1 **Impacts of multiple stressors on freshwater biota across spatial scales and ecosystems**

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76 **Abstract**

77   Climate and land-use change drive a suite of stressors that shape ecosystems and interact

78 to yield complex ecological responses, *i.e.* additive, antagonistic and synergistic effects.

79 Currently we know little about the spatial scale relevant for the outcome of such interactions

80 and about effect sizes. This knowledge gap needs to be filled to underpin future land

81 management decisions or climate mitigation interventions, for protecting and restoring

82 freshwater ecosystems. The study combines data across scales from 33 mesocosm

83 experiments with those from 14 river basins and 22 cross-basin studies in Europe producing

84 174 combinations of paired-stressor effects on a biological response variable. Generalised

85 linear models showed that only one of the two stressors had a significant effect in 39% of the

86 analysed cases, 28% of the paired-stressor combinations resulted in additive and 33% in

87 interactive (antagonistic, synergistic, opposing or reversal) effects. For lakes the frequency of

88 additive and interactive effects was similar for all spatial scales addressed, while for rivers

89 this frequency increased with scale. Nutrient enrichment was the overriding stressor for lakes,

90 generally exceeding those of secondary stressors. For rivers, the effects of nutrient enrichment

91 were dependent on the specific stressor combination and biological response variable. These

92 results vindicate the traditional focus of lake restoration and management on nutrient stress,

93 while highlighting that river management requires more bespoke management solutions.

Introduction

Multiple stressors are increasingly recognized as a major concern for aquatic ecosystems and for those organisations in charge of their management[1]. Stressors commonly interact to affect freshwater species, communities and functions, but the questions remain to which degree this evidence from experiments can be transferred to field conditions and how relevant stressor interactions are for ecosystem management[2]. Critically, no study has been conducted to systematically confirm the frequency of occurrence of multiple stressor interactions across spatial scales (*i.e.* from waterbody to continental scales) and ecosystem types (*i.e.* for rivers and lakes). Using the most comprehensive large-scale assessment of multiple stressor interactions to date, we show that dominance of a single stressor, namely nutrient enrichment, is still common in lakes, while for rivers stressor interactions are much more relevant, demanding for more complex and informed management decisions.

Formerly, single, intense and well characterised stressors, such as organic and nutrient pollution from point sources, dominated freshwater ecosystem responses[3]. However, as these formerly dominant stressors are now controlled and others emerge, recent large-scale analyses have shown that freshwater ecosystems are exhibiting novel ecological responses to different stressors[4,5,6].

For the simplest case of two stressors acting simultaneously, three main types of effects can be conceptually distinguished[7]: (i) Only one of the two stressors has notable ecological effects so that the effects of Stressor A outweigh those of Stressor B or vice versa (stressor dominance); (ii) the two stressors act independently such that their joint effect is the sum of the individual effects (additive effects); (iii) a stressor either strengthens or weakens the effects of the other (interaction). However, there is a striking lack of information on the frequency of occurrence of these effect types across spatial scales (*i.e.* from individual waterbodies to a whole continent) and ecosystem types (rivers *vs.* lakes)[8].

Here we use a combined empirical-exploratory approach and a common quantitative framework to analyse a large set of original and compiled data on combinations of stressor pairs (explanatory variables), with each of them related to a biological response variable. We build on conceptual understanding of ecological responses to stressor interactions[9,10,11] to structure an empirical modelling approach, using generalised linear modelling (GLM) and 174 stressor combinations with single biological responses from more than 18,000 observations (Figure 1). Outputs of the GLMs were interpreted to identify the frequency of cases with stressor dominance, additive stressor relationships and stressor interactions

127   (synergistic or antagonistic), stratified by ecosystem type (lake or river) and spatial scale

128   (experiments, basin studies, cross-basin studies).

129   With this approach we addressed four questions: (1) How frequent are the three different

130   types of stressor effects in lakes and rivers? We expected a high share of additive and

131   interactive relationships in both lakes and rivers, as intense stressors obscuring the effects of

132   secondary stressors rarely occur nowadays[12,13]. (2) To what extent do ecosystem type (lake *vs.*

133   river) and spatial scale influence the combined effects of two stressors? We expected more

134   frequent stressor interactions in rivers, as their greater heterogeneity increases the likelihood

135   for two stressors to have an impact[14]. We further expected more frequent stressor interactions

136   in small-scale studies (*i.e.* in mesocosms), as these are less influenced by confounding

137   factors[15,16]. (3) What is the influence of ecosystem type (lake *vs.* river) and spatial scale on the

138   explanatory power of two stressors and their interaction? We expected the explanatory power

139   to be lower for rivers because of greater heterogeneity and thus potentially confounding

140   factors in comparison to lakes[17]. We also expected a decreasing explanatory power of

141   individual stressors and their interactions with spatial scale, reflecting the increasing

142   importance of confounding factors at large scales[18,19]. (4) Is nutrient enrichment still the most

143   prominent stressor affecting European aquatic ecosystems as suggested by [20], despite the

144   progress in wastewater cleaning, and does the importance of co-stressors differ between lakes

145   and rivers? We expected a dominating effect of nutrient stress in lakes due to the dominance

146   of primary producers and a greater relevance of hydrological and morphological changes in

147   rivers[21,22].

148   Our study pursues a phenomenological approach (*sensu* [23]) and seeks to disclose stressor

149   interrelations under "real-world" conditions, contributing to solve some of the pertinent issues

150   in ecosystem management[2].

151

152   **Results and discussion**

153   *Impact of ecosystem type on stressor effect types*

154   Stressor interactions are regularly reported from the available synthesis papers on multiple

155   stressors in freshwater ecosystems[8,10]. Therefore, we hypothesised that high proportions of

156   both lake and river case studies would indicate additive or interactive paired-stressor

157   relationships – this was not supported. Among the 174 cases, 39% of models indicated single

158   stressor dominance, 28% indicated additive paired-stressor effects, and 33% indicated paired

159   stressors interacting significantly (Figure 2; see also *"Data and code availability"*).

160   We expected a higher proportion of river cases to exhibit stressor interactions, compared

161 to lakes, as a result of greater habitat heterogeneity in rivers[14] – this was supported. The

162 proportions of effect types differed between lakes (62% dominance, 16% additive, 22%

163 interactive) and rivers (28% dominance, 33% additive, 39% interactive; see Figure 2) (Chi-

164 squared test, $p < 0.001$).

165   We assumed the different frequency of effect types between lakes and rivers might have

166 been rooted in different frequencies of the stressor types investigated[8]: nutrient enrichment

167 was one of the two stressors in 95% of the lake cases, but only in 76% of the river cases.

168 However, these differences between lakes and rivers in the share of stressor dominance

169 remain if only cases with nutrient enrichment are considered: 60% (lakes) *vs.* 27% (rivers),

170 compared to 62% (lakes) *vs.* 29% (rivers) considering all cases.

171   There were also differences between lake and river cases in the frequency of organism

172 groups considered as response variables: for lakes, phytoplankton was the most frequently

173 used organism group (76% of the cases) followed by fish (22%), while in rivers benthic

174 invertebrates (52% of the cases) were dominating and fish were used in 21% of the cases.

175 However, when only regarding cases with fish as response variable, the differences in the

176 share of dominant effect types is still high with 75% (lakes) *vs.* 32% (rivers). We therefore

177 conclude that the observed differences in effect types between lakes and rivers are neither

178 rooted in differences between the stressors nor in the organism groups investigated.

179   An alternative explanation is the different exposure of organisms inhabiting rivers and

180 lakes to stressor effects. While freshwater ecosystems in general are sinks "collecting"

181 anthropogenic stressors, the much higher shoreline length of rivers multiplies the effects of

182 human activities in the catchment, such as land and water uses[24,25]. This results in an

183 increased exposure to hydrological and morphological stressors, the latter also being more

184 relevant in rivers due to their primarily benthic habitats and assemblages[26]. This is also

185 expected for toxic substances that can act more directly in (small) rivers, as much lower

186 compound quantities are needed to reach toxic concentrations[27]. Within the 58 cases where

187 models included a significant interaction term, the combinations of nutrients with toxic or

188 morphological stress represented the greatest proportion of confirmed interaction effects (ratio

189 of 0.45 or 0.43, respectively; only combinations with total number of cases > 5; no significant

190 correlation between total number of cases and share of interactive cases). All but one of the

191 cases with toxic substances as a stressor were rivers.

Impact of spatial scale on stressor effect types

We expected that the frequency of interactions would decrease with spatial scale – this was not supported. While for lakes additive and interactive effects did not differ significantly between scales, for rivers the share of additive and interactive cases increased with scale (Chi-squared test, $p < 0.01$). Two contrasting mechanisms may explain this pattern: On the one hand, increasing spatial scale implies an increase in confounding factors (including stressors not addressed in this analysis and thus not tested), limiting the likelihood of detecting additive or interactive effects between the targeted stressors, as they may be masked by other factors not under investigation. On the other hand, increasing spatial scale implies longer stressor gradients. In fact, nutrient and hydrological stressor ranges significantly increase with scale (Kruskal-Wallis H-test, $p < 0.001$), enhancing the likelihood of additive or interactive stressor effects, which may only occur at certain stressor intensities. The latter holds true only if stressors are effective over the whole gradient length, e.g. the biological response does not level off at low or intermediate stressor levels (as in case of nutrient saturation[29,30]).

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

Though we are not aware of other studies comparing the effects of spatial scale on the explanatory power of stressor interactions models, the observed differences in the frequency of stressor interactions between experiments and field studies are in line with the synthesis studies of [8] and [10]. While the study of Jackson et al.[10] included only experiments and observed interactive or additive effect types in all cases considered, the study by Nõges et al.[8] focussed on field studies and interactive or additive effect types were only given for 50% of the river and 15% of the lake cases.

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

European lakes are generally in a better condition than European rivers[20] and are affected by a lower number of stressors[31]. Therefore, we expected the explanatory power of our models to be lower for rivers because of greater impact of stressors that have not been regarded (*i.e.* confounding factors)[8,32]. Contrasting to our expectations, however, river models performed significantly better than lake models. This better performance can be explained by the specific nature of riverine ecosystems: rivers feature various niche and habitat factors that can be altered by multiple stressors (e.g. water quality, hydrology, benthic habitats), and the riverine fauna is sensitive to the impacted oxygen conditions, which may "collect" the effects

227   of a variety of stressors into a single gradient. Oxygen, however, is rarely measured in a

228   meaningful way in monitoring programs (including the daily maxima and minima) and was

229   thus not considered as a stressor in our analysis. In contrast, lake phytoplankton seems less

230   susceptible to the effects of multiple stressors, as long as nutrients are in the growth-limiting

231   concentration range.

232   We expected a decreasing explanatory power with spatial scale, reflecting the increasing

233   importance of confounding factors at large scales[18,19] – this was partly supported. The

234   variance in biological response explained by the paired-stressor models (expressed as

235   marginal R^2) ranged between 0.05 and 0.88, with a median value of 0.19. These ranges

236   differed significantly between experiments (median marginal $R^2 = 0.38$), basin (median

237   marginal $R^2 = 0.22$) and cross-basin studies (median marginal $R^2 = 0.16$) (Bonferroni-

238   corrected Mann-Whitney U-test, $p < 0.05$; Figure 3A). The marginal R^2 differed significantly

239   between lakes and rivers, with river cases showing on average slightly higher explanatory

240   power (lakes: $R^2 = 0.15$, rivers: $R^2 = 0.22$; not shown). The importance of the interaction term

241   (expressed as $\%R^2$ change) was significantly higher for lakes than for rivers. For rivers, this

242   importance tended to decrease with increasing spatial scale of investigation, but differences

243   between investigation scales were generally not significant (Figure 3B). We are not aware of

244   a single other study targeting the role of spatial scale for the explanatory power of stressor

245   interaction models.

246   For the experiments addressed in our study, the high level of control on potentially

247   confounding factors can account for the on average greater explanatory power, when

248   compared to field studies. Furthermore, the experimental studies had lower numbers of

249   observations and less complex biological communities. Compared with this, factors such as

250   temperature variation are already temporally pronounced at basin-scale and the spatial

251   variation across basins is considerable.

252

253   *Role of nutrient stress for lakes vs. rivers*

254   The recent surveys by [8,20] suggest that eutrophication is still the most prominent stressor

255   affecting the biota of Europe's water, in particular lakes, while rivers are also strongly

256   affected by hydrological and morphological stressors. We therefore expected that responses to

257   nutrient stress is retarded by the presence of secondary stressors in rivers more so than lakes

258   where responses to nutrient enrichment are strongest[21,22] – this was supported.

259   We identified eleven combinations of nutrient stress paired with another stressor, covering

260   morphological, hydrological (including hydropeaking), thermal, toxic and chemical stress

261   (brownification) (Table 1). The number of analytical cases in each stressor combination

262 ranged from four to 33, with the combinations including hydropeaking and brownification

263 stress exclusively comprising data collected at the experimental scale. All other combinations

264 comprised data from up to ten different studies, most of which originated from two or more

265 spatial scales. Best represented were the combinations of nutrient stress paired with thermal

266 stress affecting autotrophs in lakes, and nutrient stress paired with morphological stress

267 affecting heterotrophs in rivers (Figure 4).

268

269 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 A	0	1
Morphological | Toxic	0	5
Morphological | Chemical	0	2
Toxic | Chemical	0	2

270 A Connectivity disruption and morphological river alteration

271

272   Nutrient stress often had the stronger effect in the paired-stressor models. Hence, nine of

273 the eleven combinations in lakes and rivers showed a positive %AES median, implying on

274 average stronger effects of nutrients compared to the other stressor. Five combinations even

275 showed a positive 25th percentile %AES, indicating that in three quarters of the cases in these

276 combinations nutrient effects outweighed the other stressors. This was evident for all lake

277 stressor combinations except nutrients and brownification represented by a single case study.

278 The few additional lake cases, for which the non-nutrient stressor was stronger, included

279 warming affecting cyanobacterial biomass in European lakes, and lithophilous or piscivorous

280 fish abundance in French lakes.

281   The dominance of nutrients over secondary stressors in lakes applies, surprisingly, also to

282 temperature stress, which is often considered to interact in a synergistic way with

283 eutrophication in rivers and lakes[33]. One mesocosm experiment even demonstrated an

284 antagonistic relationship at high nutrient stress[34]. Water temperature may affect lake

285 communities by modifying the food-web structure, e.g. by supporting planktivorous fish[35]; the

286 two temperature-driven functional fish-trait responses mentioned above perhaps indicate the
287 emergence of such modification.

288 Brownification is a remarkable exception from this general pattern but observed here only
289 in a single case study. It strongly superimposes the effects of nutrient stress, in particular by
290 decreasing light transmission in the pelagic zone, which inhibits productivity despite excess
291 nutrient concentrations (opposing interaction) and favours mixotrophic phytoplankton
292 species. Brownification is triggered by global warming and wetter climate, and becomes
293 increasingly relevant in boreal regions, as it originates from dissolved organic carbon in
294 leachates of bogs and permafrost soils mineralising due to increasing temperatures and
295 flushing, and the recovery from acidification[36,37].

296 Rivers generally showed a more heterogeneous pattern: nutrients clearly affected
297 autotrophs more strongly when paired with hydrological or morphological stress, and
298 heterotrophs when paired with thermal stress. The few river cases in these combinations, for
299 which the non-nutrient stressor was stronger, included fine sediment influx affecting
300 macrophyte and diatoms in UK rivers, and temperature increase affecting sensitive
301 invertebrate taxa in Greek rivers. All other combinations were more ambiguous, with the
302 %AES median being almost zero, indicating stressor effects of roughly equal size.

303 The pattern of nutrient stress outweighing the effects of hydrological or morphological
304 stress for river autotrophs is similar to lakes. Here, "the response variable matters"[38] – while
305 river autotrophs have shown to be responsive to hydrological or morphological stress
306 elsewhere (e.g. [39,40]), their effect size was overruled by the nutrient signal in our study. In one
307 case, however, hydropeaking outweighed the nutrient signal on river autotrophs. The
308 immediate mechanical effect of flush flows is very pervasive, but presumably limited to short
309 river stretches downstream of a hydropower dam.

310 By contrast, river heterotrophs were equally affected by paired stressors when nutrient
311 enrichment was paired with either hydrological, morphological or (to a lesser degree) thermal
312 stress. This indicates that these paired stressors co-act on oxygen contents or habitat
313 availability. In our study, we found small but consistent antagonistic interactions, in particular
314 for channelized rivers, probably due to increased current velocities facilitating the oxygen
315 availability. In the case of toxic stress our conjectures on mechanistic pathways remain
316 speculative. The diversity of compound-specific modes of action across xenobiotics in each
317 mixture renders toxic stress a multi-stressor issue in itself[41]. Notably, the toxic effects of
318 ambient mixtures were clearly discernible in all respective paired-stressor case studies
319 (n = 17), despite the likely different stressor modes of action[42]. Given the lack of adequate

320 monitoring of xenobiotics, our findings support that toxic effects in the multiply-stressed

321 freshwaters of Europe are largely underestimated[43].

322   In summary, nutrient enrichment overrules the effects of most other stressors in lakes,

323 while the situation in rivers is more complex with plants being more strongly affected by

324 nutrients, while animals were equally affected by nutrient enrichment and other stressors.

325

326 **Conclusions**

327   Our study supports the conjecture that eutrophication is still the most relevant stressor

328 affecting many lakes, irrespective of the spatial scale considered. Other stressors are

329 subordinate but may reveal notable effects if interacting with nutrients. These deserve special

330 attention if antagonistic (e.g. lake brownification) and synergistic interactions (e.g. climate

331 warming) can be expected that control the overall nutrient effect on phytoplankton. Relevant

332 stressors and stressor combinations are more variable in rivers and more strongly affected by

333 spatial scales. While river autotrophs are mainly impacted by nutrients, heterotrophs seem to

334 be mainly influenced by oxygen availability that is impaired by a range of stressors (pollution,

335 warming, flow reduction and fine sediment entry) on top of nutrient enrichment. While

336 reduction of nutrient stress is most relevant for lakes, in particular under the conditions of

337 climate warming, rivers require mitigation measures addressing several stressors

338 simultaneously. Options include the establishment of woody riparian buffer strips that address

339 several stressors (eutrophication, hydromorphological degradation) simultaneously.

Methods

Case studies

The 45 studies analysed here covered selected European lakes and rivers (including one estuary) and addressed three spatial scales of investigation: manipulative multi-stressor experiments in mesocosms and flumes, river basin studies and cross-basin studies (see Figure 1, *Supplementary Table 1*). Several studies contributed to multiple analytical cases, depending on the available combinations of stressors and responses. The number of cases totalled 174.

The manipulative experiments were conducted within the framework of the European MARS project[44], involving three lake mesocosm facilities in Denmark, Germany and United Kingdom, and four artificial flume facilities in Norway, Denmark, Austria and Portugal. The experiments applied controlled pairs of stressors to study the effects on selected biological response variables. Overall, 30 analytical cases and 1,498 sample replicates were considered in our analysis, with a median number of 79 sample replicates per study (range: 20 to 768).

The MARS project also contributed data on 14 river basin studies selected to cover the main European regions and their representative stressor combinations[44]. Based on harmonised analytical protocols[29] the multi-stressor effects were analysed using comprehensive datasets derived from regional monitoring programs. For this study we chose the most relevant paired-stressor response combinations from four lake catchments and ten river catchments that together provided 52 analytical cases with an overall number of 2,114 samples (median number of samples per basin: 97, range: 19 to 525).

The 22 cross-basin studies included in this analysis mostly originated from research activities, in which aquatic monitoring data was collated at regional, national or international level to investigate biological effects of various stressors (e.g.[45,46]). The spatial coverage of these studies exceeded a single river basin, and commonly spanned large numbers of lakes and rivers. The number of analytical cases amounted to 92, comprising 14,486 samples (median number of samples per study: 374, range: 40 to 3,706).

Stressor variables

Within this study we considered a "stressor" as any external factor modified by human intervention, which potentially moves a receptor (*i.e.* response variable) out of its normal operating range[47]. The analysed stressor variables belonged to six stress categories (see also[31]): (1) nutrient stress (142 cases), including experimental addition or field sampling of phosphorus or nitrogen compounds in the water; (2) hydrological stress (57 cases), including

experimental manipulation or field measurement of high flow (e.g. high flow pulse duration), low flow (e.g. residual flow), water level change, non-specific flow alteration (e.g. mean summer precipitation as proxy) and hydropeaking; (3) morphological stress (61 cases), including experimental treatment or field survey of river channel, bank and floodplain modification, and river connectivity disruption; (4) thermal stress (54 cases), including experimental heating or field measurement of water temperature (or air temperature as a proxy); (5) toxic stress of mixtures of xenobiotic compounds (18 cases), expressed as the multi-substance Potentially Affected Fraction[41], Toxic Units[48] or runoff potential[49]; and (6) other chemical stress (16 cases), including experimental application of humic substances and field samples of water quality determinants (e.g. dissolved oxygen, chloride, biological oxygen demand).

We always selected the stressor combinations most relevant for the respective broad lake or river type in the particular river basin or region, *i.e.* stressors that are most likely to affect biota due to their relative strength as compared to other regions and other stressors in the same region[50] (see *Supplementary Table 1*). These included stressors prevalent in European freshwaters[20] and addressed in previous multi-stressor studies[8]. In the experimental studies, stressor intensities were applied emulating "real-life" conditions of the respective water body type. For instance, flumes mimicking nutrient-poor calcareous highland rivers were enriched by ten-fold phosphorus increase towards mesotrophic conditions – a realistic scenario in case of alpine pasture use in the floodplains. Mesocosms mimicking eutrophic shallow lowland lakes were enriched by five-fold phosphorus increase towards hypertrophic conditions – a realistic scenario in intensively used agricultural lowland landscapes. In the field studies, stressor intensities reflected the existing gradient in the particular river basin or region. Thus, the stressor "forcings" in all study cases represent conditions typical for the specific lake or river type, the river basin (featuring certain land uses) and the European region. In several of the investigated basins or cross-basins, more than two stressors were acting; in these we selected those that were assumed to affect the biota most strongly, either based on their intensity or based on previous studies on the relevance of the stressors in the region.

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

Response variables

407

408   A variety of organism groups was investigated, including phytoplankton (52 cases);

409 benthic flora, *i.e.* macrophytes or phytobenthos (22); benthic invertebrates (63 cases); and fish

410 (37 cases). Within the 174 cases, four categories of biological response variables were used:

411 (1) biodiversity (76 cases), including indices reflecting the proportion of a taxonomic group

412 within the assemblage (e.g. percentage of Chlorophyta in the benthic algal assemblage), taxon

413 richness, Ecological Quality Ratios (as derived from ecological classification tools for the

414 European Water Framework Directive) and taxon-sensitivity indices (e.g. saprobic indices,

415 ASPT); (2) biomass/abundance (51 cases), including biomasses or total abundances of

416 phytoplankton or fish, chlorophyll *a* concentrations or cyanobacterial biomass; (3) functional

417 traits (38 cases), including the absolute or relative abundance of functional groups such as

418 habitat preferences, feeding types or life cycles and trait-based quality indices (e.g.

419 $SPEAR_{50}$); and (4) behaviour (9 cases), exclusively including drift rates of invertebrates and

420 stranding rates of juvenile fish. While the response category "biodiversity" covered all

421 organism groups, the category "biomass/abundance" was limited to phytoplankton (except for

422 two cases each with benthic algae and fish), and both "functional traits" and "behaviour" were

423 limited to animals (invertebrates and fish).

424

Statistical analysis

425

426   The relationship between the biological response and the paired stressors was investigated

427 for each individual analytical case by GLM based on the general formula

428    $E(Y) = g_{-1}(a \cdot x_1 + b \cdot x_2 + c \cdot x_1 \cdot x_2)$,

429   with *E(Y)* is the expected value of the biological response variable *Y*, *g* is the link function

430 that specifies how the response relates to the linear predictors, x_1 is the standardized

431 measurement of Stressor 1, x_2 is the standardized measurement of Stressor 2 and $x_1 \cdot x_2$ is the

432 interaction of the standardized measurements of Stressor 1 and Stressor 2. Parameters *a*, *b* and

433 *c* scale the effects of Stressors 1, 2 and their interaction, respectively.

434

Data processing of stressor and response variables

435

436   For large-scale data (multi-site biomonitoring data with no, or very short, temporal

437 component), long-term average measures of stress were used. For multi-year data (single or

438 multiple site), each year provided one stress measurement per site. When data was at higher

439 temporal resolution, it was pre-processed to an annual level. Categorical stressor variables

440 (e.g. experimental flow treatment) had only two levels representing stressed *vs.* unstressed

441 conditions.

442   All continuous variables (responses and stressor variables) were standardized by

443 transformation to approach normal distribution. A version of the Box-Cox transformation was

444 used[51], including an offset to ensure strict positivity (all values > 0). Transformed data was

445 inspected for normality by plotting frequency histograms. If the data exhibited skewness

446 because of extreme outliers, these outliers were excluded from the analysis. Following Box-

447 Cox transformation, each transformed variable was centred and scaled, so they had a mean of

448 zero and a variance of one.

449

450 *Choice of regression model*

451   The type of statistical model used to fit the paired-stressor response data depended on two

452 major considerations: (1) The type of analytical case, which determined whether a GLM was

453 sufficient or if a generalised linear mixed model (GLMM) with random effects was needed

454 (see *Supplementary Table 2* for the criteria). GLMMs were used when the data structure

455 included grouping factors, such as experimental block, site or year (see *"Data availability"*).

456 In most cases the analyses included random effects in the standard way as random intercept

457 terms. However, if considered appropriate (e.g. due to large data volume) models with both

458 random intercepts and slopes were used. (2) The type of response data, which determined the

459 link function and error distribution of the model (Gaussian errors and an identity link for

460 continuous data, Poisson errors and a logarithmic link for count data). GLMs were fitted with

461 the base R libraries and GLMMs were fitted with the *lme4* and *lmerTest* R packages.

462

463 *Testing and correcting for residual autocorrelation*

464   Where necessary, we tested whether model residuals showed strong evidence of spatial or

465 temporal autocorrelation, which can cause the statistical significance of model terms to be

466 exaggerated. This was only required when the analysis used GLMs without random effects,

467 since the random effects in the mixed effects models should account for grouping in space

468 and time. Autocorrelation in space or time was identified with Moran's tests on model

469 residuals and, where substantial autocorrelation was detected, the model was re-fitted

470 including a "trend surface" generated using a smoothing spline or polynomial functions[52].

471 This is a simple and generally effective way of reducing the influence of autocorrelation on

472 the model's stressor effects of interest.

Model evaluation

473   *Model evaluation*

474   To evaluate our models, residuals were examined for correlation to the fitted values and

475   deviation from the normal distribution (Shapiro-Wilk Test). We excluded 28 models where

476   residuals were correlated with fitted values ($R > 0.35$) and non-normally distributed. Model fit

477   was evaluated as the marginal R_2, *i.e.* the proportion of variance explained by the model fixed

478   effects, ignoring the contribution of any random effects[53]. We excluded models with marginal

479   $R_2 < 0.05$. Model fixed effects (main effects of both stressors and their interactions) were

480   evaluated from the standardized partial regression coefficients and their significance (*t* Test),

481   in the following referred to as standardised effect sizes (SES) (see *"Data availability"*).

482   Several case studies allowed for analysing different response variables within the same

483   organism group or across different organism groups, using datasets from the same river

484   basin(s). To avoid redundancy in paired-stressor responses we checked that model results

485   differed in marginal R_2 and fixed effects.

486

487   *Importance of the interaction term*

488   The importance of the interaction term was estimated by the change in marginal R_2 upon

489   dropping the interaction term, considered in cases with a significant interaction term, and

490   expressed as a percentage change relative to the full model's marginal R_2 (%R_2 change).

491

492   *Interaction classification*

493   The type of interaction was characterised from the SES and only considered in case of a

494   significant interaction term. We applied a simple classification scheme to the full model,

495   referring to both stressors' main effects and their interaction. This was based on the direction

496   of the interaction effect, relative to the directions of the main effects of both stressors.

497   Synergistic interaction was assigned when the SES for both stressors and their interaction all

498   had the same sign (*i.e.* all positive or all negative). Antagonistic interaction was assigned

499   when SES for both stressors had the same sign, but their interaction had the opposite sign.

500   Opposing interaction was assigned when the signs of the SES for both stressors differed, and

501   we distinguished between opposing contributing to either Stressor 1 (*i.e.* Stressor 1 and

502   interaction with same sign) or Stressor 2 (*i.e.* Stressor 2 and interaction with same sign).

503   Reversal interaction (*sensu*[9,10]) was assigned when the SES' sum for both stressors had a

504   value smaller than and a sign different from the interaction's SES (see *"Data availability"*).

Synthesis analysis

We identified the frequency of analytical cases with a significant interaction term ("interactive"), or where one ("dominance") or both stressors ("additive") were significant but not the interaction term. The importance (share) of these three types of stressor interrelations was compared between ecosystems (from studies of lakes or rivers) and between spatial scales (from experiments, basin and cross-basin studies). These comparisons were tested using the Chi-squared test. The distribution of marginal R_2 values from full models were compared between study scales, as well as the *%R_2* change for those cases with significant interaction terms. These comparisons were tested for significant differences using pairwise Mann-Whitney U-tests with Bonferroni correction for multiple comparisons.

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

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

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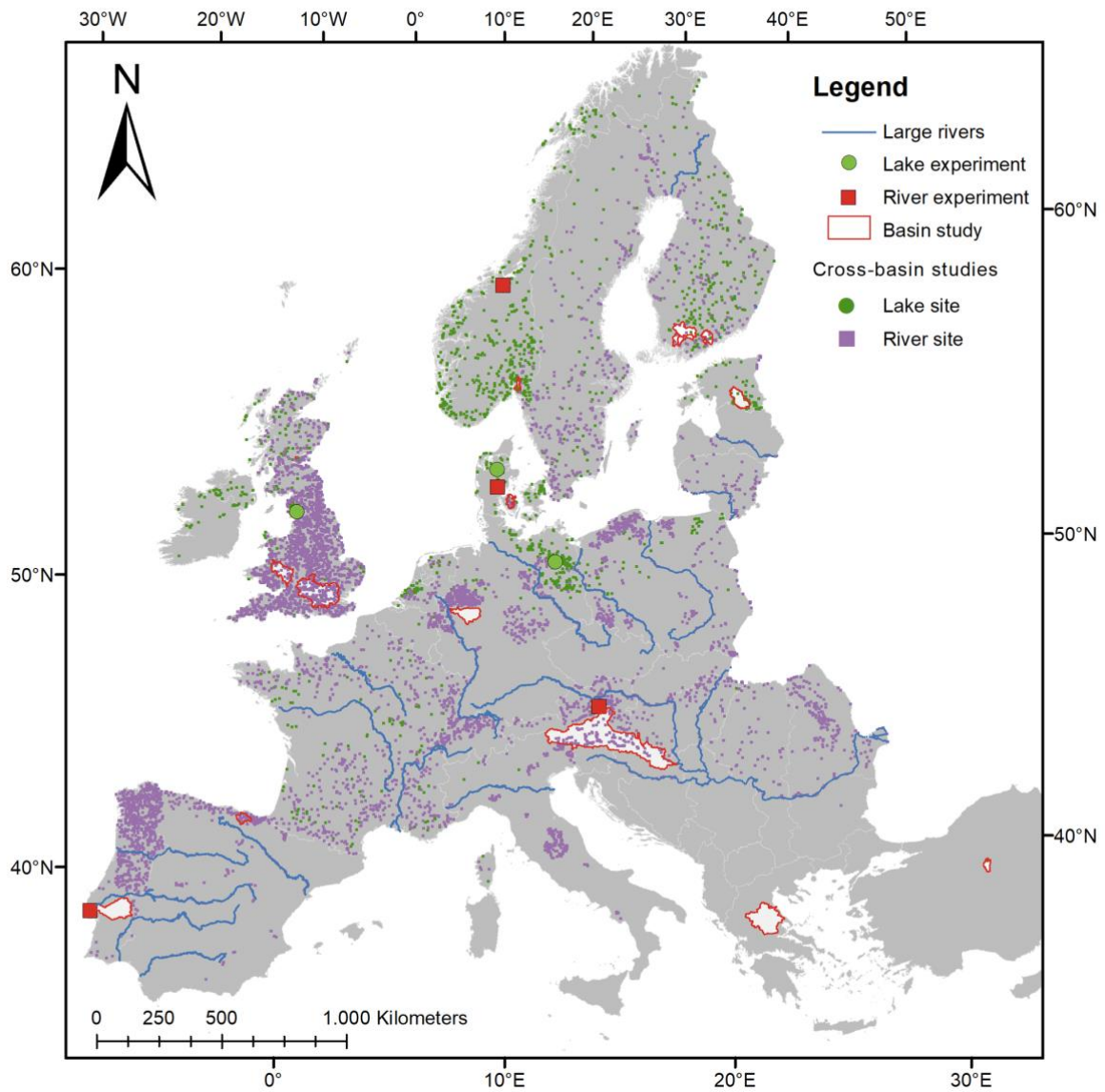
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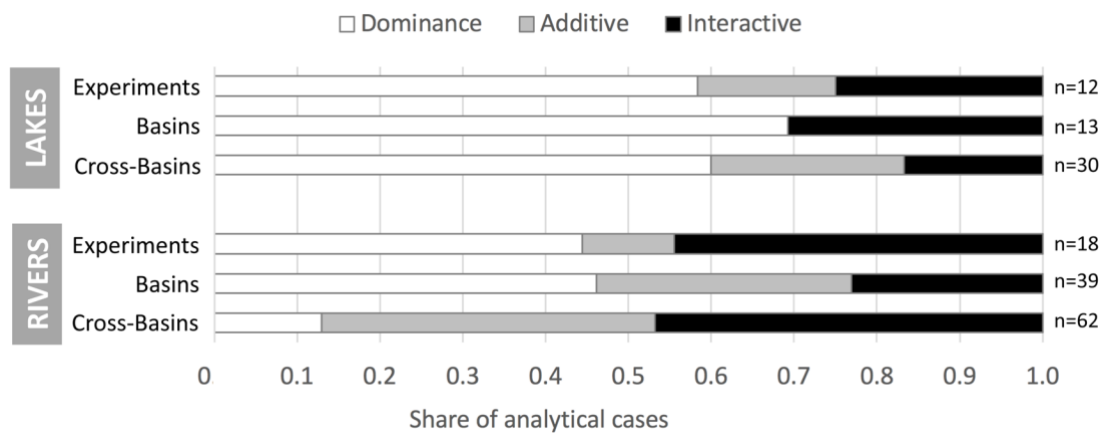
744

745 Figure 1: Location of the seven experimental facilities, 14 basin studies and sampling sites

746 (small dots) for the 22 cross-basin studies of lakes and rivers across Europe (see
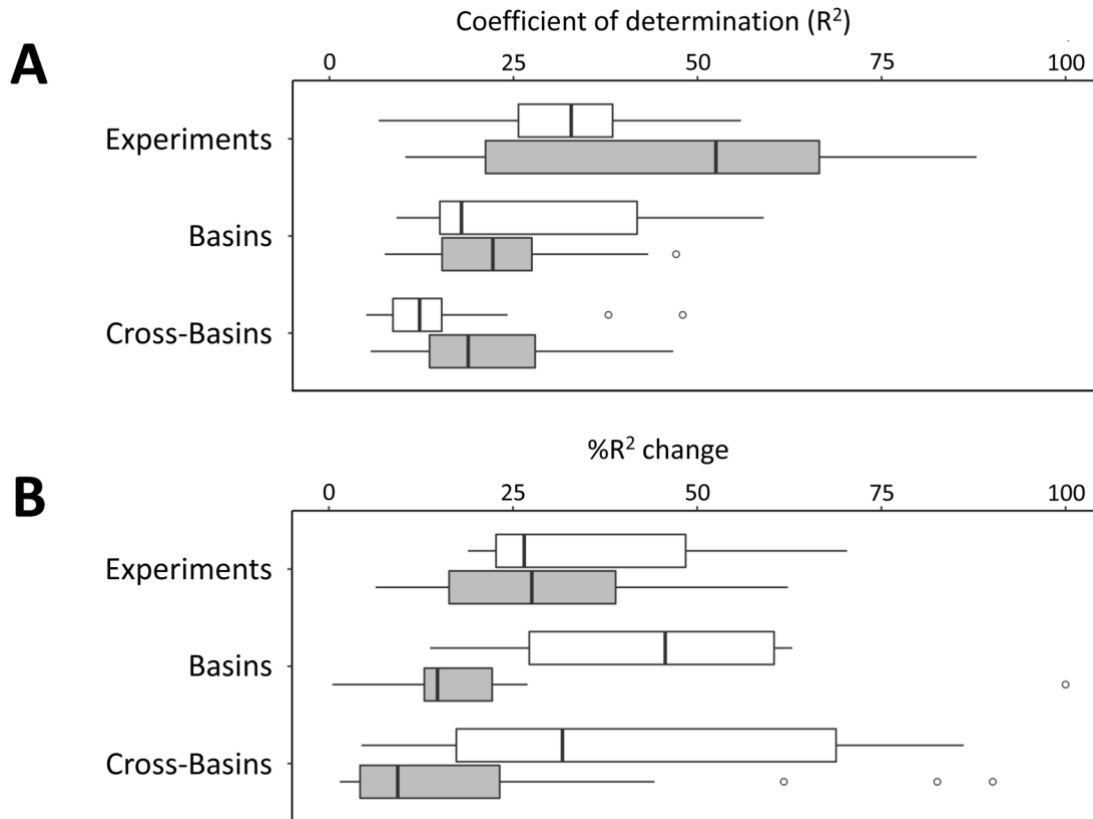
747 *Supplementary Table 1* for details).

748

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

Figure 3: (A) Percent of biological variance explained by the paired stressors including their interaction for the mesocosm experiments ($n = 30$), basin study cases ($n = 52$) and cross-basin study cases ($n = 92$), separately for lakes (white boxes) and rivers (grey boxes). Lakes and rivers differ significantly only for the cross-basin studies (pairwise Bonferroni-corrected Mann-Whitney U-test, $p = 0.001$).

(B) Percent change in explained biological variance when interaction term is removed from the model (in case of a significant interaction term) for the mesocosm experiments ($n = 11$), basin study cases ($n = 13$) and cross-basin study cases ($n = 34$), separately for lakes (white boxes) and rivers (grey boxes). None of the differences within spatial scales are significant.

Definition of box-plot elements: centre line = median; box limits = upper and lower quartiles; whiskers = 1.5x interquartile range; points = outliers.
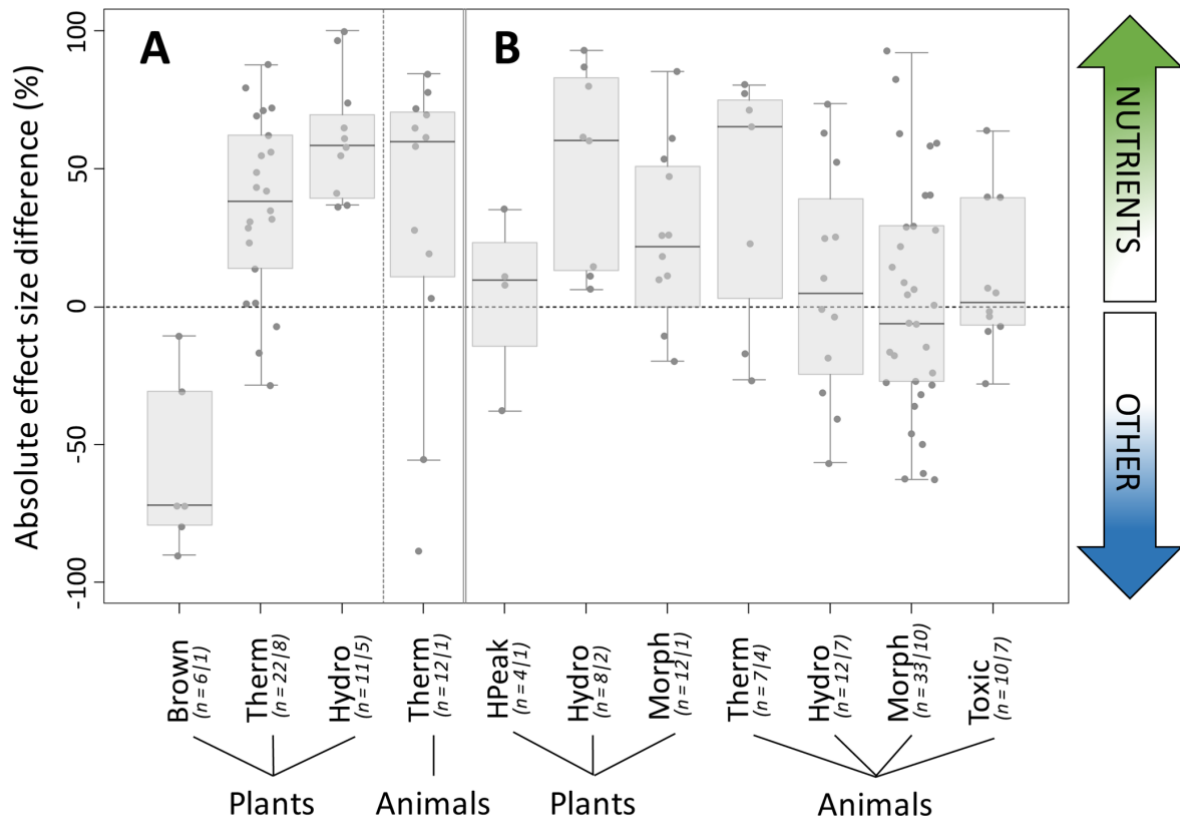
Figure 4: Range of absolute effect size differences (%AES) for nutrient stress and selected other stressors across case-studies from (A) lakes and (B) rivers. Positive %AES indicate stronger effects by nutrient stress, negative %AES indicate stronger effects by the other stressor on the biological response variable (subdivided into plants and animals) in the regression model.

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

Definition of plot elements: box centre line = median; box limits = upper and lower quartiles; whiskers = 1.5x interquartile range; points = individual analytical cases.