Meta-analysis on the effects of chemical stressors on five ecosystem functions: are there generalizable relationships?

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Abstract

Despite considerable progress in our predictive capacity for the response of different trophic levels to chemicals, generalizable relations between chemicals and ecosystem functions are lacking. We addressed this knowledge gap by conducting the largest meta-analysis to date (136 studies; ~350 observations) on the responses of ecosystem functions to chemicals. Based on this analysis, for selected classes of chemicals (pesticides, pharmaceuticals, and metals), we are the first to establish relations between chemical concentrations and the ecosystem functions organic matter decomposition and photosynthesis, showing monotonic dose-response relationships. These results concur with existing field data on chemical effects, which underpins the value of our findings. Taking into account the outlined research gaps and shortcomings, our findings and recommendations can be seen as a promising starting point to advance predictions of responses of ecosystem functions to selected chemical classes.

Keywords

Freshwater ecosystems, stress-ecosystem function relationship, context-dependency, effect modeling, chemical risk

Introduction

Freshwater habitats, despite comprising just around 0.01% of the global water resources and covering only 2% of the global surface area (Shiklomanov, 1993), hold a remarkable species biodiversity (Strayer and Dudgeon, 2010). Hosting over 140,000 species (Reid et al., 2019), with approximately 80% being invertebrate species and 15% vertebrate species (Balian et al., 2008), these ecosystems are pivotal for species biodiversity. However, a stark contrast emerges as freshwater habitats experience more pronounced declines in biodiversity compared to terrestrial ecosystems, leading to what has been labelled as the "freshwater crisis" (Harrison et al., 2018). The freshwater crisis is the result of an intricate interplay of anthropogenic disturbances: over-exploitation, toxic chemical pollution, alterations in water flow patterns, habitat destruction or degradation, and the introduction of non-native species (as comprehensively reviewed in Reid et al., 2019). Despite their paramount importance, toxic chemicals have often been marginalized in freshwater ecosystem studies, even though they play a substantial role in deteriorating these ecosystems (Bernhardt et al., 2017). Extensive meta-analyses on continental and global scales consistently reveal that a substantial proportion of water bodies are affected by toxic chemicals. In Europe, nearly half of the evaluated water bodies were identified as being at risk due to chronic chemical effects (Malaj et al., 2014). Similarly, on a global scale, insecticide contamination was observed to surpass thresholds indicating ecological impacts in nearly 70% of stream sites within agricultural landscapes (Stehle and Schulz, 2015). Recent investigations underscore the escalating chemical risk faced by freshwater organisms, both in Europe and the United States (Schulz et al., 2021; Wolfram et al., 2021), pointing toward a concerning thread of increasing contamination.

The decline in freshwater biodiversity raises considerable concerns regarding its repercussions on the functioning of aquatic ecosystems (Cardinale et al., 2012; Hooper et al., 2005; Loreau et al., 2001). Over the past few decades, substantial research efforts have been

60 dedicated to unraveling the connections between biodiversity and ecosystem functioning
61 (Naeem and Wright, 2003). Numerous empirical studies have unveiled a connection between
62 these variables, demonstrating that the loss of species generally diminishes ecosystem
63 functioning (Harrison et al., 2014; Tilman et al., 2014). Nevertheless, the interplay between
64 biodiversity and ecosystem functioning under the influence of chemical stress remains less
65 elucidated. A handful of meta-analyses and reviews (Crane et al., 2006; Kümmerer, 2009;
66 Schäfer et al., 2012b) showed that specific organisms, which are crucial for functions like
67 nutrient cycling, organic matter decomposition, and primary production, are impacted by
68 chemical stress at environmentally relevant concentrations. Nevertheless, the broader
69 implications on the functional level remain a subject of ongoing investigation. Additionally,
70 several meta-analyses and reviews have drawn connections between changes in ecosystem
71 functions and the effects of chemical stressors on specific groups of organisms (Beaumelle et
72 al. 2020; DeLorenzo et al., 2001; Feckler et al., 2015; Feckler and Bundschuh, 2020; Ferreira
73 et al., 2016; Guo et al., 2015; Kovalakova et al., 2020; Peters et al., 2013; Schäfer et al., 2012b).
74 Despite compelling evidence highlighting the impact of chemical stressors on ecosystem
75 functions, we still lack a generalizable relationship between chemical stress and ecosystem
76 functioning. Notably, a meta-analysis failed to establish a consistent dose-response relationship
77 between various groups of chemical stressors and several ecosystem functions, such as nutrient
78 cycling, organic matter decomposition and primary production (Peters et al., 2013). However,
79 Peters et al. (2013) rather aimed at identifying effect thresholds and only considered studies
80 that exceeded a defined negative effect size, whereas the responses of ecosystem functions to
81 chemical stress can be negative (e.g., Beaumelle et al., 2020; Robson et al., 2020; Rosi-
82 Marshall et al., 2013), positive (e.g., Corcoll et al., 2015; Richmond et al., 2019) or non-
83 distinguishable from a control treatment or a reference (e.g., Jepsen et al., 2019; Schäfer et al.,
84 2012a). Thus, it remains open whether an analysis of all studies, including novel studies that

85 have been conducted in the last decade, enables the derivation of generalizable relationships
86 between chemical stressors and their effects on ecosystem functions.

87 We aimed to derive such generalizable relationships to help predict chemical effects on
88 ecosystems. Therefore, we conducted a literature meta-analysis and extracted 347 observations
89 (effect sizes and respective chemical concentration) from 136 published laboratory studies that
90 cover a wide range of chemical groups (metals, pesticides, and pharmaceuticals) and five
91 ecosystem functions (community respiration, organic matter decomposition, nutrient cycling,
92 photosynthesis, and primary production). We hypothesized to find a negative association
93 between chemical stressors standardized by their toxic potency and levels of ecosystem
94 functions. Furthermore, we hypothesized that the effects of particular chemical groups would
95 be most pronounced for ecosystem functions performed by organism groups targeted by these
96 chemical stressors. For instance, we anticipated the most substantial effects of fungicides and
97 herbicides on microbially-mediated organic matter decomposition and photosynthesis,
98 respectively. Our results indicate generalizable relationships for the ecosystem functions
99 organic matter decomposition and photosynthesis, showing a decrease in the function as the
100 chemical pressure increased. The match of the results found here with data from field studies
101 suggests that the relationships are relevant for ecosystems. Finally, we distilled research gaps
102 that require further scrutiny in the future.

Materials and methods

Literature collection and inclusion criteria

We conducted an extensive search across Web of Science and Scopus to compile literature concerning the effects of chemical stressors (pesticides, pharmaceuticals, and metals) on five ecosystem functions (community respiration, nutrient cycling, organic matter decomposition, photosynthesis, and primary production). The search was limited to literature published between January 2000 and December 2020 to focus on currently used chemical stressors. Our search string was constructed using a combination of freshwater habitat-related terms (freshwater* OR stream* OR river* OR pond* OR lake*), stressor-related terms (chemical stress* OR contaminant* OR pollutant* OR toxicant* OR pesticide* OR heavy metal* OR metal* OR fungicide* OR herbicide* OR insecticide* OR pharmaceut*), and ecosystem function-related terms (ecosystem function* OR photosynth* OR primary product* OR nutrient cycl* OR organic matter decomp* OR leaf breakdown OR decomposition* OR respirat*). The asterisks (*) were employed as wildcards, capturing all terms sharing the same root. Moreover, we conducted footnote chasing by screening the reference lists of relevant articles for further literature. The selection process for studies encompassed several stages. Initially, titles were screened to identify suitable studies. Subsequently, the abstracts were evaluated, leading to the exclusion of non-matching studies. Lastly, the full texts of selected studies were assessed.

Studies were included based on a set of criteria: (1) laboratory-based investigations, as other study types (semi-field or field) yielded insufficient data points for meta-analysis (Figure A1 in the Supporting Information), (2) focus on species, populations, or communities dwelling within freshwater habitats (excluding groundwater, marshlands, coastal waters, and marine systems), (3) exposure to at least one of the pre-defined chemical stressors from the list above (excluding eutrophication, salinization, and acidification), (4) presence of a control treatment

128 (absence of chemical stress), and (5) measurement of the designated ecosystem functions as
129 well as uncertainty around the central tendency of the measurement. An overview of the
130 selected studies and moderator variables (see below) will be provided in a data repository upon
131 acceptance of the manuscript.
132
133 *Data extraction and effect size calculation*
134 We extracted central tendencies, uncertainty estimators, sample sizes, and chemical
135 concentrations from the text, tables, and figures of the selected studies. For cases where the
136 data were presented in figures, we utilized the "digitize" package (Poisot, 2011) within the
137 open-source software R (version 4.2.3 for Mac OS X; R Core Team, 2023).
138 As our response variable, we computed the ratio of the ecosystem function observed in the
139 stressor treatment to that in the control treatment. From each study, we extracted only the
140 highest effect observed per functional endpoint for the same species, population, or community.
141 This approach was adopted to derive unequivocal cause-effect relationships and prevent
142 pseudoreplication. Due to this method and the diverse test designs employed in individual
143 studies, the timing of functional measurements varied.
144 For studies investigating community respiration, the functional indicators included
145 measurements of O_2 consumption (e.g., mg O_2 g^{-1} dry weight h^{-1}) and CO_2 production (e.g., µg
146 CO_2 g^{-1} dry weight h^{-1}). In the context of organic matter decomposition, the indicators consisted
147 of breakdown rates (k (d^{-1})) and mass loss (e.g., mg d^{-1}) of leaf material, mediated by microbial
148 decomposers or a combination of microbial decomposers and detritivorous macroinvertebrates
149 (decomposer-detritivore-systems) as endpoints. Concerning nutrient cycling (primarily
150 nitrogen), the indicators encompassed anaerobic NH_4^+ oxidation rates (g-N g-volatile
151 suspended solids^{-1} d^{-1}), NO_2 and NO_3 turnover rates (e.g., mg NO_2-N mg^{-1} d^{-1}), and N_2
152 production (e.g., N_2 g^{-1} h^{-1}). In the case of photosynthesis, we determined effect sizes based on

153 maximum and effective photosystem II quantum yields (F_v/F_m and ΦPSII, respectively) and
154 O_2 production (e.g., mg O_2 g^{-1} dry weight h^{-1}). Finally, for primary production, the effect sizes
155 related to fixed carbon quantities (mg ^{14}C g^{-1} C h^{-1}) and changes in the standing biomass (net
156 primary production). Studies that exclusively reported dissolved oxygen as a response variable
157 were excluded, as net dissolved oxygen could arise from multiple sources simultaneously, such
158 as photosynthesis and the ambient atmosphere.
159
160 *Calculation of toxic units*
161 The toxic effects caused by different chemical stressors vary for a given test
162 concentration, due to differing ecotoxicological potentials, making direct comparisons
163 challenging. Therefore, we normalized the test concentrations of individual chemical stressors
164 to their acute effects by calculating toxic units (TUs). Ideally, TUs would be computed for each
165 combination of stressor and organism included in the selected literature. However, given
166 substantial data gaps, we relied on surrogate species results to facilitate comparisons between
167 different stressors. Unlike previous meta-analyses that sought to establish a generalized
168 chemical stress–ecosystem function relationship (e.g., Peters et al., 2013), we avoided
169 confining the surrogate species selection to conventional test species as stipulated by chemical
170 regulations (i.e., *Daphnia magna*, *Lemna* sp., and *Raphidocelis subcapitata*). Instead, we
171 estimated stressor toxicity based on the EC_{50} values – the concentration at which an effect is
172 observed in 50% of exposed organisms – for the most sensitive freshwater algal, plant, and
173 invertebrate species for the respective chemical stressor. We employed these EC_{50} values to
174 compute the logarithmic sum of toxic units (sumTU) as:

175 $$sumTU = log\left(\sum_{i=1}^{n}\frac{ci}{EC50i}\right)$$

176 where c represents the concentration of each toxicant i, $EC50_i$ is the median effect
177 concentration of the respective toxicant for the most sensitive species i, and n gives the number
178 of toxicants that caused an effect in the respective ecosystem function. EC_{50} values were
179 extracted from various sources including Standartox (Scharmüller et al., 2020), the Pesticide
180 Property Database (Lewis et al., 2016), the Veterinary Substance Database (Lewis et al., 2011),
181 the Norman Database (https://www.norman-network.com/nds/), and WikiPharma (Molander
182 et al., 2009). We chose EC_{50} values corresponding to a 48-hour exposure duration or, when not
183 available, the closest exposure time. It is important to note that this chosen exposure time serves
184 merely as a reference for benchmarking the relative toxicity of compounds and does not need
185 to align with the experimental exposure time. See Supplementary Data (Table A1) for an
186 overview of all EC_{50} values and their sources.
187
188 *Moderator variables*
189 Besides the sumTU, we extracted seven moderator variables: (1) ecosystem function
190 with levels: (a) community respiration, (b) organic matter decomposition, (c) nutrient cycling,
191 (d) photosynthesis, and (e) primary production; (2) detailed chemical group with levels: (a)
192 fungicide, (b) herbicide, (c) insecticide, (d) pesticide mixture, (e) pharmaceutical, (f) metal, (g)
193 metal mixture, and (h) other (such as biocides); (3) classification of the mode of toxic action
194 extracted from Lewis et al. (2016) or Molander et al. (2009); (4) organism group with levels:
195 (a) algae (including cyanobacteria), (b) aquatic plants (phytoplankton, macrophytes, etc.), (c)
196 microbial decomposers (bacteria and fungi), (d) decomposer-detritivore-systems
197 (microorganisms and macroinvertebrates); (5) exposure time as a continuous variable in days;
198 (6) pH as a continuous variable; (7) test temperature as a continuous variable in °C.
199
200 *Data analysis*

To identify the moderator variables affecting the assessed ecosystem functions and establish relationships between chemical stressors and ecosystem functions, we performed separate data analyses for each of the included ecosystem functions. We used the calculated effect size (along with the standard error of the effect size for each study) as the response variable, while including the sumTU and moderator variables as predictors. Initially, we checked for multi-collinearity, ensuring that the meta-regression coefficient estimates are robust by examining correlations ($r \geq 0.8$) among our predictors. Subsequently, we identified the best-fitting models using the "multimodel.inference" function in the R package "dmetar" (Harrer et al., 2019) based on corrected Akaike Information Criteria. Using the results of the multimodel inference, we build models to evaluate the individual impact of the identified predictors and their interactions. We achieved this by fitting meta-regression models using the R package "metafor" (Viechtbauer, 2010). We employed the "maximum likelihood" estimator to assess the amount of residual heterogeneity (τ^2) and applied the Knapp-Hartung method for random-effects meta-analysis (van Aert and Jackson, 2019). We conducted a permutation test before reporting *p*-values, as recommended for meta-regression models (Higgins and Thompson, 2004).

In a subsequent step, we fitted (quasi-)binomial Generalized Linear Models (GLMs) to explore potential dose-response relationships between ecosystem functions and sumTUs of all stressors or separately for chemical groups (fungicides, herbicides, insecticides, pharmaceuticals, and metals) or modes of toxic action (only if $n \geq 8$; Jenkins and Quintana-Ascencio, 2020). For statistical analyses and graphics, we used the open-source software R (R Core Team, 2023), along with the necessary add-on packages. Upon manuscript acceptance, we will make all code and data available in a data repository.

226 **Results**

227 *Overview of included studies*

228 Through our database query and footnote chasing, we identified a total of 7,599
229 publications, of which 7,463 publications were excluded due to various reasons including
230 violations of our predefined inclusion criteria, absence of reported effect sizes and associated
231 uncertainties, or the inability to calculate sumTUs due to a lack of toxicity data. Ultimately, we
232 focused on 136 laboratory studies that met our criteria, from which we extracted 347 effect
233 sizes, uncertainty estimators, sumTUs, and moderator variables (see Fig. A1 for details). We
234 deliberately excluded semi-field and field studies from further analyses. This decision was
235 driven by the fact that these types of studies only represented a minor share of our dataset after
236 initial screening (n = 38 and 60 for semi-field and field studies, respectively), while a major
237 fraction of these 98 studies did not meet our predefined inclusion criteria and would have been
238 excluded (see overview of studies).

239 Among the selected studies, we found that most observations (37% each) were related
240 to organic matter decomposition and photosynthesis. Additionally, there were 75 observations
241 (22%) related to primary production, while community respiration and nutrient cycling studies
242 constituted 9 observations (4%; Fig. 1a). Regarding the types of chemical stressors examined,
243 pesticides were the most extensively studied group, representing 63% of the observations (218
244 observations in total), with fungicides accounting for 56 observations, herbicides for 102,
245 insecticides for 34, and pesticide mixtures for 26. Metals and metal mixtures constituted 30%
246 of the observations (104 observations in total), while pharmaceuticals made up 7% (26
247 observations; Figs. 1a & 1b). The sumTUs ranged from -5.13 to 2.68 for metals and metal
248 mixtures, -4.54 to 3.99 for pesticides and pesticide mixtures, and -5.68 to 5.58 for
249 pharmaceuticals (Table 1). A value of 0 indicated that the toxicity corresponded to the EC_{50} of
250 the most sensitive species for which toxicity data were available. When comparing the tested

concentrations to the range of detected environmental concentrations for each stressor group, which we obtained from field measurements through meta-analyses (Deo, 2014; Wolfram et al., 2021), we observed that these two datasets only partly overlap (Fig. A2). However, this (substantial) non-overlap should be interpreted cautiously due to our specific selection procedure for effect sizes (utilizing the highest effect from each study) and potential differences between the tested and detected substances.

Influence of moderator variables and relationships between sumTU and effects on ecosystem functions

Organic matter decomposition

For the ecosystem function organic matter decomposition, our best-fit model included two predictors: ”organism group”, which categorized microbial decomposers alone and decomposer-detritivore-systems, and ”pH”. Additionally, there was a significant interaction between ”organism group” and ”sumTU”. This model accounted for a substantial portion of the variance (R^2 = 58.93%, τ^2 = 0.0434; Table 2). We observed a general dose-response relationship for organic matter decomposition across all groups of chemicals for studies examining decomposer-detritivore systems (as indicated by the GLM for sumTU and the respective ecosystem function; $p = 0.022$; $n = 62$, Fig. 2). However, when focusing on microbial decomposers alone, we did not identify a consistent relationship between sumTU and organic matter decomposition ($p = 0.801$; $n = 60$; Fig. 2). When we examined the effects of individual chemical groups or modes of toxic action on specific organism groups, no clear dose-response relationships were evident ($p \geq 0.288$; n = 8-22).

Photosynthesis

For the ecosystem function photosynthesis, our best-fit model included two predictors: ”sumTU” and ”temperature”, collectively explaining a portion of the variance (R^2 = 18.22%, τ^2 = 0.0707; Table 2). In contrast to organic matter decomposition, we did not observe an influence of the parameter ”organism group” (distinguishing between algae and aquatic plants; Fig. 3). When we examined the effects of individual chemical groups or modes of toxic action on specific organism groups, no clear dose-response relationships were evident ($p \geq 0.210$; n = 12-50).

Primary production

For the ecosystem function primary production, our best-fit model included only ”pH” as predictor, explaining a portion of the variance (R^2 = 16.06%, τ^2 = 0.0437; Fig. 4; Table 2), but not sumTU. When we examined the effects of individual chemical groups or modes of toxic action on algae (lack of data on aquatic plants), no clear dose-response relationships were evident ($p \geq 0.285$; n = 12-24).

Discussion

Chemical stressor-ecosystem function relationships

Currently, over 350,000 chemicals are registered for global production and use (Wang et al., 2020), often occurring as mixtures in surface waters. Furthermore, the Chemical Abstracts Service has records for over 100 million substances, with approximately 15,000 new substances being registered daily (Rohr et al., 2016). Considering this ever-expanding array of chemicals, undertaking comprehensive studies on all currently in use or future developmental chemicals appears insurmountable (Rohr et al., 2006). Consequently, there is a need to establish generalizable relationships between chemical exposures and their effects on the environment.

299 To the best of our knowledge, our study is pioneering in establishing relationships between
300 chemical stressors and ecosystem functions (Figs. 2 and 3). This represents a crucial initial
301 stride, as such relationships can ultimately aid in predicting the toxicity of chemicals that have
302 not yet been thoroughly studied, especially if these chemicals share similar modes of toxic
303 action. The transferability and predictive capacity of these relationships could potentially
304 reduce costs and efforts, diminish the reliance on animal testing, and mitigate the suffering of
305 living organisms – an issue that has received considerable attention in the last decade (Burden
306 et al., 2015).

307 All established relationships between chemical exposures and their effects on
308 ecosystem functioning exhibited an inverted S-shaped monotonicity (Figs. 2 and 3), signifying
309 that the measured parameters declined as the dose of the chemical stressor increased. Our
310 findings therefore align with the dose-response patterns frequently observed in the field of
311 ecotoxicology (Newman, 2009). The S-shape observed in these relationships suggests that
312 organisms carry specific defense and/or repair mechanisms that provide protection against a
313 certain amount of a chemical stressor, up to a critical threshold level. Beyond this threshold,
314 organisms become incapable of defending or repairing themselves effectively. Consequently,
315 the biological response to chemical stressors showed a graded response due to the evolutionary
316 adaptive capabilities of organisms to react to a range of low doses. The absence of additional
317 relationships between chemical exposures and their effects on ecosystem functioning, apart
318 from those identified for organic matter decomposition and photosynthesis across all chemical
319 groups, may indicate that the toxic pressure from certain chemical groups did not surpass the
320 threshold required to overcome the defense and/or repair mechanisms of enough species to
321 trigger adverse effects.

322 For the ecosystem function organic matter decomposition by decomposer-detritivore-
323 systems, for example, we observed a 50% reduction occurring at a sumTU of approximately -

0.5 (Fig. 2). This finding aligns with the sensitivity of macroinvertebrates in the field, as demonstrated in a large-scale field study involving 101 streams (Liess et al., 2021). In that study, a sumTU of -0.5 had a pronounced impact on the SPEcies At Risk index, which detects pesticide effects on the macroinvertebrate community composition in the field ($SPEAR_{pesticides}$). This indicator pointed to a poor or deteriorated environmental quality that led to the elimination of key species crucial for ecosystem functioning. These observations align well with another field study (e.g., Schäfer et al., 2012b), which similarly demonstrated a sharp decline in $SPEAR_{pesticides}$ at a sumTU of -0.5. This decline resulted in a substantial decrease of approximately 70% in organic matter decomposition by decomposer-detritivore-systems. Consequently, there is a good overlap of field data and the relationships between chemical exposures and their effect on organic matter decomposition observed in our study, despite the inherent challenges of comparing data sets (e.g., studies mainly focused on individual chemicals vs. complex mixtures in the field), highlighting the value of our results.

Despite this fairly good overlap of field data and the chemical stress-ecosystem function relationship derived here, it is essential to acknowledge that the heavy emphasis on specific chemical groups in research often hampers the broader applicability of laboratory findings to real-world conditions. Over the past two decades, a predominant focus has been on metals, pesticides (particularly herbicides), and pharmaceuticals (Figs. 1a and 1b). These chemical groups closely link to prominent anthropogenic activities such as agriculture, mining, and wastewater discharge (Bommarco et al., 2013; Nriagu, 1996; Rodriguez-Mozaz et al., 2020). Modern intensive agriculture heavily relies on pesticide application to mitigate crop losses. However, following their application, pesticides can be transported into adjacent non-target ecosystems like freshwater bodies, leading to diffuse pollution (Schulz, 2004). Mining and wastewater discharge, on the other hand, represent point sources of pollution, resulting in more localized spatial impacts primarily by metals and pharmaceuticals, though wastewater

349 treatment plants are highly abundant in densely populated regions of industrialized countries
350 (Sonter et al., 2018).

351 The predominant research focus on pesticides, metals, and pharmaceuticals can hence
352 be justified by their widespread presence and severe impacts on the environment. However, it
353 is important to recognize that these chemical groups constitute only a small fraction of the vast
354 array of chemicals present in the environment, given that approximately 350,000 chemicals are
355 registered for production and use (Wang et al., 2020). Studies employing bioassays have
356 revealed that target chemicals can only account for a small fraction (as low as < 0.1 %) of the
357 overall response to chemicals, suggesting that numerous ecotoxicologically important
358 compounds exist within freshwater ecosystems (Escher et al., 2013; Neale et al., 2020). In
359 addition to the emphasis on pesticides, metals, and pharmaceuticals, relatively few studies have
360 considered that chemicals often co-occur as mixtures (Fig. 1b). Typically, the effect of
361 chemicals in mixtures is additive, but in a relevant portion of cases, the simultaneous presence
362 of multiple chemicals can result in stronger (synergistic) or weaker (antagonistic) effects
363 compared to the assumption of additive effects. A recent review, encompassing various
364 chemical groups, indicated an average of 20% of cases with synergism and 15% of cases with
365 antagonism (Martin et al., 2021). Another review reported synergistic effects in 3% to 26% of
366 cases involving binary mixtures, with the prevalence varying by chemical group (Cedergreen,
367 2014). Furthermore, freshwater ecosystems are typically subject to multiple stressors
368 concurrently (Birk et al., 2020), which can lead to interactions between chemical and non-
369 chemical stressors (Liess et al., 2016). To provide a more comprehensive understanding of the
370 effects of chemicals in freshwater ecosystems and derive additional chemical stress-ecosystem
371 function relationships, it is essential to broaden the scope. This entails identifying
372 ecotoxicologically relevant chemical groups, assessing interactions within chemical mixtures,
373 and considering the context of multiple (non-chemical) stressors in the ecosystem.

374 Furthermore, finding chemical stressor-ecosystem function relationships can be
375 confounded by abiotic factors, such as pH and temperature, which influence the fate and
376 toxicity of metals and organic chemicals in aquatic environments (Ragnarsdottir, 2000; Soares
377 et al., 2020; Wang et al., 2016). This phenomenon is often referred to as "context dependency"
378 (Catford et al., 2022). While our findings did not reveal any significant interactions between
379 pH or temperature as moderators of the toxicity of the chemical stressors on ecosystem
380 functioning, such interactions have been observed earlier. For instance, a meta-analysis (Wang
381 et al., 2016) revealed a substantial influence of pH changes on metal toxicity. This can be
382 explained by the pH-dependency of metal speciation, which in turn affects metal
383 bioavailability. Furthermore, many pesticides are ionizable compounds (Ragnarsdottir, 2000),
384 and their degradation and subsequent toxicity are influenced by pH as well as molecular
385 structural properties (Fenner et al., 2013). Temperature also interacts with the effect of
386 chemical stressors, a concept known as climate-induced toxicant sensitivity (Noyes et al.,
387 2009). This interaction can result in lower effects due to increased biotransformation of
388 chemical stressors at higher temperatures, as well as reduced activity (and consequently, lower
389 exposure) of organisms at lower temperatures. Moreover, aside from their role in modifying
390 chemical bioavailability, speciation, and concentration, abiotic conditions have been shown to
391 increase species sensitivity by up to 30-fold in certain experiments (Liess et al., 2001; Liess
392 and Beketov, 2011).

393 Additionally, biotic factors can impede the derivation of chemical stressor-ecosystem
394 function relationships. However, the relevance of these factors largely depends on the specific
395 ecosystem function under investigation and the design of the experimental approach.
396 Laboratory experiments typically involve the use of species that are maintained in culture over
397 extended periods, often under conditions that differ from their natural habitats (e.g., variations
398 in light, temperature, and pH) and that may lay outside their niche width and optima. Thus,

these conditions affect the survival, growth, reproduction and functioning of species, and thereby the related ecosystem functions (Blackman, 1905). Indeed, pH and temperature influenced organic matter decomposition and photosynthesis, respectively (Table 2). These findings suggest a mismatch between species-specific requirements and the laboratory conditions under which the respective experiments were conducted. In addition, the acclimation to laboratory conditions can have a notable impact on the sensitivity of these species to chemicals, as highlighted in a recent review (Lavoie et al., 2017). Furthermore, the presence of cryptic species complexes and demographic variability, encompassing both demographic stochasticity (random variations in birth and death rates) and demographic heterogeneity (variations in vital rates among individuals within the same population), is frequently overlooked or inadequately considered in laboratory studies. Nevertheless, these factors have the potential to confound the results of laboratory investigations (Feckler et al., 2013; Melbourne and Hastings, 2008).

In studies that make use of complex natural communities rather than single species tests, additional biotic factors can introduce uncertainties into the derivation of chemical stressor-ecosystem function relationships. These studies typically involve communities with different compositions, and these structural differences have been demonstrated to influence the sensitivity of communities to chemical stress (Beketov et al., 2008; Feckler et al., 2018). Consequently, these variations can lead to differing effects on ecosystem functions due to differences in the capacity of communities to compensate for species loss caused by chemical stressors (Cadotte et al., 2011). Therefore, integrating theoretical advances from community ecology into the toolkit of chemical stressor effect research holds promise (Clements and Rohr, 2009). This approach can help address questions such as “Which types or structures of communities are most sensitive to pollution?”, “Which species are most vulnerable to pollution?”, and “On which [...] of the chemicals should we focus our attention?”. Community

424 ecology theory, particularly when employing methods like community viability analysis and
425 network theory, can generate testable predictions by considering factors that influence
426 community stability and contribute to the propagation of indirect effects (for further reading
427 see Rohr et al., 2006). Such an integration could enhance our understanding of the intricate
428 relationships between chemical stressors, community structure, and ecosystem functions.

429 Finally, chemical stressors have the potential to influence the shapes of biodiversity-
430 ecosystem function relationships, an effect mediated by species interactions (Baert et al., 2016).
431 In this context, ecological theory distinguishes between interspecific interactions, which can
432 change species' contributions to ecosystem functions through competitive replacement, and
433 intraspecific interactions, which can alter species' contributions to ecosystem functions but
434 without leading to competitive replacement (Fox, 2005). As the impact of chemical stress on
435 ecosystem functions is mediated through its effects on species interactions, these interactions
436 play a pivotal role not only in theories related to biodiversity-ecosystem function relationships
437 but also in the field of stress ecology (Baert et al., 2016). Consequently, understanding how
438 and to what extent chemical stress alters species interactions is crucial for developing theories
439 about stress-induced changes in biodiversity effects. However, the mechanisms behind stress-
440 induced alterations in the shapes of biodiversity-ecosystem function relationships remain
441 largely unexplored (Baert et al., 2018, 2016). Therefore, ecotoxicology can benefit from the
442 application of theoretical ecological approaches utilized in biodiversity-ecosystem function
443 research. By establishing connections between these research fields, we can work towards
444 bridging this knowledge gap and gain a mechanistic understanding of how chemical stressors
445 can modify the shapes of biodiversity-ecosystem function relationships.

446

447 *Study bias in the selection of ecosystem functions?*

448 We specifically chose biologically mediated functions for our analysis, focusing on
449 those we deemed most ecologically relevant within freshwater ecosystems. Among the five
450 selected ecosystem functions, primary production and photosynthesis by algae and aquatic
451 plants were notably prevalent (Fig. 1a). This prevalence can be attributed to several factors.
452 Firstly, these functions are likely to be highly responsive to the most studied group of
453 pesticides, namely herbicides (Hedlund et al., 2020), as well as pharmaceuticals.
454 Cyanobacteria, as photosynthetic prokaryotes, are considered sensitive to antibiotics due to
455 their close relationship with pathogenic bacteria (González-Pleiter et al., 2013), and green algae
456 and plants can also be affected by antibiotics because of the prokaryotic origin of their semi-
457 autonomous cell organelles (Nie et al., 2009). Secondly, maintaining algal cultures and plants
458 in laboratory settings is relatively straightforward, and measurement techniques for primary
459 production and photosynthesis enable rapid quantitative assessment.

460 Despite their importance for freshwater ecosystem functioning, their potential of being
461 used as indicators of streams' ecological status and in the assessment of streams' restoration
462 (Klocker et al., 2009; Udy et al., 2006; Young et al., 2008), only a minor share of studies
463 addressed chemical stressor effects on microbial community respiration and nutrient cycling
464 (Fig. 1a). Unlike for algae, standardized protocols for culturing heterotrophic microbial
465 communities (bacteria and fungi) in the laboratory are largely lacking, hindering their use in
466 research and complicating comparisons between studies. Moreover, studies assessing
467 ecosystem functions through microbial decomposers (approximately 24 ± 2 days; mean ±
468 standard error) typically required considerably longer exposure times compared to those
469 focusing on primary production and photosynthesis by algae (approximately 5 ± 0.5 days),
470 which increases the labor-intensity of these studies. Nevertheless, protocols to quantify various
471 processes related to community respiration and nutrient cycling have been developed and are
472 frequently applied in field studies to assess stream ecosystem health (von Schiller et al., 2017).

473 While these protocols are similarly complex and costly as other methods for quantifying
474 ecosystem functions, they can potentially be partially automated for use in laboratory studies
475 (von Schiller et al., 2017). Thus, we recommend their application in future laboratory studies
476 to broaden our mechanistic understanding of how chemical stressors impact ecosystem
477 functions.
478
479 **Research gaps and recommendations**
480 Along with the ever-increasing production values and usage of new chemicals, it
481 becomes insurmountable to experimentally study the effects of all chemicals alone and in
482 combination with other chemicals and stressors in a wide range of ecosystems, making
483 generalizations invaluable. In this context, our analyses revealed the potential of deriving
484 chemical stress-ecosystem function relationships using currently available data that reveal
485 adverse effects for certain ecosystem functions. Nevertheless, we identified several important
486 research gaps that should be considered in the future to support deriving more specific (e.g., in
487 terms of mode of action) chemical stress-ecosystem function relationships and ultimately
488 facilitate the prediction of chemical effects.
489 *(1) Stronger focus on underrepresented chemical stressors and ecosystem functions*
490 As we showed in our analysis, chemicals of certain groups as well as specific ecosystem
491 functions were underrepresented in the extracted literature. Therefore, data remains insufficient
492 for important but understudied chemical groups and ecosystem functions, hampering a more
493 complete evaluation of chemical stress.
494 *(2) Consideration of environmental variation and complexity of field conditions*
495 Above we showed that the context-dependency of chemical stress effects on ecosystem
496 functions is largely ignored. Therefore, future laboratory research should proactively consider
497 a series of abiotic and biotic factors as well as the composition of stressors (single stressors vs.

diverse chemical mixtures; combination of chemical and non-chemical stressors) to ensure the transferability of the data to various field situations. Ideally, such studies should simulate different climatic and geological regions as well as chemical exposure histories as both factors were shown to determine the stress response (cf. Zubrod et al., 2019). In addition, laboratory studies should be appended by semi-field and field studies as verification and validation benchmarks to assess the accuracy and reliability of laboratory data for field situations.

(3) Increased effect modeling

Given the high number of used chemicals and the predicted increase in registered chemicals in the future as well as the context-dependency of chemical effects, empirical testing will be insurmountable. In this context, modeling approaches offer useful tools to identify general findings at different levels of biological organization (e.g., microbes and invertebrates). In addition, effect modeling can help to fill data gaps.

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Conflict of interest

The authors have declared no conflict of interest.

Data accessibility statement

All data used for the analyses of the present study were extracted from previously published articles. All data and R code used in this paper will be uploaded to GitHub upon article acceptance.

Statement of authorship

Alexander Feckler: Conceptualization (equal); Methodology (equal); Investigation (lead); Formal analysis (lead); Visualization (lead); Data curation (lead); Writing – Original Draft, Review & Editing (lead). **Ralf Schulz**: Conceptualization (equal); Writing – Original Draft, Review & Editing (support); **Ralf Schäfer**: Conceptualization (equal); Methodology (equal); Investigation (support); Formal analysis (support); Writing – Original Draft, Review & Editing (support); Funding acquisition (lead).

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Figure captions

Figure 1: (a) Alluvial plot displaying the extracted effect data. The size of each box is proportional to the number of extracted observations per organism group (left) or ecosystem function (right). The link width is proportionate to the number of observations per chemical stressor type (indicated by color). (b) Cumulative number of studies on each type of stressor studied through time.

Figure 2: Dose-response relationships with 95% confidence bands (grey) between sumTU and organic matter decomposition by decomposers or decomposer-detritivore-systems, shown as the proportion of the functioning in the stressor-treatment compared to the stressor-free control. Colors indicate the chemical stressor groups (see Fig. 1). Please note the slight differences in the x-axis scale.

Figure 3: Dose-response relationships with 95% confidence bands (grey) between sumTU and photosynthesis by algae or aquatic plants, shown as the proportion of the functioning in the stressor-treatment compared to the stressor-free control. Colors indicate the chemical stressor groups (see Fig. 1). Please note the slight differences in the x-axis scale.

Figure 4: Dose-response relationships with 95% confidence bands (grey) between sumTU and primary production by algae, shown as the proportion of the functioning in the stressor-treatment compared to the stressor-free control. Colors indicate the chemical stressor groups (see Fig. 1). Please note the differences in the x-axis scale.

853 Table 1: Descriptive statistics on sumTU values for the individual chemical stressor groups.

Chemical stressor group	*n*	Mean	Min	1. Quartile	2. Quartile (Median)	3. Quartile	4. Quartile
Fungicides	56	0.20	-4.54	-0.82	0.32	1.67	3.49
Herbicides	102	1.53	-2.88	0.87	1.39	2.20	3.99
Insecticides	34	0.74	-4.25	0.43	0.86	1.86	3.60
Pesticide mixtures	26	0.10	-2.31	-1.03	-0.10	0.94	3.27
Pharmaceuticals	26	-0.18	-5.68	-1.52	0.28	0.77	5.56
Metals	94	0.41	-5.13	-0.43	0.69	1.65	2.68
Metal mixtures	10	0.86	-1.26	0.55	0.99	1.43	1.77

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855 Table 2: Outcome of meta-regression models assessing the influence of moderator variables on ecosystem functions.

Function	Predictor	Estimate	T-value	df[1]	*p*-value	Lower CI[2]	Upper CI
Organic matter decomposition	Organism group	0.3793	7.1855	85	0.001	0.2744	0.4843
	sumTU	-0.0326	-0.9749	85	0.485	-0.0992	0.0339
	pH	-0.2455	-2.7898	85	0.007	-0.4205	-0.0705
	Organism group × sumTU	0.1399	3.7094	85	0.020	0.0649	0.2149
Photosynthesis	sumTU	0.0877	4.4305	99	0.001	0.0484	0.1270
	Temperature	-0.0221	-2.8695	99	0.023	-0.0374	-0.0068
Primary production	pH	0.2932	2.8056	42	0.088	0.0823	0.5041

856 [1]df = Degrees of freedom, [2]CI = confidence interval

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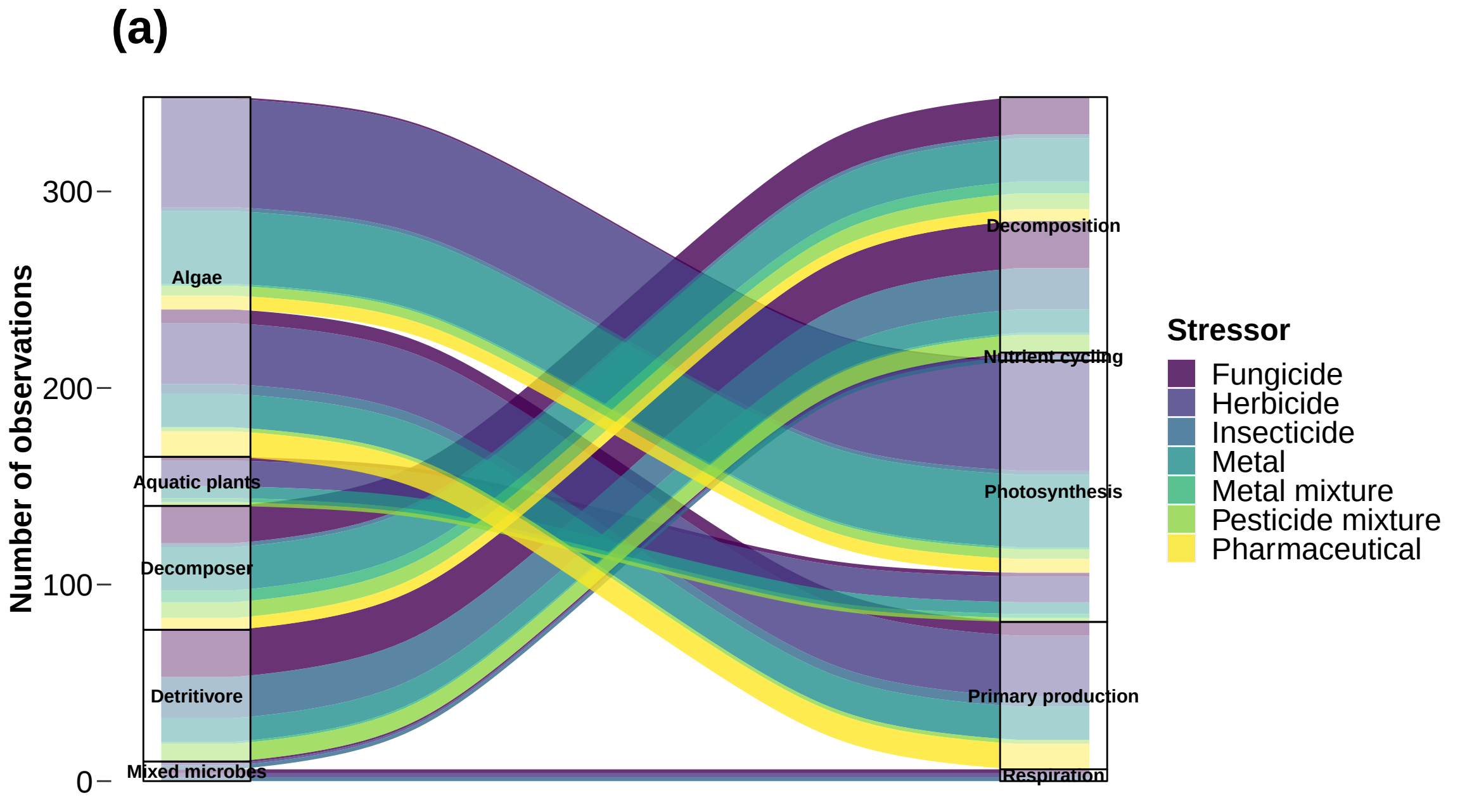
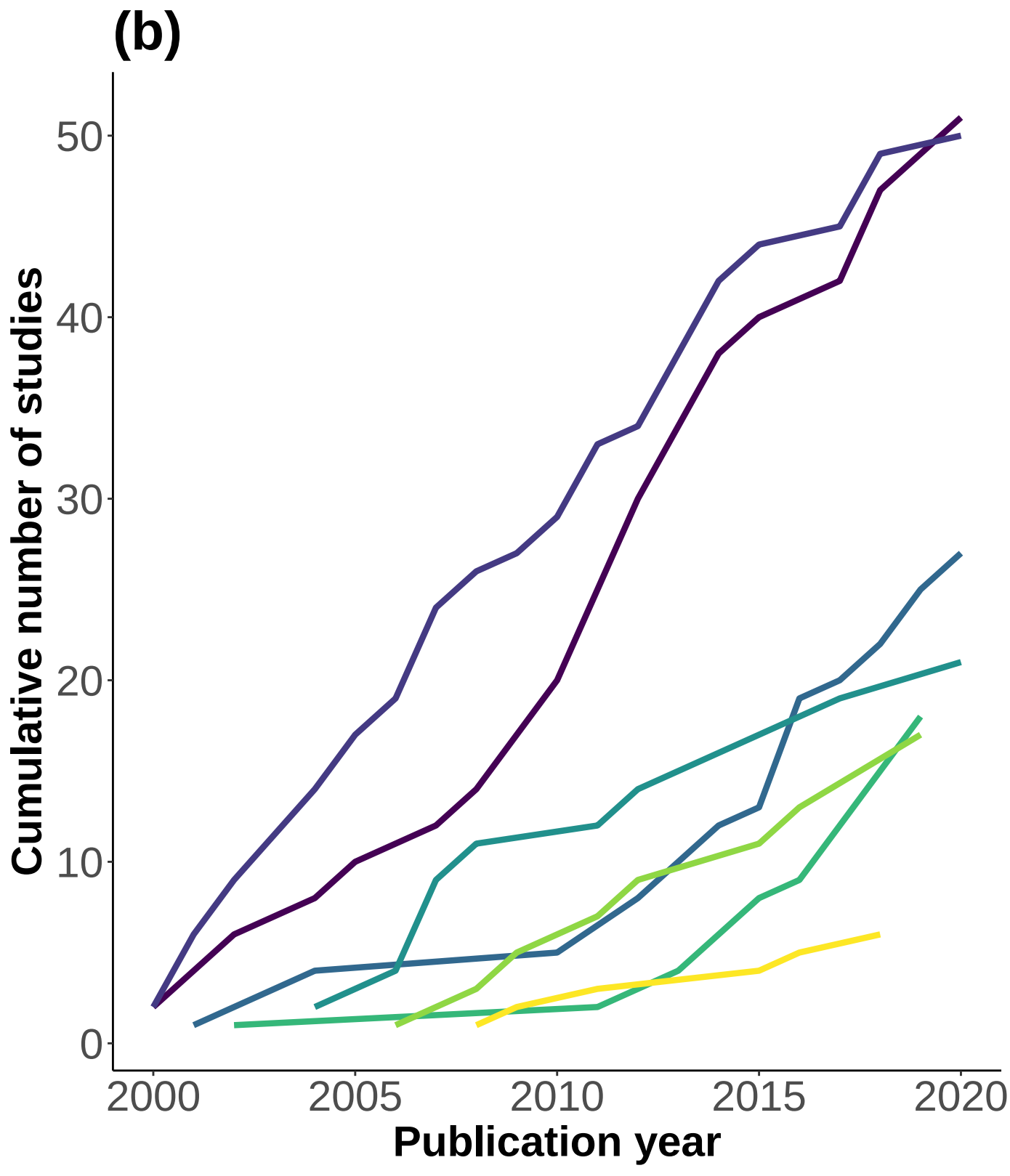
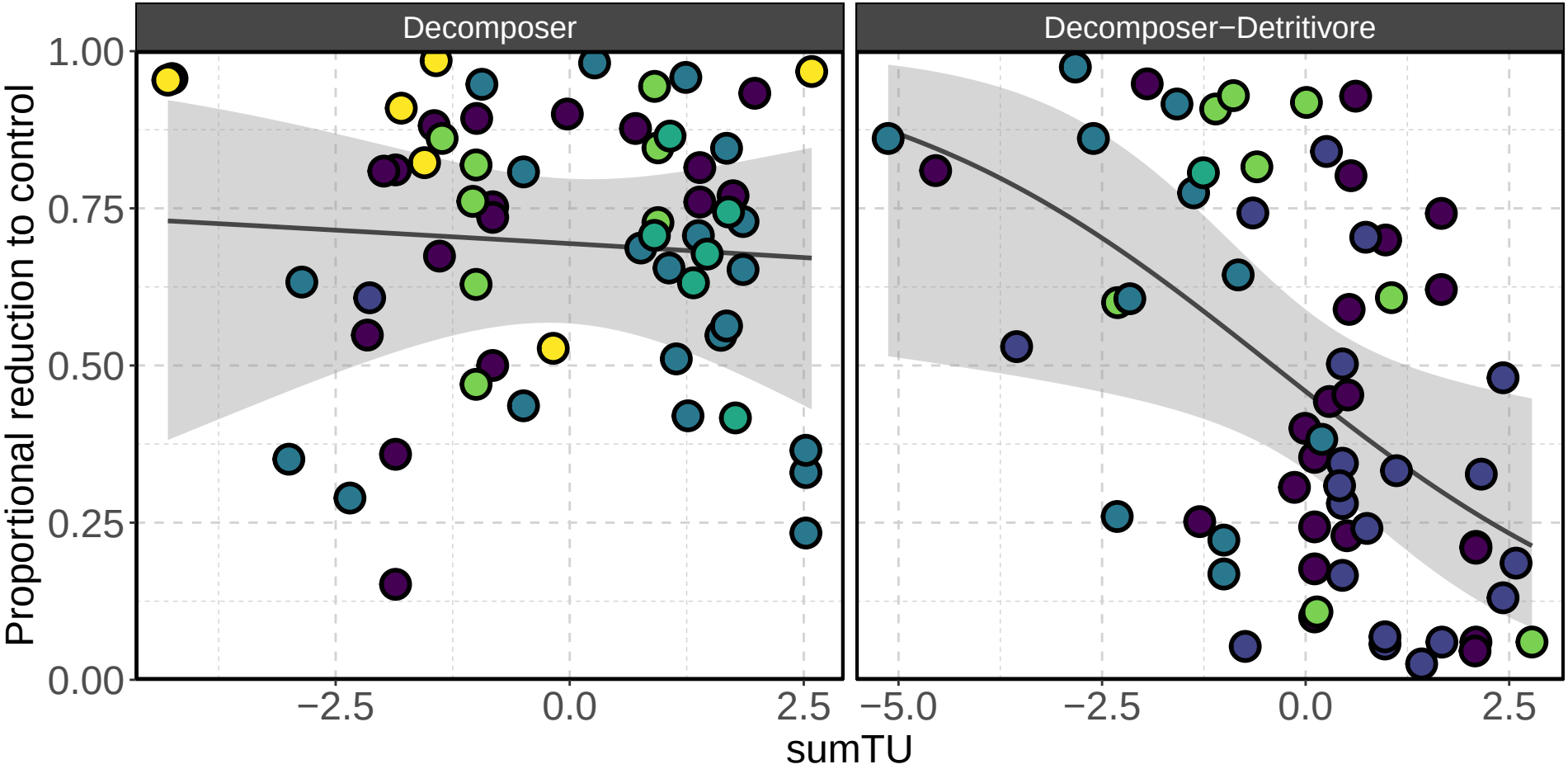

(a)
Number of observations
300
200
100
0
Algae
Aquatic plants
Decomposer
Detritivore
Mixed microbes
Decomposition
Nutrient cycling
Photosynthesis
Primary production
Respiration
Stressor
Fungicide
Herbicide
Insecticide
Metal
Metal mixture
Pesticide mixture
Pharmaceutical


(b)
Cumulative number of studies
0
10
20
30
40
50
2000
2005
2010
2015
2020
Publication year


Decomposer
Decomposer−Detritivore
Proportional reduction to control
1.00
0.75
0.50
0.25
0.00
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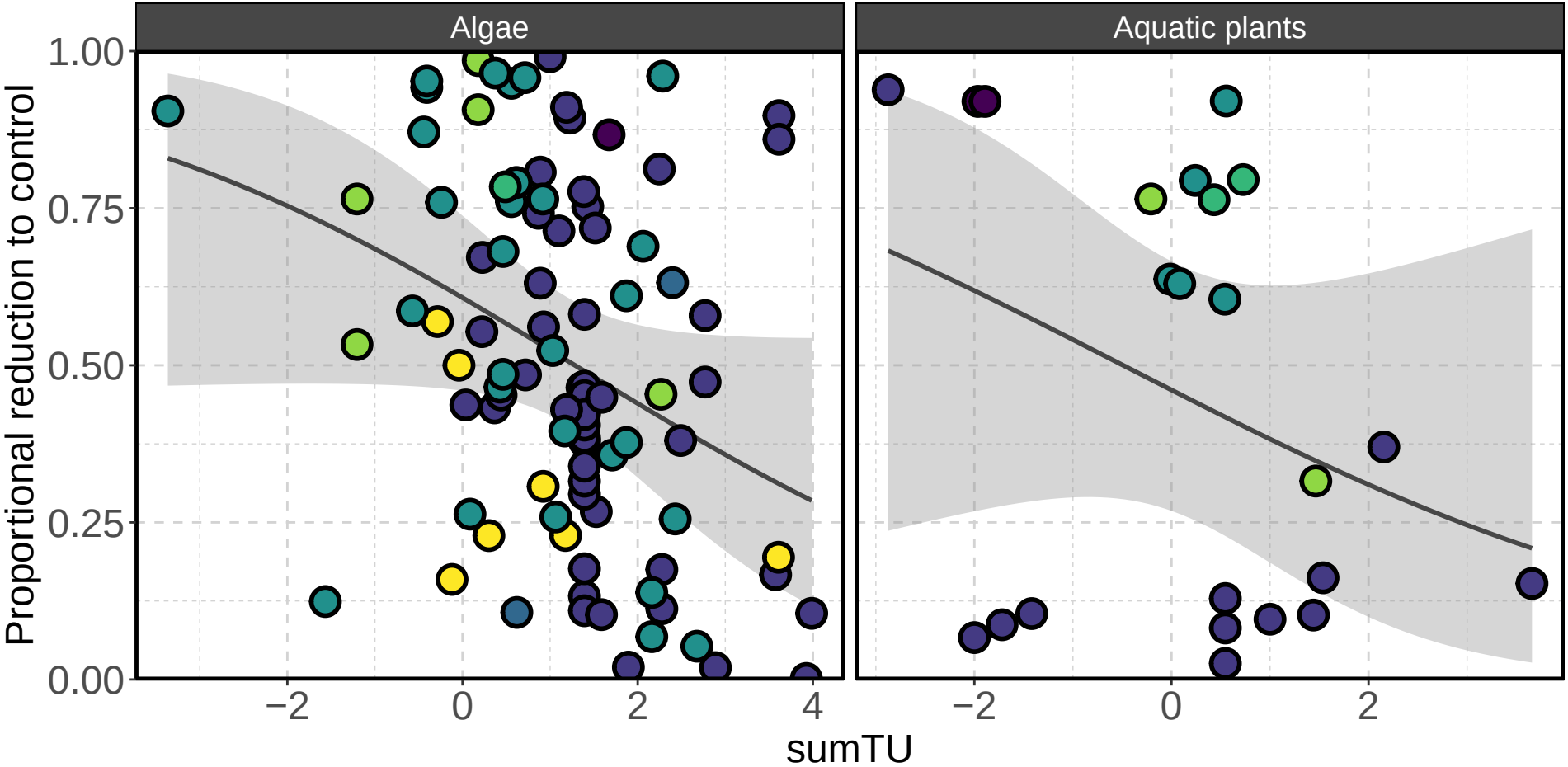
0.0
2.5
−5.0
−2.5
0.0
2.5
sumTU


Algae
Aquatic plants
Proportional reduction to control
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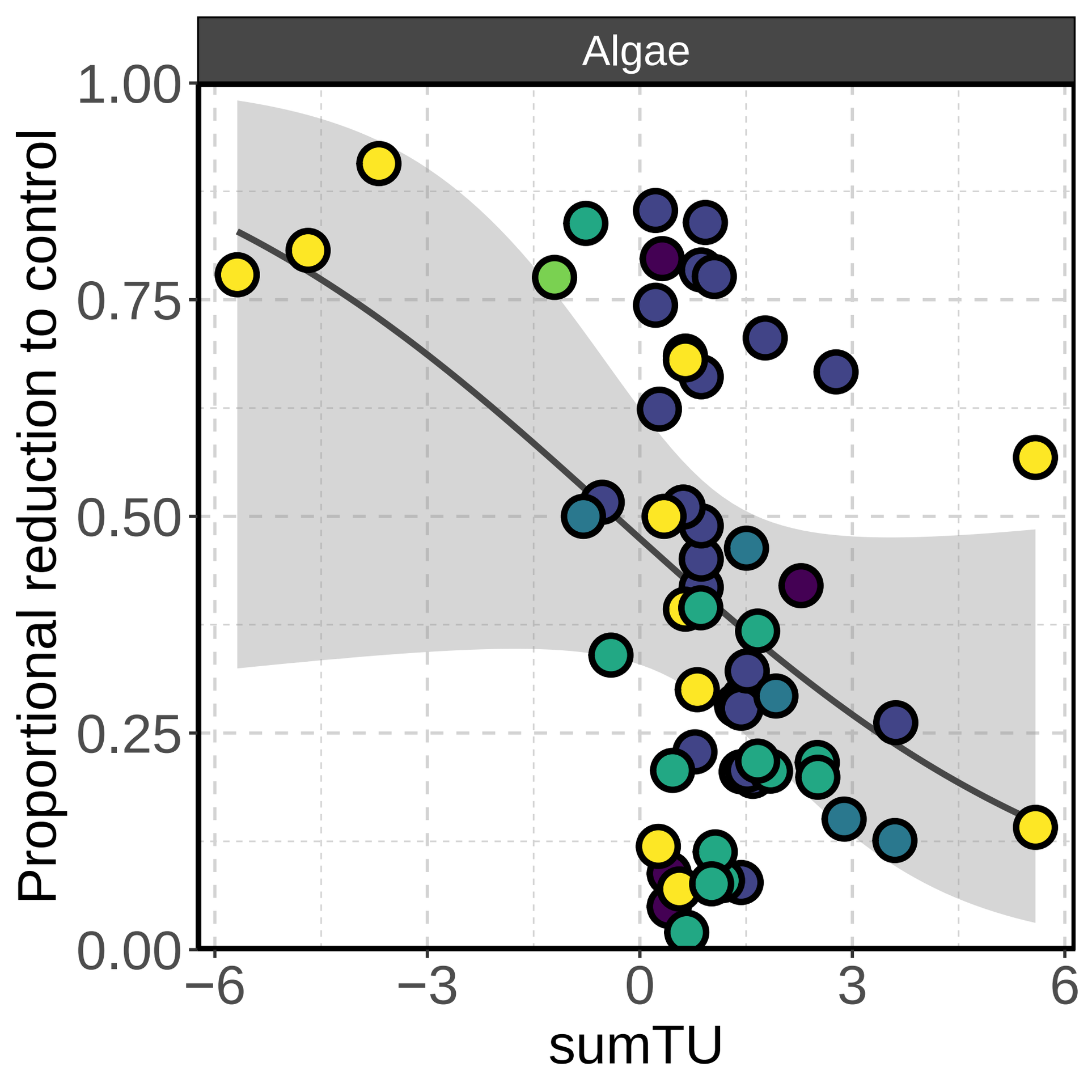
0.25
0.00
−2
0
2
4
−2
0
2
sumTU


Algae
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0.75
0.50
0.25
0.00
Proportional reduction to control
−6
−3
0
3
6
sumTU