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# 1 **Synthesizing the impacts of multiple stressors on freshwater biota across scales and** 2 **ecosystems**

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76 **Abstract**

77 Climate and land-use change drive a suite of stressors that shape ecosystems and interact to  
78 yield complex ecological responses, *i.e.* additive, antagonistic and synergistic effects.  
79 Currently we know little about the spatial extent of such interactions and about effect sizes.  
80 This knowledge gap needs to be filled to underpin future land management decisions or  
81 climate mitigation interventions, for protecting and restoring freshwater ecosystems. The  
82 study combines data across scales from 33 mesocosm experiments with those from 14 river  
83 basins and 22 cross-basin studies producing 180 combinations of paired-stressor effects on a  
84 biological response variable. Generalised linear models showed that only one of the two  
85 stressors had a significant effect in 40% of the analysed cases, 27% of the paired-stressor  
86 combinations resulted in additive and 33% in interactive (antagonistic, synergistic, opposing  
87 or reversal) effects. While for lakes the frequency of additive and interactive effects did not  
88 differ significantly between scales, for rivers this frequency increased with scale. Nutrient  
89 enrichment was the overriding stressor for lakes, generally exceeding those of secondary  
90 stressors. For rivers, the effect sizes of nutrient enrichment were dependent on the specific  
91 stressor combination and biological response variable. These results vindicate the traditional  
92 focus of lake restoration and management on nutrient stress, while highlighting that river  
93 management requires more bespoke management solutions.

94

95 **Introduction**

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

107 Formerly, single, intense and well characterised stressors, such as organic and nutrient  
108 pollution, dominated freshwater ecosystem responses (van Dijk et al., 1994). However, as  
109 these formerly dominant stressors are now controlled and others emerge, recent large scale  
110 analyses have shown that freshwater ecosystems are exhibiting novel ecological responses to  
111 different stressors (Richardson et al., 2018; Schäfer et al., 2016; Schinegger et al., 2016).  
112 For the simplest case of two stressors acting simultaneously, three main types of effects can  
113 be conceptually distinguished: (i) Only one of the two stressors has notable ecological effects  
114 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 180 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 four different types  
131 of stressor effects in lakes and rivers? We expected a high share of additive, synergistic and  
132 antagonistic relationships in both lakes and rivers, as intense stressors obscuring the effects of  
133 secondary stressors nowadays rarely occur. (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 because greater heterogeneity than in lakes increases  
136 the likelihood for two stressors to have an impact. We further expected more frequent stressor  
137 interactions in small-scale studies (*i.e.* in mesocosms), as these are less influenced by  
138 confounding factors (Vinebrooke et al., 2004; Schäfer and Piggott, 2018). (3) What is the  
139 influence of ecosystem type (lake *vs.* river) and spatial scale on the explanatory power of two  
140 stressors and their interaction? We expected the explanatory power to be lower for rivers  
141 because of greater heterogeneity and thus potentially confounding factors in comparison to  
142 lakes. We also expected a decreasing explanatory power of individual stressors and their  
143 interactions with spatial scale, reflecting the increasing importance of confounding factors at  
144 large scales. (4) Is nutrient enrichment the most prominent stressor affecting European aquatic  
145 ecosystems, and does the importance of co-stressors differ between lakes and rivers? We  
146 expected a dominating effect of nutrient stress in lakes due to the dominance of primary  
147 producers and a greater relevance of hydrological and morphological changes in rivers.  
148 Our study pursues a phenomenological approach (*sensu* Griffen et al., 2016) and seeks to  
149 disclose stressor interrelations under “real-world” conditions, contributing to solve some of  
150 the pertinent issues in ecosystem management (Côté et al., 2016).

151

## 152 **Results and discussion**

### 153 ***Frequency of stressor effect types***

154 We hypothesised that high proportions of both lake and river case studies would indicate  
155 additive or interactive paired-stressor relationships – this was not supported. Among the 180  
156 cases, 40% of models indicated single stressor dominance, 27% indicated additive paired-  
157 stressor effects, and 33% indicated paired stressors interacting significantly (Figure 2; see also  
158 *Supplementary Material Table S2*). Single stressor dominance was especially common in  
159 lakes, where responses are driven predominantly by nutrients and secondary stressor effects  
160 are not significant.

161 The observed pattern on stressor dominance may change if the stressor gradients are  
162 modified, *i.e.* the intensity of the dominant stressor is reduced or the intensity of the  
163 suppressed stressor is increased (Feld et al., 2011; Sundermann et al., 2013). The pattern of  
164 stressor dominance may further relate to the type of biological response variable. This not  
165 only holds for the fundamental distinction between autotrophs and heterotrophs regarding  
166 energy and nutrient supply, but also for the more specific differences regarding life-history  
167 and habitat preferences (Segner et al., 2014; Lange et al., 2018). For instance, the dominance  
168 of phytoplankton as a response variable in the lake cases partly explains why nutrient stress is  
169 found to be the most relevant stressor.

170

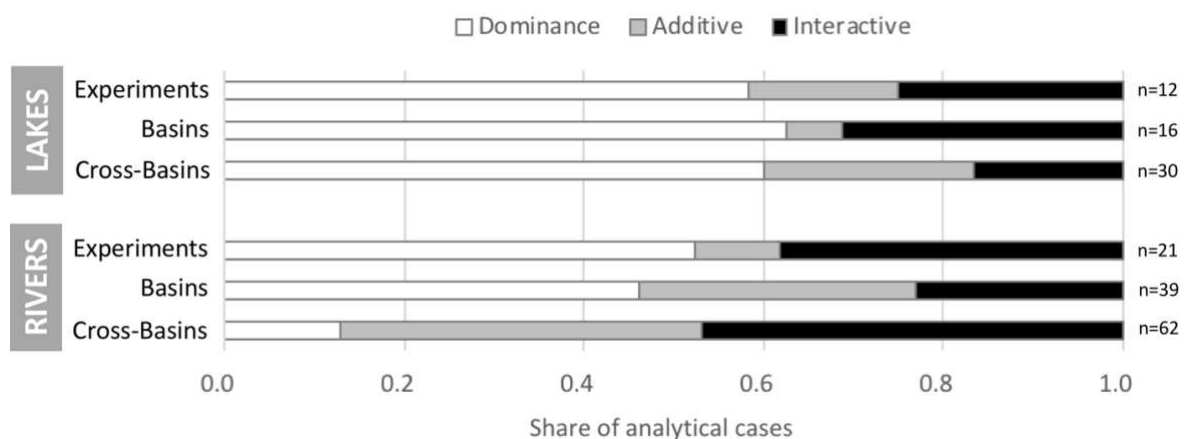
### 171 ***Impact of ecosystem type and scale on stressor effect types***

172 We expected a higher proportion of river cases to exhibit stressor interactions, compared to  
173 lakes, as a result of greater habitat heterogeneity in rivers – this was supported. The  
174 proportions of effect types differed between lakes (60% dominance, 17% additive, 22%  
175 interactive) and rivers (30% dominance, 32% additive, 38% interactive; see Figure 2) (Chi-  
176 squared test,  $p < 0.001$ ). While freshwater ecosystems in general are sinks “collecting”  
177 anthropogenic stressors, the dendritic shape of rivers multiplies human activities in the  
178 catchment, such as land and water uses. This results in an increased exposure to hydrological  
179 and morphological stressors, the latter also being more relevant in rivers due to their primarily  
180 benthic habitats and assemblages. In addition, toxic substances can act more directly in  
181 (small) rivers, as much lower compound quantities are needed to reach toxic concentrations.  
182 Most of the additive and interactive stressor effects observed in rivers are conditioned by  
183 oxygen availability, and antagonistic responses underline counter-directional stressor effects  
184 on oxygen contents (e.g. increased aeration due to faster flow in channelized rivers  
185 dampening eutrophication effects; Schinegger et al., 2018). Various other stressors act  
186 through multiple modes of action including chloride inducing osmotic stress, toxic substances  
187 obstructing metabolic processes, temperature stress increasing oxygen demand, and  
188 morphological alteration affecting physical habitat availability. These stressors are more  
189 likely to act in combination with others in rivers where toxicants can reach (sub-)lethal peak  
190 concentrations earlier and habitat disturbance is greater, when compared to lakes (Graeber et  
191 al., 2017). Notably, within the 59 cases where models included a significant interaction term,  
192 the combinations of nutrients with toxic or morphological stress represented the greatest  
193 proportion of confirmed interaction effects (ratio of 0.45 or 0.43, respectively; only

194 combinations with total number of cases > 5; no significant correlation between total number  
 195 of cases and share of interactive cases).

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,  $p < 0.001$ ). 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), limiting the likelihood of detecting additive or interactive  
 202 effects between the targeted stressors, as they may be masked by other factors not under  
 203 investigation. Conversely, unknown stressors could co-vary with the tested stressors, thus  
 204 providing false positive evidence if an unknown stressor is the true actor. On the other hand,  
 205 increasing spatial scale may imply longer stressor gradients, possibly increasing the likelihood  
 206 of additive or interactive stressor effects, which may only occur at certain stressor intensities.  
 207 The latter holds true only if stressors are effective over the whole gradient length, e.g. the  
 208 biological response does not level off at low or intermediate stressor levels (as in case of  
 209 nutrient saturation; Price & Carrick, 2016; McCall et al., 2017). In addition, rivers accumulate  
 210 stressors from their catchments.

211 As discussed above, the pattern of stressor dominance largely prevailed for lakes, irrespective  
 212 of the spatial scale. Across the 34 cases of paired nutrient-thermal stress, however, the  
 213 nutrient effects became more pronounced than the temperature effects with increasing spatial  
 214 scale. Confounding factors may have further dampened the effects of thermal stress, perhaps  
 215 connected to different stressor-specific resilience patterns across lake types (Spears et al.,  
 216 2015; Richardson et al., 2018). In addition, time series data of single water bodies, which  
 217 generally imply shorter stressor gradients, were frequently used in lake studies at basin scale  
 218 (28% of lake cases; “single-site, multi-year”; see Table 2).



219



220 Figure 2: Share of analytical cases across experiments, basin studies and cross-basin studies  
221 from lakes (n = 58) and rivers (n = 122), for which only a single stressor (dominance), both  
222 stressors (additive) or their interaction significantly contributed to the variability of the  
223 biological response.

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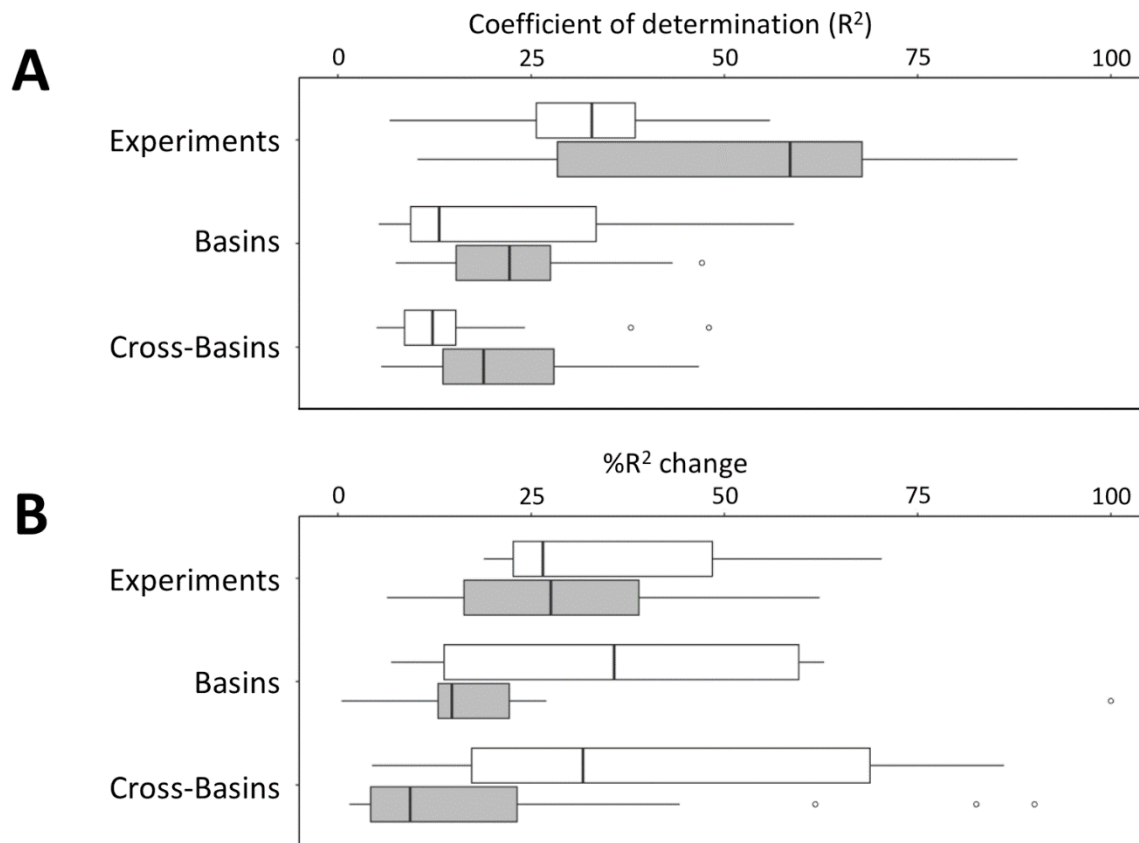
### 225 *Impact of ecosystem type and scale on the models' explanatory power*

226 Contrasting to our expectations, river models performed significantly better than lake models,  
227 in spite of a presumably higher number of confounding factors for rivers than for lakes. This  
228 better performance can be explained by the specific nature of riverine ecosystems: rivers  
229 feature various niche and habitat factors that can be altered by multiple stressors (e.g. water  
230 quality, hydrology, benthic habitats), and the riverine fauna is sensitive to the impacted  
231 oxygen conditions, which may “collect” the effects of a variety of stressors into a single  
232 gradient. Oxygen, however, is rarely measured in a meaningful way in monitoring programs  
233 (including the daily maxima and minima) and was thus not considered as a stressor in our  
234 analysis. In contrast, lake phytoplankton seems less susceptible to the effects of multiple  
235 stressors, as long as nutrients are in the growth-limiting concentration range.

236 We expected a decreasing explanatory power with spatial scale, reflecting the increasing  
237 importance of confounding factors at large scales – this was partly supported. The variance in  
238 biological response explained by the paired-stressor models (expressed as marginal  $R^2$ )  
239 ranged between 0.05 and 0.88, with a median value of 0.18. These ranges differed  
240 significantly between experiments (median marginal  $R^2 = 0.40$ ) and basin or cross-basin  
241 studies (Mann-Whitney U-test,  $p < 0.001$ ), but basin and cross-basin studies did not differ  
242 from each other (combined median marginal  $R^2 = 0.17$ ; Figure 3A). The marginal  $R^2$  differed  
243 significantly between lakes and rivers, with river cases showing on average slightly higher  
244 explanatory power (lakes:  $R^2 = 0.14$ , rivers:  $R^2 = 0.23$ ; not shown). The importance of the  
245 interaction term (expressed as % $R^2$  change) was significantly higher for lakes than for rivers.  
246 For rivers, this importance tended to decrease with increasing scale of investigation, but  
247 differences between investigation scales were generally not significant (Figure 3B).

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

253



254

255 Figure 3: (A) Percent of biological variance explained by the paired stressors including their  
 256 interaction for the mesocosm experiments ( $n = 33$ ), basin study cases ( $n = 55$ ) and cross-  
 257 basin study cases ( $n = 93$ ), separately for lakes (white boxes) and rivers (grey boxes). Lakes  
 258 and rivers differed significantly only for the cross-basin studies (pairwise Bonferroni-  
 259 corrected Mann-Whitney U-test,  $p = 0.001$ ).

260 (B) Percent change in explained biological variance when interaction term is removed from  
 261 the model (in case of significant interaction term) for the mesocosm experiments ( $n = 11$ ),  
 262 basin study cases ( $n = 14$ ) and cross-basin study cases ( $n = 34$ ), separately for lakes (white  
 263 boxes) and rivers (grey boxes). None of the differences within investigation scales are  
 264 significant.

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

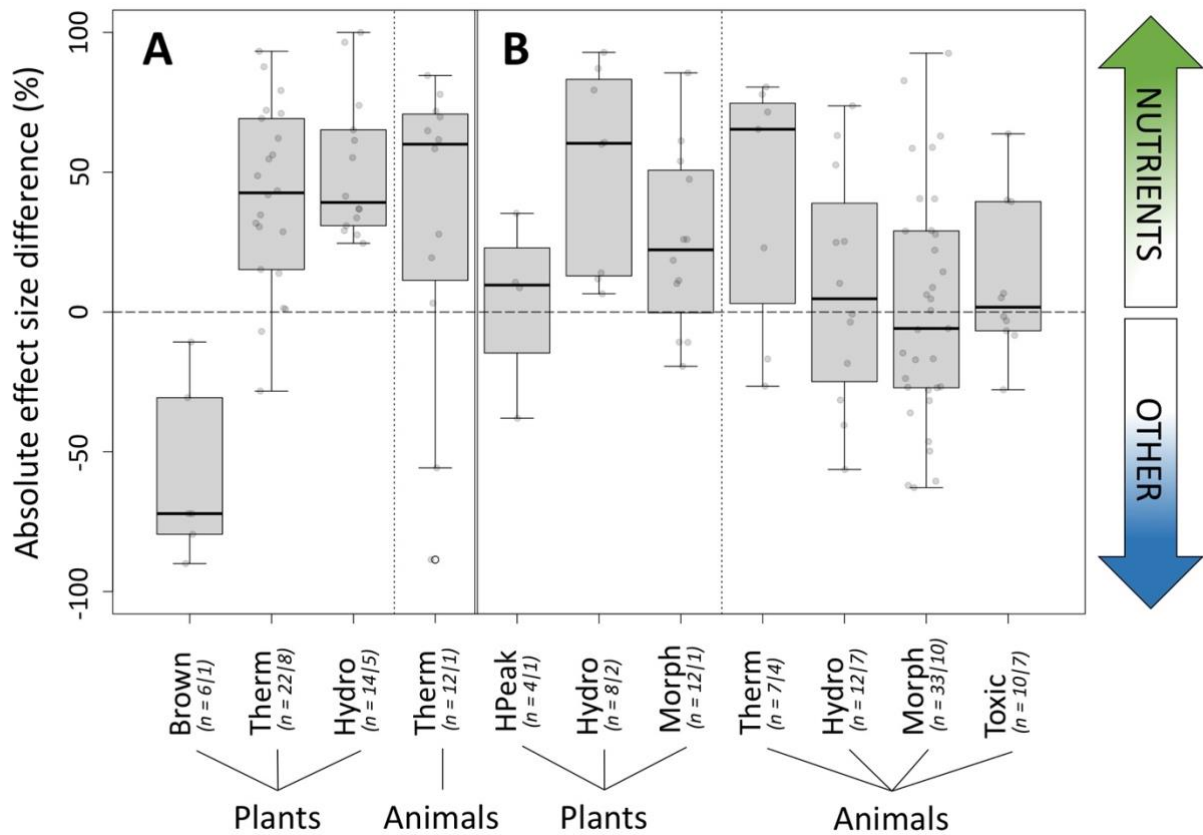
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### 268 ***Role of nutrient stress for lakes vs. rivers***

269 Finally, we supported the hypothesis that responses to nutrient stress is retarded by the  
 270 presence of secondary stressors in rivers more so than lakes where responses to nutrient  
 271 enrichment are strongest.

272 We identified eleven combinations of nutrient stress paired with another stressor, covering  
 273 morphological, hydrological (including hydropeaking), thermal, toxic and chemical stress  
 274 (brownification). The number of analytical cases in each stressor combination ranged from  
 275 four to 33, with the combinations including hydropeaking and brownification stress  
 276 exclusively comprising data collected at the experimental scale. All other combinations  
 277 comprised data from up to ten different studies, most of which originated from two or more

278 spatial scales. Best represented were the combinations of nutrient stress paired with thermal  
 279 stress affecting autotrophs in lakes, and nutrient stress paired with morphological stress  
 280 affecting heterotrophs in rivers (Figure 4).



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

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

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

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

302 The dominance of nutrients over secondary stressors in lakes applies, surprisingly, also to  
303 temperature stress, which is often considered to interact in a synergistic way with  
304 eutrophication in rivers and lakes (Moss et al., 2011). One mesocosm experiment even  
305 demonstrated an antagonistic relationship at high nutrient stress (Richardson et al., 2019).  
306 Water temperature may affect lake communities by modifying the food-web structure, e.g. by  
307 supporting planktivorous fish (Jeppesen et al., 2010); the two temperature-driven functional  
308 fish-trait responses perhaps indicate the emergence of such modification.

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

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

324 The pattern of nutrient stress outweighing the effects of hydrological or morphological stress  
325 for river autotrophs is similar to lakes. Again, “the response variable matters” (Segner et al.,  
326 2014) – while river autotrophs have shown to be responsive to hydrological or morphological  
327 stress elsewhere (e.g. Baattrup-Pedersen & Riis, 1999; Schneider et al., 2018), their effect size  
328 was overruled by the nutrient signal in our study. In one case, however, hydropeaking  
329 outweighed the nutrient signal on river autotrophs. The immediate mechanical effect of flush  
330 flows is very pervasive, but presumably limited to short river stretches downstream of a  
331 hydropower dam.

332 By contrast, river heterotrophs were equally affected by paired stressors when nutrient  
333 enrichment was paired with either hydrological, morphological or (to a lesser degree) thermal  
334 stress. This indicates that these paired stressors co-act on oxygen contents or habitat  
335 availability. In our study, we found small but consistent antagonistic interactions, in particular

336 for channelized rivers, probably due to increased current velocities facilitating the oxygen  
337 availability. In the case of toxic stress our conjectures on mechanistic pathways remain  
338 speculative. The diversity of compound-specific modes of action across xenobiotics in each  
339 mixture renders toxic stress a multi-stressor issue in itself (de Zwart & Posthuma, 2005).  
340 Notably, the toxic effects of ambient mixtures were clearly discernible in all respective  
341 paired-stressor case studies (n = 17), despite the likely different stressor modes of action  
342 (Busch et al., 2016). Given the lack of adequate monitoring of xenobiotics, our findings  
343 support that toxic effects in the multiply-stressed freshwaters of Europe are largely  
344 underestimated (Malaj et al., 2014).

345

## 346 **Conclusions**

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

360

## 361 **Author contributions**

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

368 **Data and computer code availability statement:** All data generated or analysed during this study are included  
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375

376

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529

## 530 **Methods**

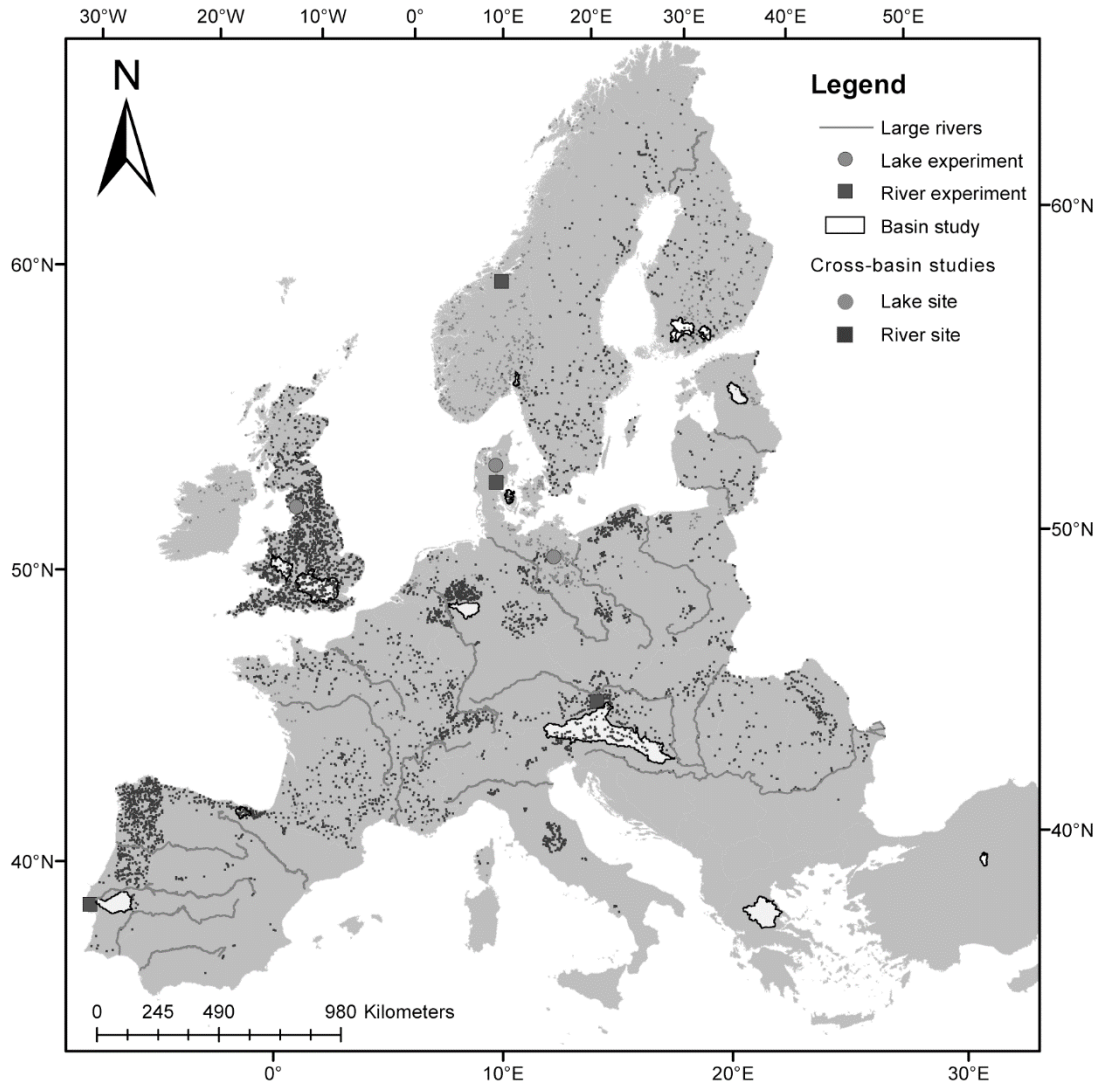
### 531 *Case studies*

532 The 45 studies analysed here covered selected European lakes and rivers (including one  
533 estuary) and addressed three spatial scales of investigation: manipulative multi-stressor  
534 experiments in mesocosms and flumes, river basin studies and cross-basin studies (Figure 1,  
535 *Supplementary Material Table S1*). Several studies contributed to multiple analytical cases,  
536 depending on the available combinations of stressors and responses. The number of cases  
537 totalled 180.

538 The manipulative experiments were conducted within the framework of the European MARS  
539 project (Hering et al., 2015), involving three lake mesocosm facilities in Denmark, Germany  
540 and United Kingdom, and four artificial flume facilities in Norway, Denmark, Austria and  
541 Portugal. The experiments applied controlled pairs of stressors to study the effects on selected  
542 biological response variables. Overall, 33 analytical cases and 1,448 sample replicates were  
543 considered in our analysis, with a median number of 79 sample replicates per study (range: 20  
544 to 768).

545 The MARS project also contributed data on 14 river basin studies selected to cover the main  
546 European regions and their representative stressor combinations (Hering et al., 2015). Based  
547 on harmonised analytical protocols (Feld et al., 2016) the multi-stressor effects were analysed  
548 using comprehensive datasets derived from regional monitoring programmes. For this study  
549 we chose the most relevant paired-stressor response combinations from four lake catchments

550 and ten river catchments that together provided 55 analytical cases with an overall number of  
551 2,086 samples (median number of samples per basin: 124, range: 19 to 525).  
552



553  
554 Figure 1: Location of the seven experimental facilities, 14 basin studies and sampling sites  
555 (small dots) for the 22 cross-basin studies of lakes and rivers across Europe (see  
556 *Supplementary Material Table S1* for details).  
557

558 The 22 cross-basin studies included in this analysis mostly originated from research activities,  
559 in which aquatic monitoring data was collated at regional, national or international scale to  
560 investigate biological effects of various stressors (e.g. Moe et al., 2008; 2013). The spatial  
561 coverage of these studies exceeded a single river basin, and commonly spanned large numbers  
562 of lakes and rivers. The number of analytical cases amounted to 92, comprising 14,486  
563 samples (median number of samples per study: 374, range: 40 to 3,706).  
564

565 ***Stressor variables***

566 Within this study we considered a “stressor” as any external factor modified by human  
567 intervention, which potentially moves a receptor (*i.e.* response variable) out of its normal  
568 operating range (Sabater et al., 2019). The analysed stressor variables belonged to six stress  
569 categories (see also Birk, 2019): (1) nutrient stress (145 cases), including experimental  
570 addition or field sampling of phosphorus or nitrogen compounds in the water; (2)  
571 hydrological stress (63 cases), including experimental manipulation or field measurement of  
572 high flow (e.g. high flow pulse duration), low flow (e.g. residual flow), water level change,  
573 non-specific flow alteration (e.g. mean summer precipitation as proxy) and hydropeaking; (3)  
574 morphological stress (61 cases), including experimental treatment or field survey of river  
575 channel, bank and floodplain modification, and river connectivity disruption; (4) thermal  
576 stress (57 cases), including experimental heating or field measurement of water temperature  
577 (or air temperature as a proxy); (5) toxic stress of mixtures of xenobiotic compounds (18  
578 cases), expressed as the multi-substance Potentially Affected Fraction (de Zwart & Posthuma,  
579 2005), Toxic Units (Liess & von der Ohe, 2005) or runoff potential (von der Ohe &  
580 Goedkoop, 2013); and (6) other chemical stress (16 cases), including experimental application  
581 of humic substances and field samples of water quality determinants (e.g. dissolved oxygen,  
582 chloride, biological oxygen demand).

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

598 most strongly, either based on their intensity or based on previous studies on the relevance of  
 599 the stressors in the region.

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

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

Paired stressors	Lakes	Rivers
Nutrient   Hydrological	14	24
Nutrient   Morphological	0	46
Nutrient   Thermal	34	9
Nutrient   Toxic	1	10
Nutrient   Chemical	6	1
Hydrological   Morphological	0	6
Hydrological   Thermal	3	11
Hydrological   Chemical	0	5
Morphological   Morphological <sup>A</sup>	0	1
Morphological   Toxic	0	5
Morphological   Chemical	0	2
Toxic   Chemical	0	2

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

607

608 ***Response variables***

609 A variety of organism groups was investigated, including phytoplankton (55 cases); benthic  
 610 flora, *i.e.* macrophytes or phytobenthos (22); benthic invertebrates (66 cases); and fish (37  
 611 cases). Within the 180 cases, four categories of biological response variables were used: (1)  
 612 biodiversity (76 cases), including indices reflecting the proportion of a taxonomic group  
 613 within the assemblage (e.g. percentage of Chlorophyta in the benthic algal assemblage), taxon  
 614 richness, Ecological Quality Ratios (as derived from ecological classification tools for the  
 615 European Water Framework Directive) and taxon-sensitivity indices (e.g. saprobic indices,  
 616 ASPT); (2) biomass/abundance (54 cases), including biomasses or total abundances of  
 617 phytoplankton or fish, chlorophyll *a* concentrations or cyanobacterial biomass; (3) functional  
 618 traits (38 cases), including the absolute or relative abundance of functional groups such as  
 619 habitat preferences, feeding types or life cycles and trait-based quality indices (e.g. SPEAR;  
 620 von der Ohe & Goedkoop, 2013); and (4) behaviour (12 cases), exclusively including drift  
 621 rates of invertebrates and stranding rates of juvenile fish. While the response category

622 “biodiversity” covered all organism groups, the category “biomass/abundance” was limited to  
623 phytoplankton (except for two cases each with benthic algae and fish), and both “functional  
624 traits” and “behaviour” were limited to animals (invertebrates and fish).

625

### 626 *Statistical analysis*

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

$$629 \quad E(Y) = g^{-1}(a \cdot x_1 + b \cdot x_2 + c \cdot x_1 \cdot x_2),$$

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

635

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

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

643 All continuous variables (responses and stressor variables) were standardized by  
644 transformation to approach normal distribution. A version of the Box-Cox transformation was  
645 used (Box & Cox, 1964), including an offset to ensure strict positivity (all values  $> 0$ ).

646 Transformed data was inspected for normality by plotting frequency histograms. If the data  
647 exhibited skewness because of extreme outliers, these outliers were excluded from the  
648 analysis. Following Box-Cox transformation, each transformed variable was centred and  
649 scaled, so they had a mean of zero and a variance of one.

650

### 651 *Choice of regression model*

652 The type of statistical model used to fit the paired-stressor response data depended on two  
653 major considerations: (1) The type of analytical case, which determined whether a GLM was  
654 sufficient or if a generalised linear mixed model (GLMM) with random effects was needed  
655 (see Table 2 for the criteria). GLMMs were used when the data structure included grouping

656 factors, such as experimental block, site or year. In most cases the analyses included random  
 657 effects in the standard way as random intercept terms. However, if considered appropriate  
 658 (e.g. due to large data volume) models with both random intercepts and slopes were used. (2)  
 659 The type of response data, which determined the link function and error distribution of the  
 660 model (Gaussian errors and an identity link for continuous data, Poisson errors and a  
 661 logarithmic link for count data). GLMs were fitted with the base R libraries and GLMMs  
 662 were fitted with the *lme4* and *lmerTest* R packages.

663

664 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.

665

666 *Testing and correcting for residual autocorrelation*

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

676

677 *Model evaluation*

678 To evaluate our models, residuals were examined for correlation to the fitted values and  
 679 deviation from the normal distribution (Shapiro-Wilk Test). We excluded 28 models where  
 680 residuals were correlated with fitted values ( $R > 0.35$ ) and non-normally distributed. Model fit  
 681 was evaluated as the marginal  $R^2$ , *i.e.* the proportion of variance explained by the models  
 682 fixed effects, ignoring the contribution of any random effects (Nakagawa & Schielzeth,  
 683 2013). We excluded only models with marginal  $R^2 < 0.05$ . Model fixed effects (main effects  
 684 of both stressors and their interactions) were evaluated from the standardized partial

685 regression coefficients and their significance (*t* Test), in the following referred to as  
686 standardised effect sizes (SES).  
687 Several case studies allowed for analysing different response variables within the same  
688 organism group or across different organism groups, using datasets from the same river  
689 basin(s). To avoid redundancy in paired-stressor responses we checked that model results  
690 differed in marginal  $R^2$  and fixed effects.

691

#### 692 *Importance of the interaction term*

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

696

#### 697 *Interaction classification*

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

711

#### 712 *Synthesis analysis*

713 We identified the frequency of analytical cases with a significant interaction term  
714 (“interactive”), or where one (“dominance”) or both stressors (“additive”) were significant but  
715 not the interaction term. The importance (share) of these three types of stressor interrelations  
716 was compared between ecosystems (from studies of lakes or rivers) and between scales (from  
717 experiments, basin and cross-basin studies). These comparisons were tested using the Chi-  
718 squared test. The range of marginal  $R^2$  values from full models were compared between study



719 scales, as well as the  $\%R^2$  change for those cases with significant interaction terms. These  
720 comparisons were tested for significant differences using pairwise Mann-Whitney U-tests  
721 with Bonferroni correction for multiple comparisons.

722

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

729

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