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# Impacts of multiple stressors on freshwater biota across scales and ecosystems

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75 **Abstract**

76 Climate and land-use change drive a suite of stressors that shape ecosystems and interact  
77 to yield complex ecological responses, *i.e.* additive, antagonistic and synergistic effects.  
78 Currently we know little about the spatial scale relevant for the outcome of such interactions  
79 and about effect sizes. This knowledge gap needs to be filled to underpin future land  
80 management decisions or climate mitigation interventions, for protecting and restoring  
81 freshwater ecosystems. The study combines data across scales from 33 mesocosm  
82 experiments with those from 14 river basins and 22 cross-basin studies producing 174  
83 combinations of paired-stressor effects on a biological response variable. Generalised linear  
84 models showed that only one of the two stressors had a significant effect in 39% of the  
85 analysed cases, 28% of the paired-stressor combinations resulted in additive and 33% in  
86 interactive (antagonistic, synergistic, opposing or reversal) effects. For lakes the frequency of  
87 additive and interactive effects was similar for all scales addressed, while for rivers this  
88 frequency increased with scale. Nutrient enrichment was the overriding stressor for lakes,  
89 generally exceeding those of secondary stressors. For rivers, the effects of nutrient enrichment  
90 were dependent on the specific stressor combination and biological response variable. These  
91 results vindicate the traditional focus of lake restoration and management on nutrient stress,  
92 while highlighting that river management requires more bespoke management solutions.  
93

94 **Introduction**

95 Multiple stressors are increasingly recognized as a major concern for aquatic ecosystems  
96 and for those organisations in charge of their management. Stressors commonly interact to  
97 affect freshwater species, communities and functions, but the questions remain to which  
98 degree this evidence from experiments can be transferred to field conditions and how relevant  
99 stressor interactions are for ecosystem management. Critically, no study has been conducted  
100 to systematically confirm the frequency of occurrence of multiple stressor interactions across  
101 spatial scales (*i.e.* from waterbody to continental scales) and ecosystem types (*i.e.* for rivers  
102 and lakes). Using the most comprehensive large-scale assessment of multiple stressor  
103 interactions to date, we show that dominance of a single stressor, namely nutrient enrichment,  
104 is still common in lakes, while for rivers stressor interactions are much more relevant,  
105 demanding for more complex and informed management decisions.

106 Formerly, single, intense and well characterised stressors, such as organic and nutrient  
107 pollution from point sources, dominated freshwater ecosystem responses (van Dijk et al.,  
108 1994). However, as these formerly dominant stressors are now controlled and others emerge,  
109 recent large-scale analyses have shown that freshwater ecosystems are exhibiting novel  
110 ecological responses to different stressors (Richardson et al., 2018; Schäfer et al., 2016;  
111 Schinegger et al., 2016).

112 For the simplest case of two stressors acting simultaneously, three main types of effects  
113 can be conceptually distinguished: (i) Only one of the two stressors has notable ecological  
114 effects so that the effects of Stressor A outweigh those of Stressor B or vice versa (stressor  
115 dominance); (ii) the two stressors act independently such that their joint effect is the sum of  
116 the individual effects (additive effects); (iii) a stressor either strengthens or weakens the  
117 effects of the other (interaction). However, there is a striking lack of information on the  
118 frequency of occurrence of these effect types across spatial scales (*i.e.* from individual  
119 waterbodies to a whole continent) and ecosystem types (rivers *vs.* lakes).

120 Here we use a combined empirical-exploratory approach and a common quantitative  
121 framework to analyse a large set of original and compiled data on combinations of stressor  
122 pairs (explanatory variables), with each of them related to a biological response variable. We  
123 build on conceptual understanding of ecological responses to stressor interactions (Piggott et  
124 al., 2015; Jackson et al., 2016; de Laender, 2018) to structure an empirical modelling  
125 approach, using generalised linear modelling (GLM) and 174 stressor combinations with  
126 single biological responses from more than 18,000 observations. Outputs of the GLMs were  
127 interpreted to identify the frequency of cases with stressor dominance, additive stressor

128 relationships and stressor interactions (synergistic or antagonistic), stratified by ecosystem  
129 type (lake or river) and spatial scale (experiments, basin studies, cross-basin studies).

130 With this approach we addressed four questions: (1) How frequent are the three different  
131 types of stressor effects in lakes and rivers? We expected a high share of additive and  
132 interactive relationships in both lakes and rivers, as intense stressors obscuring the effects of  
133 secondary stressors rarely occur nowadays. (2) To what extent do ecosystem type (lake vs.  
134 river) and spatial scale influence the combined effects of two stressors? We expected more  
135 frequent stressor interactions in rivers, as their greater heterogeneity increases the likelihood  
136 for two stressors to have an impact. We further expected more frequent stressor interactions in  
137 small-scale studies (*i.e.* in mesocosms), as these are less influenced by confounding factors  
138 (Vinebrooke et al., 2004; Schäfer and Piggott, 2018). (3) What is the influence of ecosystem  
139 type (lake vs. river) and spatial scale on the explanatory power of two stressors and their  
140 interaction? We expected the explanatory power to be lower for rivers because of greater  
141 heterogeneity and thus potentially confounding factors in comparison to lakes. We also  
142 expected a decreasing explanatory power of individual stressors and their interactions with  
143 spatial scale, reflecting the increasing importance of confounding factors at large scales. (4) Is  
144 nutrient enrichment still the most prominent stressor affecting European aquatic ecosystems  
145 as suggested by EEA (2018), despite the progress in wastewater cleaning, and does the  
146 importance of co-stressors differ between lakes and rivers? We expected a dominating effect  
147 of nutrient stress in lakes due to the dominance of primary producers and a greater relevance  
148 of hydrological and morphological changes in rivers.

149 Our study pursues a phenomenological approach (*sensu* Griffen et al., 2016) and seeks to  
150 disclose stressor interrelations under “real-world” conditions, contributing to solve some of  
151 the pertinent issues in ecosystem management (Côté et al., 2016).

152

## 153 **Results and discussion**

### 154 ***Impact of ecosystem type on stressor effect types***

155 The available synthesis papers on multiple stressors in freshwater ecosystems (Jackson et  
156 al., 2016; Nöges et al., 2016) regularly reported stressor interactions. Therefore, we  
157 hypothesised that high proportions of both lake and river case studies would indicate additive  
158 or interactive paired-stressor relationships – this was not supported. Among the 174 cases,  
159 39% of models indicated single stressor dominance, 28% indicated additive paired-stressor  
160 effects, and 33% indicated paired stressors interacting significantly (Figure 2; see also  
161 *Supplementary Material Table S2*).

162 We expected a higher proportion of river cases to exhibit stressor interactions, compared  
163 to lakes, as a result of greater habitat heterogeneity in rivers – this was supported. The  
164 proportions of effect types differed between lakes (62% dominance, 16% additive, 22%  
165 interactive) and rivers (28% dominance, 33% additive, 39% interactive; see Figure 2) (Chi-  
166 squared test,  $p < 0.001$ ).

167 We assumed the different frequency of effect types between lakes and rivers might have  
168 been rooted in different frequencies of the stressor types investigated: nutrient enrichment was  
169 one of the two stressors in 95% of the lake cases, but only in 76% of the river cases. However,  
170 these differences between lakes and rivers in the share of stressor dominance remain if only  
171 cases with nutrient enrichment are considered: 60% (lakes) vs. 27% (rivers), compared to  
172 62% (lakes) vs. 29% (rivers) considering all cases.

173 There were also differences between lake and river cases in the frequency of organism  
174 groups considered as response variables: for lakes, phytoplankton was the most frequently  
175 used organism group (76% of the cases) followed by fish (22%), while in rivers benthic  
176 invertebrates (52% of the cases) were dominating and fish were used in 21% of the cases.  
177 However, when only regarding cases with fish as response variable, the differences in the  
178 share of dominant effect types is still high with 75% (lakes) vs. 32% (rivers). We therefore  
179 conclude that the observed differences in effect types between lakes and rivers are neither  
180 rooted in differences between the stressors nor in the organism groups investigated.

181 An alternative explanation is the different exposure of organisms inhabiting river and  
182 lakes to stressor effects. While freshwater ecosystems in general are sinks “collecting”  
183 anthropogenic stressors, the much higher shoreline length of rivers multiplies the effects of  
184 human activities in the catchment, such as land and water uses. This results in an increased  
185 exposure to hydrological and morphological stressors, the latter also being more relevant in  
186 rivers due to their primarily benthic habitats and assemblages (Benda et al., 2004). This is also  
187 expected for toxic substances that can act more directly in (small) rivers, as much lower  
188 compound quantities are needed to reach toxic concentrations. Within the 58 cases where  
189 models included a significant interaction term, the combinations of nutrients with toxic or  
190 morphological stress represented the greatest proportion of confirmed interaction effects (ratio  
191 of 0.45 or 0.43, respectively; only combinations with total number of cases  $> 5$ ; no significant  
192 correlation between total number of cases and share of interactive cases). All but one of the  
193 cases with toxic substances as a stressor were rivers.

194

195 ***Impact of scale on stressor effect types***

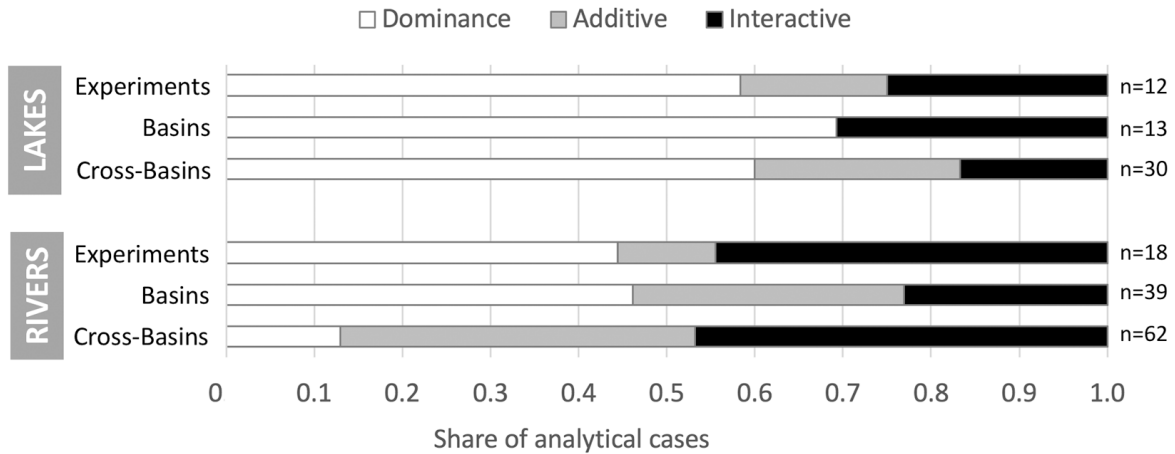
196 We expected that the frequency of interactions would increase with scale – this was only  
197 partly supported. While for lakes additive and interactive effects did not differ significantly  
198 between scales, for rivers the share of additive and interactive cases increased with scale (Chi-  
199 squared test,  $p < 0.01$ ). Two contrasting mechanisms may explain this pattern: On the one  
200 hand, increasing spatial scale implies an increase in confounding factors (including stressors  
201 not addressed in this analysis and thus not tested), limiting the likelihood of detecting additive  
202 or interactive effects between the targeted stressors, as they may be masked by other factors  
203 not under investigation. On the other hand, increasing spatial scale implies longer stressor  
204 gradients. In fact, nutrient and hydrological stressor ranges significantly increase with scale  
205 (Kruskal-Wallis H-test,  $p < 0.001$ ), enhancing the likelihood of additive or interactive stressor  
206 effects, which may only occur at certain stressor intensities. The latter holds true only if  
207 stressors are effective over the whole gradient length, e.g. the biological response does not  
208 level off at low or intermediate stressor levels (as in case of nutrient saturation; Price &  
209 Carrick, 2016; McCall et al., 2017).

210 As discussed above, the pattern of stressor dominance largely prevailed for lakes,  
211 irrespective of the spatial scale. Across the 34 cases of paired nutrient-thermal stress,  
212 however, the nutrient effects became more pronounced than the temperature effects with  
213 increasing spatial scale.

214 Though we are not aware of other studies comparing the effects of scale on the  
215 explanatory power of stressor interactions models, the observed differences in the frequency  
216 of stressors interactions between experiments and field studies are in line with the synthesis  
217 studies of Jackson et al. (2016) and Nöges et al. (2016). While the study of Jackson et al.  
218 included only experiments and observed interactive or additive effect types in all cases  
219 considered, the study by Nöges et al. focussed on field studies and interactive or additive  
220 effect types were only given for 50% of the river and 15% of the lake cases.

221





222

223 Figure 2: Share of analytical cases across experiments, basin studies and cross-basin studies  
 224 from lakes (n = 55) and rivers (n = 119), for which only a single stressor (dominance), both  
 225 stressors (additive) or their interaction significantly contributed to the variability of the  
 226 biological response.

227

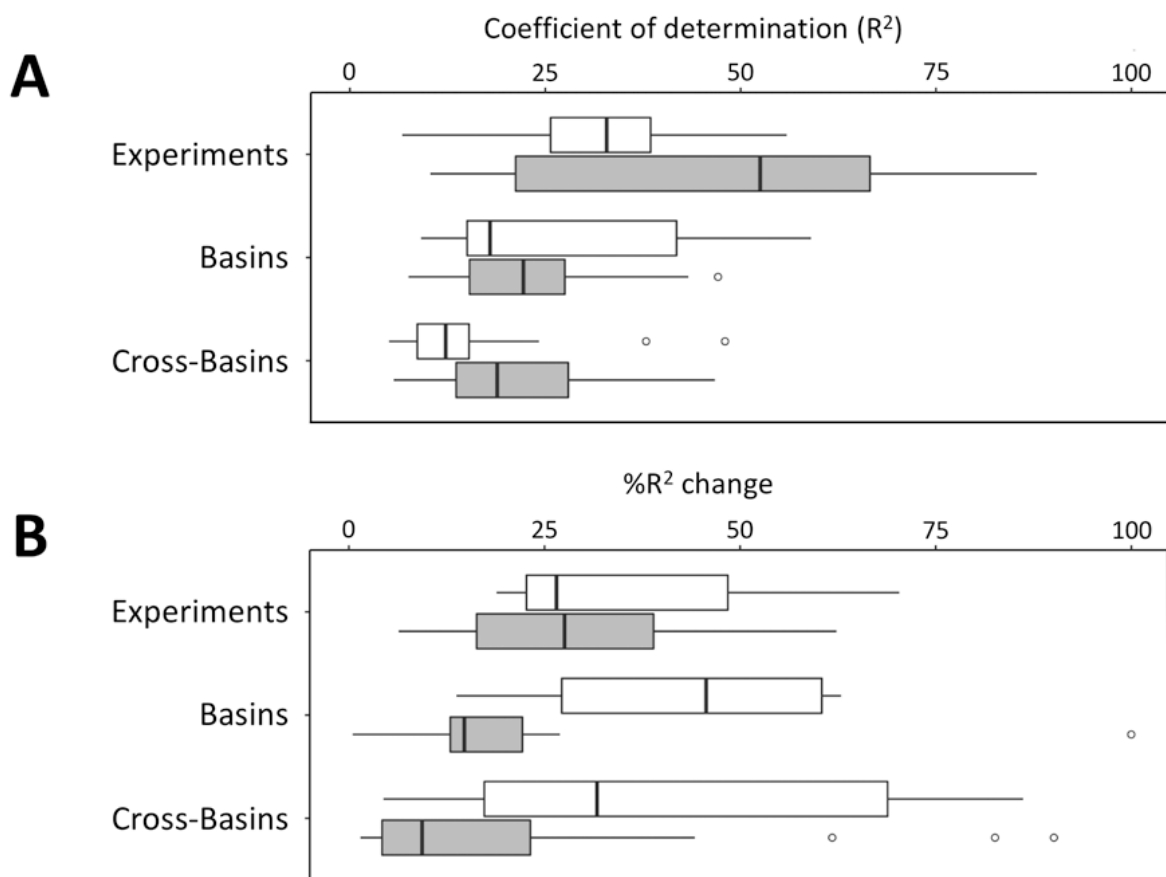
228 ***Impact of ecosystem type and scale on the models' explanatory power***

229 European lakes are generally in a better condition than European rivers (EEA, 2018) and  
 230 are affected by a lower number of stressors (EEA, 2012). Therefore, we expected the  
 231 explanatory power of our models to be lower for rivers because of greater impact of stressors  
 232 that have not been regarded (*i.e.* confounding factors). Contrasting to our expectations,  
 233 however, river models performed significantly better than lake models. This better  
 234 performance can be explained by the specific nature of riverine ecosystems: rivers feature  
 235 various niche and habitat factors that can be altered by multiple stressors (e.g. water quality,  
 236 hydrology, benthic habitats), and the riverine fauna is sensitive to the impacted oxygen  
 237 conditions, which may “collect” the effects of a variety of stressors into a single gradient.  
 238 Oxygen, however, is rarely measured in a meaningful way in monitoring programs (including  
 239 the daily maxima and minima) and was thus not considered as a stressor in our analysis. In  
 240 contrast, lake phytoplankton seems less susceptible to the effects of multiple stressors, as long  
 241 as nutrients are in the growth-limiting concentration range.

242 We expected a decreasing explanatory power with spatial scale, reflecting the increasing  
 243 importance of confounding factors at large scales – this was partly supported. The variance in  
 244 biological response explained by the paired-stressor models (expressed as marginal  $R^2$ )  
 245 ranged between 0.05 and 0.88, with a median value of 0.19. These ranges differed  
 246 significantly between experiments (median marginal  $R^2 = 0.38$ ), basin (median marginal  
 247  $R^2 = 0.22$ ) and cross-basin studies (median marginal  $R^2 = 0.16$ ) (Bonferroni-corrected Mann-  
 248 Whitney U-test,  $p < 0.05$ ; Figure 3A). The marginal  $R^2$  differed significantly between lakes

249 and rivers, with river cases showing on average slightly higher explanatory power (lakes:  
 250  $R^2 = 0.15$ , rivers:  $R^2 = 0.22$ ; not shown). The importance of the interaction term (expressed as  
 251  $\%R^2$  change) was significantly higher for lakes than for rivers. For rivers, this importance  
 252 tended to decrease with increasing scale of investigation, but differences between  
 253 investigation scales were generally not significant (Figure 3B). We are not aware of a single  
 254 other study targeting the role of scale for the explanatory power of stressor interaction  
 255 models.

256 For experiments, the high level of control on potentially confounding factors can account  
 257 for the on average greater explanatory power, when compared to field studies. Furthermore,  
 258 the experimental studies had lower numbers of observations and less complex biological  
 259 communities. Compared with this, factors such as temperature variation are already  
 260 temporally pronounced at basin-scale and the spatial variation across basins is considerable.  
 261



262  
 263 Figure 3: (A) Percent of biological variance explained by the paired stressors including their  
 264 interaction for the mesocosm experiments ( $n = 30$ ), basin study cases ( $n = 52$ ) and cross-  
 265 basin study cases ( $n = 92$ ), separately for lakes (white boxes) and rivers (grey boxes). Lakes  
 266 and rivers differ significantly only for the cross-basin studies (pairwise Bonferroni-corrected  
 267 Mann-Whitney U-test,  $p = 0.001$ ).  
 268 (B) Percent change in explained biological variance when interaction term is removed from  
 269 the model (in case of significant interaction term) for the mesocosm experiments ( $n = 11$ ),

270 basin study cases ( $n = 13$ ) and cross-basin study cases ( $n = 34$ ), separately for lakes (white  
271 boxes) and rivers (grey boxes). None of the differences within investigation scales are  
272 significant.

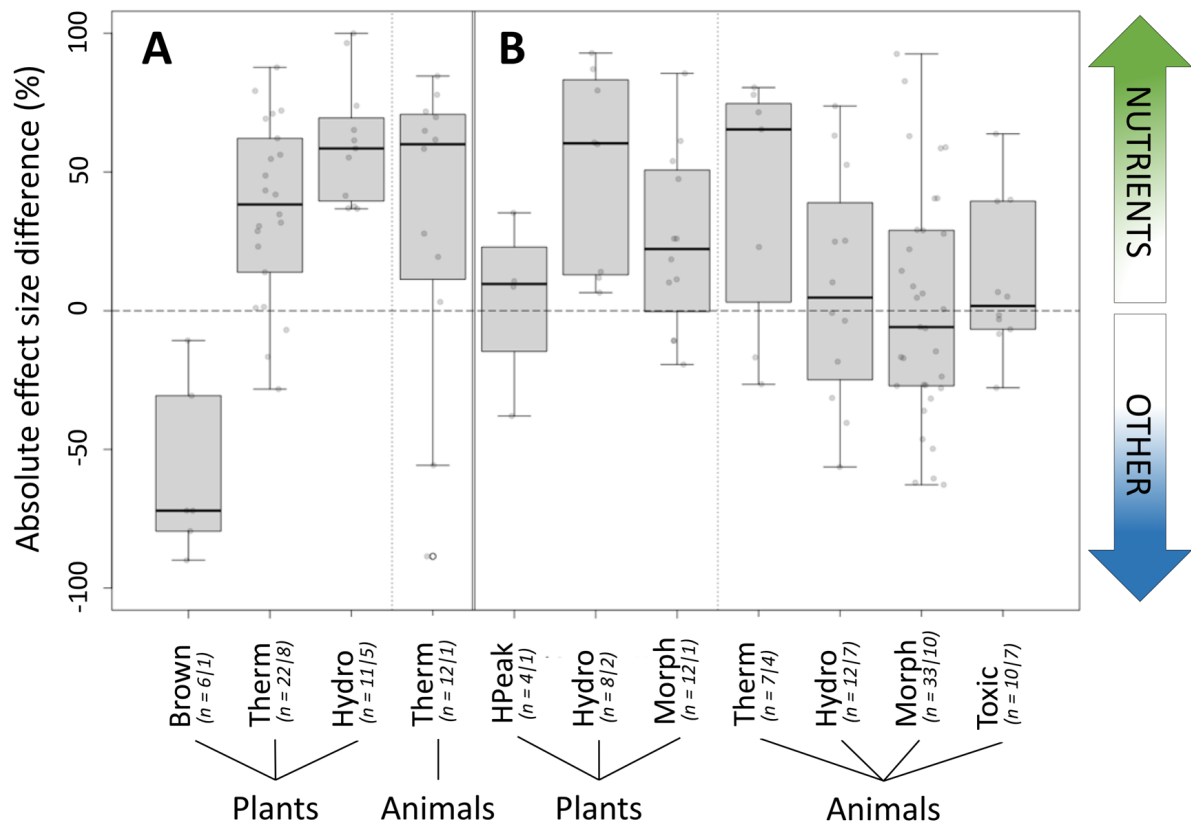
273 *Definition of box-plot elements:* centre line = median; box limits = upper and lower quartiles;  
274 whiskers = 1.5x interquartile range; points = outliers.

275

### 276 ***Role of nutrient stress for lakes vs. rivers***

277 The recent surveys by EEA (2012, 2018) suggest that eutrophication is still the most  
278 prominent stressor affecting the biota of Europe's water, in particular lakes, while rivers are  
279 also strongly affected by hydrological and morphological stressors. We therefore expected  
280 that responses to nutrient stress is retarded by the presence of secondary stressors in rivers  
281 more so than lakes where responses to nutrient enrichment are strongest – this was supported.

282 We identified eleven combinations of nutrient stress paired with another stressor, covering  
283 morphological, hydrological (including hydropeaking), thermal, toxic and chemical stress  
284 (brownification). The number of analytical cases in each stressor combination ranged from  
285 four to 33, with the combinations including hydropeaking and brownification stress  
286 exclusively comprising data collected at the experimental scale. All other combinations  
287 comprised data from up to ten different studies, most of which originated from two or more  
288 spatial scales. Best represented were the combinations of nutrient stress paired with thermal  
289 stress affecting autotrophs in lakes, and nutrient stress paired with morphological stress  
290 affecting heterotrophs in rivers (Figure 4).



291 Figure 4: Range of absolute effect size differences (%AES) for nutrient stress and selected  
 292 other stressors across case-studies from (A) lakes and (B) rivers. Positive %AES indicate  
 293 stronger effects by nutrient stress, negative %AES indicate stronger effects by the other  
 294 stressor on the biological response variable (subdivided into plants and animals) in the  
 295 regression model.  
 296

297 Brown = Brownification, Therm = Thermal stress, HPeak = Hydropeaking, Hydro = Hydrological  
 298 stress, Morph = Morphological stress, Toxic = Toxic stress; n = Number of analytical cases | case  
 299 studies.

300 *Definition of box-plot elements:* centre line = median; box limits = upper and lower quartiles; whiskers = 1.5x  
 301 interquartile range; points = outliers. Grey dots depict the individual data points.  
 302

303 Nutrient stress often had the stronger effect in the paired-stressor models. Hence, nine of  
 304 the eleven combinations in lakes and rivers showed a positive %AES median, implying on  
 305 average stronger effects of nutrients compared to the other stressor. Five combinations even  
 306 showed a positive 25<sup>th</sup> percentile %AES, indicating that in three quarters of the cases in these  
 307 combinations nutrient effects outweighed the other stressors. This was evident for all lake  
 308 stressor combinations except nutrients and brownification represented by a single case study.  
 309 The few lake cases, for which the non-nutrient stressor was stronger, included warming  
 310 affecting cyanobacterial biomass in European lakes, and lithophilous or piscivorous fish  
 311 abundance in French lakes.

312 The dominance of nutrients over secondary stressors in lakes applies, surprisingly, also to  
 313 temperature stress, which is often considered to interact in a synergistic way with

314 eutrophication in rivers and lakes (Moss et al., 2011). One mesocosm experiment even  
315 demonstrated an antagonistic relationship at high nutrient stress (Richardson et al., 2019).  
316 Water temperature may affect lake communities by modifying the food-web structure, e.g. by  
317 supporting planktivorous fish (Jeppesen et al., 2010); the two temperature-driven functional  
318 fish-trait responses perhaps indicate the emergence of such modification.

319 Brownification is a remarkable exception from this general pattern but observed here only  
320 in a single case study. It strongly superimposes the effects of nutrient stress, in particular by  
321 decreasing light transmission in the pelagic zone, which inhibits productivity despite excess  
322 nutrient concentrations (opposing interaction) and favours mixotrophic phytoplankton  
323 species. Brownification is triggered by global warming and wetter climate, and becomes  
324 increasingly relevant in boreal regions, as it originates from dissolved organic carbon in  
325 leachates of bogs and permafrost soils mineralising due to increasing temperatures and  
326 flushing, and the recovery from acidification (Monteith et al., 2007; Graneli, 2012).

327 Rivers generally showed a more heterogeneous pattern: nutrients clearly affected  
328 autotrophs more strongly when paired with hydrological or morphological stress, and  
329 heterotrophs when paired with thermal stress. The few river cases in these combinations, for  
330 which the non-nutrient stressor was stronger, included fine sediment influx affecting  
331 macrophyte and diatoms in UK rivers, and temperature increase affecting sensitive  
332 invertebrate taxa in Greek rivers. All other combinations were more ambiguous, with the  
333 %AES median being almost zero, indicating stressor effects of roughly equal size.

334 The pattern of nutrient stress outweighing the effects of hydrological or morphological  
335 stress for river autotrophs is similar to lakes. Here, “the response variable matters” (Segner et  
336 al., 2014) – while river autotrophs have shown to be responsive to hydrological or  
337 morphological stress elsewhere (e.g. Baattrup-Pedersen & Riis, 1999; Schneider et al., 2018),  
338 their effect size was overruled by the nutrient signal in our study. In one case, however,  
339 hydropeaking outweighed the nutrient signal on river autotrophs. The immediate mechanical  
340 effect of flush flows is very pervasive, but presumably limited to short river stretches  
341 downstream of a hydropower dam.

342 By contrast, river heterotrophs were equally affected by paired stressors when nutrient  
343 enrichment was paired with either hydrological, morphological or (to a lesser degree) thermal  
344 stress. This indicates that these paired stressors co-act on oxygen contents or habitat  
345 availability. In our study, we found small but consistent antagonistic interactions, in particular  
346 for channelized rivers, probably due to increased current velocities facilitating the oxygen  
347 availability. In the case of toxic stress our conjectures on mechanistic pathways remain

348 speculative. The diversity of compound-specific modes of action across xenobiotics in each  
349 mixture renders toxic stress a multi-stressor issue in itself (de Zwart & Posthuma, 2005).  
350 Notably, the toxic effects of ambient mixtures were clearly discernible in all respective  
351 paired-stressor case studies (n = 17), despite the likely different stressor modes of action  
352 (Busch et al., 2016). Given the lack of adequate monitoring of xenobiotics, our findings  
353 support that toxic effects in the multiply-stressed freshwaters of Europe are largely  
354 underestimated (Malaj et al., 2014).

355 In summary, nutrient enrichment overrules the effects of most other stressors in lakes,  
356 while the situation in rivers is more complex with plants being more strongly affected by  
357 nutrients, while animals were equally affected by nutrient enrichment and other stressors.

358

## 359 **Conclusions**

360 Our study supports the conjecture that eutrophication is still the most relevant stressor  
361 affecting many lakes, irrespective of the spatial scale considered. Other stressors are  
362 subordinate but may reveal notable effects if interacting with nutrients. These deserve special  
363 attention if antagonistic (e.g. lake brownification) and synergistic interactions (e.g. climate  
364 warming) can be expected that control the overall nutrient effect on phytoplankton. Relevant  
365 stressors and stressor combinations are more variable in rivers and more strongly affected by  
366 spatial scales. While river autotrophs are mainly impacted by nutrients, heterotrophs seem to  
367 be mainly influenced by oxygen availability that is impaired by a range of stressors (pollution,  
368 warming, flow reduction and fine sediment entry) on top of nutrient enrichment. While  
369 reduction of nutrient stress is most relevant for lakes, in particular under the conditions of  
370 climate warming, rivers require mitigation measures addressing several stressors  
371 simultaneously. Options include the establishment of woody riparian buffer strips that address  
372 several stressors (eutrophication, hydromorphological degradation) simultaneously.

373

## 374 **Author contributions**

375 Study conceptualisation: DC, LC, BMS., SB, LB, SJT, DH; data curation: DC, SB; funding acquisition and  
376 project administration: DH, LC, SB; data provision and/or formal analysis: AB, AG, AS, BMS, CA, CG-C, CP,  
377 DdZ, DG, EB-K, FC, GP, JJR, JR, JT, JUL, KR, KS, LP, LS, MCU, MJ, NK, NW, PB, PS, PvdO, RBS, R-MC,  
378 RS, SA, SB, SCS, SJM, SL, SP, SJT, TB, UI, UM; experimental investigations: AB-P, ALS, DG, EB-K, EJ, HF,  
379 JMS, JR, LC, LS, MG, PB, SA, SCS, SS, WG; manuscript writing: SB, DH, BMS, MG, DC. All authors  
380 reviewed the manuscript and included necessary amendments.

381 **Data and computer code availability statement:** All data generated or analysed during this study are included  
382 in this published article (and its supplementary information files). The R-script used for the analyses of  
383 individual case-studies is available in Chapman et al. (2017).

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388

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521

## 522 **Methods**

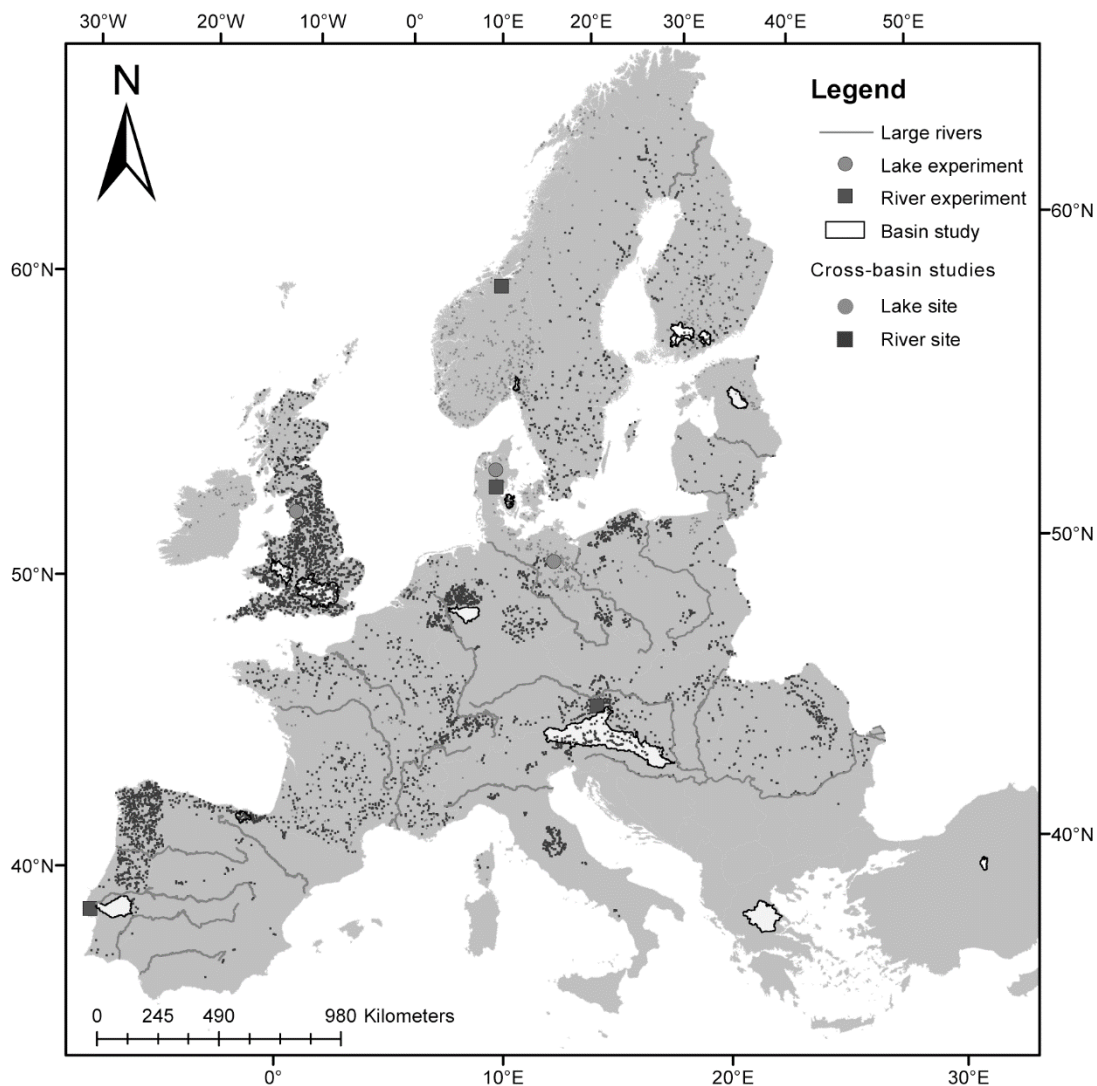
### 523 *Case studies*

524 The 45 studies analysed here covered selected European lakes and rivers (including one  
525 estuary) and addressed three spatial scales of investigation: manipulative multi-stressor  
526 experiments in mesocosms and flumes, river basin studies and cross-basin studies (Figure 1,  
527 *Supplementary Material Table S1*). Several studies contributed to multiple analytical cases,

528 depending on the available combinations of stressors and responses. The number of cases  
529 totalled 174.

530 The manipulative experiments were conducted within the framework of the European  
531 MARS project (Hering et al., 2015), involving three lake mesocosm facilities in Denmark,  
532 Germany and United Kingdom, and four artificial flume facilities in Norway, Denmark,  
533 Austria and Portugal. The experiments applied controlled pairs of stressors to study the effects  
534 on selected biological response variables. Overall, 30 analytical cases and 1,498 sample  
535 replicates were considered in our analysis, with a median number of 79 sample replicates per  
536 study (range: 20 to 768).

537 The MARS project also contributed data on 14 river basin studies selected to cover the  
538 main European regions and their representative stressor combinations (Hering et al., 2015).  
539 Based on harmonised analytical protocols (Feld et al., 2016) the multi-stressor effects were  
540 analysed using comprehensive datasets derived from regional monitoring programmes. For  
541 this study we chose the most relevant paired-stressor response combinations from four lake  
542 catchments and ten river catchments that together provided 52 analytical cases with an overall  
543 number of 2,114 samples (median number of samples per basin: 97, range: 19 to 525).



544  
 545 Figure 1: Location of the seven experimental facilities, 14 basin studies and sampling sites  
 546 (small dots) for the 22 cross-basin studies of lakes and rivers across Europe (see  
 547 *Supplementary Material Table S1* for details).

548  
 549 The 22 cross-basin studies included in this analysis mostly originated from research  
 550 activities, in which aquatic monitoring data was collated at regional, national or international  
 551 scale to investigate biological effects of various stressors (e.g. Moe et al., 2008; 2013). The  
 552 spatial coverage of these studies exceeded a single river basin, and commonly spanned large  
 553 numbers of lakes and rivers. The number of analytical cases amounted to 92, comprising  
 554 14,486 samples (median number of samples per study: 374, range: 40 to 3,706).

555  
 556 ***Stressor variables***

557 Within this study we considered a “stressor” as any external factor modified by human  
 558 intervention, which potentially moves a receptor (*i.e.* response variable) out of its normal

559 operating range (Sabater et al., 2019). The analysed stressor variables belonged to six stress  
560 categories (see also Birk, 2019): (1) nutrient stress (142 cases), including experimental  
561 addition or field sampling of phosphorus or nitrogen compounds in the water; (2)  
562 hydrological stress (57 cases), including experimental manipulation or field measurement of  
563 high flow (e.g. high flow pulse duration), low flow (e.g. residual flow), water level change,  
564 non-specific flow alteration (e.g. mean summer precipitation as proxy) and hydropeaking; (3)  
565 morphological stress (61 cases), including experimental treatment or field survey of river  
566 channel, bank and floodplain modification, and river connectivity disruption; (4) thermal  
567 stress (54 cases), including experimental heating or field measurement of water temperature  
568 (or air temperature as a proxy); (5) toxic stress of mixtures of xenobiotic compounds (18  
569 cases), expressed as the multi-substance Potentially Affected Fraction (de Zwart & Posthuma,  
570 2005), Toxic Units (Liess & von der Ohe, 2005) or runoff potential (von der Ohe &  
571 Goedkoop, 2013); and (6) other chemical stress (16 cases), including experimental application  
572 of humic substances and field samples of water quality determinants (e.g. dissolved oxygen,  
573 chloride, biological oxygen demand).

574 We always selected the stressor combinations most relevant for the respective broad lake  
575 or river type in the particular river basin or region, *i.e.* stressors that are most likely to affect  
576 biota due to their relative strength as compared to other regions and other stressors in the  
577 same region (Lyche-Solheim et al., 2019; see *Supplementary Material Table S1*). These  
578 included stressors prevalent in European freshwaters (EEA, 2018) and addressed in previous  
579 multi-stressor studies (Nõges et al., 2016). In the experimental studies, stressor intensities  
580 were applied emulating “real-life” conditions of the respective water body type. For instance,  
581 flumes mimicking nutrient-poor calcareous highland rivers were enriched by ten-fold  
582 phosphorus increase towards mesotrophic conditions – a realistic scenario in case of alpine  
583 pasture use in the floodplains. Mesocosms mimicking eutrophic shallow lowland lakes were  
584 enriched by five-fold phosphorus increase towards hypertrophic conditions – a realistic  
585 scenario in intensively used agricultural lowland landscapes. In the field studies, stressor  
586 intensities reflected the existing gradient in the particular river basin or region. Thus, the  
587 stressor “forcings” in all study cases represent conditions typical for the specific lake or river  
588 type, the river basin (featuring certain land uses) and the European region. In several of the  
589 investigated basins or cross-basins, more than two stressors were acting; in these we selected  
590 those that were assumed to affect the biota most strongly, either based on their intensity or  
591 based on previous studies on the relevance of the stressors in the region.

592 Overall, twelve paired-stressor combinations were investigated, including seven  
 593 combinations that only covered rivers (Table 1). For rivers, the combination of nutrient and  
 594 morphological stress was the most frequent, amounting to more than one-third of cases. For  
 595 lakes, the combination of nutrient and thermal stress was the most frequent, amounting to  
 596 more than half of the cases.

597 Table 1: Number of paired-stressor cases analysed across lakes and rivers

Paired stressors	Lakes	Rivers
Nutrient   Hydrological	11	24
Nutrient   Morphological	0	46
Nutrient   Thermal	34	9
Nutrient   Toxic	1	10
Nutrient   Chemical	6	1
Hydrological   Morphological	0	6
Hydrological   Thermal	3	8
Hydrological   Chemical	0	5
Morphological   Morphological <sup>A</sup>	0	1
Morphological   Toxic	0	5
Morphological   Chemical	0	2
Toxic   Chemical	0	2

598 <sup>A</sup> Connectivity disruption and morphological river alteration

599

### 600 ***Response variables***

601 A variety of organism groups was investigated, including phytoplankton (52 cases);  
 602 benthic flora, *i.e.* macrophytes or phytobenthos (22); benthic invertebrates (63 cases); and fish  
 603 (37 cases). Within the 174 cases, four categories of biological response variables were used:  
 604 (1) biodiversity (76 cases), including indices reflecting the proportion of a taxonomic group  
 605 within the assemblage (e.g. percentage of Chlorophyta in the benthic algal assemblage), taxon  
 606 richness, Ecological Quality Ratios (as derived from ecological classification tools for the  
 607 European Water Framework Directive) and taxon-sensitivity indices (e.g. saprobic indices,  
 608 ASPT); (2) biomass/abundance (51 cases), including biomasses or total abundances of  
 609 phytoplankton or fish, chlorophyll *a* concentrations or cyanobacterial biomass; (3) functional  
 610 traits (38 cases), including the absolute or relative abundance of functional groups such as  
 611 habitat preferences, feeding types or life cycles and trait-based quality indices (e.g. SPEAR;  
 612 von der Ohe & Goedkoop, 2013); and (4) behaviour (9 cases), exclusively including drift  
 613 rates of invertebrates and stranding rates of juvenile fish. While the response category  
 614 “biodiversity” covered all organism groups, the category “biomass/abundance” was limited to

615 phytoplankton (except for two cases each with benthic algae and fish), and both “functional  
616 traits” and “behaviour” were limited to animals (invertebrates and fish).

617

### 618 *Statistical analysis*

619 The relationship between the biological response and the paired stressors was investigated  
620 for each individual analytical case by GLM based on the general formula

$$621 E(Y) = g^{-1}(a \cdot x_1 + b \cdot x_2 + c \cdot x_1 \cdot x_2),$$

622 with  $E(Y)$  is the expected value of the biological response variable  $Y$ ,  $g$  is the link function  
623 that specifies how the response relates to the linear predictors,  $x_1$  is the standardized  
624 measurement of Stressor 1,  $x_2$  is the standardized measurement of Stressor 2 and  $x_1 \cdot x_2$  is the  
625 interaction of the standardized measurements of Stressor 1 and Stressor 2. Parameters  $a$ ,  $b$  and  
626  $c$  scale the effects of Stressors 1, 2 and their interaction, respectively.

627

### 628 *Data processing of stressor and response variables*

629 For large-scale data (multi-site biomonitoring data with no, or very short, temporal  
630 component), long-term average measures of stress were used. For multi-year data (single or  
631 multiple site), each year provided one stress measurement per site. When data was at higher  
632 temporal resolution, it was pre-processed to an annual level. Categorical stressor variables  
633 (e.g. experimental flow treatment) had only two levels representing stressed vs. unstressed  
634 conditions.

635 All continuous variables (responses and stressor variables) were standardized by  
636 transformation to approach normal distribution. A version of the Box-Cox transformation was  
637 used (Box & Cox, 1964), including an offset to ensure strict positivity (all values  $> 0$ ).  
638 Transformed data was inspected for normality by plotting frequency histograms. If the data  
639 exhibited skewness because of extreme outliers, these outliers were excluded from the  
640 analysis. Following Box-Cox transformation, each transformed variable was centred and  
641 scaled, so they had a mean of zero and a variance of one.

642

### 643 *Choice of regression model*

644 The type of statistical model used to fit the paired-stressor response data depended on two  
645 major considerations: (1) The type of analytical case, which determined whether a GLM was  
646 sufficient or if a generalised linear mixed model (GLMM) with random effects was needed  
647 (see Table 2 for the criteria). GLMMs were used when the data structure included grouping  
648 factors, such as experimental block, site or year. In most cases the analyses included random

649 effects in the standard way as random intercept terms. However, if considered appropriate  
 650 (e.g. due to large data volume) models with both random intercepts and slopes were used. (2)  
 651 The type of response data, which determined the link function and error distribution of the  
 652 model (Gaussian errors and an identity link for continuous data, Poisson errors and a  
 653 logarithmic link for count data). GLMs were fitted with the base R libraries and GLMMs  
 654 were fitted with the *lme4* and *lmerTest* R packages.

655

656 Table 2: Summary of model choice criteria

Analytical case	Is a mixed model (with random effect) needed?
Mesocosm experiment	Choice depending on experimental design. Grouping factors such as block or measurement period were included as random effect.
Single-site, multi-year (temporal)	No.
Multi-site, multi-year (spatio-temporal)	Yes. Random effects of site and year were included.
Multi-site, single-year (spatial)	No.

657

658 *Testing and correcting for residual autocorrelation*

659 Where necessary, we tested whether model residuals showed strong evidence of spatial or  
 660 temporal autocorrelation, which can cause the statistical significance of model terms to be  
 661 exaggerated. This was only required when the analysis used GLMs without random effects,  
 662 since the random effects in the mixed effects models should account for grouping in space  
 663 and time. Autocorrelation in space or time was identified with Moran’s tests on model  
 664 residuals and, where substantial autocorrelation was detected, the model was re-fitted  
 665 including a “trend surface” generated using a smoothing spline or polynomial functions  
 666 (Dormann et al., 2007). This is a simple and generally effective way of reducing the influence  
 667 of autocorrelation on the model’s stressor effects of interest.

668

669 *Model evaluation*

670 To evaluate our models, residuals were examined for correlation to the fitted values and  
 671 deviation from the normal distribution (Shapiro-Wilk Test). We excluded 28 models where  
 672 residuals were correlated with fitted values ( $R > 0.35$ ) and non-normally distributed. Model fit  
 673 was evaluated as the marginal  $R^2$ , *i.e.* the proportion of variance explained by the models  
 674 fixed effects, ignoring the contribution of any random effects (Nakagawa & Schielzeth,  
 675 2013). We excluded models with marginal  $R^2 < 0.05$ . Model fixed effects (main effects of  
 676 both stressors and their interactions) were evaluated from the standardized partial regression  
 677 coefficients and their significance ( $t$  Test), in the following referred to as standardised effect  
 678 sizes (SES).



679 Several case studies allowed for analysing different response variables within the same  
680 organism group or across different organism groups, using datasets from the same river  
681 basin(s). To avoid redundancy in paired-stressor responses we checked that model results  
682 differed in marginal  $R^2$  and fixed effects.

683

#### 684 *Importance of the interaction term*

685 The importance of the interaction term was estimated by the change in marginal  $R^2$  upon  
686 dropping the interaction term, considered in cases with a significant interaction term,  
687 expressed as a percentage change relative to the full model's marginal  $R^2$  ( $\%R^2$  change).

688

#### 689 *Interaction classification*

690 The type of interaction was characterised from the SES and only considered in case of a  
691 significant interaction term. We applied a simple classification scheme to the full model,  
692 referring to both stressors' main effects and their interaction. This was based on the direction  
693 of the interaction effect, relative to the directions of the main effects of both stressors.  
694 Synergistic interaction was assigned when the SES for both stressors and their interaction all  
695 had the same sign (*i.e.* all positive or all negative). Antagonistic interaction was assigned  
696 when SES for both stressors had the same sign, but their interaction had the opposite sign.  
697 Opposing interaction was assigned when the signs of the SES for both stressors differed, and  
698 we distinguished between opposing contributing to either Stressor 1 (*i.e.* Stressor 1 and  
699 interaction with same sign) or Stressor 2 (*i.e.* Stressor 2 and interaction with same sign).  
700 Reversal interaction (*sensu* Piggott et al., 2015; Jackson et al., 2016) was assigned when the  
701 SES' sum for both stressors had a value smaller than and a sign different from the  
702 interaction's SES.

703

#### 704 *Synthesis analysis*

705 We identified the frequency of analytical cases with a significant interaction term  
706 ("interactive"), or where one ("dominance") or both stressors ("additive") were significant but  
707 not the interaction term. The importance (share) of these three types of stressor interrelations  
708 was compared between ecosystems (from studies of lakes or rivers) and between scales (from  
709 experiments, basin and cross-basin studies). These comparisons were tested using the Chi-  
710 squared test. The range of marginal  $R^2$  values from full models were compared between study  
711 scales, as well as the  $\%R^2$  change for those cases with significant interaction terms. These

712 comparisons were tested for significant differences using pairwise Mann-Whitney U-tests  
713 with Bonferroni correction for multiple comparisons.

714 To evaluate the relevance of nutrient enrichment in the paired-stressor context, we  
715 selected a subset of cases that included both nutrient stress paired with another stressor. The  
716 strength of their effect sizes was compared, distinguishing between effects on autotrophs and  
717 heterotrophs across lakes and rivers. In this analysis we simply considered the magnitude of  
718 the absolute effect sizes of the two stressors (and their interaction) rather than whether they  
719 had positive, negative or opposing effects on the response variable.

720 We calculated the relative absolute effect sizes per analytical case (%AES) by setting the  
721 sum of the absolute SES of Stressor 1, Stressor 2 and their interaction to 100 % (irrespective  
722 of their statistical significance in the regression analysis), and expressing the individual SES  
723 as a percentage. The difference between %AES of the nutrient stressor and %AES of the other  
724 stressor revealed which stressor had the stronger effect on the biological response, with  
725 positive values indicating stronger effects of nutrient enrichment, and negative values  
726 indicating stronger effects of the other stressors. In the case of an opposing interaction, the  
727 %AES of the interaction term was added to the stressor's %AES with which the interaction  
728 SES shared the sign (e.g. the %AES of a positive interaction SES was added to the %AES of  
729 the nutrient stressor if its SES was also positive). In case of a synergistic or antagonistic  
730 interaction, we considered the interaction effect to be equally relevant for both stressors with  
731 no implications for the difference in the individual stressor effects.