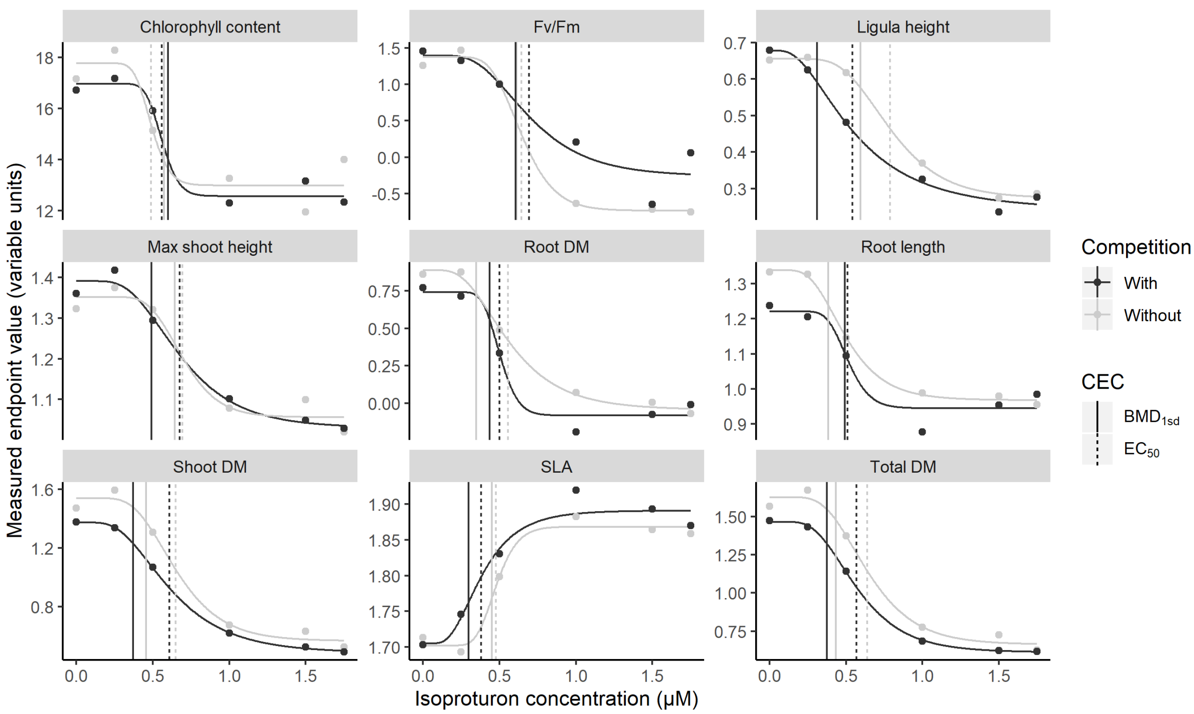
We calculated for each $\mathrm{BMD_{Zsd}}$ value the corresponding percentage of response (i.e. the x in $\mathrm{EC_x}$ corresponding to each $\mathrm{BMD_{Zsd}}$ value). $\mathrm{BMD_{1sd}}$ corresponded in average to $\mathrm{EC_{29.7}}$ values (interquartile range of x equivalent for $\mathrm{BMD_{1sd}}$: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

Figure 3 summarizes the CEC values calculated for the different CEC types, species and endpoints and displays the value with competition against the value without competition. All CEC being pooled, about 10% of points are between the two dashed lines, thus showing that, in this case, competition had here minor or no effect (less than a 5% variation in one way or another). Many more points are below the dashed lines (70%) than above (20%), thus showing that interspecific competition had mostly a negative effect on organism tolerance to chemical stress in our experiment. This is consistent with the hypothesis stated in introduction. This trend toward negative effect can be seen for every calculated CECs and every species and endpoints tested even if it is not systematic. This trend toward negative effect however seems less visible for $\mathrm{EC_{50}}$ (44%) than for other CECs (69% for $\mathrm{EC_{10}}$, 73% for $\mathrm{BMD_{1sd}}$ and 74% for $\mathrm{BMD_{2sd}}$).

3.5. ESD and SSD modelling

An example of the fitted ESD for the different species for $\mathrm{BMD_{1sd}}$ is shown in Figure 4. An exemple of figure for another CEC can be found in SI (D). Figure 5 shows examples of SSDs calculated from $\mathrm{BMD_{1sd}}$ and using the different methods for handling multiple endpoints (minimum, mean, total dry mass and "ESD"). An example of SSD obtained for another CEC is shown in SI (E). Table 2 summarizes the shifts between $\mathrm{HC_5}$ values with and without competition through the ratios between $\mathrm{HC_5}$ values without competition and $\mathrm{HC_5}$ values 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_{50} levels and the plain vertical lines the BMD_{1sd} levels. EC_{10} and BMD_{2sd} are not displayed here for reasons of clarity. DM stands for dry mass.



thus showing that the interspecific competition tended to lower plant tolerance at the community level. We can also see that the competition effect can lower the HC_5 up to 11-fold, which strongly surpasses the Assessment Factor of 5 typically used in SSD-based ERA. This however happened only once and for the least robust method.

	EC_{10}	**EC_{50}**	**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 HC_5 values (in µM) for the different CEC and multiple endpoints handling methods. The value before the slash is the HC_5 without competition, the value after the slash the HC_5 with competition and the value between brackets the ratio between the two HC_5.

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.

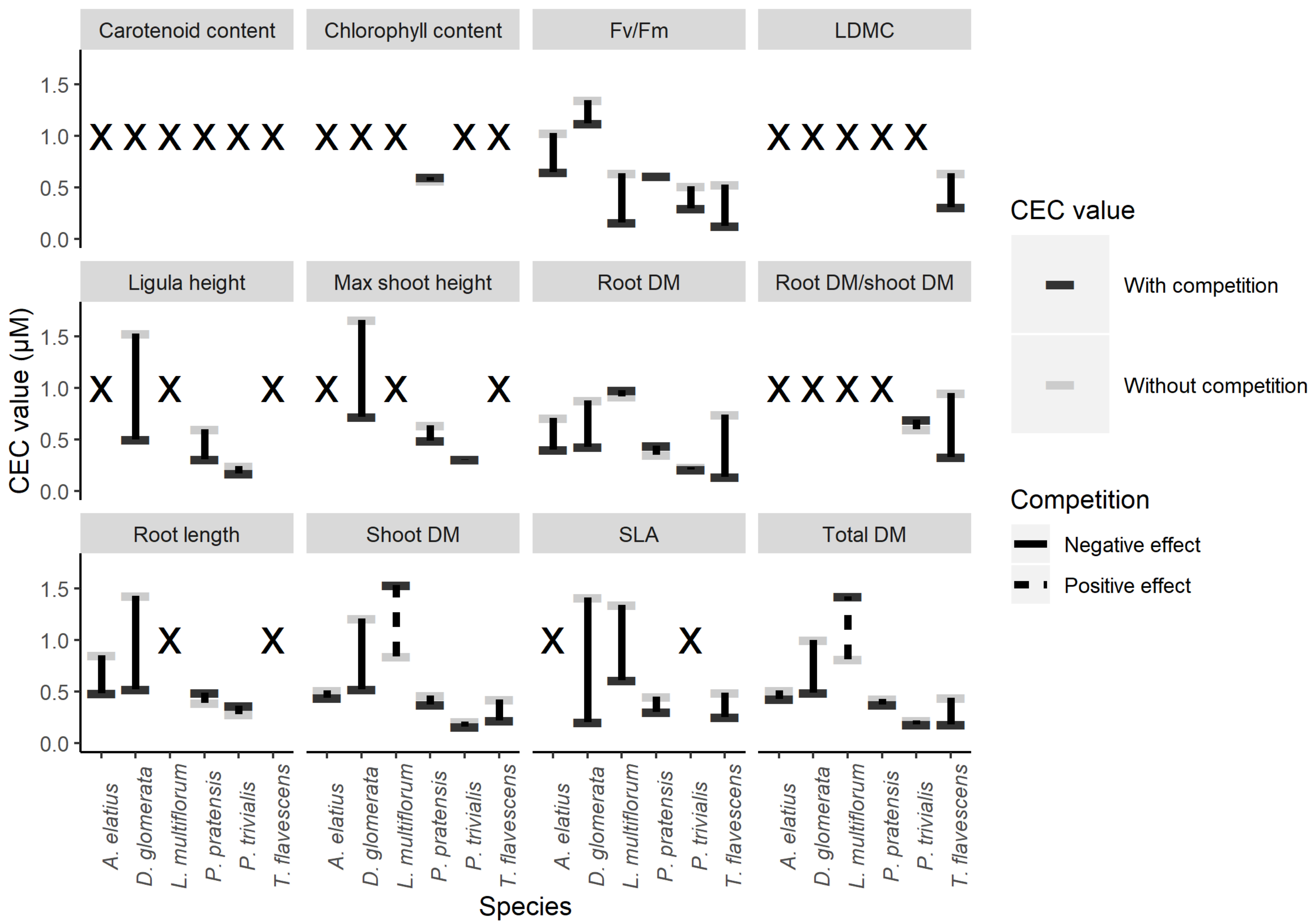


Figure 3: Comparison of calculated CEC values in presence of a competitor (y-axis) against the CEC values in absence of a competitor (x-axis) for the four types of calculated CECs. The different species and the different endpoints are respectively described by different point shapes and by their shade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

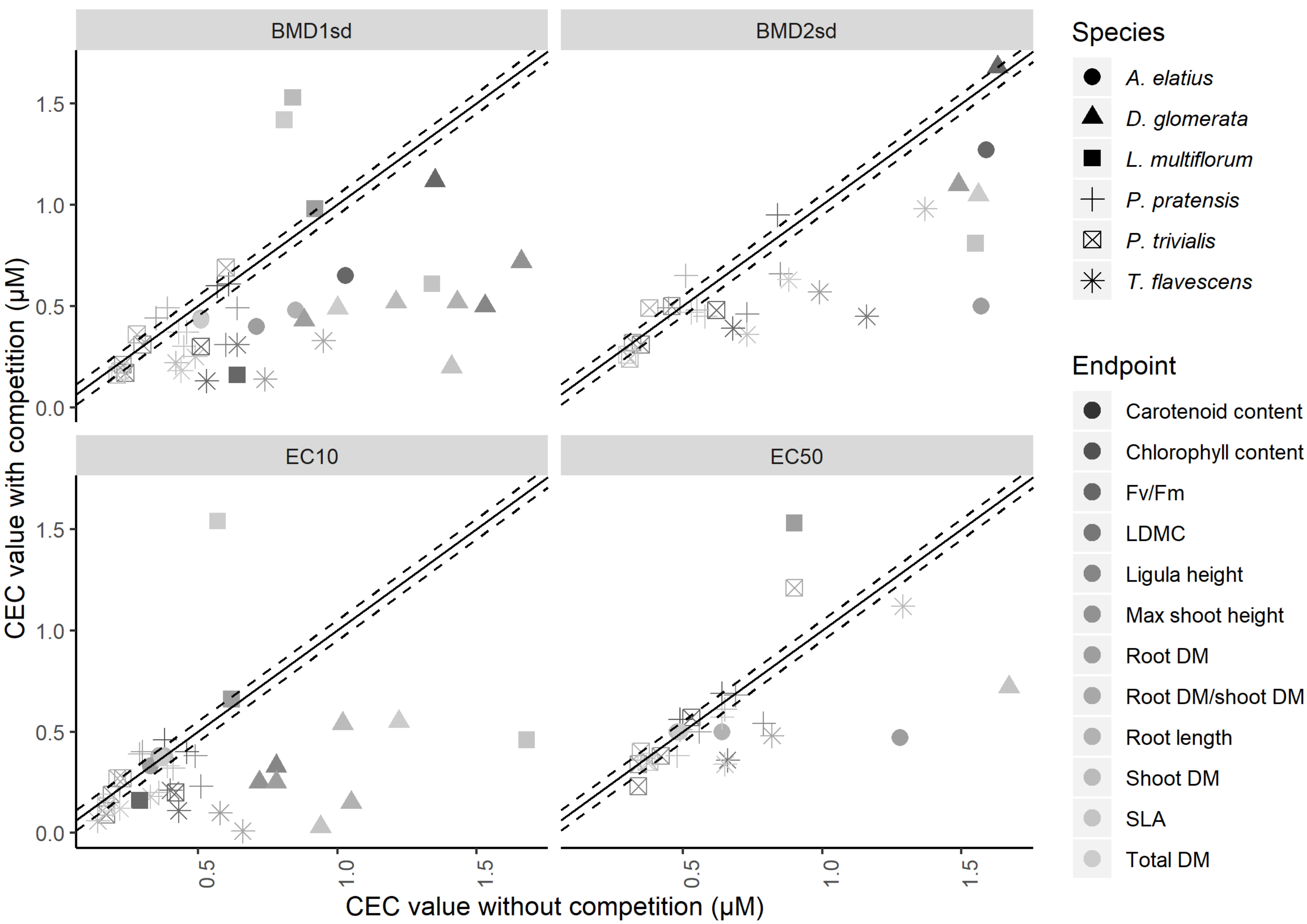


Figure 4: Example of Endpoint Sensitivity Distribution fits for BMD_{1sd} and the six tested species. The stairs represents the empirical cumulative distribution function of the data used to model the ESDs and the curves gives the ESDs themselves. In grey are the data without competition and in black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

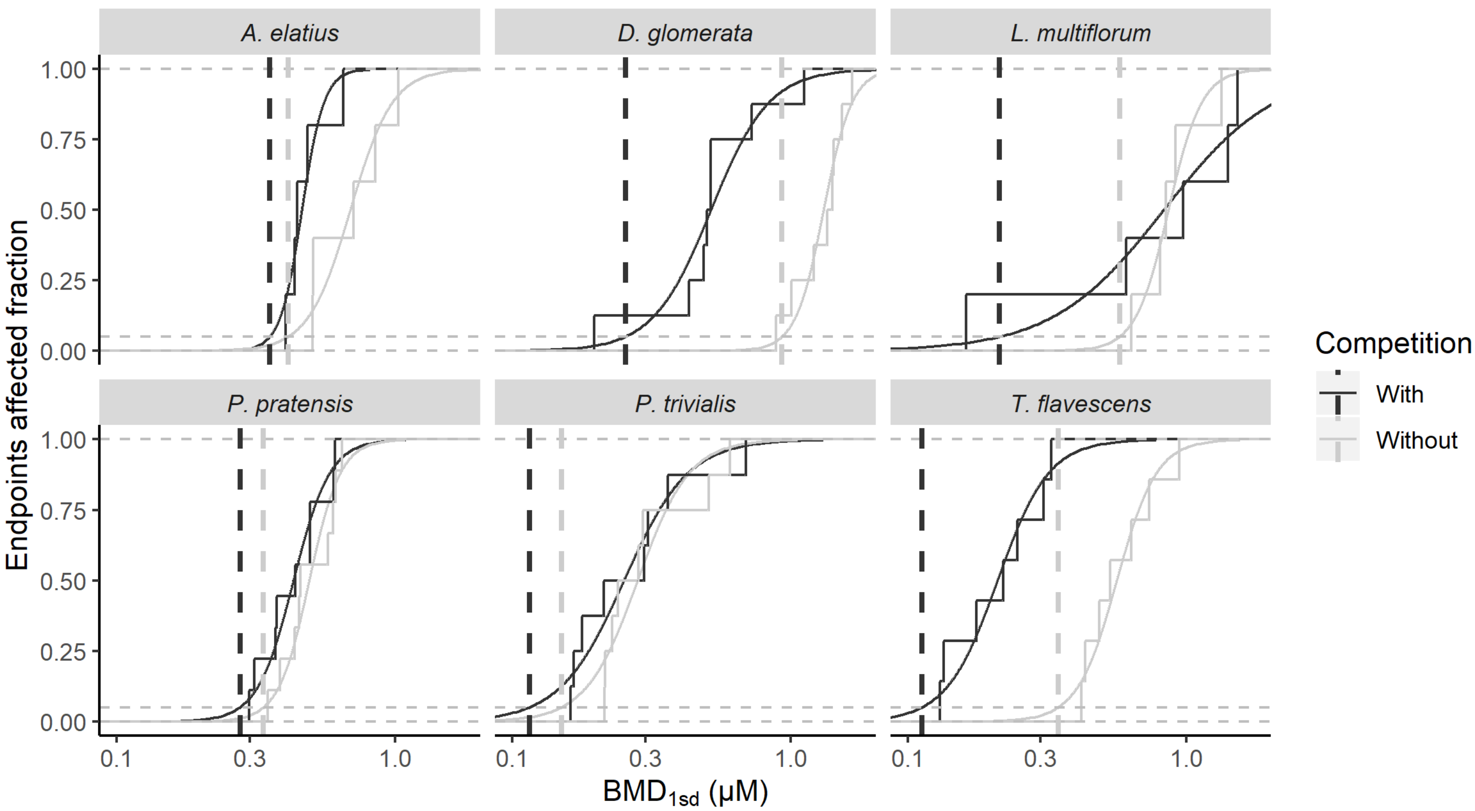
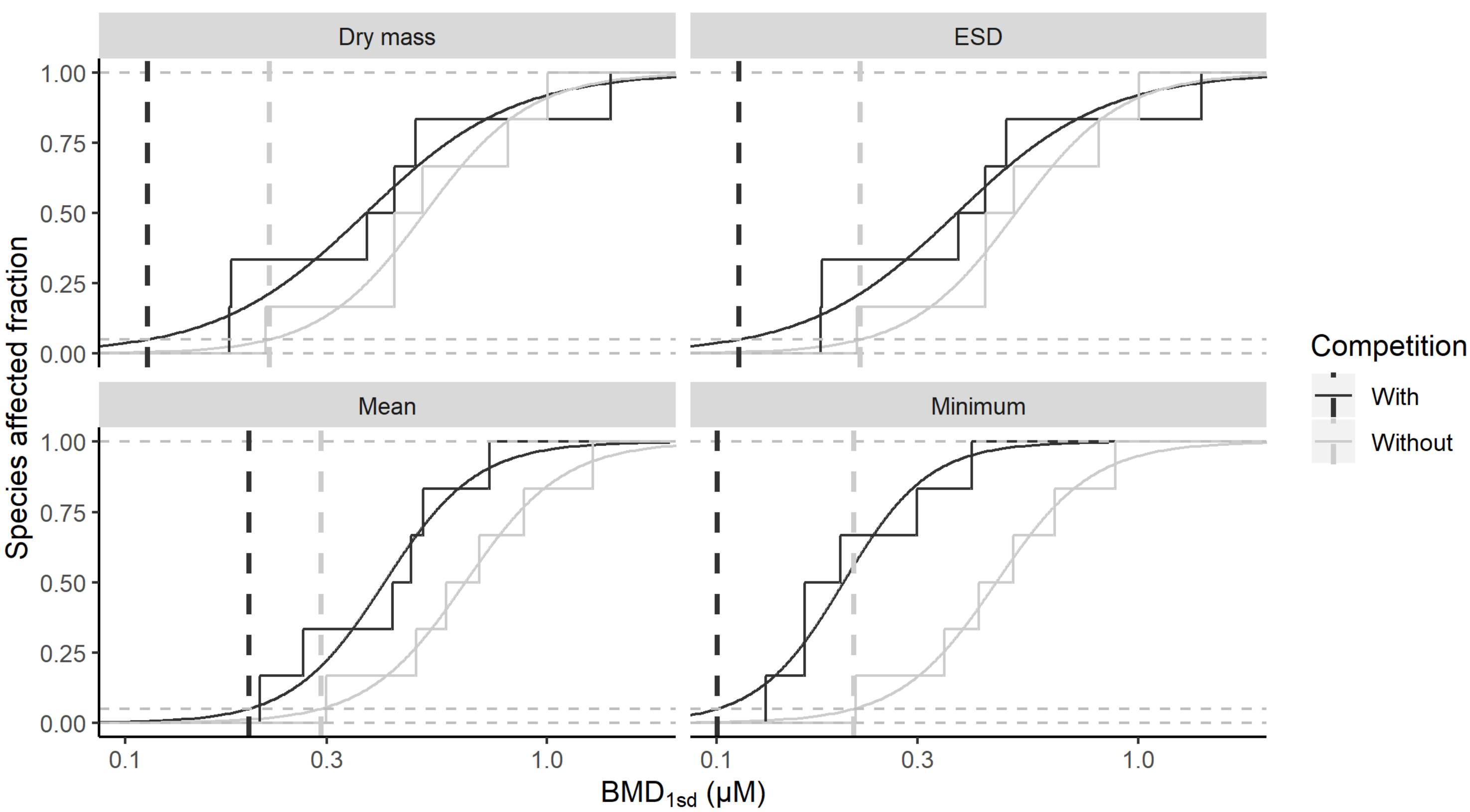


Figure 5: Example of Species Sensitivity Distribution fits produced with BMD_{1sd}. The stairs represents the empirical cumulative distribution function of the data used to model the SSDs and the curves give the SSDs themselves. In grey are the data without competition and in black the data with competition.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



4. Discussion

4.1. Ecotoxicological relevance of bioassays

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

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

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

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

4.2. Relevance of SSD approaches in the context of ERA

The number of species used in this study is under the classical SSD standards. However, 6 species are enough regarding regulatory guidance of some countries such as Australia and New Zealand (Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand [2000]; minimum 5 species). Forbes and Calow [2002] have also proposed that the quality of data is at least as important as its quantity and the fact that the six species studied here come from a consistent community and that several endpoints have been measured for each may be arguments in favor of the quality of our dataset. Finally, the built SSDs are not intended to have a direct regulatory use but to address the effect of interspecific competition on SSD. Even with only six species, our study was able to provide some insight about this question.

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

Another issue is the question of the "non-responsive" endpoints. We made the choice to exclude them from the analysis, considering that they would not present a response to the isoproturon concentration, even above the maximum concentration of our experimental design. Excluding those data from our dataset could lead to possible biases. Indeed, this exclusion implies that they are not relevant for our study whereas they may be only sparsely or not at all sensitive, thus meaning we may underestimate the total tolerance of tested species and related community.

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

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

5. Conclusion

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

Associated content

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

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

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