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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

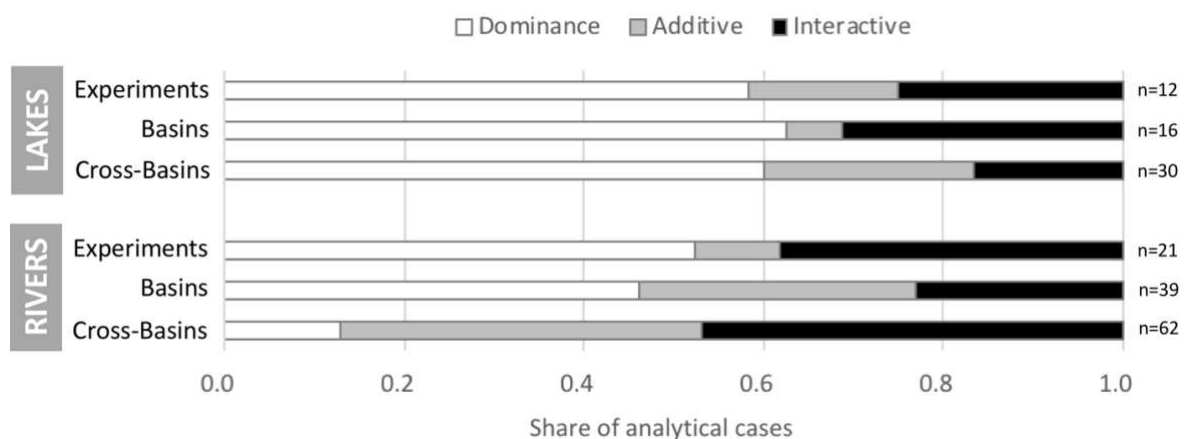
171 ***Impact of ecosystem type and scale on stressor effect types***

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.

224

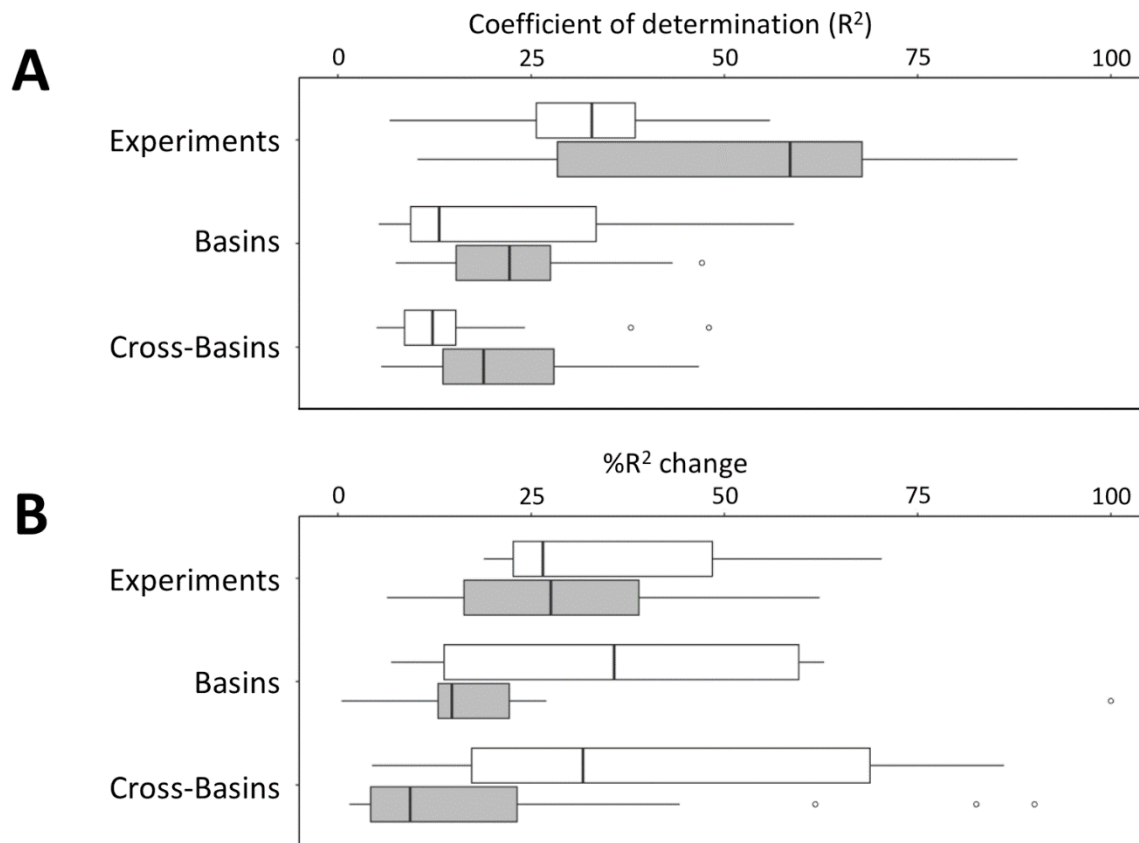
225 *Impact of ecosystem type and scale on the models' explanatory power*

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.

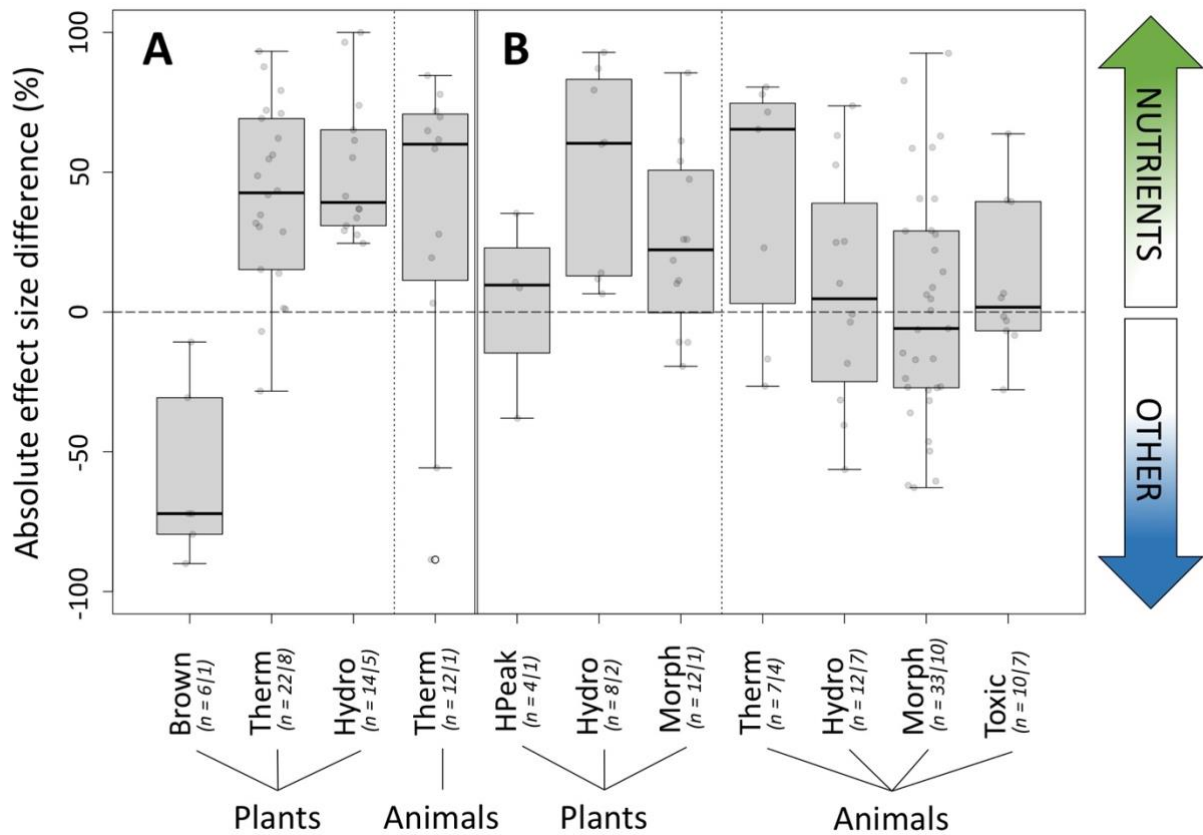
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268 ***Role of nutrient stress for lakes vs. rivers***

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
 282 Figure 4: Range of absolute effect size differences (%AES) for nutrient stress and selected
 283 other stressors across case-studies from (A) lakes and (B) rivers. Positive %AES indicate
 284 stronger effects by nutrient stress, negative %AES indicate stronger effects by the other
 285 stressor on the biological response variable (subdivided into plants and animals) in the
 286 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.

292

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 25th 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,
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375

376

377 **References**

- 378 Baattrup-Pedersen, A., Riis, T., 1999. Macrophyte diversity and composition in relation to
379 substratum characteristics in regulated and unregulated Danish streams. *Freshw. Biol.* 42,
380 375–385. <https://doi.org/10.1046/j.1365-2427.1999.444487.x>
- 381 Birk, S., 2019. Detecting and quantifying the impact of multiple stress on river ecosystems.
382 In: Sabater, S., Ludwig, R., Eloise, A. (Eds.), *Multiple Stress in River Ecosystems. Status,*
383 *Impacts and Prospects for the Future.* Academic Press, Oxford, pp. 235–253.
384 <https://doi.org/10.1016/B978-0-12-811713-2.00014-5>
- 385 Box, G.E.P., Cox, D.R., 1964. An analysis of transformations. *J. R. Stat. Soc. Ser. B* 26, 211–
386 252. <https://www.jstor.org/stable/2984418>
- 387 Busch, W., Schmidt, S., Kühne, R., Schulze, T., Krauss, M., Altenburger, R., 2016.
388 Micropollutants in European rivers: A mode of action survey to support the development of
389 effect-based tools for water monitoring. *Environmental Toxicology and Chemistry* 35, 1887–
390 1899. <https://doi.org/10.1002/etc.3460>
- 391 Chapman, D., Banin, L., Thackeray, S., Richardson, J., Henrys, P., Spears, B.M., Carvalho,
392 L., 2017. Analysing stressor-response relationships and interactions in multi-stressor
393 situations: a WP6 guidance document. Internal working document of the EU research project
394 MARS. CEH Edinburgh. <https://tinyurl.com/y4ac5wus>
- 395 Côté, I.M., Darling, E.S., Brown, C.J., 2016. Interactions among ecosystem stressors and their
396 importance in conservation. *Proc. R. Soc. B Biol. Sci.* 283, 20152592.
397 <https://doi.org/10.1098/rspb.2015.2592>
- 398 de Laender, F., 2018. Community- and ecosystem-level effects of multiple environmental
399 change drivers: beyond null model testing. *Glob. Chang. Biol.* 1–10.
400 <https://doi.org/10.1111/gcb.14382>
- 401 de Zwart, D., Posthuma, L., 2005. Complex mixture toxicity for single and multiple species:
402 Proposed methodologies. *Environ. Toxicol. Chem.* 24, 2665–2676.
403 <https://doi.org/10.1897/04-639R.1>
- 404 Dormann, C.F., McPherson, J., Araújo, M.B., Bivand, R., Bolliger, J., Carl, G., Davies, R.G.,
405 Hirzel, A., Jetz, W., Kissling, D., Kühn, I., Ohlemüller, R., Peres-Neto, P.R., Reineking, B.,
406 Schröder, B., M. Schurr, F., Wilson, R., 2007. Methods to account for spatial autocorrelation
407 in the analysis of species distributional data: A review. *Ecography (Cop.)*. 30, 609–628.
408 <https://doi.org/10.1111/j.2007.0906-7590.05171.x>
- 409 [EEA, 2018. European waters: Assessment of status and pressures 2018. EEA Report 7/2018,](https://www.eea.europa.eu/publications/state-of-water/)
410 [European Environment Agency, Copenhagen, 90 pp.](https://www.eea.europa.eu/publications/state-of-water/)
411 <https://www.eea.europa.eu/publications/state-of-water/>

- 412 Feld, C.K., Birk, S., Bradley, D.C., Hering, D., Kail, J., Marzin, A., Melcher, A., Nemitz, D.,
413 Pedersen, M.L., Pletterbauer, F., Pont, D., Verdonschot, P.F.M., Friberg, N., 2011. From
414 Natural to Degraded Rivers and Back Again: a test of restoration ecology theory and practice.
415 *Adv. Ecol. Res.* 44, 119–209. <https://doi.org/10.1016/B978-0-12-374794-5.00003-1>
- 416 Feld, C.K., Segurado, P., Gutiérrez-Cánovas, C., 2016. Analysing the impact of multiple
417 stressors in aquatic biomonitoring data: A ‘cookbook’ with applications in R. *Sci. Total*
418 *Environ.* 573, 1320–1339. <https://doi.org/10.1016/j.scitotenv.2016.06.243>
- 419 Graeber, D., Jensen, T.M., Rasmussen, J.J., Riis, T., Wiberg-Larsen, P., Baattrup-Pedersen,
420 A., 2017. Multiple stress response of lowland stream benthic macroinvertebrates depends on
421 habitat type. *Sci. Total Environ.* 599–600, 1517–1523.
422 <https://doi.org/10.1016/j.scitotenv.2017.05.102>
- 423 Graneli, W., 2012. Brownification of Lakes, in: Bengtsson, L., Herschy, R.W., Fairbridge,
424 R.W. (Eds.), *Encyclopedia of Lakes and Reservoirs*. Springer Netherlands, Dordrecht, pp.
425 117–119. https://doi.org/10.1007/978-1-4020-4410-6_256
- 426 Griffen, B.D., Belgrad, B.A., Cannizzo, Z.J., Knotts, E.R., Hancock, E.R., 2016. Rethinking
427 our approach to multiple stressor studies in marine environments. *Mar. Ecol. Prog. Ser.* 543,
428 273–281. <https://doi.org/10.3354/meps11595>
- 429 Hering, D., Carvalho, L., Argillier, C., Beklioglu, M., Borja, A., Cardoso, A.C., Duel, H.,
430 Ferreira, T., Globevnik, L., Hanganu, J., Hellsten, S., Jeppesen, E., Kodeš, V., Solheim, A.L.,
431 Nöges, T., Ormerod, S., Panagopoulos, Y., Schmutz, S., Venohr, M., Birk, S., 2015.
432 *Managing aquatic ecosystems and water resources under multiple stress - An introduction to*
433 *the MARS project*. *Sci. Total Environ.* 503, 10–21.
434 <https://doi.org/10.1016/j.scitotenv.2014.06.106>
- 435 Jackson, M.C., Loewen, C.J.G., Vinebrooke, R.D., Chimimba, C.T., 2016. Net effects of
436 multiple stressors in freshwater ecosystems: a meta-analysis. *Glob. Chang. Biol.* 22, 180–189.
437 <https://doi.org/10.1111/gcb.13028>
- 438 Jeppesen, E., Meerhoff, M., Holmgren, K., González-Bergonzoni, I., Teixeira-de Mello, F.,
439 Declerck, S.A.J., De Meester, L., Søndergaard, M., Lauridsen, T.L., Bjerring, R., Conde-
440 Porcuna, J.M., Mazzeo, N., Iglesias, C., Reizenstein, M., Malmquist, H.J., Liu, Z., Balayla,
441 D., Lazzaro, X., 2010. Impacts of climate warming on lake fish community structure and
442 potential effects on ecosystem function. *Hydrobiologia* 646, 73–90.
443 <https://doi.org/10.1007/s10750-010-0171-5>
- 444 Lange, K., Bruder, A., Matthaei, C.D., Brodersen, J., Paterson, R.A., 2018. Multiple-stressor
445 effects on freshwater fish: Importance of taxonomy and life stage. *Fish Fish.* 19, 974–983.
446 <https://doi.org/10.1111/faf.12305>

- 447 Liess, M., von der Ohe, P.C., 2005. Analyzing Effects of Pesticides on Invertebrate
448 Communities in Streams. *Environ. Toxicol.* 24, 954–965. <https://doi.org/10.1897/03-652.1>
- 449 Malaj, E., von der Ohe, P.C., Grote, M., Kühne, R., Mondy, C.P., Usseglio-Polatera, P.,
450 Brack, W., Schäfer, R.B., 2014. Organic chemicals jeopardize the health of freshwater
451 ecosystems on the continental scale. *Proc. Natl. Acad. Sci.* 111, 9549–9554.
452 <https://doi.org/10.1073/pnas.1321082111>
- 453 McCall, S.J., Hale, M.S., Smith, J.T., Read, D.S., Bowes, M.J., 2017. Impacts of phosphorus
454 concentration and light intensity on river periphyton biomass and community structure.
455 *Hydrobiologia* 792, 315–330. <https://doi.org/10.1007/s10750-016-3067-1>
- 456 Moe, S.J., Dudley, B., Ptacnik, R., 2008. REBECCA databases: experiences from compilation
457 and analyses of monitoring data from 5,000 lakes in 20 European countries. *Aquat. Ecol.* 42,
458 183–201. <https://doi.org/10.1007/s10452-008-9190-y>
- 459 Moe, S.J., Schmidt-Kloiber, A., Dudley, B.J., Hering, D., 2013. The WISER way of
460 organising ecological data from European rivers, lakes, transitional and coastal waters.
461 *Hydrobiologia* 704, 11–28. <https://doi.org/10.1007/s10750-012-1337-0>
- 462 Monteith, D.T., Stoddard, J.L., Evans, C.D., De Wit, H.A., Forsius, M., Høgåsen, T.,
463 Wilander, A., Skjelkvåle, B.L., Jeffries, D.S., Vuorenmaa, J., Keller, B., Kopécek, J., Vesely,
464 J., 2007. Dissolved organic carbon trends resulting from changes in atmospheric deposition
465 chemistry. *Nature* 450, 537–540. <https://doi.org/10.1038/nature06316>
- 466 Moss, B., Kosten, S., Meerhoff, M., Battarbee, R.W., Jeppesen, E., Mazzeo, N., Havens, K.,
467 Lacerot, G., Liu, Z., Meester, L. De, Paerl, H., Scheffer, M., 2011. Allied attack: climate
468 change and eutrophication. *Inl. Waters* 1, 101–105. <https://doi.org/10.5268/IW-1.2.359>
- 469 Nakagawa, S., Schielzeth, H., 2013. A general and simple method for obtaining R^2 from
470 generalized linear mixed-effects models. *Methods in Ecology and Evolution* 4, 133–142.
471 <https://doi.org/10.1111/j.2041-210x.2012.00261.x>
- 472 Nöges, P., Argillier, C., Borja, Á., Garmendia, J.M., Hanganu, J., Kodeš, V., Pletterbauer, F.,
473 Sagouis, A., Birk, S., 2016. Quantified biotic and abiotic responses to multiple stress in
474 freshwater, marine and ground waters. *Sci. Total Environ.* 540, 43–52.
475 <https://doi.org/10.1016/j.scitotenv.2015.06.045>
- 476 Piggott, J.J., Townsend, C.R., Matthaei, C.D., 2015. Reconceptualizing synergism and
477 antagonism among multiple stressors. *Ecol. Evol.* 5, 1538–1547.
478 <https://doi.org/10.1002/ece3.1465>
- 479 Price, K.J., Carrick, H.J., 2016. Effects of experimental nutrient loading on phosphorus
480 uptake by biofilms: Evidence for nutrient saturation in mid-Atlantic streams. *Freshw. Sci.* 35,
481 503–517. <https://doi.org/10.1086/686269>

482 Richardson, J., Miller, C., Maberly, S.C., Taylor, P., Globevnik, L., Hunter, P., Jeppesen, E.,
483 Mischke, U., Moe, S.J., Pasztaleniec, A., Søndergaard, M., Carvalho, L., 2018. Effects of
484 multiple stressors on cyanobacteria abundance varies with lake type. *Glob. Chang. Biol.* 24,
485 5044–5055. <https://doi.org/10.1111/gcb.14396>

486 Richardson, J., Feuchtmayr, H., Miller, C., Hunter, P.D., Maberly, S.C., Carvalho L., 2019.
487 The response of cyanobacteria and phytoplankton abundance to warming, extreme rainfall
488 events and nutrient enrichment. *Glob. Chang. Biol.*, in print. www.doi.org/10.1111/gcb.14701

489 Sabater, S., Ludwig, R., Elozegi, A., 2019. Defining multiple stressor implications. In:
490 Sabater, S., Ludwig, R., Elozegi, A. (Eds.), *Multiple Stress in River Ecosystems. Status,*
491 *Impacts and Prospects for the Future.* Academic Press, Oxford, pp. 1–22.
492 <https://doi.org/10.1016/B978-0-12-811713-2.00001-7>

493 Schäfer, R.B., Kühn, B., Malaj, E., König, A., Gergs, R., 2016. Contribution of organic
494 toxicants to multiple stress in river ecosystems. *Freshw. Biol.* 61, 2116–2128.
495 <https://doi.org/10.1111/fwb.12811>

496 Schäfer, R.B., Piggott, J.J., 2018. Advancing understanding and prediction in multiple
497 stressor research through a mechanistic basis for null models. *Glob. Chang. Biol.* 24, 1817–
498 1826 <https://doi.org/10.1111/gcb.14073>

499 Schinegger, R., Palt, M., Segurado, P., Schmutz, S., 2016. Untangling the effects of multiple
500 human stressors and their impacts on fish assemblages in European running waters. *Sci. Total*
501 *Environ.* 573, 1079–1088. <https://doi.org/10.1016/j.scitotenv.2016.08.143>

502 Schinegger, R., Borja, A., Buijse, T., Chrzanowski, C., Feld, C., Globevnik, L., Hering, D.,
503 Kuijper, M., Lyche Solheim, A., Schmutz, S., Schülting, L., Venohr, M., Birk, S., 2018.
504 MARS recommendations on how to best assess and mitigate impacts of multiple stressors in
505 aquatic ecosystems. Deliverable 8.2 in the EU research project MARS.
506 <https://tinyurl.com/y3f8psu8>

507 Schneider, S.C., Sample, J.E., Moe, J.S., Petrin, Z., Meissner, T., Hering, D., 2018.
508 Unravelling the effect of flow regime on macroinvertebrates and benthic algae in regulated
509 versus unregulated streams. *Ecology* 11, e1996. <https://doi.org/10.1002/eco.1996>

510 Segner, H., Schmitt-Jansen, M., Sabater, S., 2014. Assessing the Impact of Multiple Stressors
511 on Aquatic Biota: The Receptor's Side Matters. *Environ. Sci. Technol.* 48, 7690–7696.
512 <https://doi.org/10.1021/es405082t>

513 Spears, B.M., Ives, S.C., Angeler, D.G., Allen, C.R., Birk, S., Carvalho, L., Cavers, S., Daunt,
514 F., Morton, R.D., Pocock, M.J.O., Rhodes, G., Thackeray, S.J., 2015. Effective management
515 of ecological resilience - are we there yet? *J. Appl. Ecol.* 52, 1311–1315.
516 <https://doi.org/10.1111/1365-2664.12497>

517 Sundermann, A., Gerhardt, M., Kappes, H., Haase, P., 2013. Stressor prioritisation in riverine
518 ecosystems: Which environmental factors shape benthic invertebrate assemblage metrics?
519 *Ecol. Indic.* 27, 83–96. <https://doi.org/10.1016/j.ecolind.2012.12.003>

520 Vinebrooke, R., Cottingham, K., Norberg, M., 2004. Impacts of multiple stressors on
521 biodiversity and ecosystem functioning: the role of species co-tolerance. *Oikos* 3, 451–457.
522 <https://doi.org/10.1111/j.0030-1299.2004.13255.x>

523 von der Ohe, P.C., Goedkoop, W., 2013. Distinguishing the effects of habitat degradation and
524 pesticide stress on benthic invertebrates using stressor-specific metrics. *Sci. Total Environ.*
525 444, 480–490. <https://doi.org/10.1016/j.scitotenv.2012.12.001>

526 van Dijk, G.M., van Liere, L., Admiraal, W., Bannink, B.A., Cappon, J.J., 1994. Present state
527 of the water quality of European rivers and implications for management. *Sci. Total Environ.*
528 145, 187–195. [https://doi.org/10.1016/0048-9697\(94\)90309-3](https://doi.org/10.1016/0048-9697(94)90309-3)

529

530 **Methods**

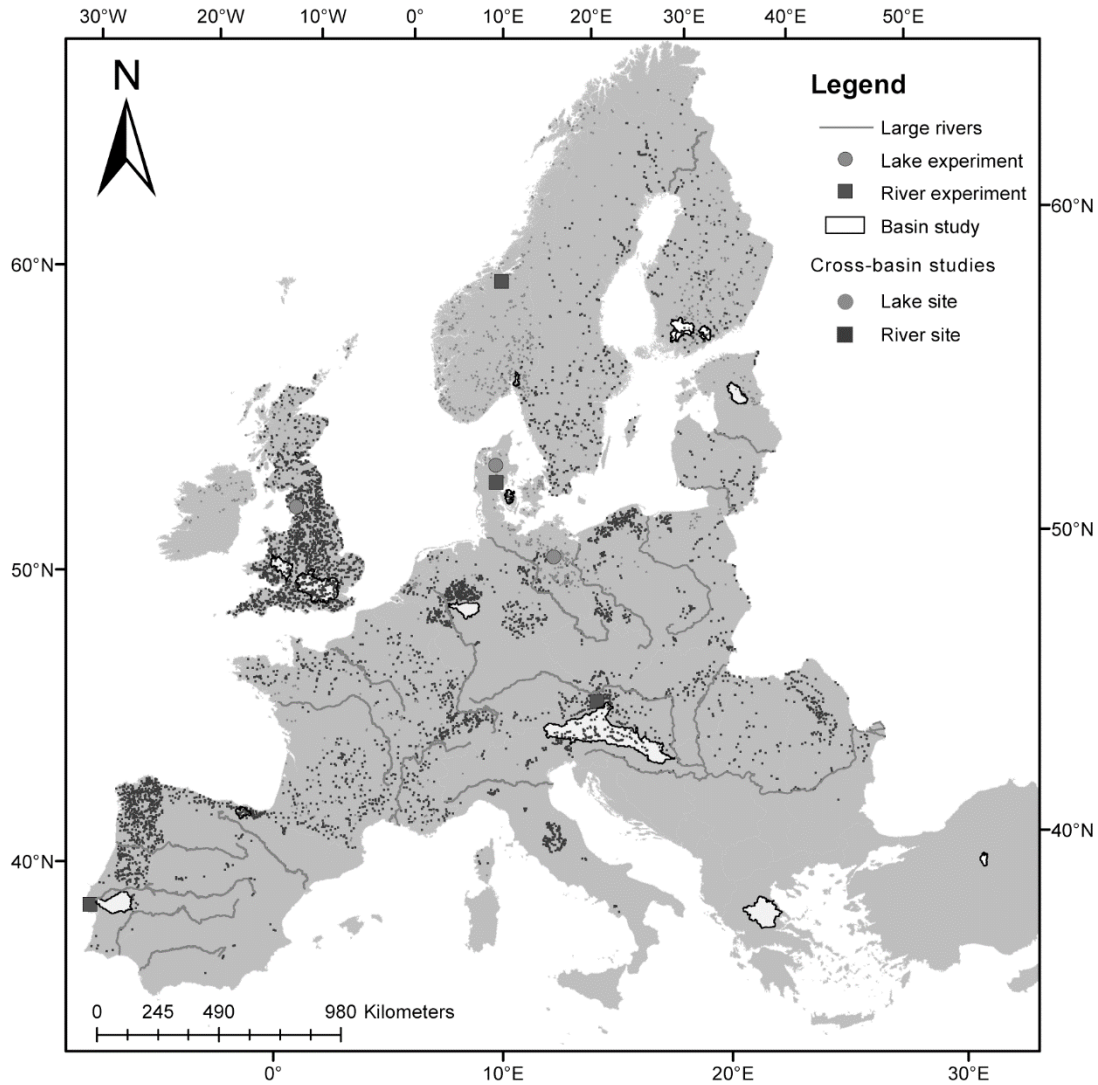
531 *Case studies*

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 (Nöges 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 ^A	0	1
Morphological Toxic	0	5
Morphological Chemical	0	2
Toxic Chemical	0	2

606 ^A 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.