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How does interspecific competition modify the response of grass plants against herbicide treatment? A hierarchical concentration-response approach.

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## ABSTRACT

Ecological interactions are rarely taken into account in environmental risk assessment. The objective of this work was to assess how interspecific competition affects the way plant species react to herbicides and more specifically how it modifies the concentration-response curves that can be built using ecotoxicological bioassays. To do this, we relied on the results of ecotoxicological bioassays on six herbaceous species exposed to isoproturon under two conditions: in presence and in absence of a competitor. At the end of the experiments, eleven endpoints were measured. We modelled these data using a hierarchical modelling framework designed to assess the effects of competition on each of the four parameters of the concentration response curves (*e.g.* the level of response at the control or the concentration at the inflexion point of the curve) simultaneously for the six species. The modelled effects could be of three types, 1) competition had no effect on the parameter, 2) competition had the same effect on the parameter for all species and 3) competition had a different effect on the parameter for each species. Our main hypothesis was that different species would react differently to competition. Results showed that about a half of the estimated parameters showed a modification under competition pressure among which only a fourth showed a species-specific effect, the three other fourth showing the same effect between the different species. Our initial hypothesis was thus not supported as species

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tended to react in the same way to competition. The competition effect on plants was mainly negative, thus showing that they were more affected by isoproturon under competition pressure. This study therefore establishes how competition modifies plant responses to chemical stress and how this interaction varies from one species to the other.

*Keywords:* hierarchical modelling, environmental risk assessment, isoproturon, herbicide, biotic interaction, multi-stress

## **1. Introduction**

Environmental Risk Assessment (ERA) is mainly based on data from monospecific bioassays. The obtained effect concentrations are usually divided by an Assessment Factor (AF) to compensate for processes not taken into account [Aagaard et al., 2013] such as biotic interactions, intraspecific variability or interlaboratory variability. The value of the applied AF depends on the method applied to calculate predicted no effect concentrations, the quality of the used data, the ecological relevance of these data and the type of ecosystem considered. For ERA in freshwater, the AF value is for example comprised between 1 (for data with high ecological relevance and method including numerous species and modelling process such as species sensitivity distributions) and 1,000 (for basic procedure keeping the lowest critical effect concentration value from three bioassays) [Amiard and Amiard-Triquet].

Interspecific competition is one of the biotic interactions that can have an important effect on plant development. It is defined by Aschehoug et al. as “the ability of individuals to usurp resources or otherwise suppress their neighbor’s fitness and include both resource and interference competition” [Aschehoug et al., 2016]. Even if this biotic constraint has been largely studied [Aschehoug et al., 2016], studies of its interactions with chemical stress remain scarce. For example, Boutin et al. studied how sublethal doses of two herbicides (glyphosate and metsulfuron methyl) could modify the competitive interactions between two plants (*Centaurea cyanus* L. and *Silene noctiflora* L.) [Boutin et al., 2019]. The results showed that competitive ability of *S. noctiflora* was negatively affected by glyphosate, leading to a competitive advantage of *C. cyanus*. Concerning metsulfuron methyl, interspecific competition was found to have an important effect on the herbicide effects on seed production. Another study, from Damgaard et al. tested the effects of glyphosate on growth and competitive effect of two grass species (*Festuca ovina* and *Agrostis capillaris*) in a semi-field experiment [Damgaard et al., 2014]. The results showed that competitive relationships were modified as the competitive effect of *F. ovina* increased whereas

that of *A. capillaris* decreased with increasing doses of glyphosate. It is important to emphasise that the study of the interaction between interspecific competition and chemical stress encompasses two questions: whether the competitive ability is affected by the chemical stress or whether the tolerance to the chemical stress is affected by competition. Anyway, the previously mentioned studies show that monospecific bioassays can hardly encompass the true effects of herbicides on natural communities and that environmental risk assessment procedures can thus sometimes not be suitable enough to protect entirely these communities. Those studies however focus on only two species, thus limiting generalisations to other species. This assessment is further complicated by the fact that the applied modelling method usually considers the datasets obtained for the different species and measured endpoints independently. Independent concentration-response curves are built and a critical effect concentration (CEC) as the x percent effect concentration ( $EC_x$ ) is derived from each curve to summarize it. Those CEC are finally modelled into species sensitivity distributions (SSD) as for example in Baillard et al. [2020].

Some works however tried to implement more complex modelling techniques. For example, Kon Kam King et al. implemented a hierarchical model for six herbicides SSDs that include every concentration-response models [Kon Kam King et al., 2015]. One of the main advantages of this approach was that it integrated the whole information of the data in the models and not only a statistical summary as the  $EC_{10}$ . It thus propagated the uncertainty in the different levels of organization of the data (the different species and contaminant concentrations used) and it gave insights about a global response of the community against the used contaminants. In this work, this approach was carried out further on a dataset previously used in Baillard et al. where herbaceous plants were exposed to isoproturon in presence/absence of a competitor [Baillard et al., 2020]. We indeed tried to assess the effects of competition on plant responses to an herbicide by comparing the entirety of modelled concentration-response curves and analysing if and how the different parameters of said concentration-response curves (the level of the two asymptotes, the steepness of the curve and the position of the inflexion point) were modified by competition. To do this, we modelled at once the concentration-response curves for the different tested species and the two competition modalities to assess in an integrated manner the effects of competition on plant response against chemical stress. Our hypotheses were that, 1) such a modelling framework would be suitable to help understand the interaction between chemical stress and interspecific competition and 2) based on Baillard et al., interspecific competition would negatively affect plant

responses to isoproturon [Baillard et al., 2020].

## **2. Materials and methods**

### **2.1. Experimental design**

The dataset used in this work has previously been published in Baillard et al., where further details can be found [Baillard et al., 2020]. Briefly, a series of ecotoxicological bioassays on herbaceous plants were carried out in two situations: 1) with plants isolated in each experimental device (*i.e.* without competition) and 2) with plants included in a standardized hexagonal matrix of 37 seedlings of a competitor species *Bromus erectus* [Birch et al., 2007]. Six species of the Poaceae family, representative of plant diversity of grassland communities, were used for these experiments: *Arrhenatherum elatius*, *Dactylis glomerata*, *Lolium multiflorum*, *Poa pratensis*, *Poa trivialis* and *Trisetum flavescens*. Microcosms (three-liter-round microcosms, 20 cm diameter, 12.9 cm height containing inert sterilized vermiculite as substrate) were used for the experiments and vegetalized by transferring seedlings at 2-leaves phenological stage, previously grown from seeds in absence of chemical stress. Exposure to isoproturon began after a 4-days acclimatization. Isoproturon was added to the microcosms *via* watering to induce chemical exposure to the roots. The watering protocol was as follows: 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 concentrations (0.25, 0.5, 1, 1.5 and 1.75  $\mu\text{M}$ , corresponding to, respectively, 51.5, 103, 206, 309, and 360  $\mu\text{g/L}$ ) plus a control were used and for each concentration (six), competition modality (two) and species (six), eight replicates were realized, giving a total of 576 experimental devices.

### **2.2. Endpoint analysis and dataset description**

After the exposure, a set of eleven endpoints were measured on each organism. These endpoints were chosen to assess the effects of the two tested factors (interspecific competition and chemical stress) on the species under study. A list of publications used to set up this endpoint list can be found in SI (A). Some endpoints were chosen for their expected response to both competition and herbicide. This was the case of maximal shoot height, root length, root dry mass (DM) and shoot DM, which represent global endpoints of plant performance as an outcome of functional trait responses. Some other endpoints were chosen mainly for their response to competition as functional responsive traits: Leaf Dry Matter Content (LDMC), ligula height (corresponding to stem height) and Specific Leaf Area (SLA). A third group of endpoints was chosen for its response

mainly to herbicide: carotenoid and chlorophyll contents and maximum efficiency of photosystem II determined from Fv/Fm (later called Fv/Fm). A final performance endpoint, total DM, was calculated by adding together root and shoot DM. This was considered as a performance trait indicating plant ability to tolerate isoproturon and competition. We also measured *B. erectus* shoot DM at the end of the experiment to ensure that this DM was constant between the different isoproturon concentrations for each species and that competition pressure was thus constant all along each concentration-response curve.

A log-transformation was applied on every endpoint but Fv/Fm. For this latter, as it is expressed as a proportion, a logit transformation was applied. Such transformations helped improve data normality and homoscedasticity as shown in Chiarini et al. [1998] and Baillard et al. [2020].

### **2.3. Data modelling**

#### **2.3.1. Rationale**

The main objective was to derive parameter values for the concentration-response curves (CRCs) and to estimate the modification of those parameter values due to interspecific competition. When looking at CRCs independently, it is impossible to draw a global and statistically significant conclusion concerning the effects of competition. Here, we built a modelling framework allowing to take into account every data simultaneously and to produce, for each endpoint, a global answer to the questions “What is the effect of interspecific competition on herbaceous plant response toward an herbicide?” and “Are the effects of competition similar between the different tested species?”. For each endpoint, twelve CRCs were described within one single hierarchical model (six species \* two competition modalities), allowing to describe every data point (*i.e.* for every species, competition modality, isoproturon concentration and replicate) and to study the effects of interspecific competition on the CRCs of all species at once. The modelling framework was built to assess the effects of competition on different elements of the CRC as for example the modification of the level of response at the control, *i.e.* without chemical stress, or the shift in EC<sub>50</sub> values.

#### **2.3.2. Presentation of the core model**

For each endpoint, a modelling framework was designed to encompass twelve CRCs. We used a log-probit model to describe those curves:

$$\mu_{i,j,k} = d_{i,j} - \Delta dc_{i,j} * \phi\left(\frac{\log(x_k/e_{i,j})}{b_{i,j}}\right) * resp_i \quad (1)$$

$$y_{i,j,k,l} = \mu_{i,j,k} + \varepsilon_{i,j,k,l} \text{ with } \varepsilon_{i,j,k,l} \sim \mathcal{N}(0, S_i)$$

where  $i$  indicates the species,  $j$  indicates the competition modality (0 means there is no competition and 1 means there is competition),  $k$  indicates the isotruron concentration and  $l$  indicates the replicate.  $y_{i,j,k,l}$  is the measured endpoint value,  $d_{i,j}$  is the asymptotic response for low concentrations,  $\Delta dc_{i,j}$  is the difference between  $d_{i,j}$  and the response value at high concentrations,  $e_{i,j}$  is the concentration of the curve's inflection point (corresponding to the EC<sub>50</sub> value) and  $b_{i,j}$  is a shape parameter. More precisely,  $b_{i,j}$  corresponds to the standard deviation of the law of concentrations in log scale of which  $\phi$  is the cumulative distribution function (assumed Gaussian). Hence, the lower is  $b_{i,j}$ , the steeper is the log-probit model. A summary of the role of these different parameters on the log-probit curves can be found in Figure 1. Because  $b_{i,j}$  and  $e_{i,j}$  values could take various orders of magnitude, they were manipulated in log scale. The notations  $\log b_{i,j}$  and  $\log e_{i,j}$  will thus be used hereafter. For each species, replicate spreading around CRC is considered to be Gaussian and is characterized by a standard deviation  $S_i$  supposed to be constant inside a given species and thus independent of concentration and competition. The  $resp_i$  parameter indicates whether the log-probit model is necessary to describe the concentration-response relationship or if a constant model at the  $d_{i,j}$  level describes the data sufficiently well. We considered that  $resp_i$  should be the same with and without competition for a given endpoint, so that this parameter is defined at the species level.

Figure 1: Scheme of the role of the different parameters of the basic log-probit model, their modification by the competition parameters and how this is translated on theoretical CRCs showing a visible variation of parameters value with competition: the blue curve and the parameters named with the index 0 refers to the situation without competition and the red curve and the parameters named with the index 1 to the situation with interspecific competition.

### 2.3.3. Modelling of competition effects, parameter definition and model choice

For each species (index  $i$ ), two sets of basic log-probit parameters ( $d_{i,j}$ ,  $\Delta dc_{i,j}$ ,  $loge_{i,j}$  and  $logb_{i,j}$ , this group of parameters later denoted as  $\theta_{i,j}$ ) were estimated, one in absence ( $j = 0$ ) and the other in presence ( $j = 1$ ) of competition, these two sets of parameters later denoted as  $\theta_{i,0}$  and  $\theta_{i,1}$ . For each parameter, the difference of value between the two competition modalities (the difference between  $d_{i,0}$  and  $d_{i,1}$  for example) were defined by competition parameters named *compd* for  $d_{i,j}$ , *comp $\Delta$ dc* for  $\Delta dc_{i,j}$ , *complog $e$*  for  $loge_{i,j}$  and *complog $b$*  for  $logb_{i,j}$  (this group of parameters later defined as *comp $\theta$* , see SI B). For a given endpoint, we considered that each *comp $\theta$*  parameter could be of three different types. First, it could be a null effect and thus be equal to 0 for every species as it was possible for interspecific competition to have no visible effects on the parameter for the given endpoint. Second, it could have a fixed value among the different species (generic effect) meaning that modelling the same non-null effect of competition on CRC best described the data. Finally, the competition parameter could have a different value for each species (random effect, *i.e.* species-specific) as reaction to competition is not necessarily the same for every species. In that last case, competition parameters became respectively *comp $d_i$* , *comp $\Delta$ dc $_i$* , *complog $e_i$*  and *complog $b_i$*  (this group of parameters later defined as *comp $\theta_i$*  parameters) as they had different values for the different species.

The effects of those competition parameters on the CRC can be highlighted and translated into biological terms. *compd* translates the modification of the response level at the control (*i.e.* without chemical stress) and thus shows the effects of competition alone on the monitored endpoint. The three other *comp $\theta$*  parameters (*comp $\Delta$ dc*, *complog $e$*  and *complog $b$* ) are for their part translating a modification of the toxic effects by competition ( $\Delta dc_{i,j}$ ,  $loge_{i,j}$  and  $logb_{i,j}$  being contaminant-related parameters) as *comp $\Delta$ dc* translates a change in the amplitude of the toxic response, *complog $e$*  a change in the concentration at which is situated the inflection point of the curve (and thus the effects of competition on EC<sub>50</sub> which is commonly used in ERA) and *complog $b$*  a change in the concentration-response curve steepness.

$\theta_{i,j}$  parameters were defined at the species level, meaning that each parameter had a different values for each species, but these values were assumed to follow a same Gaussian law, as frequently hypothesized in Species Sensitivity Distribution modelling [Posthuma et al., 2001].



Likewise, when  $comp\theta$  parameters were defined as random (*i.e.* species-specific parameters), they were defined at the species level and assumed to follow Normal laws.

The type of each competition parameter was defined by calculating the DIC (Deviance Information Criterion, a criterion of fit quality for hierarchical models using Bayesian inference penalized by the model complexity) of models built using every different combination types for each competition parameter and selecting the model with the lowest DIC. When DIC values were close (with a difference of less than two, as commonly applied for the AIC criterion in frequentist inferences), the simplest model was kept. For a global view of the built models, a summary of the defined nodes and their definition and an example of a probabilistic Directed Acyclic Graph (DAG) can be found in SI B.

#### **2.3.4. Inference and parameter estimation**

Bayesian inference was carried out to estimate model parameters. This method provides estimates as posterior distributions, based on prior distributions and observations. In this work, mainly weakly informative priors based on the experimental design were chosen to keep inferences in a reasonable range. The details of how those priors were defined and the explanations of these values can be found in SI C. Posterior parameter estimation by Bayesian inference were realized with Markov Chains Monte-Carlo techniques [Gilks et al., 1996] using JAGS [Plummer, 2003] and Rjags [Plummer, 2018] under the R environment [R Core Team, 2019]. Three independent chains were generated. For each model, a Raftery and Lewis diagnostic [Raftery and Lewis, 1991] was done to define the number of iterations necessary to reach a good convergence in our simulations. For each endpoint, chain convergence was checked visually and using Gelman and Rubin convergence diagnostic [Gelman and Rubin, 1992]. To take into account potential autocorrelation of iterations, we applied a “thin” on the chains, consisting in keeping only one simulated point every thin number. The precise procedure and settings used for simulations can be found in SI D. DIC values were calculated using the same number of iterations and thin. Parameter estimation was realized in two steps since, because of its boolean nature, the estimation of  $resp_i$  at the same time of the other parameters caused convergence difficulties for these parameters. First, the simulations were run as described above but only the estimated values of the  $resp_i$  parameter were kept. Those values were considered to be of good quality after a visual inspection of the CRCs. In a second time, the simulations were run again with  $resp_i$  values fixed at 0 or 1

according to the results of the first simulation.

### 3. Results

#### 3.1. Modelling quality indicators

For a majority of endpoints, the procedure was able to return CRCs that described well the data. The exceptions were for chlorophyll and carotenoid contents that exhibited high variability and for which responses varied very little against the isoproturon concentration. Those endpoints were thus excluded from the analysis (raw data plots for those two endpoints can be found in SI E), thus leaving nine remaining endpoints for the modelling.

With a maximum value of about 1.04, the results obtained for the Gelman and Rubin indicator were low enough to indicate a good convergence of the Bayesian inference. Detailed results are provided in SI F. Table 1 shows which effect type was applied on each  $\theta_{i,0}$  parameter (in absence of competition) to obtain its  $\theta_{i,1}$  counterpart (in presence of competitors). This table shows that, for every endpoint, at least one  $comp\theta$  parameter was defined to have a fixed or random effect, evidencing that an effect of interspecific competition was visible for each endpoint. However for two endpoints (root length and root DM) this competition only modified the response at the control ( $compd$ ), meaning that the other aspects of the modelled CECs were not significantly affected by the competition.

Table 1: In the first part: models chosen by the procedure described in this article. It is indicated for each endpoint which was the effect type for each  $comp\theta$  or  $comp\theta_i$  between null (represented by a “-” sign), fixed and random effect. In the second part: summary of the chosen competition effect type of each estimated  $comp\theta$  or  $comp\theta_i$  parameters.

Endpoint	Competition effect on parameter			
	$compd$ or $compd_i$	$comp\Delta dc$ or $comp\Delta dc_i$	$complog e$ or $complog e_i$	$complog b$ or $complog b_i$
<b>Fv/Fm</b>	-	-	-	Fixed
<b>LDMC</b>	-	-	Fixed	Fixed
<b>Ligula height</b>	-	Fixed	Fixed	Fixed
<b>Max shoot</b>	Random	Fixed	-	-

<b>height</b>				
<b>Root DM</b>	Fixed	-	-	-
<b>Root length</b>	Fixed	-	-	-
<b>Shoot DM</b>	Random	-	Fixed	-
<b>SLA</b>	Fixed	-	Random	-
<b>Total DM</b>	Random	-	Fixed	-
<b>Effect type</b>				
<b>Total null</b>	3	7	4	6
<b>Total fixed</b>	3	2	4	3
<b>Total random</b>	3	0	1	0

Two examples of fit can be found in Figure 2, for the performance endpoint root DM in Figure 2a and for SLA in Figure 2b (the CRCs fitted for every other endpoint are provided in SI G). These two examples were chosen because they were good examples of how the different effect types applied by the modelling framework were translated in CRCs. For root DM (and for root length), the data were better described with a null effect of competition on  $\Delta dc_{i,j}$ ,  $loge_{i,j}$  and  $logb_{i,j}$  and with a fixed effect on  $d_{i,j}$ . We can see on Figure 2a that this is visible on the CRC with only a vertical shift between curves with and without competition. For the competition responsive functional endpoint SLA, the data were better described with a null effect of competition on  $\Delta dc_{i,j}$  and  $logb_{i,j}$ , a fixed effect on  $d_{i,j}$  and a random effect on  $loge_{i,j}$ . The effect on  $loge_{i,j}$  is translated on Figure 2b by an horizontal shift of the curve to the left traducing a decrease of the  $EC_{50}$  values, this shift being different for each species contrary to the vertical one, the same vertical shift that for root DM being also visible. These two shifts mean that for SLA, interspecific competition modified the way plant responded to isoproturon by making the different species more sensitive to herbicide ( *complog<sub>e<sub>i</sub></sub>* parameter). The only exception is for *P. trivialis* which exhibited no response against isoproturon with or without competition (and therefore had a *resp<sub>i</sub>* value of zero, corresponding to a constant curve). Shoot and total DM performance endpoints had a pattern close from the one displayed by SLA. Indeed, competition had an effect on the same two parameters, but here, this effect was random on *compd<sub>i</sub>* meaning that competition alone had a

different effect on the different species, and a fixed effect on *comploge* meaning that the horizontal shift of the curves with and without competition was the same from one species to another (SI G). On the four remaining endpoints, three of them, Fv/Fm, LDMC and ligula height, had respectively 1, 2 and 3 parameters with a fixed effect. They had a fixed effect on *complogb*. In addition, for LDMC and ligula height, a fixed effect was applied for *comploge*. The *comploge* and *complogb* parameters together define the species sensibility against the contaminant and are the most important to assess the effects of competition on species response. Finally, ligula height also had a fixed effect on *comp $\Delta$ dc*. This parameter is more complicated to interpret as it reflects a change in response amplitude between the two asymptotes of the curves. However, as there was no effect on the *compd* parameter, it meant that the species were more strongly impacted by high doses of isoproturon when the competition constraint was present. The remaining endpoint is the maximum shoot height on which were applied a random effect for *compd<sub>i</sub>* as for shoot and total DM endpoints and a fixed effect on *comp $\Delta$ dc*. It is difficult to interpret this situation directly. The fitted CRCs for this endpoint (SI G) shows that some species are less affected by high concentrations of isoproturon in presence of a competition constraint.

Figure 2: Example of concentration-response curves produced using parameters of the model built for the Root DM and SLA endpoints. Open circles represent the data point, plain circles represent the mean of data points for each concentration, the lines are the CRCs drawn using the mean estimated value of each parameter and the vertical plain lines are the 95% Credible Interval (CI) of the model response at each tested concentration (abscissa values were modified for those lines not to superimpose on the data points). In blue are the data/model without competition and in red the data/model with competition.

The second part of Table 1 summarizes the number of null, fixed and random effects of competition on each of the four parameters of CRCs for the nine endpoints. We can see in this table that adding a competition effect in the concentration response curves described the data better for 16 parameters on 36. These effects were mainly fixed effects (12 parameters on 36) and random effects were rarely selected by the modelling framework (4 parameters on 36). A small majority of competition parameters was determined as corresponding to null effects (20

parameters on 36), meaning that data were not described better when effects due to interspecific competition was taken into account for these parameters.

Graphs showing the estimated competition effects for each  $comp\theta$  and  $comp\theta_i$  parameter can be found in Figure 3a for fixed effects (*i.e.* generic effects) and 3b for random effects (*i.e.* specific effects). We can see on these two figures that observed competition effects could be positive or negative depending on the measured endpoints. In some cases, for example for  $compd_i$  of shoot DM (Figure 3b), this effect was positive for some species (*L. multiflorum*) and negative for the others, showing a variety of reaction against competition not only in intensity, but sometimes also in direction. Another important point was that for every random effect on  $compd_i$  (this trend was less visible on  $complog_e_i$ ), the rank of intensity of this effect between the different species was similar: *D. glomerata* and *P. trivialis* showed the most negative effect, *A. elatius*, *P. pratensis* and *T. flavescens* an intermediate effect and *L. multiflorum* the most positive effect, thus providing information about the difference of response to competitor of these species (species have been ordered on Figure 3b to highlight this point).

Figure 3: Figures showing median (point) and 95% IC (solid line) of the estimated  $comp\theta$  and  $comp\theta_i$  parameter value according to the effect type, the endpoint and the species (for random effect only). The vertical grey dashed lines highlight the zero value. For the fixed effect parameters (a), each graphic represents the information for a given  $comp\theta$  parameter and each line give the associated value for a specific endpoint. The word “random” means that the parameter was defined to be random and is thus represented on Figure 3b. No information means that the parameter was defined to be of the null type. For the random effect parameters (b), each graphic represents the information for a given  $comp\theta_i$  parameter and endpoint combination. On each line is shown the informations for a specific species.

#### 4. Discussion

Despite recent advances [Boutin et al., 2019; Damgaard et al., 2014], the effects of competition on plant response to chemical stress remain poorly characterised. In this work, we modelled in a single modelling framework data from monospecific bioassays in presence and absence of competitor for six phylogenetically close species. The aim was to see how competition modifies

the CRCs of those species for the studied contaminant. This hierarchical modelling showed that an effect of interspecific competition on plant response to herbicide was visible for every species and selected endpoint, highlighting the importance of using multi endpoint and/or multi species approaches in ERA. This effect was applied by the modelling framework on close to a half of the total estimated CRC parameters and only 25% of these effects varied from one species to another, the others being defined as constant between the different species.

This approach had the advantage to estimate the values of every parameters for a given endpoint at once. The CRCs of the six species and two competition modalities are defined all together and are thus interdependent. This is not the case in classical ERA approaches as in Baillard et al. where CRCs were defined independently[Baillard et al., 2020]. In our study, this approach had the advantage to integrate the competition effect for the different species in the four parameters of the sigmoid CRCs, allowing to track down on which aspect the interspecific competition had an effect. An interesting approach would have been to model the whole available dataset inside a unique modelling framework. It was however impossible in those conditions as the different endpoints exhibited a range of values that were each too different from the other, thus limiting the shrinkage possibilities. It would have been possible to transform the data to bring them in the same range, for example by calculating percentage of variation compared to the control, but we made the choice not to superimpose different transformation types as we already applied one to improve residuals normality and homoscedasticity. This would also have been problematic for SLA that varies in the opposed direction against isoproturon compared to the other tested endpoints.

In our modelling process, integrating the competition effect in the models better described the dataset. Indeed, for each endpoint analyzed, at least one parameter varied under competition, which led to a significant effect of competition on half of the 36 parameters tested, each of the nine endpoint being associated to 4 parameters (Table 1). In particular, among the 16 parameters modified by competition, ten corresponded to parameters involved in plant responses to isoproturon ( $comp\Delta dc$  or  $comp\Delta dc_i$ ,  $complog_e$  or  $complog_e_i$  and  $complog_b$  or  $complog_b_i$  parameters), whereas six were independent from herbicide exposure ( $compd$  or  $compd_i$  parameters). In addition to confirm the existence of interspecific competition in the experimental design used, these results thus showed that the presence of competitors modified the way plants respond to chemical stress. Moreover, the responses of shoot-related endpoints to competitors in

the absence of chemical stress (*compd* or *compd<sub>i</sub>* parameters) indicated that competition mainly acted on aerial parts of plants. Indeed, the increase of both shoot height and SLA under competition (Figure 3) is characteristic of response to shading, allowing increasing light interception [Fan et al., 2019, Yang et al., 2019]. This was also relevant with the fact that the experiments were conducted under non-limiting water and mineral nutrient conditions at the root level.

The responses of plant endpoints to chemical stress can depend on the mode of action of the contaminant. Although the existence of multiple targets cannot be ruled out [Ramel et al., 2012], the phenylurea herbicide isoproturon is known to target photosynthesis through PSII, with subsequent generation of triplet chlorophyll, singlet oxygen and ROS cascades [Grouselle et al., 1995, Rutherford and Krieger-Liszky, 2001]. Moreover, root-level application of herbicides can lead to shoot impact by whole-plant distribution through the xylem flow [Sulmon et al., 2007]. Given its effects on PSII, the present isoproturon exposure can therefore be seen as a situation of leaf PSII perturbation and photooxidative stress with a cascade of regulatory and developmental consequences involving metabolic, hormonal and shoot-root signalling mechanisms. Aerial parts of the plants were thus directly targeted by both isoproturon treatment and interspecific competition (through shading). This explains the results of Table 1 where isoproturon-related parameters exhibited a competition effect only for the shoot-related endpoints. In the model, the  $e_{i,j}$  parameter corresponds to the  $EC_{50}$  value of the concentration-response curve. The *complog<sub>e</sub>* or *complog<sub>e<sub>i</sub></sub>* parameters, estimating the modification of CRC  $EC_{50}$  values under competition, can thus be taken as a relevant marker of chemical stress – competition interaction. These parameters were found to be significant for the shoot-related endpoints LDMC, SLA, ligula height, and shoot and total DM (Table 1), competition inducing in each case a decrease of  $EC_{50}$  values (Figure 3, SI G). As functional traits directly involved in competition response [Fan et al., 2019, Yang et al., 2019], the leaf-dedicated LDMC and SLA endpoints were particularly affected by competition. This makes them particularly relevant endpoints to conclude that interspecific competition increases the inhibition effect of isoproturon on plant species or makes them more sensitive to isoproturon. In contrast, shoot and total DM, which also exhibited a competition effect on  $log e_{i,j}$ , consisted in more global endpoints of plant performance, resulting from the integration of functional trait responses to competition and chemical stress exposure. The *complog<sub>b</sub>* and

*complogb<sub>i</sub>* parameters also demonstrated interesting effects of interspecific competition on mainly leaf-related endpoints (Table 1). Competition was thus found to increase CRC steepness of Fv/Fm whereas decreasing it for LDMC (SI G). These results thus suggest that competition relieves isoproturon effect on photosynthesis, as herbicide-targeted process, and emphasizes its effect on a more global leaf physiology-related endpoint (LDMC). Competition, by inducing leaf shading, has been shown to change leaf physiology, leading to an increase of chloroplast number and grana layer thickness [Fan et al., 2019]. As these changes may increase the number of PSII and consequently the number of D1 proteins targeted by isoproturon, the herbicide would thus be less efficient in inhibiting a higher number of D1 targets. LDMC represents a proxy of leaf density [Ryser, 1996], which is strongly related to its biochemical composition and in particular to its protein content [Wilson et al., 1999]. Yet, this endpoint has been shown to be decreased by shading under competition [Yang et al., 2019]. Thus, changes in leaf components, and especially the decrease in leaf protein content suggested by LDMC decrease under competition, may explain the increase of plant sensitivity to isoproturon since plants need numerous cell compounds (antioxidants, chaperone proteins, antioxidant enzymes) to respond to chemical stress and particularly to PSII inhibiting herbicides [Ramel et al., 2009, 2007]. The  $\Delta dc_{i,j}$  parameter was the least affected by competition. Indeed, this parameter, which estimates the inhibitory effect of the highest isoproturon treatment (1.75 $\mu$ M), exhibited a positive competition effect only for the shoot-related height endpoints (ligula height, max shoot height; Table 1, Figure 3a), which were more inhibited by isoproturon under competition than without. It thus seemed that competition weakly affected plant responses at high chemical stress intensity, in accordance with previous studies on abiotic stresses demonstrating that competition acts mainly under low stress intensity [Callaway and Walker, 1997, Choler et al., 2001, Jung et al., 2009, Liancourt et al., 2005]. Moreover, the fact that competition had reversed effects on isoproturon response for shoot height, adverse for low concentrations and advantageous for high concentrations (SI G) strongly suggested the involvement of energetic trade-offs under combined chemical and biotic stresses.

This study was carried out by using six species of the Poaceae family in order to be representative of some plant biodiversity in grass communities and in order to consider the potential diversity of responses to chemical stress and to competitors. This experimental design thus showed that most competition effects identified for parameters related to chemical stress



responses (*i.e.* effects on  $\Delta dc_{i,j}$ ,  $\log e_{i,j}$  and  $\log b_{i,j}$ ) were characterized as fixed (*i.e.* as *comp* $\Delta dc$ , *comp* $\log e$  and *comp* $\log b$ ), and thus species-independent. In contrast, three out of the four random competition effects were applied on  $d_{i,j}$  parameter, which is chemical stress-unrelated. For most of the endpoints analyzed, it thus seemed that, under chemical stress, the competition effect remained constant whatever the species targeted, and whatever its effect in the absence of chemical stress, thus suggesting a role of chemical stress in homogenizing competition effects. Given the focus of our study on Poaceae, the characterization of generic parameters of chemical stress-competition interactions will be particularly useful for ERA and plant dynamics modelling of grazed pasture or grassland plant communities, which cover a large fraction of the Earth's land surface [Barbehenn et al., 2004]. Moreover, induction of situation of PSII perturbation is also known to occur in a number of abiotic stresses, such as high light, drought, salinity, or heat [Pospíšil and Prasad, 2014], which are known to be significant components of climate change constraints [Bigot et al., 2018]. Competition-herbicide interactions at the level of PSII therefore also suggest the potential interference of competition with climate change responses [Bigot et al., 2018, D'Alessandro and Havaux, 2019, Munné-Bosch et al., 2013, Noctor and Mhamdi, 2017].

## 5. Conclusion

This study helped understand better how competition modifies the way that herbaceous plants respond to chemical stress and how this response varies from one species to another. Competition effects were observable on every endpoint and these effects could be tracked down on the different parameters of the fitted concentration-response curves. This innovative approach had the major advantage to integrate every information given by the dataset and to propagate uncertainty from one hierarchical level to another. This work thus gives new insights for the integration of realistic supplementary factors, such as competition, into ecotoxicological bioassays and environmental risk assessments.

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## Declaration of competing interests


The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:



### Credit Author Statement

VB analyzed and interpreted the data and wrote the manuscript, CS, AKB, CM, IC and GG designed the experiment and collected the data, MLDM analyzed and interpreted the data, CS, SD and EB supervised the analysis and interpretation of data and helped writing the manuscript. All authors revised this manuscript and approved the submission of this work.



## Graphical abstract

### Highlights

In ecotoxicology, dose-response curves are used as species- and condition-specific  
We modelled curves for six grassland plant species and two competition modalities  
Hierarchical modelling integrated competition to species and contaminant effects  
Competition effect was significant and mostly generic rather than species-specific  
Modelling is a way to handle multiple factor effects in ecotoxicological bioassays



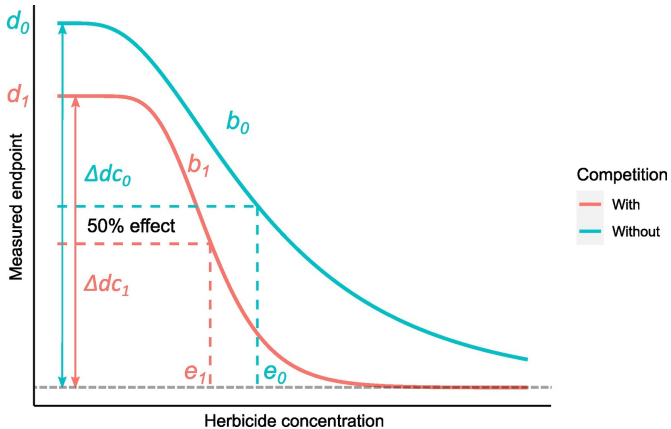
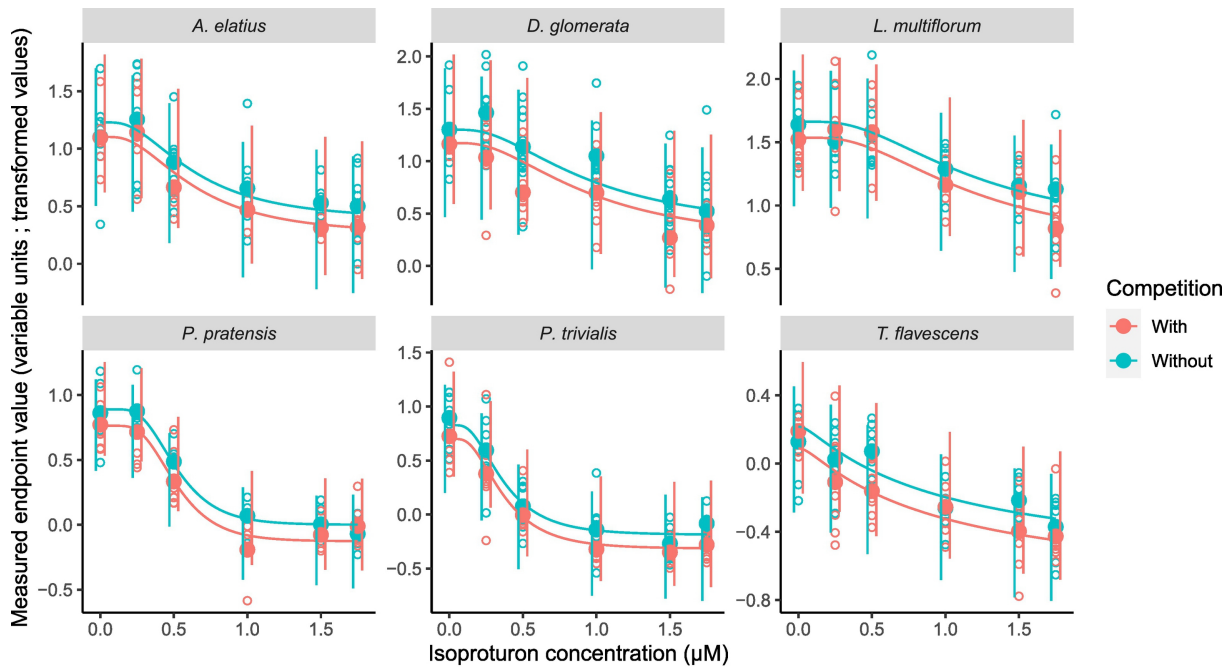
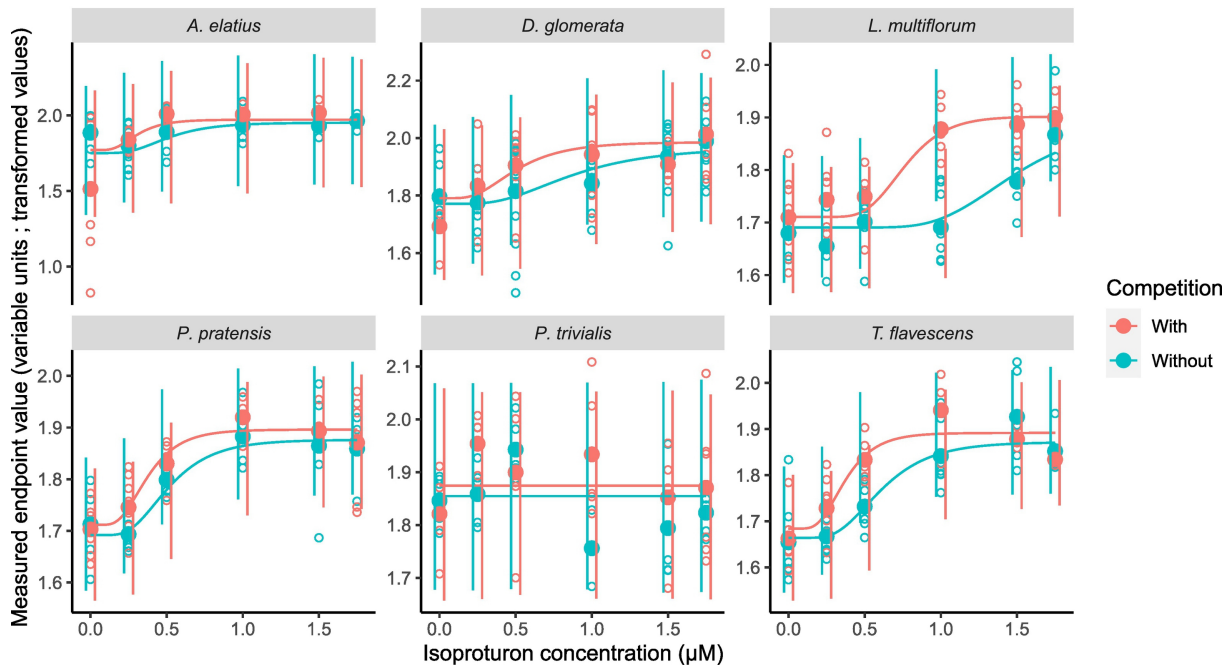


Figure 1

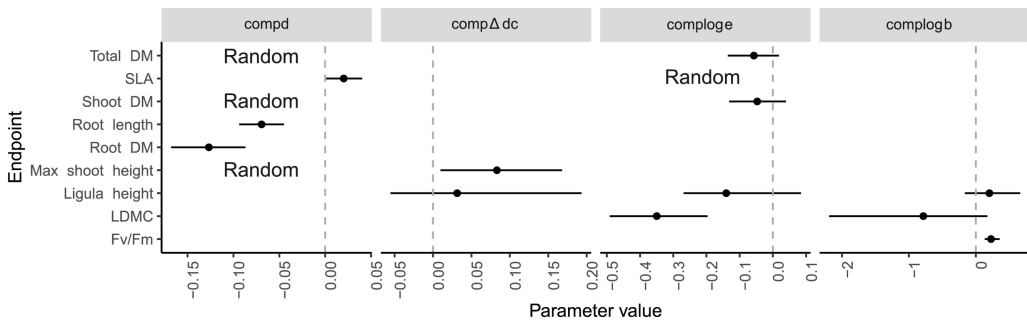


(a) Root DM endpoint

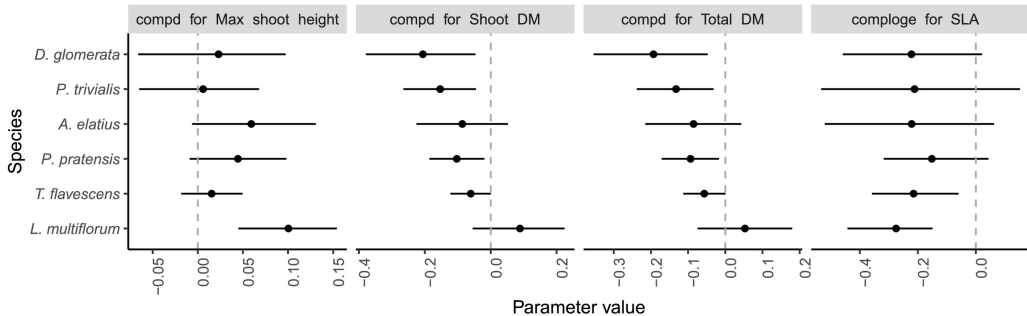


(b) SLA endpoint

Figure 2



(a) For the fixed effect parameters.



(b) For the random effect parameters.

Figure 3