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

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Abstract

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

Introduction

Environmental risk assessment (ERA) of chemicals aims to derive environmental threshold concentrations that protect the structure and function of ecosystems. Most risk assessment procedures worldwide, however, still rely on single-species bioassays[1–5]. Hence the reliability of the ecosystem-level effects that are inferred from the species-level effects measured in these bioassays, strongly depends on the assumptions made on how species-level and ecosystem-level effects are linked[6–8]. Environmental risk assessment procedures generally need to balance pragmatism and environmental realism due to time or monetary constraints[7,9]. Therefore, simple theoretical models, such as the cumulative species sensitivity distribution (SSD), have increasingly been used for both regulatory and scientific purposes since the 1990s[10–12]. SSDs are obtained by fitting a statistical distribution, generally a lognormal or log-logistic distribution, to the single-species toxicity data[10,12]. Environmental threshold concentrations are subsequently derived based on the adversely affected fraction of species that is considered acceptable, i.e. without putting the structure and functions of ecosystems at risk (e.g. 5% in EU legislation)[1,2,4]. The SSD approach hence requires that the species from which it is derived are representative for all species in the system, and that a certain degree of functional redundancy between species exists so that ecosystem-level effects do not exceed species-level effects[10,11,13]. A variety of statistical and ecological effects can cause violations of these assumptions, which can consequently cause observed effects on ecosystem structure and function to deviate from those expected based on single species bioassays[7,8,14–16].

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

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

Effects of chemical exposure on ecosystem function, in contrast, do not necessarily exceed species-level effects[7,23,24]. Many ecosystem functions comprise the sum of the individual species contributions (e.g. total biomass, productivity or nutrient retention). When functional reductions in one species are (partly) compensated by other species, this reduces the effect of chemicals on ecosystem functioning compared to their direct species-level effects[23,27]. Whether, and to what extent, functional compensation occurs depends on the type of species interactions[8,24,27] and the degree of functional redundancy between species[18]. Positive interactions invariably increase ecosystem-level effects compared to species-level effects, as reductions in one species will result in additional reductions in other species. Negative interactions, in contrast, can reduce ecosystem-level effects as decreases in one species are compensated by increases in other species. The extent by which loss in function in one species can be compensated for by other species thereby depends on the degree of functional redundancy between species[28]. Depending on whether the replacing species are functionally less, equal or better than the affected species, ecosystem function can respectively

decrease, remain unaffected or even increase[18]. An accurate prediction of ecosystem-level effects from species level effects thus assumes limited indirect effects (Figure 1, dashed black line). Several concepts in ERA are based on the assumption of a certain degree of functional redundancy between species (Figure 1, yellow line)[7,15]. Under this assumption, protecting ecosystem structure is also protective for ecosystem functioning. The SSD concept, for example, therefore generally assumes that avoiding effects of chemical on 95% of the species is generally considered sufficiently protective for the structure and function of ecosystems[1,2,4,10,12].

There is now mounting evidence that ecosystems have indeed a certain degree of functional redundancy[24]. Hence, most microcosm studies report an ecosystem-level no observed effect concentrations (NOECs) that exceeds the species-level NOECs, and so allowing effects in 5% of the species (expressed as the 5% hazardous concentration, HC_5) is protective for the system[19,29–33]. However, effects on function can exceed effects on structure when functional redundancy between species is low. This is for example the case when keystone species or ecosystem engineers are present, for which any loss of function will result in a disproportional effect on ecosystem functions[7,34,35]. This is particularly important when keystone species rank among the most sensitive species in the system (Figure 1, red line).

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

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

Here, we explore how correlations between the trait value under control conditions and the sensitivity of the trait to chemical stress affect how species-level effects of chemicals correspond to ecosystem-level effects. It is hypothesized that strong correlations between species sensitivities and functional traits should strongly affect the likelihood of functional compensation, and thus the effects of chemicals on ecosystem functioning. Chemical effects on two functional traits (the per capita growth rate and the monoculture yield) are considered here. Note that, as the monoculture yields cannot be measured at the species level, it is not a true functional trait [40]. Both are however measures of species fitness under given environmental conditions, and are commonly used as endpoints of single-species bioassays[6]. Hence, for simplicity we will adhere to the term traits when referring to both endpoints. While effects on the per capita growth rate determines the speed at which species and the system can respond to chemical stress, changes in the monoculture yield relate to long term effects of chemicals[41]. First, a community model is introduced to demonstrate how correlations in both traits can be expected to alter the speed and extent of functional compensation, and thus the effect on ecosystem functioning for temporal and constant chemical exposure. Next these model predictions are validated against a 3-week microcosm experiment in which 8 communities of marine diatoms with different correlations between sensitivity and per capita growth rate and monoculture yield were exposed for one (temporary) or three weeks (constant) of exposure to two concentrations of the herbicide atrazine (100 and 250 µg L^{-1}).

Materials and Methods

Ecosystem model

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

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

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

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

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

Where c is the chemical concentration (µg L^{-1}), and $\mu_i(0)$ and $K_i(0)$ are the per capita growth rate and carrying capacity under control conditions, respectively. The EC_{50} (g L^{-1}) is the concentration at which a 50% reduction occurs, and the parameter s (-) determines the steepness of the slope of the concentration-effect relationship. Per capita interaction strengths were assumed to be unaffected by chemical exporsure[25].

Model simulations

Two different scenarios of chemical exposure were simulated. Identical to the microcosm experiment, communities were first allowed to develop for one week before exposure to a chemical. In the first scenario, communities were temporarily exposed to a chemical for 1 week and left to recover in unstressed conditions for two more weeks. In the second scenario, communities were 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 model[24]. 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 tolerance[42]. 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

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

Algal strains

Eight species of marine diatoms (*Bacillariophyceae*) were isolated from a single phytoplankton sample taken in the Belgian part of the North Sea during the 2015 March spring bloom (Supplementary Table 1). Single cells were isolated with a micropipette, rinsed 3 times with growth medium and grown to monoclonal stock cultures based on the protocol by Andersen[43]. F2 medium supplemented with Si at a 30 µg L^{-1} final concentration was used as growth medium[44]. Cultures were kept at 20±1°C and a 12 h photoperiod at a 35±5 µmol photons m^{-2} s^{-1} light intensity. New stock cultures were started weekly to keep stock cultures in the exponential growth phase.

Single-species toxicity tests

All species were exposed to 5 levels of atrazine (0, 50, 100, 250 and 250 µg L^{-1}), which comprise an environmental relevant range[45]. Toxicity tests ran for two weeks, with three replicates per treatment. This timespan largely exceeds the 72h used in standard alga growth inhibition tests[46,47]. However, division rates of the species used are typically 0.5-1 d^{-1} (Supplementary Table 1). Hence, two weeks were required to measure atrazine effects on the per capita growth rate and equilibrium monoculture yield or carrying capacity. Cultures were kept at the same temperature, photoperiod and light intensity as the stock cultures. Twice a week, 25 ml of the growth medium was replaced to avoid a decrease in the atrazine concentration through photolysis. Because diatoms settle at the bottom, the growth medium was removed from the upper part of the water column and contained less than 1% of the total biovolume. Cell densities were determined at day 4, 7, 9, 11 and 14 from 1 ml samples using a Whipple Grid. Biovolumes of each species were determined based on the method proposed by Hillebrand et al.[48] The average volume of 50 cells was used for converting cell densities to biovolumes. Atrazine concentrations in the new growth medium were measured using a GC-MS

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

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

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

Next, a log-logistic dose response curve was fitted to both the per capita growth rates (equation 2) and carrying capacities (equation 3) to estimate the EC_{50} and slope (s) of the dose response curve for each species (Supplementary Figures 2 and 3, Supplementary Table 1). All calculations were carried out in R[49] and parameters were estimated based on the least squares estimates.

Microcosm experiment

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

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

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

$$\%\, loss = cor(\mu, EC_{50}) \times S \times D + cor(\mu, EC_{50}) \times S \times C + cor(\mu, EC_{50}) \times C \times D + cor(K, EC_{50}) \times S \times D + cor(K, EC_{50}) \times S \times C + cor(K, EC_{50}) \times C \times D + \varepsilon \quad (5)$$

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

Results

Community model

The correlation between species' sensitivity and the per capita growth rate of a species under control conditions affected the extent of ecosystem-level effects only during changes in environmental 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 ITable 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

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

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

Discussion

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

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

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

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

These results thus stress the importance of using suitable endpoints in singe-species bioassays. Different endpoints are regularly being used in single-species bioassays, measuring chemical effects on species growth, reproduction or survival, which may not all be representative for the ecosystem-level effects under assement[6,7,36]. When multiple endpoints are available for a species, the most sensitive endpoint is generally used in the SSD. This results in a combination of different endpoints for different species based on their taxonomic and trophic position. However, not all of them may be representative for the ecosystem function under consideration. In particular when endpoints used in bioassays may not directly link to, or affect species demographic rate such as the prevalence of developmental abnormalities. Even when bioassay endpoints measure effects on demographic rates, our results show that mismatches between species and ecosystem level effects can easily arise. For example, effects on the per capita growth rate are commonly used as an endpoint in bioassays[47]. Reductions in the per-capita growth rate only affect the rate at which the system responds, and are hence generally a bad predictor of changes in species equilibrium density and long-term effects[52] (Figure 2B and 3C). Empirical studies have indeed reported a better correspondence between observed ecosystem level effects and those expected from the SSD in studies using a single, short-term exposure to a chemical[53,54], compared to studies using a chronic exposure[30,31]. Still, current risk assessment routinely uses reductions in the per capita growth rate as an endpoint in algal toxicity tests[47,48].

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

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

Associated Content

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

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548 Figures

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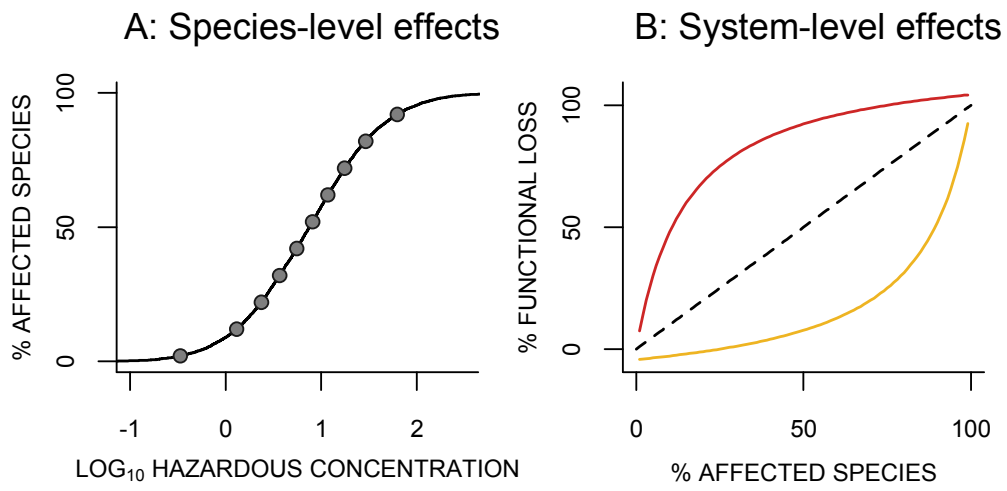

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551 **Figure 1:** Extrapolating species-level effects to ecosystem-level effects. In ERA, the SSD (A) is often used
552 to set environmental threshold values based on the fraction of species that is allowed to be put at risk. How
553 these species-level 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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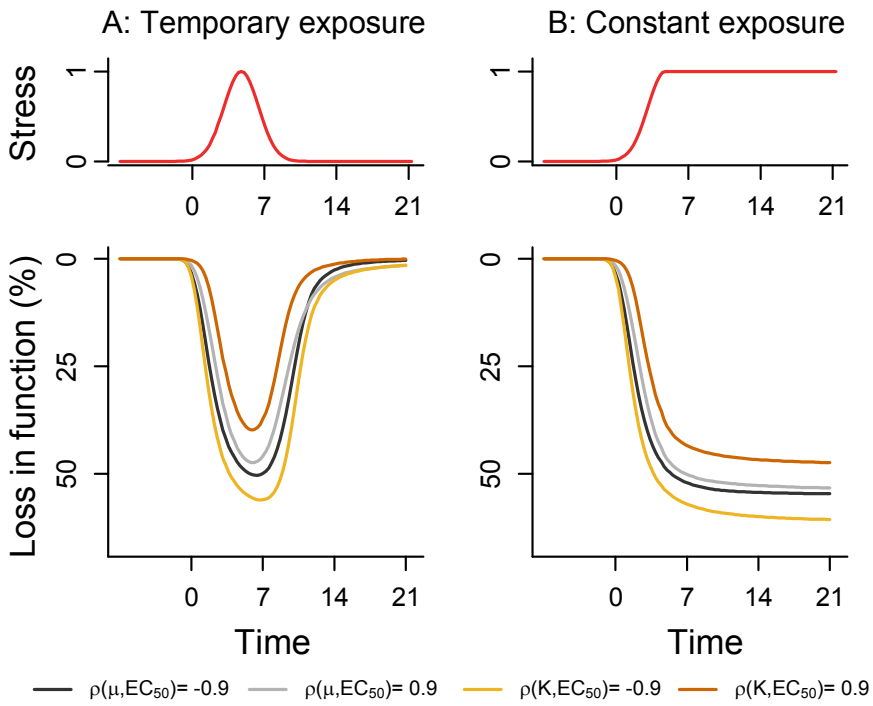

Figure 2: Ecosystem-level effects of environmental stress. Loss in total biovolume for different correlations between species sensitivity (expressed as the EC_{50}) and per capita growth rate (μ) or equilibrium monoculture yield (K) during temporary (A) and constant exposure (B) to environmental stress.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

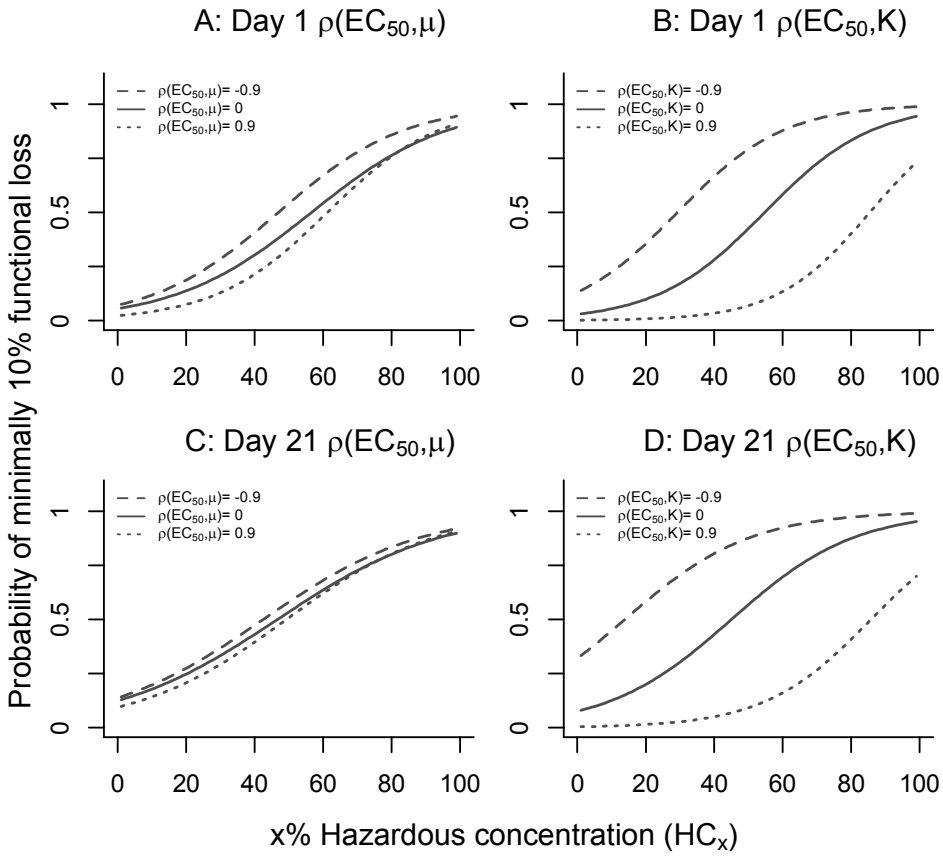

Figure 3: Probability of observing minimally 5% loss in ecosystem function. The environmental stress imposed by chemical is expressed as the hazardous concentration (HC_x), whereby x refers to the percentage of species in which direct effects occur. All simulations represent the constant exposure scenario.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

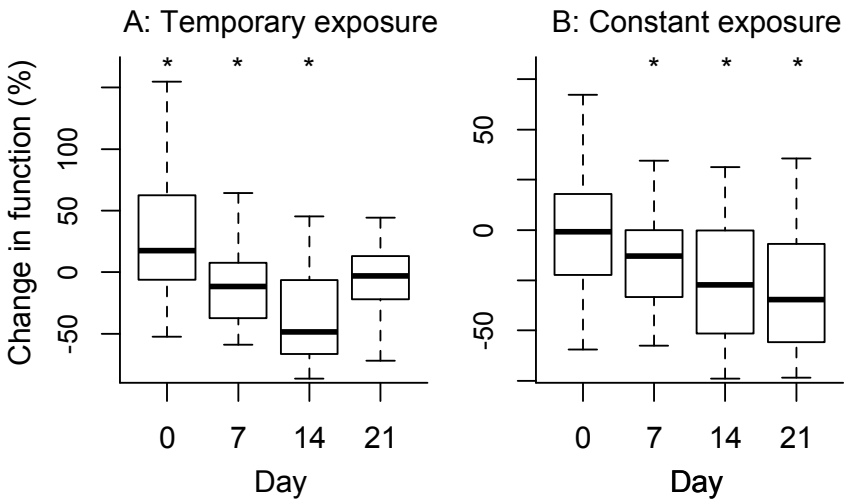

Figure 4: Boxplots for changes in productivity compared to the control treatment during (A) temporary and (B) constant exposure to atrazine. Asterisks indicate significant effects on ecosystem function. P-values were calculated from the t-statistics obtained from a linear mixed effect including only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

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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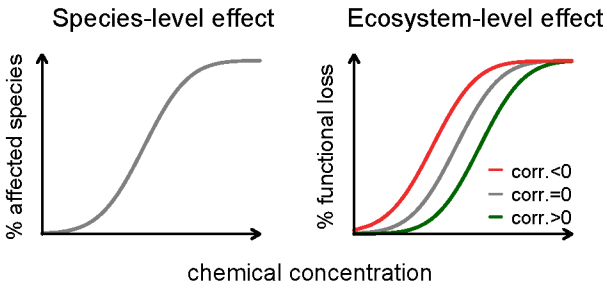
Species-level effect
Ecosystem-level effect
% affected species
% functional loss
corr.<0
corr.=0
corr.>0
chemical concentration