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1 The consequences of non-randomness in species-sensitivity in relation to
2 functional traits for ecosystem-level effects of chemicals

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24 Abstract

25 Estimating ecosystem-level effects from single-species bioassays is a major challenge in
26 environmental risk assessment. Most extrapolation procedures are based on the implicit assumption
27 that species sensitivities are random with regard to their functional traits. Here, we explore how non-
28 randomness in species sensitivities affects how species-level and ecosystem level effects of chemical
29 exposure correspond. The effect of a correlation between the trait value under control conditions
30 and the sensitivity of the trait to chemical stress is studied for two traits (per capita growth rate and
31 monoculture yield) under constant and temporary exposure. Theoretical model predictions are
32 thereby validated against a 3-week microcosm experiment, in which 8 marine diatoms systems with
33 different correlations between trait values and sensitivities were temporary (1 week) or constantly (3
34 weeks) exposed to two concentrations of the herbicide atrazine (100 and $250 \mu\text{g L}^{-1}$). Negative
35 correlations increased the reduction in ecosystem functioning (productivity) by atrazine for both
36 traits. However, correlations in the per capita growth rate affected productivity only shortly following
37 changes in environmental conditions, whereas correlations in the monoculture yield affected
38 productivity throughout exposure. Correlations between species sensitivities and functional trait
39 values can thus help to identify when ecosystem-level effects are likely to exceed species-level
40 effects.

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49 Introduction

50 Environmental risk assessment (ERA) of chemicals aims to derive environmental threshold
51 concentrations that protect the structure and function of ecosystems. Most risk assessment
52 procedures worldwide, however, still rely on single-species bioassays¹⁻⁵. Hence the reliability of the
53 ecosystem-level effects that are inferred from the species-level effects measured in these bioassays,
54 strongly depends on the assumptions made on how species-level and ecosystem-level effects are
55 linked⁶⁻⁸. Environmental risk assessment procedures generally need to balance pragmatism and
56 environmental realism due to time or monetary constraints^{7,9}. Therefore, simple theoretical models,
57 such as the cumulative species sensitivity distribution (SSD), have increasingly been used for both
58 regulatory and scientific purposes since the 1990s¹⁰⁻¹². SSDs are obtained by fitting a statistical
59 distribution, generally a lognormal or log-logistic distribution, to the single-species toxicity data^{10,12}.
60 Environmental threshold concentrations are subsequently derived based on the adversely affected
61 fraction of species that is considered acceptable, i.e. without putting the structure and functions of
62 ecosystems at risk (e.g. 5% in EU legislation)^{1,2,4}. The SSD approach hence requires that the species
63 from which it is derived are representative for all species in the system, and that a certain degree of
64 functional redundancy between species exists so that ecosystem-level effects do not exceed species-
65 level effects^{10,11,13}. A variety of statistical and ecological effects can cause violations of these
66 assumptions, which can consequently cause observed effects on ecosystem structure and function to
67 deviate from those expected based on single species bioassays^{7,8,14-16}.

68

69 Ecosystem structure comprises the number and densities of species within the system. Changes in
70 ecosystem structure by chemical exposure can arise through both direct effects on reproduction or
71 survival rates, as well as through indirect effects by density changes in other species as a result of
72 species interactions^{8,17,18}. The direct effects measured in single-species bioassays thereby allow
73 inferring the concentration of the chemical at which species start to become affected, and changes in
74 ecosystem structure thus start to arise. Still, the correct inference of direct species-level effects in

75 the system requires that the set of species exposed in bioassays in lab conditions is a sufficiently
76 large, random sample of the species present in the ecosystem^{6,13,19,20}. If not, changes in ecosystem
77 structure may start to occur at lower or higher chemical concentrations than expected. In addition,
78 species-level effects observed in bioassays can also be unrepresentative because of differences in
79 sensitivity between lab and field conditions^{21,22}. However, due to indirect effects through species
80 interactions, effects on ecosystem structure can exceed the direct species-level effects measured in
81 bioassays^{17,23,24}. The magnitude of indirect effects, and thus the overall change in ecosystem
82 structure due to chemicals exposure, thereby depends on the type and strength of species
83 interactions within the system^{25,26}. Positive interactions (e.g. facilitation or mutualism) cause direct
84 negative effects on one species to result in additional indirect negative effects on another species.
85 Similarly, negative interactions (e.g. resource competition or predation) result in positive indirect
86 effects.

87

88 Effects of chemical exposure on ecosystem function, in contrast, do not necessarily exceed species-
89 level effects^{7,23,24}. Many ecosystem functions comprise the sum of the individual species
90 contributions (e.g. total biomass, productivity or nutrient retention). When functional reductions in
91 one species are (partly) compensated by other species, this reduces the effect of chemicals on
92 ecosystem functioning compared to their direct species-level effects^{23,27}. Whether, and to what
93 extent, functional compensation occurs depends on the type of species interactions^{8,24,27} and the
94 degree of functional redundancy between species¹⁸. Positive interactions invariably increase
95 ecosystem-level effects compared to species-level effects, as reductions in one species will result in
96 additional reductions in other species. Negative interactions, in contrast, can reduce ecosystem-level
97 effects as decreases in one species are compensated by increases in other species. The extent by
98 which loss in function in one species can be compensated for by other species thereby depends on
99 the degree of functional redundancy between species²⁸. Depending on whether the replacing species
100 are functionally less, equal or better than the affected species, ecosystem function can respectively

101 decrease, remain unaffected or even increase¹⁸. An accurate prediction of ecosystem-level effects
102 from species level effects thus assumes limited indirect effects (Figure 1, dashed black line). Several
103 concepts in ERA are based on the assumption of a certain degree of functional redundancy between
104 species (Figure 1, yellow line)^{7,15}. Under this assumption, protecting ecosystem structure is also
105 protective for ecosystem functioning. The SSD concept, for example, therefore generally assumes
106 that avoiding effects of chemical on 95% of the species is generally considered sufficiently protective
107 for the structure and function of ecosystems^{1,2,4,10,12}.

108

109 There is now mounting evidence that ecosystems have indeed a certain degree of functional
110 redundancy²⁴. Hence, most microcosm studies report an ecosystem-level no observed effect
111 concentrations (NOECs) that exceeds the species-level NOECs, and so allowing effects in 5% of the
112 species (expressed as the 5% hazardous concentration, HC₅) is protective for the system^{19,29–33}.
113 However, effects on function can exceed effects on structure when functional redundancy between
114 species is low. This is for example the case when keystone species or ecosystem engineers are
115 present, for which any loss of function will result in a disproportional effect on ecosystem
116 functions^{7,34,35}. This is particularly important when keystone species rank among the most sensitive
117 species in the system (Figure 1, red line).

118

119 Environmental risk assessment procedures, such as the SSD, thus not only assume that the species
120 from which ecosystem level-effects are derived are a random sample of the species sensitivities in
121 the system, but also implicitly assume that species sensitivities are randomly related to the species'
122 functional traits^{7,36}. Ecological theory provides important insights in how the type of species
123 interactions and the degree of functional redundancy between species determines how effects on
124 structure and function are linked. Including target or keystone species (i.e. non-random sampling)
125 has been proposed as ways to account for non-randomness in species sensitivities^{10,12,32}. However,
126 detailed knowledge of species interactions and functional redundancy is often not available at the

127 ecosystem level^{8,37-39}. Identifying these systems where protecting ecosystem structure based on the
128 species-level effects measured in bioassays is insufficient to preserve ecosystem functions is
129 therefore an important objective for environmental risk assessment^{7,15}.

130

131 Here, we explore how correlations between the trait value under control conditions and the
132 sensitivity of the trait to chemical stress affect how species-level effects of chemicals correspond to
133 ecosystem-level effects. It is hypothesized that strong correlations between species sensitivities and
134 functional traits should strongly affect the likelihood of functional compensation, and thus the
135 effects of chemicals on ecosystem functioning. Chemical effects on two functional traits (the per
136 capita growth rate and the monoculture yield) are considered here. Note that, as the monoculture
137 yields cannot be measured at the species level, it is not a true functional trait⁴⁰. Both are however
138 measures of species fitness under given environmental conditions, and are commonly used as
139 endpoints of single-species bioassays⁶. Hence, for simplicity we will adhere to the term traits when
140 referring to both endpoints. While effects on the per capita growth rate determines the speed at
141 which species and the system can respond to chemical stress, changes in the monoculture yield
142 relate to long term effects of chemicals⁴¹. First, a community model is introduced to demonstrate
143 how correlations in both traits can be expected to alter the speed and extent of functional
144 compensation, and thus the effect on ecosystem functioning for temporal and constant chemical
145 exposure. Next these model predictions are validated against a 3-week microcosm experiment in
146 which 8 communities of marine diatoms with different correlations between sensitivity and per
147 capita growth rate and monoculture yield were exposed for one (temporary) or three weeks
148 (constant) of exposure to two concentrations of the herbicide atrazine (100 and 250 $\mu\text{g L}^{-1}$).

149

150 Materials and Methods

151 *Ecosystem model*

152 System-level effects of chemical stress were simulated using a generic Lotka-Volterra competition
153 model for a system of n species:

$$154 \quad \frac{dN_i}{dt} = \mu_i(c) N_i \left(1 - \frac{\sum_{j=1}^n \alpha_{ij} N_j}{K_i(c)} \right) \quad (1)$$

155 N_i is the biovolume density ($\text{mm}^3 \text{L}^{-1}$), μ_i is the per capita growth rate (d^{-1}) and K_i is the carrying
156 capacity ($\text{mm}^3 \text{L}^{-1}$) of species i . The interaction strength between species pairs in the system is
157 quantified by the parameters α_{ij} (-). Larger values of α_{ij} denote stronger competition between
158 species i and j . Intraspecific interaction coefficients, α_{ii} , were set to 1. Hence growth rates and
159 carrying capacities are identical between the community model (equation 1) and single species
160 logistic growth curves (equation 4). Chemical stress was assumed to reduce both the per capita
161 growth rate μ_i and equilibrium biovolume density K_i . Log-logistic dose response relationships were
162 used to simulate stress effects on both parameters:

$$163 \quad \mu_i(c) = \frac{\mu_i(0)}{1 + \left(\frac{c}{EC_{50}}\right)^s} \quad (2)$$

$$164 \quad K_i(c) = \frac{K_i(0)}{1 + \left(\frac{c}{EC_{50}}\right)^s} \quad (3)$$

165 Where c is the chemical concentration ($\mu\text{g L}^{-1}$), and $\mu_i(0)$ and $K_i(0)$ are the per capita growth rate
166 and carrying capacity under control conditions, respectively. The EC_{50} (g L^{-1}) is the concentration at
167 which a 50% reduction occurs, and the parameter s (-) determines the steepness of the slope of the
168 concentration-effect relationship. Per capita interaction strengths were assumed to be unaffected by
169 chemical exposure²⁵.

170

171 *Model simulations*

172 Two different scenarios of chemical exposure were simulated. Identical to the microcosm
173 experiment, communities were first allowed to develop for one week before exposure to a chemical.
174 In the first scenario, communities were temporarily exposed to a chemical for 1 week and left to
175 recover in unstressed conditions for two more weeks. In the second scenario, communities were
176 continuously exposed during 3 weeks. Simulations were run for 28 days in total for both scenarios.

177

178 A Monte-Carlo simulation procedure was used to quantify the effect of the correlation between
179 species sensitivities (EC_{50}) and functional traits, μ_i and $K_i(0)$, on system level-effects of chemical
180 exposure. For each simulation run, model parameters were drawn from a proposed parameter
181 distribution, covering a range of ecologically relevant scenarios. Carrying capacities under control
182 conditions were sampled from a generic uniform distribution $U(1,100)$. Per capita growth rates
183 under control conditions were sampled from the uniform distribution $U(0,1)$, corresponding to the
184 range of growth rates observed in the microcosm experiment (Supplementary Table 1). Interspecific
185 interactions strengths were restricted to the $U(0.75,1.25)$ range. This includes both strong
186 competitive interactions that exceed the strength intraspecific completion ($\alpha_{i,j}>1$) as well as weak
187 competitive interactions ($\alpha_{i,j}<1$). A larger parameter range, however, would result in too strong
188 competitive differences causing many control treatments to become monocultures. Note that as
189 negative interactions are essential for functional compensation, facilitative interactions ($\alpha_{i,j}<0$) were
190 not considered in the model²⁴. Species EC_{50} were drawn from a lognormal distribution \log_{10}
191 $N(50,30)$, corresponding to the most commonly used statistical distribution for SSDs^{10,12}. The
192 standard deviation was set at 30 to ensure a sufficiently large variation in species sensitivities. The
193 slope parameter s was sampled from the uniform distribution $U(1,5)$, allowing for both small and
194 large intraspecific variability in stress tolerance⁴². For simplicity, the same slope value was used for
195 both stress effects on the growth rate and carrying capacity (equations 2 and 3). In analogy to the
196 microcosm experiment, the number of species was set to 4. Systems were simulated under
197 unstressed conditions and the two scenarios of chemical exposure for each Monte-Carlo run. Next,
198 ecosystem-level effects on function were calculated as the percentage total biovolume lost
199 compared to the control treatment. A total of 1000 simulations were run. Note that all simulations
200 represent systems being sampled from the same SSD, differing only in their correlation between
201 sensitivity and the per capita growth rate or carrying capacity, and the strength of species

202 interactions. Using different, ecologically relevant, parameter distributions did not alter results
203 (Supplementary Figure 1).

204

205 *Algal strains*

206 Eight species of marine diatoms (*Bacillariophyceae*) were isolated from a single phytoplankton
207 sample taken in the Belgian part of the North Sea during the 2015 March spring bloom
208 (Supplementary Table 1). Single cells were isolated with a micropipette, rinsed 3 times with growth
209 medium and grown to monoclonal stock cultures based on the protocol by Andersen⁴³. F2 medium
210 supplemented with Si at a 30 $\mu\text{g L}^{-1}$ final concentration was used as growth medium⁴⁴. Cultures were
211 kept at $20\pm 1^\circ\text{C}$ and a 12 h photoperiod at a $35\pm 5 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ light intensity. New stock
212 cultures were started weekly to keep stock cultures in the exponential growth phase.

213

214 *Single-species toxicity tests*

215 All species were exposed to 5 levels of atrazine (0, 50, 100, 250 and 250 $\mu\text{g L}^{-1}$), which comprise an
216 environmental relevant range⁴⁵. Toxicity tests ran for two weeks, with three replicates per
217 treatment. This timespan largely exceeds the 72h used in standard alga growth inhibition tests^{46,47}.
218 However, division rates of the species used are typically 0.5-1 d^{-1} (Supplementary Table 1). Hence,
219 two weeks were required to measure atrazine effects on the per capita growth rate and equilibrium
220 monoculture yield or carrying capacity. Cultures were kept at the same temperature, photoperiod
221 and light intensity as the stock cultures. Twice a week, 25 ml of the growth medium was replaced to
222 avoid a decrease in the atrazine concentration through photolysis. Because diatoms settle at the
223 bottom, the growth medium was removed from the upper part of the water column and contained
224 less than 1% of the total biovolume. Cell densities were determined at day 4, 7, 9, 11 and 14 from 1
225 ml samples using a Whipple Grid. Biovolumes of each species were determined based on the method
226 proposed by Hillebrand et al.⁴⁸ The average volume of 50 cells was used for converting cell densities
227 to biovolumes. Atrazine concentrations in the new growth medium were measured using a GC-MS

228 (Thermo Quest Finnigan Trace DSQ coupled to Thermo Quest Trace 2000 series, Supplementary
229 Table 2).

230

231 Per capita growth rates and carrying capacities for each atrazine concentration were estimated by
232 fitting a logistic growth curve to the cell density data.

$$\frac{dN_i}{dt} = \mu_i(c) N_i \left(1 - \frac{N_i}{K_i(c)}\right) \quad (4)$$

233 Next, a log-logistic dose response curve was fitted to both the per capita growth rates (equation 2)
234 and carrying capacities (equation 3) to estimate the EC_{50} and slope (s) of the dose response curve for
235 each species (Supplementary Figures 2 and 3, Supplementary Table 1). All calculations were carried
236 out in R⁴⁹ and parameters were estimated based on the least squares estimates.

237

238 *Microcosm experiment*

239 Eight communities of 4 species were randomly composed from the 8 stock cultures (Supplementary
240 Table 3). Communities were allowed to develop for one week prior to the start of the experiment.
241 Communities were exposed to the herbicide atrazine in two different exposure concentrations in a 3-
242 week microcosm experiment: a constant exposure and a temporary exposure during the first week of
243 the experiment. Based on the species' sensitivities to atrazine, species were exposed to 100 and 250
244 $\mu\text{g L}^{-1}$ atrazine in both scenarios. This corresponds to mild and severe stress, and ensures clear
245 effects compared to the control treatment. Communities were exposed in 100 ml glass Erlenmeyer
246 flasks filled with 35 ml of growth medium of the appropriate atrazine concentration and fitted with
247 cellulose plugs. All 4 species were inoculated at an initial volume of $10^7 \mu\text{m}^3 \text{ ml}^{-1}$. To minimize
248 variability between communities, species were inoculated from the same stock culture. Communities
249 were established in three replicates for each exposure scenario. Cultures were kept under the same
250 temperature and light conditions as the stock cultures. Twice a week, 25 ml of the growth medium
251 was replaced to maintain atrazine concentrations and avoid nutrient limitation. Atrazine

252 concentrations in the new growth medium were measured using a GC-MS (Thermo Quest Finnigan
253 Trace DSQ coupled to Thermo Quest Trace 2000 series; Supplementary Table 4). At day 0, 7, 14 and
254 21, 1 ml samples were taken, fixed with formaldehyde at a 6% final concentration and stored at 4°C
255 until analysis. Cell densities were determined using as Whipple grid and converted to biovolumes
256 using the same average volumes calculated for the single-species toxicity tests.

257

258 A linear mixed effect model was used to test if the % reduction in total biovolume compared to the
259 control treatment over the course of the experiment depended on the correlation between species
260 sensitivity and the per capita growth rate, $cor(\mu, EC_{50})$, or the carrying capacity, $cor(K, EC_{50})$, the
261 exposure scenario (S , with 'constant' and 'temporary' as levels), atrazine concentration (C) and day
262 (D). The exposure scenario and day were thereby included as factor variables. Including community
263 composition as a random effect to account for the dependence of observations between exposure
264 scenarios did not improve the model (ANOVA, $p=.82$), nor did fitting a temporal correlation structure
265 to account for repeated measurements (ANOVA, $p=1$) as model residual remained uncorrelated after
266 removing random effects and autocorrelation structures (Supplementary Figure 5). Up to three-way
267 interactions were considered in the initial model:

$$\begin{aligned} 268 \quad \% \text{ loss} = & cor(\mu, EC_{50}) \times S \times D + cor(\mu, EC_{50}) \times S \times C + cor(\mu, EC_{50}) \times C \times D + \\ 269 \quad & cor(K, EC_{50}) \times S \times D + cor(K, EC_{50}) \times S \times C + cor(K, EC_{50}) \times C \times D + \varepsilon \end{aligned} \quad (5)$$

270 The optimal model structure was obtained by a backward selection. Normality and independence of
271 model residuals was tested for the optimal model (Supplementary Figures 4 and 5).

272

273 Results

274 *Community model*

275 The correlation between species' sensitivity and the per capita growth rate of a species under control
276 conditions affected the extent of ecosystem-level effects only during changes in environmental
277 conditions (i.e. the first week of atrazine exposure, Figure 2). In contrast, the correlation between

278 species' sensitivity and the equilibrium monoculture yield or carrying capacity under control
279 conditions affected ecosystem-level effects throughout exposure. When systems have identical SSDs,
280 the likelihood that function loss will occur following chemical exposure is higher when fast growing
281 species are more sensitive, i.e. when the per capita growth rate and EC_{50} are negatively correlated
282 (Figure 3A and C). This effect of the correlation between the per capita growth rate and EC_{50} is
283 particularly strong at intermediate chemical stress levels (expressed as the hazardous concentration,
284 Figure 3A). At very low or high chemical stress levels, none or all of the species are respectively
285 affected, which causes a reduction in the effect of the correlation. However, this effect of the
286 correlation between species' sensitivity and the per capita growth rate disappears if chemical
287 exposure persists long enough (Figure 2B and 3C). The effect of the correlation between species'
288 sensitivity and the monoculture yield, in contrast, remains throughout chemical exposure (Figure 2B
289 and 3D). For systems having an equal SSD, a more severe loss of ecosystem function by chemical
290 exposure is thereby more likely to occur when the species sensitivity and the monoculture yield are
291 negatively correlated, compared to systems where both are positively correlated (Figure 3B and D).
292 Similarly the correlation between the EC_{50} and the per capita growth rate, the effects of correlation
293 between the EC_{50} and monoculture yield is most pronounced at intermediate chemical stress levels
294 (expressed as the hazardous concentration, Figure 3A).

295

296 *Microcosm experiment*

297 Temporary exposure to atrazine reduced the average productivity at day 7, i.e. after one week of
298 exposure. Atrazine effects on productivity even increased at day 14, one week after exposure had
299 ceased, but community productivity fully recovered at day 21 (Figure 4A, Table 1 and Supplementary
300 Table 5). Constant exposure to atrazine, in contrast, increasingly reduced the average productivity
301 throughout the experiment (Figure 4B, Table 1 and Supplementary Table 5). Changes in productivity
302 by atrazine exposure depended on the correlation between species' sensitivity and both per capita
303 growth and monoculture yield under unstressed conditions (Table 1 and Supplementary Information

304 Figure 5). At the beginning of the experiment, the correlation between growth rate and sensitivity
305 had a negative effect on the productivity as more sensitive species dominate the system under
306 unstressed conditions (Supplementary Figures 6 and 7). However, as predicted by the model,
307 reductions in productivity by atrazine exposure were more severe during a temporary exposure
308 when fast growing species were more tolerant (Table 1, Supplementary Table 5). This effect of a
309 positive correlation between the per capita growth rate under unstressed conditions and sensitivity
310 was positive after one week of atrazine exposure and increased at day 14. Although atrazine
311 exposure had ceased at this point, effects on productivity were maximal at day 14 (Figure 4A). At day
312 21, the correlation between per capita growth rate and the monoculture yield capacity had again a
313 negative effect. At this point, atrazine effects had disappeared (Figure 4A) and effects of the
314 correlation between the per capita growth rate and sensitivity were similar to systems prior to
315 atrazine exposure. Throughout the experiment, a positive correlation between the monoculture yield
316 under control condition and its sensitivity to atrazine had a positive effect on productivity (Table 1,
317 Supplementary Table 5).

318

319 In the constant exposure scenario, the correlation between the per capita growth rate and sensitivity
320 did not alter the effect of atrazine on productivity (Table 1, Supplementary Table 5). Instead,
321 differences in the effect of atrazine solely depended on the correlation between the carrying
322 capacity and sensitivity. Atrazine effects on productivity were reduced when more productive species
323 were more tolerant (Table 1, Supplementary Table 5). However, this effect was slightly less after one
324 week of exposure. This was caused by the absence of measurements for the 250 $\mu\text{g L}^{-1}$ constant
325 exposure treatment at day 7, where atrazine effects are more severe.

326 Discussion

327 In this study, it is explored how correlations between functional trait values under unstressed
328 conditions and their sensitivity to a chemical could affect ecosystem-level effects of chemical

329 exposure. These correlations between species' sensitivity and functional traits can cause ecosystem-
330 level effects of chemicals to differ strongly between systems with similar species sensitivity
331 distributions, affecting the representativeness of species-level effects for ecosystem-level effects.
332 Methods that infer ecosystem-level effects from single-species bioassays, such as the SSD approach,
333 have often been criticized for ignoring potential effects of species interactions^{6,7,13,36,51}. Here, it is
334 shown that the correlation between species sensitivities and functional traits can partly account for
335 this lack of information (Table 1 and Supplementary Table 5). In addition, inferring ecosystem-level
336 effects requires measuring species-level effects that are relevant to both the aggregated ecosystem
337 function and exposure scenario under assessment (Figures 2 and 3).

338

339 Environmental risk assessment generally requires the estimation of ecosystem-level effects from
340 incomplete knowledge, as species-level toxicity data are often only available for a limited number of
341 species. Reliable assessment of ecosystem-level effects thus strongly depends on how well the
342 species-level effects in bioassays correspond to species-level and ecosystem-level effects under field
343 conditions. The results presented here demonstrate how the probability that indirect effects on
344 species interactions cause chemical effects on ecosystem function to exceed species-level effects can
345 also be directly related to the non-randomness in species sensitivity (Figures 2 and 3). The correlation
346 between species sensitivity and per capita growth rate alters chemical effects on ecosystem
347 functions through the speed at which functional compensation can occur. When fast growing species
348 are more sensitive, i.e. when the per capita growth rate and EC_{50} are negatively correlated, their
349 replacement by tolerant species will be slow. This consequently results in stronger effects on
350 ecosystem functioning following environmental changes (Figure 2A and 3A). The correlation between
351 species sensitivity and the per capita growth rate, however, only affect the magnitude of effects
352 following environmental changes. Hence, these effects are transient. The correlation between
353 species' sensitivity and the equilibrium monoculture yield or carrying capacity, in contrast,
354 determines the extent by which density reductions in sensitive species can be compensated for by

355 tolerant species. When species' carrying capacities under unstressed conditions and sensitivities are
356 negatively correlated, species with the highest functional abilities are the most sensitive to chemical
357 exposure. This increases the likelihood that reductions in sensitive species will be so severe that they
358 cannot be fully compensated by tolerant species with lower functional abilities, causing species-level
359 effects to result in larger effects on ecosystem functioning (Figure 2B and 3D). Consequently, the
360 effect of the correlation between species sensitivities and carrying capacities remains throughout
361 chemical exposure.

362

363 These results thus stress the importance of using suitable endpoints in single-species bioassays.
364 Different endpoints are regularly being used in single-species bioassays, measuring chemical effects
365 on species growth, reproduction or survival, which may not all be representative for the ecosystem-
366 level effects under assessment^{6,7,36}. When multiple endpoints are available for a species, the most
367 sensitive endpoint is generally used in the SSD. This results in a combination of different endpoints
368 for different species based on their taxonomic and trophic position. However, not all of them may
369 be representative for the ecosystem function under consideration. In particular when endpoints used
370 in bioassays may not directly link to, or affect species demographic rate such as the prevalence of
371 developmental abnormalities. Even when bioassay endpoints measure effects on demographic rates,
372 our results show that mismatches between species and ecosystem level effects can easily arise. For
373 example, effects on the per capita growth rate are commonly used as an endpoint in bioassays⁴⁷.
374 Reductions in the per-capita growth rate only affect the rate at which the system responds, and are
375 hence generally a bad predictor of changes in species equilibrium density and long-term effects⁵²
376 (Figure 2B and 3C). Empirical studies have indeed reported a better correspondence between
377 observed ecosystem level effects and those expected from the SSD in studies using a single, short-
378 term exposure to a chemical^{53,54}, compared to studies using a chronic exposure^{30,31}. Still, current risk
379 assessment routinely uses reductions in the per capita growth rate as an endpoint in algal toxicity
380 tests^{47,48}.

381 The probability that effects on ecosystem functioning exceed effects on structure however decreases
382 as biodiversity increases^{55,56}. More diverse systems have a greater chance that several species are
383 functionally redundant, so that stress-tolerant species are able to (partly) compensate for the
384 functional loss in sensitive species, and reduce ecosystem-level effects of chemicals^{23,28}. Moreover,
385 as diversity increases the number of functional responses, this decreases the likelihood of strong
386 correlations between sensitivities and functional traits in the system. Preserving ecosystem structure
387 by preventing direct effects on species is therefore particularly important for low diverse systems.

388

389 The results presented here thus demonstrate that, when occurring, strong correlations could indicate
390 when indirect chemical effects through species interactions can result in effects on aggregated
391 ecosystem functions that are equal to, or exceed effects on structure. Current risk assessment
392 procedures still rely on the assumption that species interactions result in a certain degree of
393 functional compensation between species so that protecting ecosystem structure suffices to also
394 protect ecosystem functions^{10,36}. Therefore weighing the species sensitivities for their relative
395 abundances and including target or keystone species (i.e. non-random sampling) have been
396 proposed as ways to account for non-randomness in species sensitivities^{10,12,32}. These methods
397 thereby aim to lower threshold concentrations derived from the SSD to ensure their protectiveness
398 for the structure and functions of ecosystems. The occurrence of strong correlations between species
399 functional and functional traits can thus be a first indicator, based on the information gathered in
400 bioassays, indicating when more scrutiny is needed for extrapolating species-level to ecosystem-level
401 effects.

402

403 Associated Content

404 The Supplementary Information is available free of charge on the ACS Publications website

405

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409

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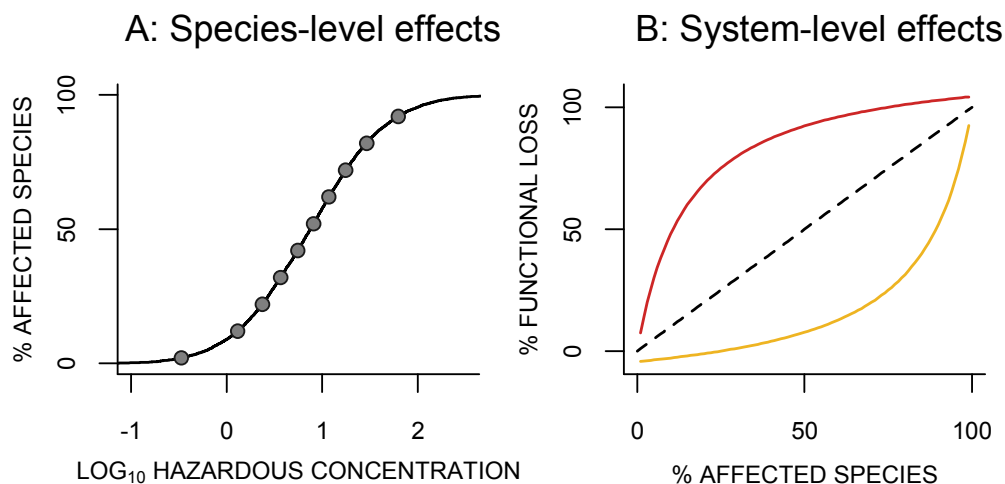
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548 Figures

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551 **Figure 1:** Extrapolating species-level effects to ecosystem-level effects. In ERA, the SSD (A) is often used
552 to set environmental threshold values based on the fraction of species that is allowed to be put at risk. How
553 these species-level effects correspond to ecosystem-level effects depends on species interactions, driving
554 indirect chemical effects B). In the absence of indirect effects, species-level effects correspond to ecosystem-
555 level effects (dashed line). Functional compensation can cause ecosystem-level effects to be smaller (red line)
556 than species-level effects, whereas low functional redundancy can cause ecosystem-level effects to exceed
557 (yellow line) species-level effects.

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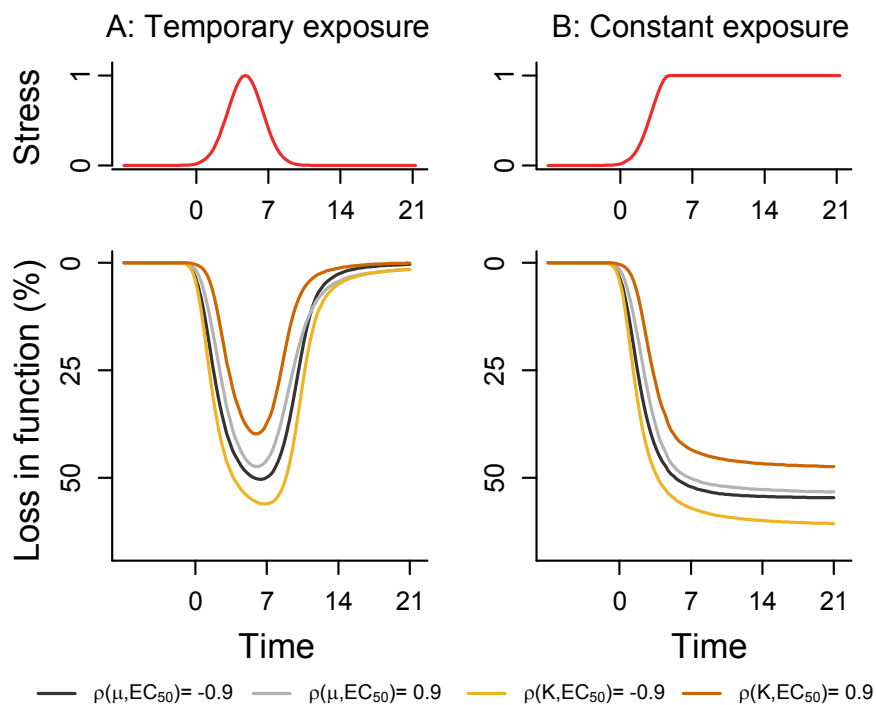
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568 **Figure 2:** Ecosystem-level effects of environmental stress. Loss in total biovolume for different
 569 correlations between species sensitivity (expressed as the EC_{50}) and per capita growth rate (μ) or equilibrium
 570 monoculture yield (K) during temporary (A) and constant exposure (B) to environmental stress. Lines represent
 571 the average for 1000 Monte-Carlo simulations for systems with identical SSDs. Stress intensity was expressed
 572 as a normalized value.

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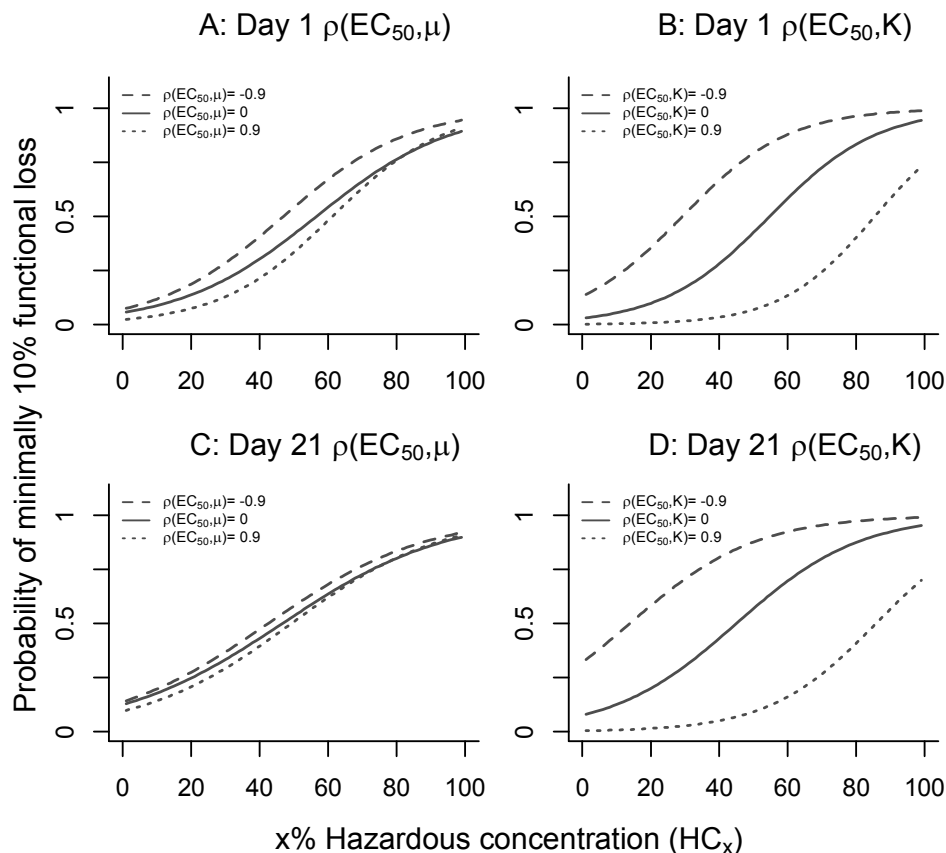
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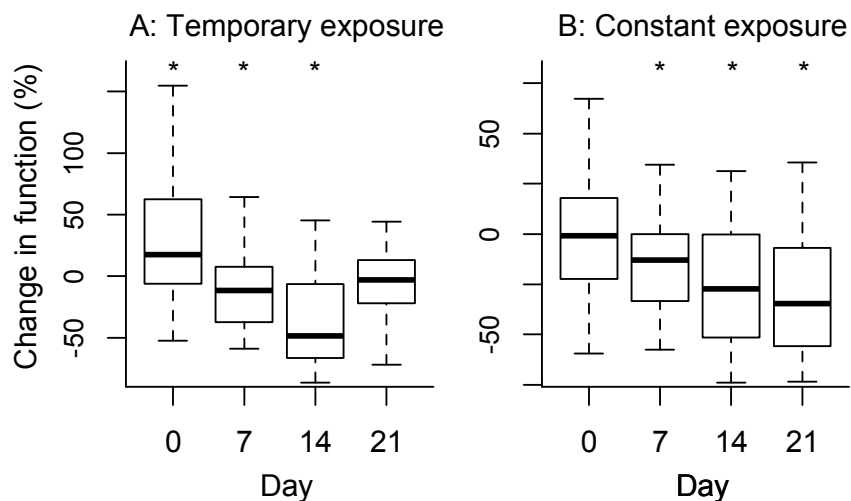
581 **Figure 3:** Probability of observing minimally 5% loss in ecosystem function. The environmental stress
582 imposed by chemical is expressed as the hazardous concentration (HC_x), whereby x refers to the
583 percentage of species in which direct effects occur. All simulations represent the constant exposure
584 scenario. Upper panels represent the probability of observing effects for a given correlation between
585 species sensitivity and the growth rate (A) or the carrying capacity (B) after 1 day of atrazine
586 exposure. Lower panels represent the probability of observing effects for a given correlation
587 between species sensitivity and the growth rate (C) or the carrying capacity (D) after 21 days of
588 atrazine exposure. Curves were obtained by fitting a binomial regression model to the model
589 predictions.

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595 **Figure 4:** Boxplots for changes in productivity compared to the control treatment during (A)
596 temporary and (B) constant exposure to atrazine. Asterisks indicate significant effects on ecosystem
597 function. P-values were calculated from the t-statistics obtained from a linear mixed effect including
598 only day and scenario as fixed effect, and community composition as a random effect. Boxplot
599 whiskers correspond to maximal 1.5 times the interquartile range.

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612 Tables

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614 **Table 1:** Estimates of fixed effects of the linear mixed effects model. Note that regression coefficients

615 and p-values are expressed against day 0 and constant exposure as a baseline.

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Constant exposure				
	Day 0	Day 7	Day 14	Day 21
Intercept	44.44**	-60.43*	-48.96*	-64.15**
Cor(μ ,EC ₅₀)	NS	NS	NS	NS
Cor(K,EC ₅₀)	67.31*	-15.59*	67.31*	67.31*
Temporary exposure				
	Day 0	Day 7	Day 14	Day 21
Intercept	40.57**	-20.05*	-8.13*	36.79*
Cor(μ ,EC ₅₀)	-107.37***	15.75***	50.03***	-31.82***
Cor(K,EC ₅₀)	67.40*	129.09*	163.66*	146.75*

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