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Ecotoxicity effects on functional diversity – A proof of concept using a sensitivity distribution approach☆

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ABSTRACT

Current approaches for estimating the effects of chemical exposure commonly rely on species sensitivity distributions, which are well-established in ecotoxicological assessments for chemical registration and authorisation. However, this method does not consider the functional roles of species within ecosystems, an aspect captured by functional diversity. In this paper, we present a method to estimate the toxic effects of chemicals on functional diversity, with an emphasis on functional richness. Our approach integrates ecotoxicity data with abundance and trait data to determine the potentially affected fraction of functional diversity across chemical concentrations. For this purpose, we fitted a functional sensitivity distribution, similar to a species sensitivity distribution, and derived the concentration–response slope factor for a given species group and chemical. We demonstrate our method using the terrestrial plant order Poales (including grasses such as wheat) and the aquatic fish order Cypriniformes (ray-finned fish such as carp). Our results show increasing negative effects on both functional and species richness with increasing chemical concentrations. Notably, a toxic effect on species richness did not always lead to an effect on functional richness, highlighting the added value of considering functional traits. A key challenge of this method is the limited availability of trait and ecotoxicity data for many species and chemicals. Nevertheless, as data availability improves, integrating functional sensitivity distributions into chemical risk assessment offers a promising tool for evaluating chemical-induced ecological effects, supporting authorisation and registration decisions, and triggering risk management measures for chemicals already on the market.

1. Introduction

Chemical pollution poses considerable threats to biodiversity. It encompasses many different chemical and material groups and has a heterogeneous nature, being captured under the concept of ‘novel entities’, one of the nine planetary boundaries which is already largely exceeded (Persson et al., 2022; Richardson et al., 2023). Together with climate change and biodiversity loss, chemical pollution is a key component of the’triple planetary crisis’, caused by human activities threatening the stability and health of life on Earth (Persson et al., 2022; Richardson et al., 2023).

Despite the multifaceted nature of biodiversity, it is most commonly measured using species diversity indices, such as species richness: the number of species within an ecosystem (Cadotte et al., 2011). Such indices do not consider the functional roles that species play in the ecosystem and, therefore, provide limited insight into ecosystem functioning (Peña-Lara et al., 2022; Scherer et al., 2023; Wordley et al., 2017). In contrast, functional diversity captures the variation in species’ functional traits (e.g., body size, dispersion), which influence species’ roles within ecosystems and their responses to environmental changes (Ahmed et al., 2019; Mason et al., 2005; Villéger et al., 2008). These traits are considered adaptations of organisms or populations to their environments and can serve as indicators of how populations respond to stressors, including chemical pollution (Kunz et al., 2022). Although functional diversity provides greater explanatory power regarding ecosystem functioning, conventional biodiversity metrics, such as species richness, are still predominantly used when assessing the effects of chemicals on ecosystems in higher-tier studies. As a result, conclusions are often drawn with limited insight into how chemical exposure affects ecosystem processes and functioning, resulting in limited information in the biodiversity assessment of chemicals by risk assessors.

The meta-analysis by Ahmed et al. (2019) highlighted the scarcity of

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research directly linking functional diversity to chemical exposure. One notable exception is the study by Arenas-Sánchez et al. (2021), who explored the toxic effects of seasonal hydrological variation and pollution levels on functional diversity in an aquatic ecosystem. In contrast, other studies mainly investigated the toxic effects of chemicals on ecosystem processes, focusing on ecosystem services. For instance, Awuah et al. (2020) related the effect of toxic elements on soil ecosystem functions to ecosystem services. Similarly, Syberg et al. (2017) analysed the effect of pesticide runoff in coastal water on ecosystem services. Fajana et al. (2024) broadened this idea by incorporating the functional roles of soil organisms and soil functions into an approach building on the species sensitivity distribution (SSD). An SSD is a method to estimate the fraction of species affected at the relevant concentration range of a chemical (Posthuma et al., 2001, 2019). This estimation is achieved by ranking and fitting species based on their sensitivity to the chemical. From this SSD, acceptable effect thresholds can be derived for monitoring, risk assessment, and impact assessment. Even though this study and Fajana et al. (2024) build on the SSD approach, the application is different. Fajana et al. (2024) derived "eco-indicators", which are related to ecosystem services. In this study, we use functional diversity, which is related to ecosystem functioning. On top of that, their method does not consider the relative importance of each species, which we include by using the change in functional trait space, with carefully selected traits related to certain functions within the ecosystem. In short, while recent studies investigate the toxic effects of chemicals on ecosystem services, a systematic method to investigate the toxic effects of chemicals on functional diversity, and thus relating the effect to ecosystem functioning, is currently lacking. Yet, this added knowledge will improve the risk assessment of chemicals, which will benefit policymakers.

In this study, we aim to fill this research gap and develop such a method that links the toxic effects of chemicals to functional diversity. Moreover, we provide a proof of concept for this method with two case studies: the terrestrial plant order Poales (grasses like wheat) exposed to the herbicide atrazine and the aquatic fish order Cypriniformes (ray-finned fish like carps) exposed to the insecticide malathion. We started with all terrestrial species present in the databases and then, after filtering steps, selected the Poales case study, as this combination best met the criteria for deriving the functional sensitivity distribution under limited data availability. We also added an aquatic case study to enhance the proof of concept. With these two case studies, we demonstrate the applicability of the method with accessible data, thereby enhancing practical relevance. These two case studies showcase the ability to scale up to other chemicals and species groups and identify limitations that may not be apparent through theoretical analysis alone. We build on and compare our method to the established approach of a species sensitivity distribution, extending this approach to functional richness. We expect responses by functional richness to differ from those by species richness, at least in some cases, as some species are functionally redundant, while others are functionally unique. With this method, the toxic effects of chemicals on biodiversity can be assessed not only through an effect on species richness but also through an effect on functional richness. This combination is useful as a prospective risk assessment tool under various chemical legislations and, therefore, provides additional insights for practitioners concerned with pollution screening and/or chemical risk assessment. Therefore, this method, combined with the species sensitivity distribution, will strengthen the information about ecotoxicity to aid the registration of chemicals and the derivation of Environmental Quality Standards.

2. Materials and methods

2.1. Study overview

We developed a method to assess the toxic effects of chemicals on functional diversity by integrating ecotoxicity data with species functional traits and, where relevant, species abundance. Species abundance is used in the calculation of two out of the three functional diversity indices, namely functional evenness and functional divergence. We started broadly with all species available in the databases, and, after filtering, selected two case studies that had sufficient overlapping species available in the ecotoxicity, trait, and abundance datasets. Thus, we show a proof of concept for this method with the order Cypriniformes and the order Poales. The framework of the methods can be found in Fig. 1. First, the undisturbed functional diversity at zero chemical concentration was established using only the trait and abundance datasets. Second, ecotoxicological effect data were incorporated to estimate the effect of increasing chemical concentration on functional diversity (FD). To model this relationship, we calculated the potentially affected fraction of functional diversity (PAF_{FD}) and used this PAF_{FD} to construct a functional sensitivity distribution (FSD) as an adaptation of a species sensitivity distribution (SSD). This distribution was then used to determine the PAF_{FD} at the hazardous concentration affecting at least 10 % of the individuals of a species ($EC_{10}eq$) for 20 % of the species in the species group ($HC20_{EC10eq}$). Finally, we derived a concentration–response slope factor from the $HC20_{EC10eq}$, enabling comparison of different chemicals and species assemblages. All analyses were conducted using R version 4.4.0.

2.2. Functional diversity

2.2.1. Functional diversity indices

In this paper, we use functional diversity and its associated indices as proposed by Villéger et al. (2008), following the recommendation of Ahmed et al. (2019), which is based on their evaluation of multiple functional diversity indices. Here, functional diversity consists of three independent and complementary indices: functional richness, functional evenness, and functional divergence (Villéger et al., 2008). These indices are calculated by mapping species' functional traits (their characteristics, such as length) within a community onto a multidimensional plane known as the functional trait space. Functional richness refers to the volume of this functional trait space occupied by the community. Because this index is based on extreme traits, it is heavily influenced by outliers (Laliberté and Legendre, 2010; Legras et al., 2018; Villéger et al., 2008). Communities with higher functional richness can exploit a broader range of resources, thereby enhancing their resistance to environmental change, pressure, and invasion (Ahmed et al., 2019; Díaz and Cabido, 2001). Notably, functional richness can only be calculated when the number of species exceeds the number of traits, as trait volume cannot be defined otherwise. For functional evenness and functional divergence, species abundance is needed for the calculation and incorporated into the functional trait space. Functional evenness measures the regularity of species' abundances in the functional trait space (Villéger et al., 2008; Weisse, 2017). Communities with higher functional evenness use resources more effectively, as abundances are more evenly distributed, leading to greater productivity and reducing invasion opportunities (Keeney and Poulin, 2007; Mason et al., 2005). Lastly, functional divergence relates to the divergence in the distribution of species abundances in the functional trait space (Villéger et al., 2008). In communities with high functional divergence, abundant species tend to exhibit extreme trait values, which enhances resource use efficiency and reduces resource competition (Keeney and Poulin, 2007; Mason et al., 2005).

2.2.2. Functional sensitivity distribution

Functional diversity (FD) was estimated using the fundiversity package (Grenié and Gruson, 2023). We selected at least seven species, as this number allows for five estimates of functional diversity, considering that at least three species are needed to estimate functional diversity and five data points are considered sufficient to derive the HC_{20} from an SSD, following the recommendations of Owsianiak et al. (2023). We first calculated the FD under undisturbed conditions, without

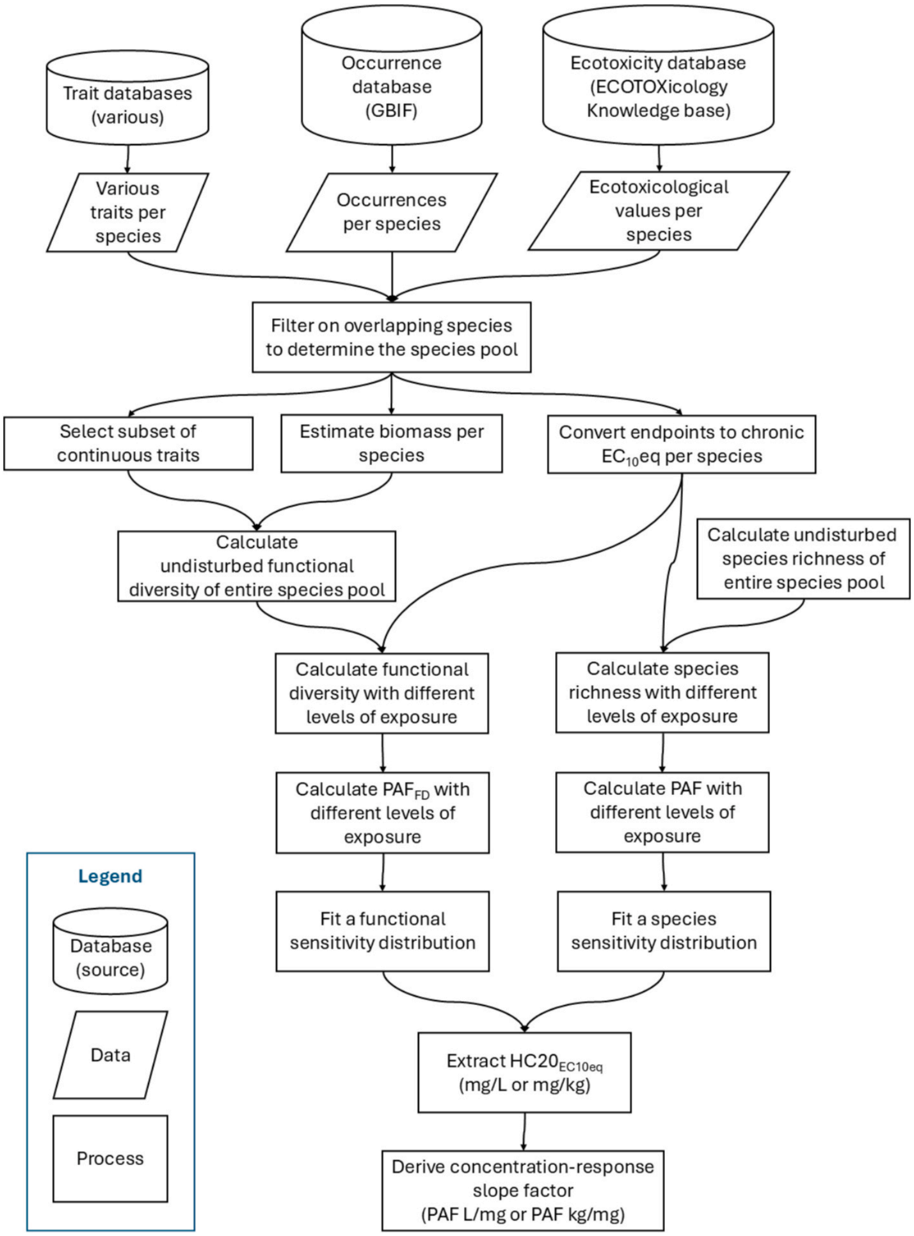

Fig. 1. Conceptual framework for the derivation of the concentration–response slope factors from the functional and species sensitivity distributions. EC_x = effect concentration, HC_x = hazardous concentration, PAF = potentially affected fraction.

chemical exposure. This undisturbed FD is calculated with the trait dataset and, where relevant, the abundance dataset, using all given species of the taxonomic order. Subsequently, ecotoxicity data were incorporated to estimate the disturbed FD at increasing chemical concentrations. The ecotoxicity data provided the effect on individual species at a given chemical concentration. When the concentration in our model exceeds the critical threshold, the species no longer adds to the functional trait space. As this threshold, we used the equivalent endpoint EC_{10}, as this endpoint is closest to realistic environmental concentrations, following Owsianiak et al. (2023). We assumed that exposure at or above species' EC_{10} equivalent (EC_{10}eq) concentrations resulted in a change in community composition. Specifically, once a species reached its EC_{10}eq threshold, it was considered affected and excluded from the FD calculation by setting the species to absent for functional richness or setting the abundance to zero for functional divergence and functional evenness. As direct changes in trait expression due to the chemical exposure are typically unknown (e.g., a chemical that reduces a bird's wingspan), we did not alter the trait values for increasing chemical concentrations in the FD estimation. We then iteratively build the cumulative distribution for the FSD by recalculating the FD only including the species whose EC_{10}eq fell below a given concentration. We determined the PAF_{FD} at that concentration with the disturbed FD and the undisturbed FD, the latter of which was determined at a concentration of zero (Equation (1)). These PAF_{FD} were determined for increasing concentrations until all species were affected, providing us with PAF_{FD}-concentration pairs for every affected species. More information on the calculation of the functional diversity indices can be found in the supplementary information Section S9.

$$PAF_{FD} = \frac{Undisturbed\ FD - Disturbed\ FD}{Undisturbed\ FD} \tag{1}$$

A PAF_{FD} value of zero indicates that functional diversity at that concentration remained unchanged compared to the undisturbed FD. A positive PAF_{FD} indicates a decrease in FD, whereas a negative value indicates an increase compared to the undisturbed FD.

PAF_{FD} –concentration pairs were used to construct an FSD for functional richness. For this purpose, we followed the same ranking approach applied in traditional SSDs, but substituting species richness for functional richness. In this way, our method replaces the traditional PAF with the PAF_{FD} to build a sensitivity distribution. The FSD was constructed by fitting a logistic curve using the drm() function from the drc package (Ritz et al., 2015). Likewise, we also constructed the SSD based on the same EC_{10}eqs and species for comparison purposes, which resulted in a loss of two species (6 %) for fish compared to using all available EC_{10}eqs and no loss for plants. Supplementary information Table S4.1 provides the parameters for the fitted logistic curves, and supplementary information Section S9 provides information about the calculation of the PAF. For both the FSD and SSD, we determined the $HC20_{EC10eq}$ (Owsianiak et al., 2023). This $HC20_{EC10eq}$ can be used instead of the actual, but unknown, background concentration to calculate the concentration–response slope factor (CRF; Equation (2); Saouter et al., 2017).

$$CRF = \frac{PAF_{(FD)}\ at\ HC20_{EC10eq}\ of\ the\ SSD}{HC20_{EC10eq}\ of\ the\ SSD} \tag{2}$$

The CRF assumes linearity between concentration and response, with higher values indicating stronger toxic effects of chemicals on a biodiversity metric (Saouter et al., 2017). For both the SSD and FSD, we used the $HC20_{EC10eq}$ of the SSD to replace the unknown background concentration as the working point for the CRF calculation. This $HC20_{EC10eq}$ corresponds to a PAF of 0.2 for the SSD, while the corresponding PAF_{FD} was extracted from the FSD. This method for determining the CRF allows for direct comparison across different chemicals, species groups, and biodiversity metrics, as the same working point was used to calculate the CRF for both the SSD and FSD.

This method could only be applied to the functional diversity index of functional richness, for which the PAF monotonically decreased with increasing chemical concentrations, in line with a logistic curve. In contrast, functional evenness and functional divergence showed bidirectional responses to chemical exposure, preventing the fitting of a sensitivity distribution. These bidirectional responses result from their dependence on the distribution within the functional trait space rather than its size. Therefore, the PAF can both increase and decrease depending on the abundance and location of the affected species within the trait space.

2.3. Data

2.3.1. Ecotoxicity

Ecotoxicity data were retrieved from the ECOTOXicology Knowledgebase ASCII, version 12–12-2024 (Olker et al., 2022). This dataset was cleaned and merged following the procedure described by Schür et al. (2023). Briefly, their method outlines the required input files, keys for matching these files, and steps for harmonisation and filtering of the dataset. Subsequently, all effect concentrations were standardised to a common unit: mg/kg dry soil for plants and mg/L water for fish. Ecotoxicity data have been published using both measured and nominal concentrations. For example, Shao-nan and De-fang (1996) reported their data as nominal and Beyers et al. (1994) as measured for fish. This discrepancy also becomes clear from the OECD guidelines. For fish, the OECD recommends measured concentrations, but results may also be based on the nominal concentration if the deviation is less than twenty percent. For plants, the OECD recommends using appropriate analytical verification for the concentration (OECD, 2006, 2025). Since the ECOTOXicology Knowledgebase does not document the type of concentration, we could not apply any filter or correction and assumed that the concentrations were comparable. The dataset was then filtered to keep the most common effect that directly influences populations: population size for plants and mortality for fish. Subsequently, the endpoints were classified into equivalent standard endpoints, either EC_{10}eq or EC_{50}eq (Table 1, supplementary data). Since the lowest observed effect (LOEC, LOEL, LOER) and no observable effect (NOEC, NOEL, NOER) are not distinguished from EC_{10} in regulatory frameworks, we classified them also directly as EC_{10}eq (Aggarwal et al., 2024). The main endpoints, before classification, were NOEL (47 %) and LOEL (47 %) for plants and LC_{50} (88 %) for fish.

Table 1

Ecotoxicological endpoints equivalent to EC_{10} or EC_{50}. The first column indicates the equivalent into which the endpoints in the second column can be classified, for which ‘x’ corresponds to the range provided in the third column.

Endpoint equivalent	Endpoint original[*]	Range of x
EC_{10}eq	EC_x[†], ED_x, LC_x[†], LD_x[†], LOEC[†], LOEL[†], LOER, MATC[†], NOEC[†], NOEL[†], NOER, NR-ZERO[†], BMC_x, IC_x, ID_x	0–15
EC_{50}eq	EC_x, ED_x, IC_x, ID_x, LC_x, LD_x, LR_x	45–55

[*] Abbreviations of the endpoints are described in the supplementary information Section S1.

[†] Indicates the endpoints used for fish and/or plants.

Finally, acute EC_{50}eq, chronic EC_{50}eq, and acute EC_{10}eq were converted to chronic EC_{10}eq using the regression equations of Aggarwal et al. (2024). The researchers constructed regression lines through all logarithmic equivalent endpoints-logarithmic chronic EC_{10}eq pairs, providing equations to convert various equivalent endpoints, both chronic and acute, into chronic EC_{10}eq. As their conversion method is for aquatic species, the species group *algae* was used as a surrogate for terrestrial plants, and the species group *fish* for fish. For plants, concentrations were classified as chronic when test durations were longer than 21 days or not reported and acute when shorter than 21 days (OECD, 2006). Conversely, fish tests were classified as acute when the test duration was less than 96 h and chronic when the test duration was more than 96 h (OECD, 2025). These assumptions were then used to standardise the effect concentrations to chronic EC_{10}eq. For plants, acute EC_{50}eq values were converted to chronic EC_{10}eq (Equation (3). For fish, acute EC_{50}eq, chronic EC_{50}eq, and acute EC_{10}eq values were converted to chronic EC_{10}eq (Equations (4), 5, and 6). When multiple chronic EC_{10}eq values for the same species, chemical, and effect were found after the classification and conversion, the geometric mean was taken to ensure a single concentration per species per chemical per effect. The resulting ecotoxicity data were incorporated into the functional diversity calculations.

$$Plant:\ \ EC_{10}eq\ chronic = 0.30 EC_{50}eq\ acute^{0.53} \tag{3}$$

$$Fish:\ \ EC_{10}eq\ chronic = 0.09 EC_{50}eq\ acute^{0.84} \tag{4}$$

$$Fish:\ \ EC_{10}eq\ chronic = 0.28 EC_{50}eq\ chronic^{0.75} \tag{5}$$

$$Fish:\ \ EC_{10}eq\ chronic = 0.23 EC_{10}eq\ acute^{0.77} \tag{6}$$

2.3.2. Abundance

To estimate species abundance, we followed the method of Callaghan et al. (2023), which uses occurrence data from the Global Biodiversity Information Facility (GBIF) over ten years as a proxy for global species abundance. Occurrence counts for each species were retrieved using the rgbif package and its occ_count() function (Chamberlain et al., 2024). This function provides occurrence records mediated by GBIF for specified species and periods, in our case, between 2015 and 2024 (GBIF, 2025a, 2025b). More information about the abundance calculation can be found in the supplementary information Section S9. To account for size differences among species, the species’ occurrence-based abundance was multiplied by the species’ body mass to estimate the biomass for each species. Biomass provides a better picture, as larger species tend to play stronger functional roles compared to smaller species (Keeney and Poulin, 2007; Saint-Germain et al., 2007). These body mass data were obtained from trait databases (see

Section 2.3.3). For fish, we used the adult body weight (g), and for plants, the dry weight of the whole plant (g). The resulting biomass was used in the functional diversity calculations.

2.3.3. Traits

For the trait selection, we followed the approach of Scherer et al. (2020). Although categorical traits would sometimes yield broader species coverage, for example, for the order Insecta, we first filtered for continuous traits, since this format provides the most information and variation in traits and makes functional diversity indices easier to calculate. Traits were selected based on four criteria: species coverage, variation in trait values, inter-trait correlation, and trait classification.

Trait data for plants were obtained from the TRY Plant Trait Database (Kattge et al., 2020) and for fish from FishBase (Froese and Pauly, 2024). Similar traits were merged, and multiple trait values per species were averaged to obtain a single value per species per trait. To ensure sufficient species coverage, we selected traits available for at least seven plant species. Contrarily, more data were available for fish, so a stricter initial filter was applied: only traits with at least 50 % species coverage were retained, ensuring both high coverage and manageable trait numbers for further selection steps. Subsequent trait selection was based on trait classification, which associates traits with trait types and ecological functions (Martini et al., 2021; Rosa et al., 2025; Scherer et al., 2020). There are four ecological functions: resource acquisition, growth, reproduction/regeneration, and survival/persistence, and four trait types: morphology, life history, physiology, and behaviour (Martini et al., 2021). The final trait combination was selected to 1) minimise missing data, 2) maximise the number of ecological functions and trait types, 3) minimise inter-trait correlation, and 4) select traits with at least three unique trait values. We ensured that the number of species was at least two to the power of the number of traits, as falling below this threshold can introduce bias ($S > 2^T$; Villéger et al., 2008).

Lastly, all traits were standardised (mean = 0, SD = 1) using the scale () function in base R (Grenié and Gruson, 2023; Petchey and Gaston, 2006; Villéger et al., 2008). Standardisation ensures that all traits contribute equally and removes the unit-based differences, which equalises the biological variation (Petchey and Gaston, 2006; Villéger et al., 2008). These scaled functional traits were used in the functional diversity calculations.

All methods were repeated using all trait combinations that fulfilled the requirements, after which we manually selected the most suitable trait combination. Traits were selected from the subset with sufficient species coverage and at least three unique values. Within this trait selection, we ensured that the number of trait types and ecological functions was maximised for each case study, and that the inter-trait correlation was low. Due to data limitations, it was not feasible to cover all four trait types and ecological functions, especially when limiting the trait set to two or three traits to ensure $S > 2^T$ (Villéger et al., 2008). For each trait combination, we used the residual standard error (RSE) as a measure of performance for the functional sensitivity distribution. The final trait combination was selected by balancing the three criteria: low inter-trait correlation, low RSE, and high species coverage. This selection does not represent a global optimal solution for each criterion but rather reflects a compromise. For example, a trait set with greater species coverage was preferred over one with a slightly lower RSE or correlation. We then qualitatively selected the most suitable trait combination for both case studies. Supplementary information Section S2 provides more information on the selected traits and the methods used.

2.3.4. Further species groups

We wanted to demonstrate the proof of concept for both an aquatic and a terrestrial case study. The aquatic case study was straightforward, as there is abundant data available in both the trait and ecotoxicity data for fish (41 % of ecotoxicity data is on fish, crustaceans, and algae; Schür et al., 2023). In contrast, the terrestrial case study proved more challenging, as both trait and ecotoxicity data were substantially less available. To overcome this limitation, we collected, merged, and processed data from 41 sources (supplementary information Table S3.1 for all sources and species groups). Trait data were collected from both large online databases and smaller datasets in published papers. We initially focused on terrestrial species groups with either known comprehensive trait datasets or a strong representation in the ecotoxicity database, namely, worms, beetles, butterflies, spiders, mammals, birds, and plants. To maximise species coverage, we harmonised and merged similar traits across datasets. This included both directly comparable traits (e.g., body weight) and functionally analogous traits presented in different formats. For instance, the trait “trophic group” was reported by Webb et al. (2018) using broad feeding types (e.g., algivore, parasitoid), while Middleton-Welling et al. (2020) used a numeric food type coding scheme (0, 1, NA). These data were harmonised into standardised trophic categories: omnivore, detritivore, herbivore, and carnivore. When merging resulted in multiple trait values per species, we averaged them again to obtain a single value per species. An exception was the only categorical trait used, feeding group, which was used to group species in the SSD and FSD. For this trait, we classified the species as an omnivore when multiple trophic groups were reported and as a detritivore, carnivore, or herbivore when this was consistently the only option within all trait datasets.

We then combined this trait dataset with the ecotoxicity dataset to find the orders with sufficient species overlap that we could thus use for the proof of concept. For this purpose, we separately analysed the trait dataset and the ecotoxicity dataset for each order by obtaining the median number of traits per species, the median number of species per trait, and the maximum number of species per chemical per endpoint. We also analysed the maximum number of species per chemical per endpoint for the combined dataset per order, as the number of species per chemical was, in most cases, the limiting factor.

2.3.5. Data curation

The scientific names of species across all databases were harmonised to the accepted name in GBIF using the rgbif package (Chamberlain et al., 2024). Taxonomic information, authorship, and common names provided within the databases were used to improve matching accuracy, which is needed when the same scientific name is historically used for multiple species. When scientific names could not be resolved at the species level, matches were made at the genus level where possible. This genus-level dataset was used to impute trait values for the order Cypriniformes, while ecotoxicity data always remained at the species level. Entries with a taxonomic precision below the genus level or with unmatched scientific names were removed from the datasets. These harmonised scientific names were then used to match species across the different datasets.

2.4. Case studies

Two case studies were selected from the compiled comprehensive dataset to demonstrate the proof of concept to link the toxic effects of chemicals to functional diversity by building on the SSD approach. These case studies were the terrestrial plant taxonomic order Poales exposed to the herbicide atrazine (CAS registry number: 1912–24-9), and the aquatic fish taxonomic order Cypriniformes exposed to the insecticide malathion (CAS registry number 121–75-5). These combinations were chosen because they each included more than seven overlapping species belonging to the same taxonomic order across the trait, ecotoxicity, and abundance datasets, providing sufficient data while ensuring a comparable niche space. Functional diversity indices require species to coexist within a comparable ecological community and niche space; for instance, traits such as bird wing length and fish fin length are not ecologically or functionally comparable. Furthermore, species from distinct environments (e.g., terrestrial birds and aquatic fish) do not interact within the same ecosystem and thus cannot be evaluated together in functional diversity analyses. Among the terrestrial species groups, Poales was the only taxonomic order that met all criteria, while the aquatic fish had multiple species groups and chemical combinations fulfilling the requirements. For the latter, we selected the assemblage with the most balanced representation of trophic levels and the lowest residual standard error for the functional sensitivity distribution. The final case studies consisted of eight species from the order Poales and 30 species from the order Cypriniformes.

2.4.1. *Case study 1: Order Poales and herbicide atrazine*

Atrazine is a commonly applied herbicide that inhibits electron transfer in photosystem II, thereby impairing photosynthesis, enzymatic processes, and growth (Diagboya and Düring, 2024; Ramezanpoor et al., 2022; Rostami et al., 2021). Additionally, atrazine alters plant metabolism by changing the amino acid content (Barchanska et al., 2024). Due to its widespread application and environmental persistence, atrazine accumulates in soils and groundwater, leaves residues in crops, and is transported via runoff into aquatic ecosystems, where it affects non-target organisms (M. T. Huang et al., 2016; Rostami et al., 2021; Singh et al., 2018). Although atrazine was banned in the European Union in 2004, it is still widely found, as atrazine is environmentally persistent (European Commission, 2004). Residues have been detected in soils more than 21 years after the last application (Vonberg et al., 2014). In other parts of the world, the use of this herbicide is still permitted, including the United States. Here, its usage is restricted by the Environmental Protection Agency with a maximum annual application rate and prohibition of application during rain (U.S. Environmental Protection Agency, 2024).

Poales is a taxonomic order of grasses, sedges, and allies, many of which are vital to humans as crops, feed, or ornamental plants. Some crops within the order Poales possess an enzymatic detoxification system that makes them largely unaffected by atrazine. In contrast, other crops show substantial inhibition in growth and germination upon exposure to atrazine (Burhan and Shaukat, 2000; Lamoureux et al., 1970).

2.4.2. *Case study 2: Order Cypriniformes and insecticide malathion*

Malathion is among the most widely used insecticides globally (Karmakar et al., 2016; Shahbazi Naserabad et al., 2015). This insecticide is commonly applied in agriculture, residential landscaping, and public recreation areas to control soft-bodied insects (Karmakar et al., 2016; Shahbazi Naserabad et al., 2015; Sharmin et al., 2015). Malathion, an organophosphate, exerts its neurotoxic effects by inhibiting the enzyme acetylcholinesterase, which is responsible for breaking down and removing the neurotransmitter acetylcholine at synaptic junctions (Karmakar et al., 2016; Patil and David, 2010; Shahbazi Naserabad et al., 2015). This inhibition leads to the accumulation of acetylcholine, resulting in nervous system disruption, manifested as hyperactivity, tremors, convulsions, and potentially death (David et al., 2007). Aquatic ecosystems are exposed to malathion through agricultural use via spray drift, drainage, runoff, and discharge (Karmakar et al., 2016; Patil and David, 2010; Sharmin et al., 2015). Due to its high toxicity, the use of malathion is restricted in the European Union; it is only permitted in permanent greenhouses (European Commission, 2023).

Cypriniformes is a taxonomic order of ray-finned fish, many of which humans consume. As a result, the accumulation of chemicals in these species poses direct risks to human health. Although Cypriniformes are not the target organisms, malathion can cause extensive harm by altering organ function, morphology, and behaviour (Akter et al., 2023; Karmakar et al., 2016; Verma et al., 2024). Ultimately, the cumulative effects can result in mortality (Akter et al., 2023; Patil and David, 2010; Shahbazi Naserabad et al., 2015).

2.4.3. *Trait selection in case studies*

Two functional traits were selected for the order Poales and three for Cypriniformes, ensuring that the condition $S > 2^T$ was met (Villéger et al., 2008). For the order Poales, the selected traits were specific leaf area (unit: mm^2/mg) and flowering period (season) (unit: total number of months), representing the trait types morphology and life history, and the ecological functions resource acquisition, growth, reproduction, and survival (supplementary information Table S2.1). The flowering period is important for plant reproduction, resource availability, and pollination (Martins et al., 2021). The specific leaf area reflects the whole plant growth and resource acquisition and plays a role in biogeochemical processes (M. Liu et al., 2017; Z. Liu et al., 2023). The two traits were weakly correlated (Spearman's ρ = -0.15; supplementary information Table S2.3) and showed no outliers. Although whole plant dry mass (unit: g) included a potential outlier (*Triticum aestivum L.*), it was retained in the analysis due to the absence of a reference value. No missing data were present for any of the plant traits.

For the order Cypriniformes, three functional traits were selected: predator–prey ratio (unit: unitless), body length (unit: cm), and preferred temperature (unit: °C), representing the trait types morphology, physiology, and behaviour. These traits collectively covered all four ecological functions (supplementary information Table S2.1). Predator-prey ratio is related to trophic structure and community dynamics influencing predation and predator kill rates (Hossie and Murray, 2016; Perkins et al., 2022). Body length is related to extinction risks, growth, reproduction, and mortality, forecasting responses to environmental change (Fritschie and Olden, 2016; Séguin et al., 2014). Preferred temperature is related to the response and performance of species to changes in temperature and to thermal stress (García et al., 2018). Correlations between traits were low (Spearman's ρ range = 0.10 – 0.27; supplementary information Table S2.4). The trait body length contained three outliers, but these values were retained, as they appeared valid. Similarly, one potential outlier was identified in body weight (unit: g), but this value was also retained due to a lack of validation data. To address missing data in the fish trait dataset, two approaches were used: 1) removing all species with missing trait values and 2) imputing missing values. For the second approach, missing values were first imputed using, when present, genus-level data, followed by further imputation using the impute() function from the funspace package, which implements the missForest algorithm (Carmona et al., 2024). The first approach resulted in a subset of 18 species, while the second retained all 30 species.

3. Results

3.1. *Case study 1: Order Poales and herbicide atrazine*

The $EC_{10}eq$ concentrations for the order Poales exposed to the herbicide atrazine ranged from 0.01 to 1.8 mg/kg dry weight soil. Changes in functional richness could only be assessed when the first five species were affected, as afterwards the number of traits exceeded the number of species. These first six affected species had $EC_{10}eq$ concentrations between 0.01 and 0.5 mg/kg dry weight soil. The corresponding dataset can be found in the supplementary data.

From the species sensitivity distribution, it follows that *Avena sativa* L. (oat) is the first species affected as chemical concentrations increase, followed sequentially by *Bromus inermis* Leyss. (brome), *Dactylis glomerata* L. (cock's foot), *Hordeum vulgare* L. (barley), *Panicum virgatum* L. (switchgrass), *Phleum pratense* L. (timothy), *Secale cereale* L. (rye), and *Triticum aestivum* L. (wheat; Fig. 2a).

Functional richness remained nearly unchanged when *A. sativa* was affected (Fig. 2b). While *A. sativa* is located on the edge of the functional trait space, nearby species such as *B. inermis* and *P. pratense* maintained the functional trait space. Afterwards, a noticeable increase in the potentially affected fraction of functional richness occurred as more species were affected. Firstly, both *D. glomerata, B. inermis,* and *H. vulgare* were affected around the same chemical concentration. However, only *B. inermis* and *H. vulgare* affected functional richness. *B. inermis* was located on the edge of the functional trait space,

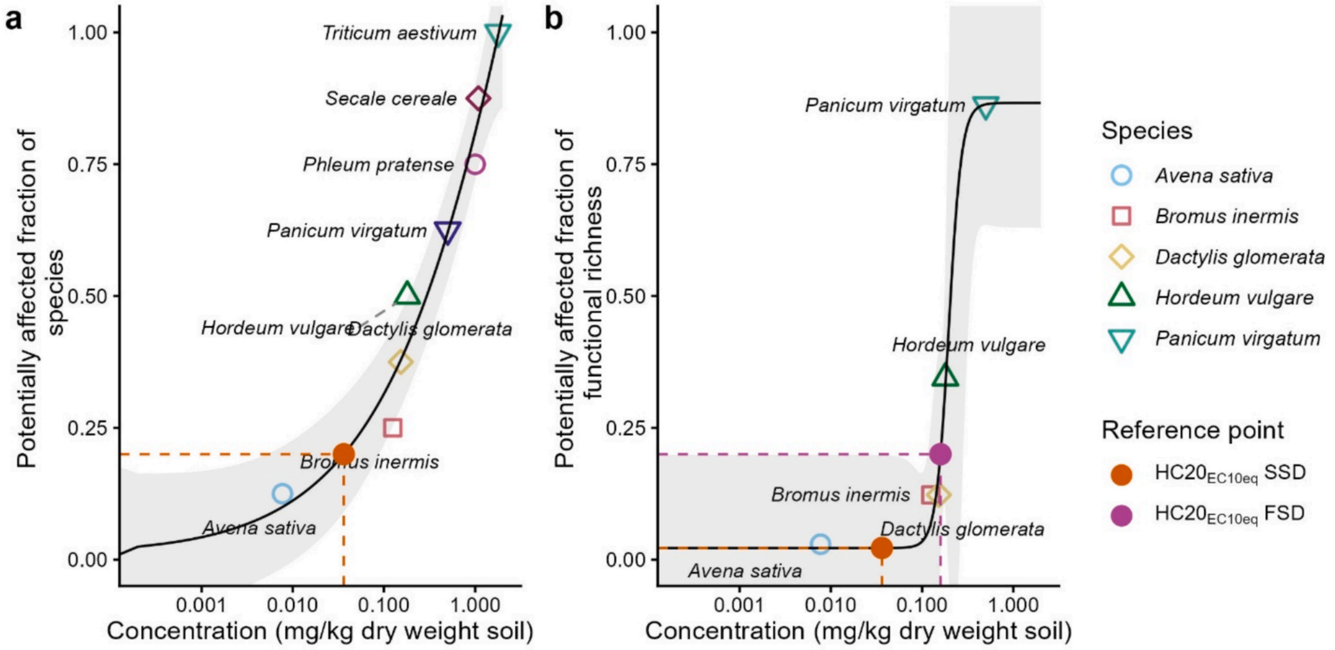

Fig. 2. Sensitivity distributions for the order Poales exposed to the herbicide atrazine. (a) Species sensitivity distribution and (b) functional sensitivity distribution for functional richness. The datapoints correspond to the species that is/are affected by the chemical at that concentration. For model parameters, see the supplementary information Section S4.

exhibiting more extreme trait values with a longer flowering period and lower specific leaf area than the other unaffected species. Then, *H. vulgare* was affected, which also caused a jump, as it possesses the highest specific leaf area in the group and an average flowering period, contributing substantially to trait diversity. Lastly, *P. virgatum* was affected, which greatly changed the functional richness. This species is located at the edge of the functional trait space with both the lowest flowering time and the lowest specific leaf area.

Species richness and functional richness were correlated (adjusted $R^2 = 0.69$, supplementary information Fig. S5.1), and both showed increasing effects with increasing chemical concentrations. However, the $HC20_{EC10eq}$ was lower for species richness than for functional richness (Table 2). Both models showed a good model fit with a low RSE. At the $HC20_{EC10eq}$ for the species sensitivity distribution, 2 % of functional richness is affected. At this concentration, the CRF for species richness was seven times higher than for functional richness (Table 2). At the $HC20_{EC10eq}$ for the species sensitivity distribution, the potentially affected fraction increases for both functional diversity indices (supplementary information Figs. S6.1 and S6.2). At the $HC20_{EC10eq}$ for the species sensitivity distribution, the potentially affected fraction increases for functional evenness and decreases for functional divergence.

Table 2
$HC20_{EC10eq}$ for two biodiversity indices, potentially affected fraction (PAF) at $HC20_{EC10eq}$ for species richness, concentration–response slope factor (CRF) at $HC20_{EC10eq}$ for species richness, and residual standard error (RSE) for the order Poales exposed to the herbicide atrazine for two biodiversity indices.

Species group	Chemical	Biodiversity index	$HC20_{EC10eq}$	PAF	CRF	RSE
Poales	Atrazine	Species richness	0.036 mg/kg	0.200	5.509 PAF kg/mg	0.07
		Functional richness	0.159 mg/kg	0.022	0.608 PAF kg/mg	0.05

3.2. *Case study 2: Order Cypriniformes and insecticide malathion*

The $EC_{10}eq$ concentrations ranged from 0.01 to 1.5 mg/L for the order Cypriniformes exposed to the insecticide malathion. However, functional richness could not be calculated for the last four affected species, limiting the range for functional richness analysis to 0.01 to 0.7 mg/L. The corresponding dataset can be found in the supplementary data.

The species sensitivity distribution shows that detritivores, herbivores, and carnivores are affected by the chemical across the entire concentration range (Fig. 3a).

The functional sensitivity distribution of the order Cypriniformes revealed more patterns compared to the order Poales, due to the larger number of species (Fig. 3b). The first notable change occurred after the second species was affected, which was a detritivore with the highest trait value for body length and relatively high trait values for both predatory-prey ratio and preferred temperature. The second jump happened when a cluster of two detritivores and two carnivores was affected, located at the edge of the functional trait space, with extreme values for predator–prey ratio and body length. Another jump occurred when the next herbivore was affected. This species was on the edge of the functional trait space with the lowest preferred temperature. Afterwards, a cluster of six herbivores and five carnivores was affected. These species had the lowest predator–prey ratios. This jump was followed by a smaller cluster of two carnivores, with extreme values for the preferred temperature and relatively low predator–prey ratios. The last cluster contained two detritivores, three carnivores, and one herbivore. These species had the highest and lowest values for predator–prey ratio and preferred temperature.

Species richness and functional richness were highly correlated (adjusted $R^2 = 0.95$, supplementary information Fig. S5.2), both showing a decrease in the potentially affected fraction with increasing chemical concentrations. The two different approaches of dealing with missing trait values presented similar results, all with a good model fit shown by the low RSE (Table 3; supplementary information Figs. S7.1 and S7.2). Therefore, all subsequent analyses were conducted using the imputed dataset, which provided more data points and, consequently,

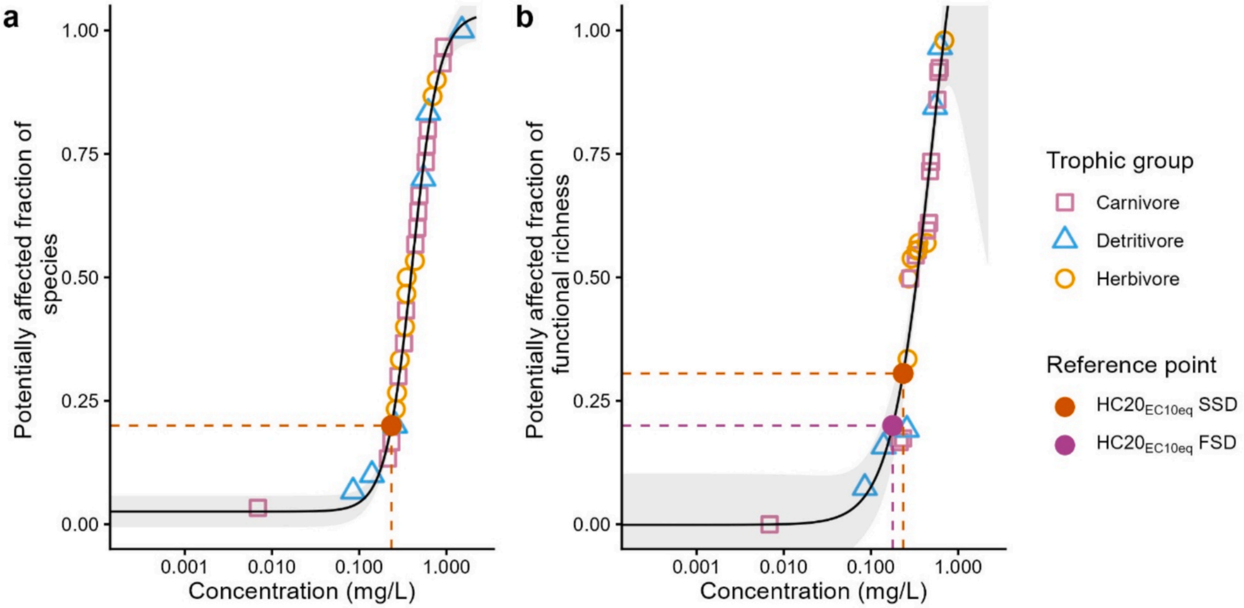

Fig. 3. Sensitivity distribution for the order Cypriniformes exposed to the insecticide malathion. (a) Species sensitivity distribution and (b) functional sensitivity distribution for functional richness, both calculated with the imputed trait dataset. The data points correspond to the species that is/are affected by the chemical at that concentration. For model parameters, see the supplementary information Section S4.

Table 3

$HC20_{EC10eq}$ for two biodiversity indices and two data processing approaches, potentially affected fraction (PAF) at $HC20_{EC10eq}$ for species richness, concentration–response slope factor (CRF) at $HC20_{EC10eq}$ for species richness, and residual standard error (RSE) for the order Cypriniformes exposed to the insecticide malathion for two biodiversity indices and two data processing approaches.

Species group	Chemical	Biodiversity index	Data processing	$HC20_{EC10eq}$	PAF	CRF	RSE
Cypriniformes	Malathion	Species richness	Removal	0.216 mg/L	0.200	0.927 PAF L/mg	0.04
			Imputation	0.234 mg/L	0.200	0.855 PAF L/mg	0.03
		Functional richness	Removal	0.215 mg/L	0.201	0.933 PAF L/mg	0.07
			Imputation	0.178 mg/L	0.305	1.310 PAF L/mg	0.08

more information, whilst having a similar performance. The $HC20_{EC10eq}$ was slightly higher for species richness than for functional richness (Table 3). At the $HC20_{EC10eq}$ for the species sensitivity distribution, only an additional 0.1 of the potentially affected fraction of functional richness was affected. Therefore, the CRF for functional richness is slightly higher (Table 3). At the $HC20_{EC10eq}$ concentrations for both sensitivity distributions, the potentially affected fraction decreases for both functional divergence and functional evenness (supplementary information Figs. S8.1 and S8.2).

The main difference between the species sensitivity distribution and functional sensitivity distribution lies in the clustering of species along the curve. In the conventional species sensitivity distribution, each affected species resulted in an equal increase in the potentially affected fraction of species, so no clusters could be identified. In contrast, in the functional sensitivity distribution, the influence of each affected species varied. Some species were functionally redundant, causing minor changes when this species was affected. Other species had unique extreme trait values, resulting in noticeable jumps in the potentially affected fraction of functional richness.

3.3. Data challenges

During this research, we identified substantial data gaps in most species group-chemical combinations, limiting the number of usable combinations. Missing information was found in either ecotoxicity data, trait data, or their overlap. Even when both datasets contained many species, the actual overlap between species, with sufficient coverage and variation, was often limited.

The cleaned ecotoxicity dataset for terrestrial species contained 7669 unique ‘Latin names’, referring to various levels such as subspecies, species, genus, family, or community. Of these ‘Latin names’, for the taxonomic rank genus and higher taxonomic precision, 96 % were matched exactly to the GBIF database, 2 % were fuzzy matched and included, 2 % matched at a higher taxonomic rank and used, either after changing to a lower rank or after accepting the higher rank, and 0.1 % was removed, as they were of a higher rank than genus or they could not be matched. Of the fuzzy and higher rank matches, 98 % were resolved. Ultimately, a dataset containing 6001 unique species and a dataset containing 3529 unique genera were retained for further filtering. During the filtering process, the number of species decreased substantially. After filtering for the medium soil, 56 % of the species remained. Filtering on endpoints that can be classified or converted into EC_{10}eq endpoints removed another 5 % of the species. The next filtering step focused on the most common effects within the species groups: mortality (19 %) and population size (70 %). For population size, only 31 % of species had units that could be standardised to mg/kg dry weight soil, whereas for mortality, this number was higher at 49 %. Afterwards, we assessed the number of species group-chemical combinations across several groups (Table 4). The order Araneae could not be used for analyses since each effect contained fewer than seven species per chemical. The order Poales could not be analysed for mortality, and all species groups within the classes Mammalia and Aves, and the phylum Annelida, lacked sufficient data for population size. The other species groups contained sufficient species for both effects.

The cleaned ecotoxicity dataset for fish contained 968 unique ‘Latin names’ for the taxonomic rank genus or higher taxonomic precision, all

Table 4

Data availability in the trait and ecotoxicity datasets. For columns with a gradient, green indicates high data availability and red indicates low. The values below the threshold of seven are presented in bold. Species with a '-' before the scientific name are of the taxonomic rank order, the rank used as a species group for the functional sensitivity distribution.

	Traits				Ecotoxicity		Trait Ecotoxicity overlap	
Species group (- order)	Number of Traits	Number of Species	Median number of species per trait	Median number of traits per species	Mortality, max. number of species per chemical	Population, max. number of species per chemical	Mortality, max. number of species per chemical	Population, max. number of species per chemical
Mammalia	136	5,361	721	17	8	3	8	**3**
- Rodentia	118	2,273	227	13	7	3	7	**3**
Aves	164	10,954	1,002	33	14	2	14	**0**
- Passeriformes	152	6,572	368	31	**5**	1	**5**	**0**
- Galliformes	124	297	36	26	7	1	7	**0**
Insecta	370	21,379	65	4	132	89	30	20
- Coleoptera	99	12,447	43	3	37	32	10	11
- Lepidoptera	144	6,927	294	5	40	26	13	11
Arachnida	128	1,566	7	29	21	10	8	**5**
- Araneae	60	1,361	813	31	**6**	**4**	**0**	**0**
Plantae	182	29,678	123	4	11	39	11	36
- Poales	86	2,238	42	4	**6**	9	**6**	9
- Pinales	64	355	40	8	9	**2**	9	**2**
- Malpighiales	80	1,506	49	4	**1**	**2**	**1**	**2**
- Fabales	94	2,784	51	3	**2**	7	**2**	7
- Solanes	46	378	20	3	**1**	**5**	**1**	**5**
Annelida	95	92	19	21	10	**5**	7	**0**
Fish	108	35,414	485	4	163	11	161	11
- Cypriniformes	61	4,600	684	3	47	**4**	47	**4**
- Perciformes	100	11,749	1,351	11	46	**3**	46	**3**
- Salmoniformes	56	226	41	3	13	**1**	12	**1**
- Siluriformes	58	3,946	342	3	10	**2**	10	**2**
- Atheriniformes	52	381	42	3	7	**1**	7	**1**
- Cyprinodontiformes	52	1,402	207	3	13	**3**	12	**3**

of which could be matched to GBIF. Of these matches, 96 % were exact, 3 % fuzzy and included, and 1 % on a higher taxonomic rank and used, either after changing to a lower rank or after accepting the higher rank. After this matching, a dataset with 852 species and a dataset with 493 genera were retained for the filtering steps. First, we filtered on aquatic medium and filtered on endpoints that can be classified or converted into EC_{10}eq endpoints, leaving 84 % of species. Here, the effects considered were also mortality (83 %) and population (7 %). The units could be standardised to mg/L for 87 % of species for mortality and 63 % for population. Ultimately, only the effect mortality could be used for the analyses, as the effect population lacked sufficient species for each species group-chemical combination (Table 4).

Trait data presented challenges for the terrestrial species, especially for the class Insecta, requiring the merging of 35 trait databases to achieve acceptable species coverage. Conversely, the class Mammalia was covered by eight databases, the class Aves by ten, the class Arachnida by 11, and both the kingdom Plantae and the aquatic species fish by one each. Still, the median number of traits per species remained low for Insecta, Plantae, and fish (Table 4). However, both Arachnida and Insecta had a relatively low median of seven and 65 species per trait, compared to 123 – 1002 for Plantae, fish, Mammalia, and Aves (Table 4).

Finally, overlapping species across the trait and ecotoxicity datasets were grouped by species group and chemical. This step drastically reduced the number of species such that fewer than seven species remained; the trait dataset suffered from excessive gaps or lacked variation, or the ecotoxicity dataset lacked variation. As a result, Mammalia, Aves, Annelida, and Insecta could not be included in the proof of concept, leaving one species group of Plantae suitable for the analyses. This limitation is not immediately evident from the number of species in the separate databases. For example, the order Araneae had a median of 31 traits per species and 813 species per trait in the comprehensive trait dataset, but fewer than seven species per chemical in the ecotoxicity dataset, resulting in no overlap. For the order Coleoptera, although there was a median of 43 species per trait and approximately 30 species per chemical per effect, only ten species overlapped between the datasets. Due to this limited overlap, combined with low variation and species coverage, we could not use this order in the analyses. Notably, the order Poales had the second lowest median number of species per trait among all species groups and only nine species in the ecotoxicity dataset, yet the overlap with the ecotoxicity dataset was sufficient for a proof of concept, with a maximum of nine species for the effect population size (Table 4).

4. Discussion

In this study, we developed a method to link the toxic effects of chemicals to functional diversity using a functional sensitivity distribution, which builds upon the well-established species sensitivity distribution by incorporating trait data. Both our case studies demonstrated a loss of biodiversity with increasing chemical concentration for both the functional sensitivity distribution and species sensitivity distribution. However, for the order Poales exposed to the herbicide atrazine, the $HC20_{EC10eq}$ was almost five times higher for the functional sensitivity distribution compared to the species sensitivity distribution. These findings indicate that the negative effect of atrazine on biodiversity appears less severe when assessed through functional richness rather than species richness. The CRF for functional richness was tenfold smaller than the CRF for species richness at the same concentration. These differences may reflect actual differences in the ecotoxicological effects between species groups and chemicals, or they could be an artefact of limited data availability. A similar, though less pronounced, pattern was observed in the case of the order Cypriniformes exposed to the insecticide malathion, with only a minor difference between the $HC20_{EC10eq}$s, both corresponding to a loss of biodiversity due to the chemical. At the same malathion concentration, both sensitivity distributions show a similar CRF. All functional traits used within this research, for both Poales and Cypriniformes, are shown to be related to ecosystem functioning (Hossie and Murray, 2016; Z. Liu et al., 2023; Martins et al., 2021; Perkins et al., 2022). Yet, only the trait body length was found to affect population vulnerability due to its link to ecotoxicology (Rubach et al., 2012). Even though we have used only two case studies, the negative effects of chemicals on functional richness become clear. While both the species and functional sensitivity distribution rely on ranking species by sensitivity, the additional ecological information that the functional sensitivity distribution incorporates by including functional traits provides insight into the toxic effects of chemicals on ecosystem functioning. Therefore, this proof of concept highlights the value of using the functional sensitivity distribution as a more comprehensive approach for assessing the toxic effects of chemicals on biodiversity. Future studies are needed to substantiate our methodologies, which currently assessed two restricted case studies due to data availability.

Species richness and functional richness are both used to assess biodiversity, but they capture different aspects thereof. While species richness reflects the number of species, functional richness measures the volume of functional trait space that those species occupy. Thus, an increase in species richness does not mean that this ecosystem is performing more functions, something that functional richness can indicate (Resende et al., 2022; Su et al., 2022). In both our case studies, species and functional richness were positively correlated, a relationship frequently found in ecological studies (Huang et al., 2022; Peña-Lara et al., 2022; Resende et al., 2022; supplementary information Section S5). New species often occupy new functional trait space. However, despite the high correlation, the two richness indices are not always aligned; species richness can be affected when functional richness is not (Scherer et al., 2023). This trend is also demonstrated in our case studies by the different CRFs derived from the functional and species sensitivity distributions (Tables 2 and 3). Species can have redundant trait values, meaning that their loss does not reduce the volume of functional trait space. As a result, species richness may decline without a corresponding loss in functional richness. In some studies, functional richness even reached a plateau, where adding species did not expand the trait space, but merely packed it more tightly (Huang et al., 2022; Peña-Lara et al., 2022; Resende et al., 2022). Functional redundancy is important for ecosystem stability, as losing a species does not drastically affect ecosystem functioning (Resende et al., 2022). In our analysis, we demonstrated the negative effect on functional richness and saw the plateau effect, as it took several species until the functional richness showed a noticeable change. However, instances where functional richness remained stable despite species loss suggested redundancy. Overall, our findings show similar patterns for both species and functional richness, a negative effect on biodiversity with increasing chemical concentrations. Despite the similar patterns that we found for species richness and functional richness, we still found differences between the two indices. Both methods together can complement each other, which helps regulators to assess the toxic effects of chemicals on a broader scale, and subsequently could help ensure safe chemical background concentrations by regulating chemicals. Policymakers, in turn, can use this additional information to strengthen the evaluation of chemical risks and decisions.

While functional richness showed that an increasing fraction of biodiversity became affected, no such clear pattern emerged for functional evenness and functional divergence. For these indices, the potentially affected fraction increased and decreased across the concentration gradient, preventing the fitting of a curve through the data. At the $HC20_{EC10eq}$ derived from both sensitivity distributions, contrasting trends were observed. For the order Poales exposed to the herbicide atrazine, functional evenness showed increasing effects at the $HC20_{EC10eq}$ from both sensitivity distributions. Functional divergence showed increasing effects at the $HC20_{EC10eq}$ from the species sensitivity distribution and decreasing effects from the functional sensitivity distribution. For the order Cypriniformes exposed to the insecticide malathion, both functional evenness and functional divergence showed decreasing effects from both sensitivity distributions. Such variability is consistent with patterns reported in the literature. For instance, Rosa et al. (2025) found that anthropogenic land use reduced functional richness and divergence but increased functional evenness. Similarly, Scherer et al. (2023) reported that under climate change, functional richness either became more affected or remained constant, whereas functional evenness and divergence responded in both directions. Deng et al. (2018) observed comparable outcomes during forest recovery: functional richness increased, but functional evenness and divergence changed bidirectionally. These examples suggest that changes in functional evenness and divergence are context-dependent and do not follow a monotonic, directional trend after environmental disturbance. As a result, our method cannot be extended to these two indices using the ranking approach of the functional sensitivity distribution. Consequently, we advise not to use these indices in the assessment of chemical risks in the same way as functional richness. Nevertheless, functional evenness and functional divergence remain valuable indicators of biodiversity, as each of the three functional diversity indices captures a distinct facet of biodiversity.

In this research, we encountered several data-related challenges that must be addressed to extend the functional sensitivity distribution approach to more chemicals and taxa and to validate this method. The three primary challenges were 1) limited data overlap, 2) lack of standardised methods, and 3) species scientific name harmonisation. The first challenge of limited data overlap mainly lies within the overlap of species between the different datasets. Even when individual datasets contained sufficient species, their overlap was often limited. Therefore, many species groups and chemical combinations could not be used to construct functional sensitivity distributions. The second challenge, a lack of standardised methods, hinders data usage. In the ecotoxicity dataset, inconsistencies in units, endpoints, effects, chemicals, and species reduced the number of comparable EC_{10}eq values. For example, endpoints were often reported in incompatible units, such as concentration per body weight versus concentration per diet. Additionally, many studies reported only a single concentration, offering no variability for modelling, affecting the construction of both the species and functional sensitivity distribution. Another issue arose from the two exposure types, acute and chronic, which were both converted to a chronic endpoint. However, research shows that comparison between exposure types is more consistent than across exposure types and requires analysis at the chemical and species level (Aggarwal et al., 2024; Aurisano et al., 2019). A standardised method should ensure that this issue is solved by requiring the use of multiple concentrations until mortality with acute and chronic exposure. The need for standardised reporting in ecotoxicology has already been recognised by the developers of the ECOTOXicology Knowledgebase, who created a dedicated vocabulary to translate data from diverse sources (Olker et al., 2022). The trait dataset faced similar issues: traits from multiple sources were expressed using inconsistent terminology, formats, and measurement protocols, complicating integration (Kunz et al., 2022; Martini et al., 2021). The last challenge was the scientific name harmonisation. Often, additional taxonomic information, such as authorship and year, was missing from the datasets. This absence was especially problematic when a name had historically referred to multiple taxa. Without this information, reliable species matching was not possible, leading to unnecessary data loss. Together, these challenges highlight the current limitations in trait and ecotoxicity research. These issues frequently and substantially reduced the number of usable species in our analyses to below the required threshold of seven. With standardised methods and harmonised and comprehensive datasets in the future, our method can be expanded to include more organisms and chemicals, which, in turn, will help to validate our model so it can be used by regulators. Even more, we can separate this risk assessment method into multiple trophic levels to inform policymakers about the toxic effects of chemicals on ecosystem functioning of a trophic chain and not just one taxon. On top of that, specific traits and orders can be selected to relate the effect of chemicals to specific ecosystem functions, which are connected to ecosystem services. Thus, with more data, the functional sensitivity distribution can inform policymakers on the effect of chemicals on a trophic chain and relate that effect to specific ecosystem services.

Within our case studies and methods, we have demonstrated the effect of the chemical based on the EC_{10} and HC_{20} values. Nonetheless, other endpoints could also be used when the data challenges are addressed, which will safeguard a different level of species and show a different level of effect of the chemical. We have used the EC_{10}eq values instead of other EC_x values, as this is closer to realistic concentrations following Owsianiak et al. (2023). With the EC_{10}, we can thus predict the ecological effects at environmentally relevant background concentrations. In the future, it would be interesting to see the effect of using other, higher endpoints, which will be closer to changes in community structure, as those endpoints are closer to lethal effects on the organisms. Similarly, we used the HC_{20} values, which provide ecologically relevant predictions of the effect of the chemical concentration on the ecosystem and are recommended for use in life cycle assessment (Owsianiak et al., 2023). With the current data challenges, it would not have been possible to provide a terrestrial example for, for example, the HC_5, which is an even more conservative approach to look at the toxic effects of chemicals on the ecosystem, as that would require a minimum of 20 data points when following the reasoning of Owsianiak et al. (2023). The data challenges must be addressed before the functional sensitivity distribution can be used with other endpoints, depending on the objective of the study. With different endpoints, policymakers can be advised on diverse effects of the chemical related to ecosystem functioning and help reduce the chemical exposure to levels that are not harmful to biodiversity.

Currently, the Poales case study is relatively weak, due to its low number of species, and only two case studies have been used for the proof of concept. Therefore, these two case studies alone cannot be accurately used to link the toxic effects of chemicals to ecosystem functioning beyond the proof of concept provided here. The Cypriniformes case study showed promising results with a sufficient number of species, yet more case studies are required to further validate our method. Thus, when the data challenges are addressed, we can increase the number of species to strengthen these results and increase the number of case studies to compare their results. This method, together with the species sensitivity distribution, can help regulators to understand and compare the effect of chemicals on both the community and the functioning of the ecosystem and can, therefore, offer a broader ecological view than currently possible. For multiple assessments, information on the effect of chemicals on biodiversity and community has been lacking. A first example is the unfinished risk assessment on the effect of glyphosate caused by a lack of information on biodiversity (EFSA, 2023). Another example is the risk assessment on the effect of neonicotinoids on non-target ecosystems, primarily focusing on bees and consequently overlooking the protection goals for aquatic species (EASAC 2015). These two examples highlight the need for research on the complex and multifaceted biodiversity and to align the diversity of methodologies and protection goals. The species sensitivity distribution relates the effect of the chemical to species, whereas the functional sensitivity distribution relates the effect of the chemical to ecosystem functioning. Therefore, using both these methods together would have added information related to the change in species and ecosystem functioning and, consequently, aid the integration of biodiversity in decision-making.

5. Conclusion

We developed a method to assess the toxic effects of chemicals on biodiversity by integrating functional traits into the established species sensitivity distribution. This approach, the functional sensitivity distribution, revealed patterns similar to the species sensitivity distribution in our two case studies, both indicating a negative effect of chemicals on biodiversity. Functional richness has the advantage of providing additional information by relating the reduction in species to a potential change in ecosystem functioning. Functional divergence and functional evenness should be added to provide more information since each metric indicates a different, yet complementary, component of functional diversity. The case studies from the proof of concept show that our method faces challenges with available data, mainly for terrestrial species. When these current data challenges are addressed through further (standardised) research, our method can easily be applied to more species groups and chemicals to further validate our approach. With this method, regulators can assess the toxic effects of chemicals on ecosystems because this method also informs about ecosystem functioning. In the future, this method can support decisions on risk management measures for chemicals, even though the effects of chemicals on biodiversity are currently not routinely assessed.

CRediT authorship contribution statement

Annetrude Boeije: Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Conceptualization. **S. Henrik Barmentlo:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Martina G. Vijver:** Writing – review & editing, Supervision, Funding acquisition. **Laura Scherer:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

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.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2026.110078.

Data availability

The data that support the findings of this study are available in the article and its supporting information and supporting data.

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